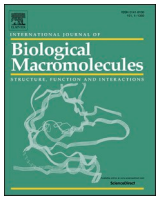
International Journal of Biological Macromolecules 234 (2023) 123675

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac



Effects of high acyl gellan gum on the rheological properties, stability, and salt ion stress of sodium caseinate emulsion

Xingfen He [a], Bin Wang [b], Yuhang Xue [a], Yanhua Li [a], Mingxiang Hu [c], Xingwang He [c], Jie Chen [a,*], Yuecheng Meng [a,*]

[a] School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310018, People's Republic of China
[b] College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, People's Republic of China
[c] Zhejiang Tech-way Biotechnology Co., Ltd., Shaoxing 311811, People's Republic of China

ARTICLE INFO

Keywords:
High acyl gellan gum
Sodium caseinate
Emulsion
Stability
Rheological behaviour
NaCl

ABSTRACT

Sodium caseinate (SC) is widely used as a biological macromolecular emulsifier in oil-in-water (O/W) emulsions. However, the SC-stabilized emulsions were unstable. High-acyl gellan gum (HA) is an anionic macromolecular polysaccharide that improves emulsion stability. This study aimed to investigate the effects of HA addition on the stability and rheological properties of SC-stabilized emulsions. Study results revealed that HA concentrations >0.1 % could increase Turbiscan stability, reduce the volume average particle size, and increase the zeta-potential absolute value of the SC-stabilized emulsions. In addition, HA increased the triple-phase contact angle of SC, transformed SC-stabilized emulsions into non-Newtonian fluids, and effectively inhibited the movement of emulsion droplets. The effect of 0.125 % HA concentration was the most effective, allowing SC-stabilized emulsions to maintain good kinetic stability over a 30-d period. NaCl destabilized SC-stabilized emulsions but had no significant effect on HA-SC emulsions. In summary, HA concentration had a significant effect on the stability of SC-stabilized emulsions. HA altered the rheological properties and reduced creaming and coalescence by forming a three-dimensional network structure, increasing the electrostatic repulsion of the emulsion and the adsorption capacity of SC at the oil-water interface, and thereby improving the stability of SC-stabilized emulsions during storage and in the presence of NaCl.

1. Introduction

The emulsions exhibit thermodynamic instability. During processing and storage, food emulsions destabilise owing to factors such as gravity, composition, and emulsifiers, resulting in phase separation phenomena such as creaming and precipitation of the aqueous phase [1,2]. Therefore, emulsifiers are widely used in food emulsification systems to improve the kinetic stability of the emulsions. On the one hand, an emulsifier can be rapidly adsorbed to the oil-water interface, thereby reducing the interfacial tension and accelerating the splitting of droplets. On the other hand, it can form a resistant interfacial film to produce electrostatic or spatial repulsion, thereby preventing system destabilization due to droplet aggregation [3]. Two major classes of emulsifiers are used in food processing: synthetic emulsifiers (monoacylglycerides, polysorbates, lecithin, etc.) and natural emulsifiers (usually proteins and polysaccharides) [4–7]. Natural food ingredients are in high demand because of the importance of a healthy diet and environmental sustainability [8]. Consequently, natural emulsifiers, such as proteins and polysaccharides of biological macromolecular polymers, have replaced synthetic chemical emulsifiers in increasing numbers.

Proteins contain hydrophobic and hydrophilic groups that can be quickly adsorbed onto the oil-water interface as emulsifiers to increase the stability of the emulsion [8]. Sodium caseinate (SC) has been widely used as an emulsifier for various O/W emulsions [9]. However, SC-stabilized emulsions are prone to destabilization, such as flocculation, when subjected to high temperatures and high ionic strengths [5]. Several studies have investigated the effects of Ca^{2+} and Na^{+} on the stability of SC-stabilized emulsions. The binding of casein to divalent Ca^{2+} results in droplet aggregation and flocculation. Aggregate formation induced by Ca^{2+} affects the stability and adsorption of protein-coated oil-in-water emulsions [12]. NaCl significantly reduced the stability of lemon oil nanoemulsions prepared using SC and Tween 20 [10]. With increasing salt concentration, the zeta potential values of the SC and β-carotene composite emulsions decreased, as did the emulsion

* Corresponding authors.
E-mail addresses: chenjie@zjgsu.edu.cn (J. Chen), myczjgsu@gmail.com (Y. Meng).

https://doi.org/10.1016/j.ijbiomac.2023.123675
Received 5 November 2022; Received in revised form 6 February 2023; Accepted 10 February 2023
Available online 16 February 2023

stability [11]. NaCl is widely used in the preparation of emulsions. Therefore, NaCl was selected to investigate the effect of ionic strength on the stability of SC-stabilized emulsions in our study. Because of the destabilization of SC single emulsions, SC is typically combined with other emulsion stabilisers to improve emulsion stability in the food industry [13]. Recent studies have shown that polysaccharides such as sodium alginate [14], chitosan [15], pectin [16], and λ-carrageenan [17] can improve the stability of SC emulsions at different ionic strengths and pH values. Polysaccharides are added to the emulsion system to prevent emulsion stratification caused by phase separation or gravity by increasing the viscosity of the continuous phase, slowing the speed of droplet movement, and forming a network structure to fix the oil droplets [18,19].

High-acyl gellan gum (HA) is a novel polysaccharide produced by *Sphingomonas elodea*. Its main chain structure is composed of repeating units of four monosaccharides and two substituents: acetyl and glyceroyl [20]. Recently, HA has been gradually studied as an emulsion stabiliser owing to its low concentration (0.05–0.2 % w/w), low viscosity, high stability, and good biocompatibility [3,21,22]. Studies have shown that HA concentrations higher than 0.05 % (w/w) can maintain the stability of the O/W emulsions, and its zeta potential ranges from −50 to −59 mV. This stable state is unaffected by system pH or ionic strength, whereas low-acyl gellan gum (LA) prepared under the same conditions cannot stabilise the emulsion [3]. In addition, high-acyl gellan gum significantly improved the stability of the *Flos Sophorae Immaturus* beverage system by modifying the interface properties [23].

Although HA has been increasingly applied to the stabilization emulsion systems, the effects of different HA concentrations on the properties of SC-stabilized emulsions and the mechanism of emulsion stability from the perspectives of macro-rheology, micro-rheology, interface characteristics, and kinetic destabilization have not been reported. In addition, the effect of HA addition on the rheological properties and stability of SC-stabilized emulsions in the presence of varying NaCl concentrations remains unclear. Therefore, this study aimed to (1) explore the effect of HA on the macro-rheological and micro-rheological properties of SC-stabilized emulsions, (2) screen the optimal HA concentration to improve the stability of SC-stabilized emulsions, (3) determine the kinetic destabilization and storage stability of emulsions, (4) analyse the changes in zeta potential, particle size, and interfacial properties of colloidal materials in emulsions, (5) ascertain the effect of HA on the interfacial microstructure of SC-stabilized emulsions and the emulsification mechanism, and (6) evaluate the effects of different concentrations of NaCl on the rheology and stability of the emulsions. This study will generate new insights for improving the stability of SC-based food emulsion systems and provide technical support and theoretical guidance for the use of HA in emulsification.

2. Materials and methods

2.1. Materials

HA (LT100-P) (weight-average molecular weight: 244,129 Da) was supplied by CP Kelco Inc. (Shanghai, China). SC (weight-average molecular weight: 920782 Da), Oil Red O, Nile red, fluorescein isothiocyanate (FITC), and sodium azide were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Sodium chloride (NaCl; analytical grade), 1,2-propanediol solution (analytical grade), and dimethyl sulfoxide (HPLC grade) were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). Sunflower oil was purchased from a local supermarket (Zhejiang, China).

2.2. Preparation of SC and HA solutions

SC was dissolved in phosphate buffer solution (0.01 M, pH 7.0) and magnetically stirred at room temperature (25 ± 1 °C) for 2 h until completely dissolved. A 2.0 % (w/w) protein solution was obtained by diluting to 100 mL with phosphate buffer (0.01 M, pH 7.0) and incubating overnight at 4 °C to ensure complete hydration of the protein. HA was mixed with phosphate buffer solution (0.01 M, pH 7.0) and dissolved overnight at 25 °C to obtain a 0.35 % (w/w) HA solution. The following day, it was fully swollen by magnetic stirring at 90 °C for 0.5 h (water evaporation loss was compensated for by adding purified water) and cooled to room temperature (25 ± 1 °C) for later use. The prepared HA solution was diluted with purified water to different concentrations, mixed with the SC solution, and stirred magnetically at room temperature (25 ± 1 °C) for 2 h until complete mixing. The final SC concentration in the prepared HA-SC mixture was 0.25 % (w/w), and the HA concentrations were 0, 0.0125, 0.025, 0.05, 0.1, 0.125, 0.15, and 0.175 % (w/w), respectively. The concentrations of HA and SC were fixed at 0.125 % and 0.25 % (w/w), respectively. The samples for salt stability were prepared by adding different amounts of NaCl (0, 50, 100, 150, and 300 mM).

2.3. Preparation of emulsion

The emulsions were prepared according to the method described by Cui et al. [24]. Oil Red O (0.5 %) was dissolved in sunflower seed oil and stirred in darkness to completely dissolve it in the oil phase. An HA-SC mixed solution containing different concentrations of HA was used as the aqueous phase. The water and oil phases were mixed at a volume ratio of 9:1 and pre-emulsified with high-speed shear (FA25, FLUKO, Shanghai, China) at 10,000 rpm for 2 min to prepare a crude emulsion. The crude emulsion was then homogenised at 200 bar for 3 min using a high-pressure homogeniser (APV-1000, Homotech, Beijing, China) to form a fresh emulsion. Approximately 15 mL of fresh emulsion was put into a glass bottle, placed in a constant temperature and humidity incubator (50 % RH, 25.0 ± 0.5 °C), and stored under normal lighting for subsequent testing.

2.4. Rheological measurement

The macro-rheological properties of the emulsions were measured at 25 °C according to a previously described method [25] using a stress-controlled rheometer (AR-G2, TA Instruments, New Castle, Delaware, USA) equipped with a parallel plate (40 mm diameter, 1 mm gap between two plates). The steady-state flow test parameters were set as follows: the shear rate was 0.1–10 s^{-1}, and the number of points per order of magnitude was 10. In addition, the frequency scan test parameters were set as follows: a frequency of 0.1–10 rad/s, logarithmic mode, 10 points per order of magnitude, and 1 % strain. Dynamic stress scanning measurements were used to determine a linear viscoelastic region for the process.

2.5. Diffusing wave spectroscopy (DWS)

The micro-rheological properties of the emulsion were measured in a DWS research laboratory (LS Instruments Inc., Fribourg, Switzerland) equipped with a red laser source (λ = 685 nm, 45 mW), according to the previously described method [26]. The parameters were set as follows: the acquisition and echo durations were both 30 s, the temperature was 25 °C, a cuvette with an optical length of 2 mm was used, and all samples were balanced in a measuring chamber for 60 s before measurement. Data processing software was provided by LS Instruments, Inc. (Fribourg, Switzerland).

2.6. Determination of particle size

According to the previously described method, emulsion particle size and distribution were measured using a laser diffraction particle analyser (Mastersizer 2000, Malvern Instruments, Malvern, UK) [23]. The analysis was performed at room temperature (25 °C). Pure water was used as the dispersant, and the following parameters were set: particle

refractive index of 1.461; particle absorption rate of 0.000, and dispersant refractive index of dispersant 1.330. $D_{[4, 3]}$, or the volume-weighted mean diameter, was used uniformly as a characterisation parameter.

2.7. Triple-phase contact angle measurement

The triple-phase contact angles of the SC and HA-SC complexes were evaluated using the pendent drop method (LSA-100, LAUDA Scientific GmbH, Lauda-Königshofen, Germany). Small amounts of SC and freeze-dried HA-SC composite powders were weighed, and the samples were cut into circular slices with a thickness of 2 mm and a diameter of 13 mm using a tableting machine. The method for obtaining HA-SC composite powders was as follows: the HA-SC mixed solution was prepared as described in Section 2.2 and the mass ratio of HA to SC in the mixed solution was 1:2. The mixture was then freeze-dried for 48 h to obtain HA-SC composite powders. The round flakes were placed in a glass dish containing 15 mL of sunflower oil. Pure water (2 μL) was gently dripped through a high-precision syringe while paying careful attention to removing any air bubbles. The contact angle (θ) was calculated by automatically fitting the profile data of the droplets using OCA 20 software [27].

2.8. Turbiscan test

The Raikos method was used to determine the storage stability of emulsions [28]. Turbiscan Lab (Formulation, Lab expert, France) parameters were set to 25 ± 0.5 °C, scan mode: interval 3 h, and scan duration: five days. The parameters of stability against salt were set as follows: temperature: 25 ± 0.5 °C; scan mode: interval 0.5 h; scan duration: 12 h. To ensure the validity of the data, the emulsion was allowed to stand for 1 h after preparation before data collection began. Approximately 15 mL of the freshly prepared emulsion was put into a transparent glass bottle with a lid and placed in a constant temperature and humidity incubator (SPX-150B-Z, Boxun, China, 50 % RH, 25.0 ± 0.5 °C). The emulsions were left under normal lighting for 30 days, and their stability was observed with a digital camera (Leica, D-LUX7, Germany) and photographed at days 0, 5, 10, 15, 20, and 30.

2.9. Microstructure observation of emulsion

According to the previously described method, the microstructure of the emulsion was observed using an optical microscope (Eclipse E100, Nikon, Minato City, Japan) equipped with a digital camera (KUY NICE, CECCD 01400KPA, China), software (EZ-MET), and a laser confocal microscope (LSM880, Zeiss, Jena, Germany) [29]. First, 1 mL of emulsion was added to 10 μL of Nile red (10 ppm 1,2-propanediol solution) and 10 μL of FITC (10 ppm DMSO solution), vortexed for 5 s, and then incubated for 0.5 h at 30 °C in the dark. The oil and water phases were stained with Nile red and FITC, respectively. Then, 10 μL of the emulsion was dropped onto a 24 × 24 mm cover glass, and Nile red was excited by a 514 nm laser in the range of 549–753 nm. FITC was excited by a 488 nm laser with a receiving range of 490–561 nm.

2.10. Cryogenic-scanning electron microscopy (Cryo-SEM)

A scanning electron microscope (SEM, SU8100, Hitachi High Technologies Corporation, Tokyo, Japan) equipped with cryogenic devices (Alto2500, Gatan UK, Abingdon, Oxfordshire, UK) was used to observe the interfacial microstructure of the emulsion according to the previously described method [30]. First, the emulsion was filled into a metal rivet and quickly frozen in liquid nitrogen (−210 °C). The emulsion was then transferred to a low-temperature preparation chamber for sublimation (−95 °C for 15 min). The inside of the frozen droplets was exposed by slicing them and ejecting them with platinum for 60 s using a 5 mA current. The emulsion was scanned at a temperature of −140 °C and an accelerating voltage of 3 kV.

2.11. Measurement of zeta-potential

The zeta potential of the emulsion was measured using a Zetasizer potentiometer (Nano-ZS, Malvern Instruments, Malvern, UK) according to the method described by Xu et al. [31] with slight modifications. Using the Smoluschwsky function, the instrument software converts electrophoretic mobility measurements of the sample into zeta potential values. Measurement conditions: 633 nm He—Ne laser source, temperature of 25 °C, angle of 173°, and sample refractive index of 1.460.

2.12. Measurement of creaming index

The freshly prepared emulsion (15 mL) was immediately transferred to a transparent glass vial with a diameter of 15 mm and a height of 100 mm. The solution was sealed immediately to prevent evaporation. It was kept in a constant temperature and humidity incubator (50 % RH, 25.0 ± 0.5 °C) under normal lighting for 30 days. The change in the creaming index of the emulsion over 30 days was recorded using a digital Vernier calliper (MNT929310; Minette Industrial, China) [32]. The creaming index (CI) was calculated as follows:

$$CI\Big/\% = \frac{H_S}{H_T} \times 100 \quad (1)$$

where, H_S is the serum layer height (mm) and H_T is the total emulsion height (mm).

2.13. Statistical analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). The results were analysed by one-way ANOVA using SPSS (version 17.0; SPSS Inc., Chicago, IL, USA). Statistical significance was set at a p-value < 0.05. Additional calculations for the analysis of measured data were performed using Origin 2021, the student version (Microcal, Piscataway, NJ, USA).

3. Results and discussion

3.1. Effect of HA on the creaming index of SC-stabilized emulsions

The stability of an emulsion system can be directly assessed by observing and recording its images during storage. By observing the changes in the emulsion's appearance over the course of seven days, it was found that for samples with a HA concentration exceeding 0.1 %, the emulsion system always maintained good stability, with no stratification or sedimentation. After one day of storage, the emulsions with HA concentrations of 0.0125 %, 0.025 %, and 0.05 % began to exhibit slight stratification. The stratification was aggravated on day seven. The emulsion without HA demonstrated obvious stratification and oil floating after one day of storage (Fig. 1A). Previous studies have shown that, at a lower concentration of Na-caseinate (0.1–0.4 wt%), a clear oil-off layer was observed in the o/w primary emulsions [33]. This phenomenon was quantified. The results demonstrated that the creaming index of the emulsion without HA increased gradually with storage time, reaching a maximum after seven days. The creaming index was significantly reduced when 0.1 % or more HA was added, and the creaming index of the emulsion was always 0 within seven days. However, adding 0.0125 %, 0.025 %, and 0.05 % HA increased the creaming index, with the maximum value reaching 50.12 % when HA was added at a concentration of 0.05 % (Fig. 1B). Previous studies have revealed that adding a low concentration of kappa-carrageenan (0.05 % to 0.1 %) destabilizes SC-stabilized emulsions and causes the phenomenon of creaming. Furthermore, it has been reported that carrageenan can form covalent or noncovalent complexes with NaCas to improve emulsion stability [17]. Carrageenan and high-acyl gellan gum are similar anionic

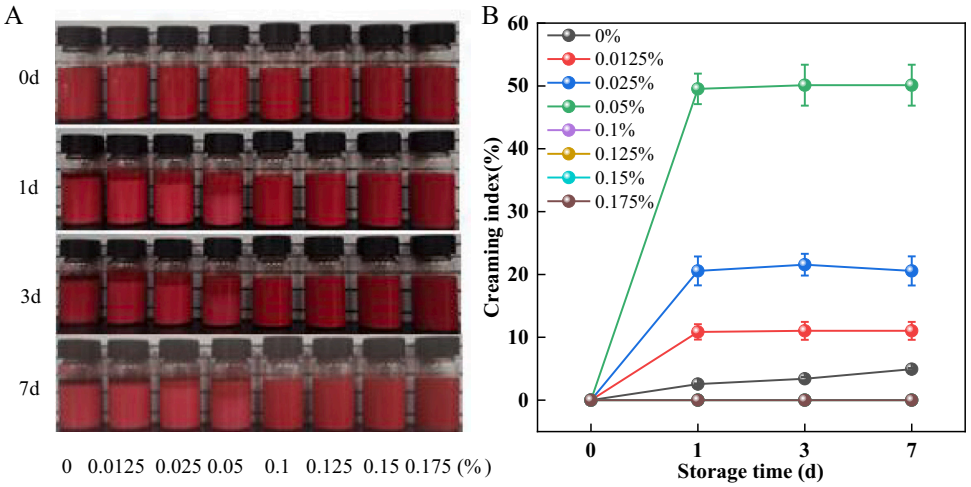


Fig. 1. Effect of HA concentration on the (A) morphological appearance and (B) creaming index of SC emulsion. Bars indicate standard error.

polysaccharides. In addition, this phenomenon was prevented by the addition of HA at the optimal concentration. However, the mechanism underlying these phenomena requires further investigation.

3.2. *Effect of HA concentration on the stability of emulsions*

The reference spectra of backscattered light produced by the Turbiscan Lab are often used to measure the stability of emulsion systems [34]. There was no significant change in the ΔBS of HA-SC emulsions when HA concentrations >0.05 % were added (Fig. 2A). The ΔBS of the emulsions was further analysed to determine their Turbiscan stability index (TSI). The smaller the TSI, the more stable the emulsion [35]. When the HA concentration exceeded 0.1 %, the TSI values of the entire, top, and bottom regions of the emulsions were significantly reduced (Fig. 2B). Furthermore, the slope of the kinetic destabilization curve was significantly lower for emulsions with HA concentrations >0.1 % (Fig. 2C). Similar results were obtained by Kartal et al. [33] in their study of the effect of pectin concentration on the stability of SC-

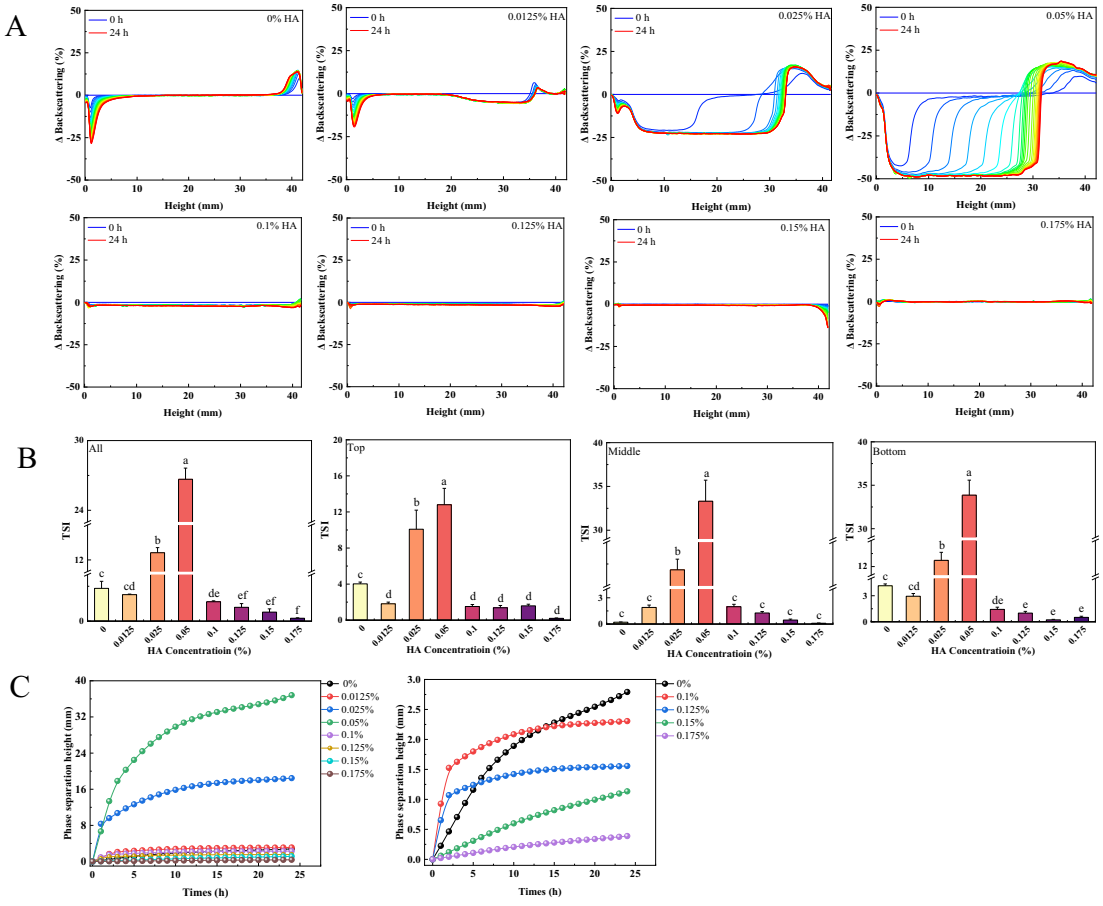


Fig. 2. Effect of HA concentration on the (A) ΔBS, (B) TSI, and (C) kinetic instability curves of SC emulsions. Bars indicate standard error. Different letters indicate significant differences ($P < 0.05$).

stabilized emulsions, i.e., higher concentrations of pectin improved emulsion stability, whereas substantial bridging flocculation and emulsification of droplets were observed when the pectin concentration was below 0.5 %. This is due to the fact that, at medium concentrations, increasing the effective volume of the particles through droplet flocculation is greater than increasing the viscosity of the continuous phase, which leads to a creaming phenomenon in the system. At pH 7.0, due to the negative charges of HA and SC, repulsive interactions developed between HA and SC, and little HA adsorbed on the droplet surface; hence, unabsorbed HA might induce depletion flocculation at lower HA concentrations (0.01 %–0.05 %). At higher polysaccharide concentrations, droplet movement was blocked, and creaming was inhibited owing to the increase in viscosity and formation of the gel network structure. This demonstrates that creaming varied when the HA concentration was <0.1 %. When the concentration of HA was greater than or equal to 0.1 %, creaming was effectively inhibited, and emulsion stability was significantly increased. The emulsion stability was highest at a 0.125 % concentration.

3.3. Effect of HA on particle size, zeta-potential and interfacial properties of emulsions

The particle size of an emulsion is an important index for evaluating its stability. The smaller the particle size, the more uniform the distribution, and the better the emulsion stability. When the particle size and variation increase, the stability of the emulsion system decreases, making it more prone to flocculation, creaming, and other unstable phenomena [36]. The particle size distributions of the SC-stabilized and HA-SC emulsions were bimodal. In addition, compared with the SC-stabilized emulsions, except for the HA emulsion with a concentration of 0.05 %, the peak particle size distribution of the HA-SC emulsion shifted to the left (Fig. 3A). In addition, the $D_{[4,\ 3]}$ of the SC-stabilized emulsions was 1.93 μm. When the addition of HA was >0.1 %, the emulsion particle size decreased significantly. When the amount of HA added was 0.125 %, the $D_{[4,\ 3]}$ of the emulsion decreased to a minimum of 1.00 μm, which was significantly lower than that of the control group 48.19 % ($P < 0.05$) (Fig. 3B). This result is similar to that of emulsions formed by welan gum polymers [37]. Previous studies have shown that the average particle size of the prepared SC-stabilized emulsions gradually decreases as the concentration of xanthan gum increases in the medium. However, as the pectin concentration increased from 0.1 % to 0.5 %, the average size of SC-stabilized emulsions increased slightly, which could be attributed to the slight flocculation that occurred in the original samples [38]. These contradictory results may be related to the emulsifier used, polysaccharide type, formulation, and emulsion preparation method. Therefore, HA is preferred over pectin for improving the stability of SC emulsions under neutral conditions. This study also found that HA addition inhibited the aggregation and sedimentation of droplets by increasing the viscosity of the emulsion and promoting the formation of a gel network structure, which helped increase the proportion of small droplets in the emulsion.

The zeta potential is a vital index for characterising the stability of an emulsion system. The results revealed that the absolute value of the zeta potential of the HA-SC emulsion increased with increasing HA concentration. For example, when the additional amount of HA was 0.175 %, the maximum value was 47.93 mV, which was significantly higher than the 18.21 % of SC-stabilized emulsions (Fig. 3C). This result is similar to the previous anionic polysaccharide alginate increase in emulsion zeta potential [18]. In addition, the addition of gellan gum increased the zeta potential of soy protein isolate, mung bean protein isolate, and pea protein isolate-stabilized emulsions [39]. The emulsion stability against creaming depends on the strength of the interaction between the droplets, which is determined by the charge on their surface. Previous studies have shown that the smaller the molecules or dispersed particles in an emulsion system, the more charge the molecules carry on the surface, the greater the repulsive force between the particles, and the more stable the system [40]. In this study, we also found that the addition of HA significantly decreased the average particle size of the emulsion, which helped increase the repulsive force between the molecular particles. Furthermore, at high HA concentrations, the net charge on the protein–polysaccharide complexes becomes sufficiently large such that electrostatic repulsion between the adsorbed complexes stabilises the emulsion system. The results indicated that the addition of HA increased the zeta potential of SC-stabilized emulsions and improved their stability.

The wettability of the hydrocolloid material at the oil-water interface, that is, the triple-phase contact angle, has a significant effect on the stability of the emulsion. In this study, we found that the triple-phase

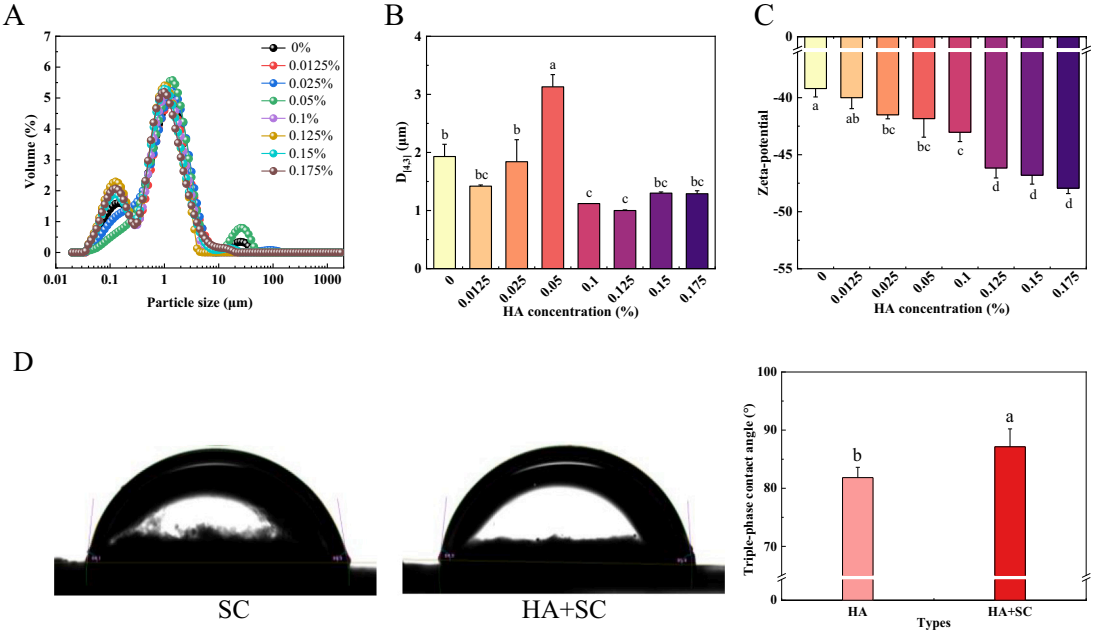


Fig. 3. Effect of HA concentration on (A) particle size distribution, (B) volume-weighted mean diameter ($D_{[4,\ 3]}$), (C) zeta potential, and (D) three-phase contact angle of SC emulsion. Bars indicate standard error. Different letters indicate significant differences ($P < 0.05$).

contact angle of SC was 81.83°, whereas that of HA-SC complex was 87.13°, significantly higher than that of SC by 6.5 % (Fig. 3D). These results are similar to those of previous studies on the effect of gum Arabic on the triple-phase contact angle of corn gluten [41]. According to previous studies, the closer the triple-phase contact angle is to 90°, the stronger is the particle adsorption, which can effectively prevent the aggregation of oil droplets and stabilise the prepared emulsion [30]. Moreover, HA is a hydrocolloid that regulates the wettability of HA-SC colloidal materials via electrostatic interactions [42]. Therefore, the results indicate that the addition of HA facilitates the adsorption of SC to the water-oil interface. The high contact angle of HA-SC endowed it with a balanced amphiphilicity.

3.4. Rheological properties of emulsions

3.4.1. Effect of HA on the macro-rheological properties of emulsions

The rheological behaviour of emulsions significantly affects their physicochemical properties, processing, and utilisation. The macro-rheological properties of SC-stabilized emulsions demonstrated that the viscosity of SC-stabilized emulsions hardly changed with shear rate and was always close to zero. However, after the addition of HA, the viscosity of the HA-SC emulsions gradually decreased with increasing shear rates (Fig. 4A). As the shear rate increased sufficiently to overcome Brownian motion, the emulsion droplets were arranged in an orderly manner along the flow field, resulting in less resistance to the flow and a lower viscosity. However, with an increasing shear rate, the decrease in viscosity could indicate floc disruption. Additionally, it has been reported that droplet deformation or deflocculation on the dispersed phase, as well as non-Newtonian behaviour of the continuous phase, can cause shear-thinning behaviour in emulsions [43]. Small and uniform droplets in emulsions with higher HA concentrations resulted in a more structured system, which increased consistency and shear-sensitive behaviour. Previous studies have shown that in the absence of xanthan, SC-stabilized emulsions exhibit Newtonian-like behaviour with low apparent viscosities as a function of shear rate, indicating that the SC single emulsion is a Newtonian fluid, whereas the HA-SC emulsion is a non-Newtonian fluid [44]. In addition, at a relatively high shear rate, the emulsion polymer network disentangled and individual macromolecules aligned in the direction of shear occurred, resulting in a decrease in emulsion viscosity, which increased with increasing HA concentration (Fig. 4A). This indicates that the HA concentration significantly affects the viscosity of the continuous phase. Previous studies have shown that the protein on the surface of the oil droplets interacts with the polysaccharide in the continuous phase, increasing the viscosity of the emulsion. In addition, our study found that the mobility of HA-SC emulsion droplets was significantly reduced and that they were more densely distributed, resulting in greater deformation resistance and increased viscosity [15].

The emulsion modulus results demonstrated that the storage modulus (G′) of all samples was higher than the loss modulus (G′) in the frequency range of 0.1–10 rad/s, and the G′ and G″ of the emulsion increased with increasing HA concentration (Fig. 4B and C). This indicates that adding HA forms a gel-like network structure in the emulsion, and the solid-like elastic behaviour dominates the emulsion rather than the liquid-like viscous behaviour. Liu et al. [45] also observed this viscoelastic property when studying the effects of flaxseed gum and NaCl concentration on the stability of O/W emulsions. Previous studies have shown that polysaccharides can reduce the mobility of macromolecules in networks [46]. In addition, anionic polysaccharides can promote continuous adsorption of protein molecules at the oil-water interface by increasing negative charge repulsive interactions and forming more rigid elastic interfacial films [47]. Therefore, we speculated that the addition of HA may help form a stronger emulsion network against coalescence and flocculation. In summary, the increase in HA concentration enhanced the gel-like network structure and increased the emulsion viscosity.

3.4.2. Effect of HA on the micro-rheological properties of emulsions

Diffusion-wave spectroscopy (DWS) fills the gap in macro-rheology by preserving the fine structure of the emulsion [48]. This study found that the addition of HA shifted the correlation function to shift to the right, and the higher the HA concentration, the longer the decay time (Fig. 5A). This corresponded to the shift in the correlation function of the whey protein emulsion after the addition of flaxseed gum [49]. This shows that an increase in HA concentration decreases the diffusion of droplets in an emulsion. Previous studies have shown that an increase in the continuous phase viscosity causes a rightward shift in the emulsion correlation function and a prolongation of the decay time [50]. Considering the above macroscopic rheological findings, we believe that HA inhibits droplet diffusion in emulsions by increasing the viscosity of the continuous phase. The mean square displacement (MSD) of the sample during the lag time can be used to characterise the Brownian motion of the particles within the sample [51]. The results demonstrated that the MSD of all samples increased with the decay time and demonstrated a linear relationship with time (Fig. 5B).Furthermore, the MSD curve of the emulsion gradually decreased from 0.99 to 0.52 with the increase in HA concentration (Table S1). Previous studies on the effect of preparation methods on the micro-rheological properties of ovalbumin/carboxymethyl cellulose composite emulsions found that when the slope of the MSD curve was <1, the emulsion droplets exhibited sub-diffusion behaviour in the system and their Brownian motion was inhibited [26]. Therefore, we believe that HA effectively suppresses the Brownian motion of emulsion droplets.

DWS was used to measure the viscosity of the emulsion in the dynamic mode. The results showed that the viscosity of the HA-SC emulsion decreased with increasing frequency, whereas that of the SC-stabilized emulsions remained unchanged. In addition, the HA concentration increased the viscosity of the emulsion (Fig. 5C). This is

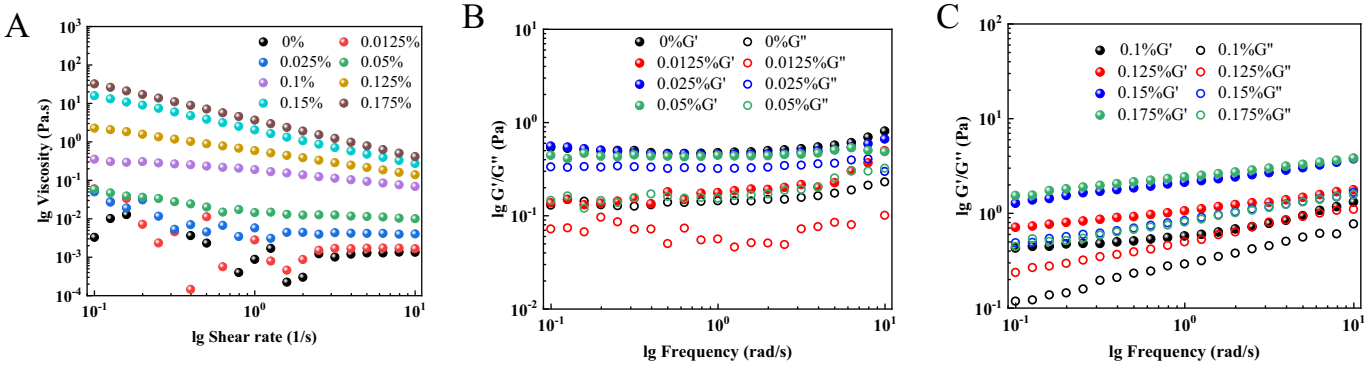


Fig. 4. Effect of HA concentration on (A, B) viscosity and (C, D) modulus of SC emulsion.

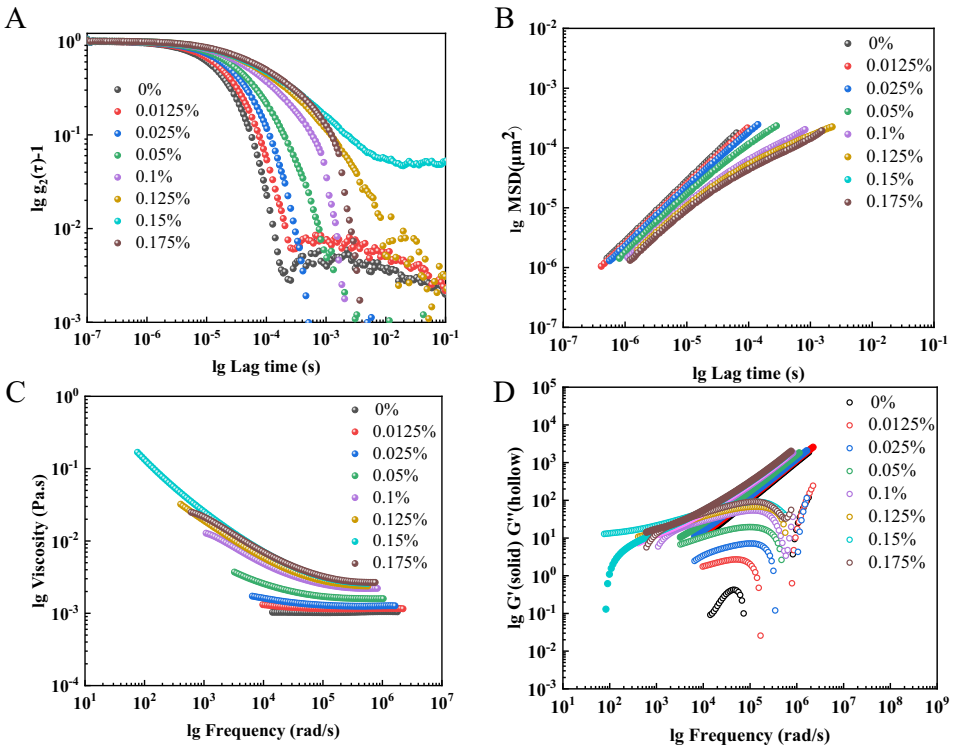


Fig. 5. Effect of HA concentration on (A) intensity correlation function $g^2(\tau) - 1$ vs. time, (B) mean square displacements curve, (C) viscosity as a function of frequency, and (D) viscoelastic parameters as a function of the frequency of SC emulsion.

consistent with the macro-rheological results. The SC-stabilized emulsions are Newtonian fluids, whereas the HA-SC emulsions are non-Newtonian fluids [52]. Furthermore, when the amount of HA added was <0.1 %, the G″ of the emulsion was always greater than G′, and there was no crossover. When the added amounts of HA were 0.1 %, 0.125 %, 0.155 %, and 0.175 %, the two moduli of the emulsion crossed at a high frequency (>10^3), and G′ was always greater than G″ after the crossing point (Fig. 5D). When DWS was used to characterise an O/W emulsion stabilized by sodium dodecyl sulphate (SDS), it was discovered that the emulsion exhibited elastic properties (G′ > G″) when the angular frequency was <10 rad/s. In contrast, above the threshold frequency, the emulsion exhibited viscous behaviour (G″ > G′) [53]. This finding differs from the results of the present study. This could be because the emulsion with <0.1 % HA is flocculated inside, resulting in viscous behaviour at high frequencies. It was concluded that adding HA below 0.1 % was insufficient to stabilise the HA-SC emulsion. In order to stabilise the HA-SC emulsion, it was determined that adding <0.1 % HA was insufficient.

3.5. *Effect of HA on the interfacial microstructure of emulsions*

Based on the above results and the fact that 0.125 % HA stabilized

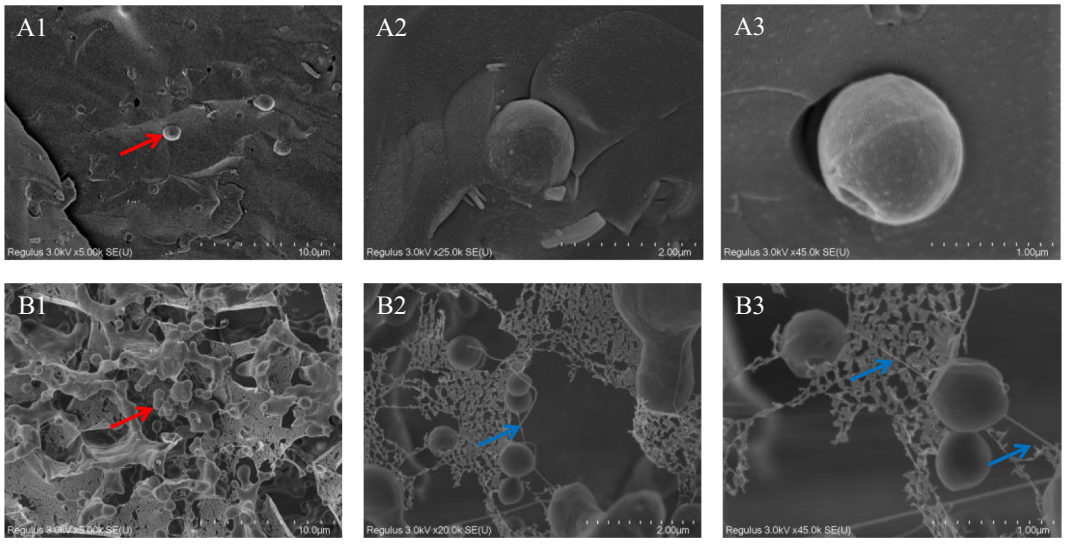


Fig. 6. Cryo-scanning electron microscope morphology of (A) SC emulsion and (B) HA-SC emulsion (the Sub-index 1, 2 to 3 indicated an increase in the magnification. Red and blue arrows pointed to emulsion droplets and network structure, respectively). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the emulsion, this concentration was chosen for further research. The distribution of emulsifiers on the surfaces of emulsion droplets was observed using cryo-SEM [54]. This study demonstrated that the droplets of SC and HA-SC emulsions containing 0.125 % HA were approximately smooth spheres, but that there were significant differences in their sizes and interfacial structures. The SC-stabilized emulsion droplets were significantly larger than the HA-SC emulsion droplets, and there was no distinct network structure around the droplets. However, in the HA-SC emulsion, the sugar chains of HA were tightly adsorbed on the surface film of the emulsion droplets, and the adjacent droplets formed a dense three-dimensional (3D) network structure around the droplets (Fig. 6A, B). Previous studies have shown that the stabilization mechanism in oil-in-water emulsions prepared with SC/gellan gum mixtures is attributed to the three-dimensional network formed by the polysaccharide in the aqueous phase, which is sufficient to trap oil particles, thus avoiding particle aggregation [55]. The three-dimensional network structure of HA prevented the adjacent emulsion droplets from flocculating and coalescing by acting as a space barrier.

3.6. Effect of HA on the storage stability of emulsions

Storage stability is an important factor that affects the shelf life of emulsified foods. Changes in the appearance of emulsions were recorded during the 30 days of storage. The results showed that the creaming and water layers appeared on the fifth day of storage for SC-stabilized emulsions, and they were aggravated by prolonged storage time. During the 30-day storage period, however, the HA-SC emulsion maintained excellent stability, with no stratification, creaming, or sedimentation occurring (Fig. 7A). The microscopic morphology of the emulsions was observed. On day 0, the droplets of the SC-stabilized emulsions were unevenly distributed and varied in size, whereas those of the HA-SC emulsions were uniformly distributed and evenly sized. After 30 days of storage, the droplet distribution of the SC-stabilized emulsions became more uneven and increased significantly, whereas the droplet distribution of the HA-SC emulsion remained uniform and increased slightly, but by a far lesser amount than that of SC (Fig. 7B). This is probably because protein molecules are large and have a complex structure, their hydrophobic amino acids are locally unfolded and rearranged, and the exposure of protein hydrophobic groups is increased

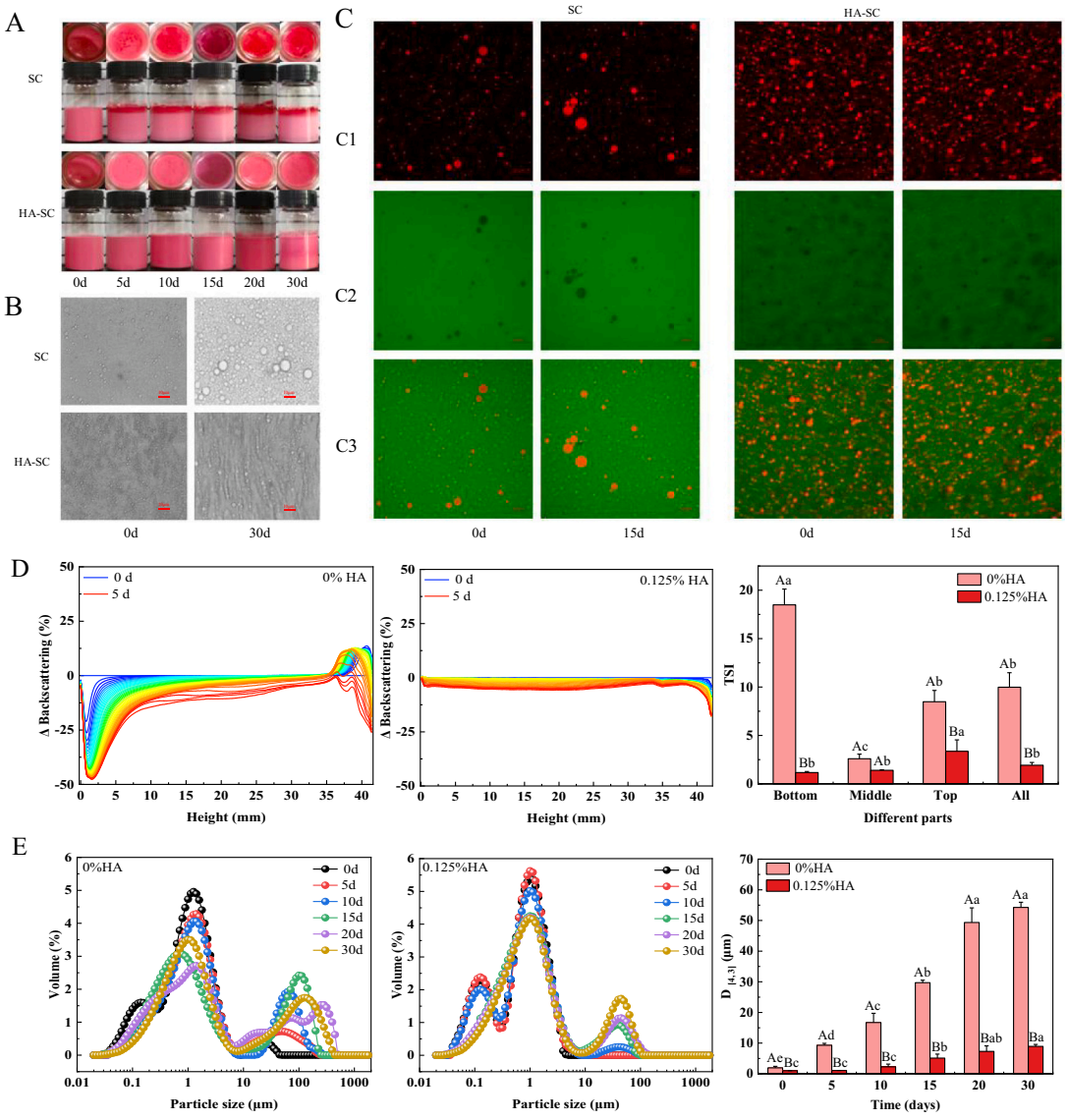


Fig. 7. Effect of HA concentration on (A) the morphological appearance in 30 d, (B) the droplet distribution in optical microscope field, (C) the droplet distribution in confocal laser scanning microscope (CLSM) field, (D) the dynamic instability in 5 d, and (E) the particle size in 30 d of SC emulsion. Bars indicate standard error. Different letters indicate significant differences ($P < 0.05$).

by the synergistic effect of the protein with the polysaccharide, thereby increasing the storage time. The creaming, damping, and aggregation of the emulsion led to an increase in particle size.

Sunflower oil was stained with Nile red, and visible red droplets were observed using confocal laser scanning microscopy (CLSM) (Fig. 6C1). SC was dissolved in the aqueous phase and stained green by FITC (Fig. 7C2). These results illustrated that the prepared emulsions were typical O/W emulsions [56]. All the emulsion droplets remained spherical throughout the storage period. In the SC-stabilized emulsions, the oil droplets were relatively large and unevenly distributed, and their size increased significantly after 15 days of storage. However, the oil droplets of the HA-SC fresh emulsion and the emulsion stored for 15 days were evenly distributed, and the size of the oil droplets was significantly smaller than that of the SC-stabilized emulsions (Fig. 7C3). In addition, there were obvious black irregular areas in the HA-SC emulsion, which may have been caused by the presence of sugar chains that led to the flocculation of droplets by bridging [57]. These results indicate that the addition of HA significantly affects droplet distribution and size.

The storage stability of the emulsions was evaluated using a Turbiscan. During a five-day storage period, a concave peak appeared at the bottom of the ΔBS spectrum of the SC-stabilized emulsions, a convex peak appeared at the top, and the ΔBS value in the middle region decreased with time. The ΔBS spectrum of the HA-SC emulsion showed no obvious fluctuations, and the ΔBS value decreased slightly with time. The TSI values for the top, middle, bottom, and all HA-SC emulsions during the five-day storage period were significantly lower than those of the SC-stabilized emulsions, as calculated from the BS values (Fig. 7D). This indicated that the addition of HA significantly improved the 30-day storage stability of the SC-stabilized emulsions. It has been demonstrated that the electrostatic adsorption of colloids onto SC can form a thick layer, resulting in spatial/electrostatic repulsion and an increase in emulsion viscosity, thereby improving emulsion stability [5].

The emulsion particle size is also a key factor affecting emulsion stability [58]. During the 30-day storage period, the particle size distribution of both SC and HA-SC emulsions shifted to the right, but the right shift of the HA-SC emulsion was significantly smaller than that of the SC-stabilized emulsions. During the 30-day storage period, the $D_{[4,3]}$ of SC-stabilized emulsions increased by 27.1 times, from 1.93 μm to 54.25 μm. However, the $D_{[4,3]}$ of HA-SC emulsion increased from 1.0 μm to 8.94 μm, only increasing by 7.94 times (Fig. 7E). This indicated that the addition of HA effectively inhibited the increase in particle size of the SC-based emulsion over a 30-day storage period. Previous studies have demonstrated that the formation of protective coatings by anionic polysaccharides can increase the aggregation stability of protein-coated oil droplets [59]. Under neutral pH conditions, both HA and SC were negatively charged, generating electrostatic repulsion. As a thickener, HA can increase the viscosity of the continuous phase and form a weak gel structure, thereby reducing the flocculation of droplets and effectively reducing the increase in the particle size of the emulsion over a long storage period [17]. Therefore, we speculate that HA's inhibition of the emulsion particle size increase during storage is related to the electrostatic repulsion between HA and SC, and the gel structure formed.

3.7. Effect of NaCl on the stability and rheological properties of emulsions

3.7.1. Effect of NaCl on the creaming index and zeta-potential of emulsions

SC-stabilized emulsions are susceptible to NaCl, resulting in unstable phenomena such as creaming and flocculation [5]. As illustrated in Fig. 8A, the SC emulsion exhibited an obvious creaming phenomenon during the storage period of 30 days. A distinct separation of the cream layer at the top and a transparent serum layer at the bottom was observed in the SC-stabilized emulsions. The occurrence of creaming was exacerbated by the addition of NaCl, and the severity increased with increasing NaCl concentration. On the 20th day of storage, the emulsions containing NaCl completely separated the oil and water phases. However, in the presence of 0.125 % HA, different NaCl concentrations did not cause creaming of the emulsion during the 30-day storage period. The results demonstrated that adding NaCl increased the creaming index of SC-stabilized emulsions in the first 10 days of storage and then gradually stabilized. Different NaCl concentrations significantly affected the creaming index ($P < 0.05$). The SC-stabilized

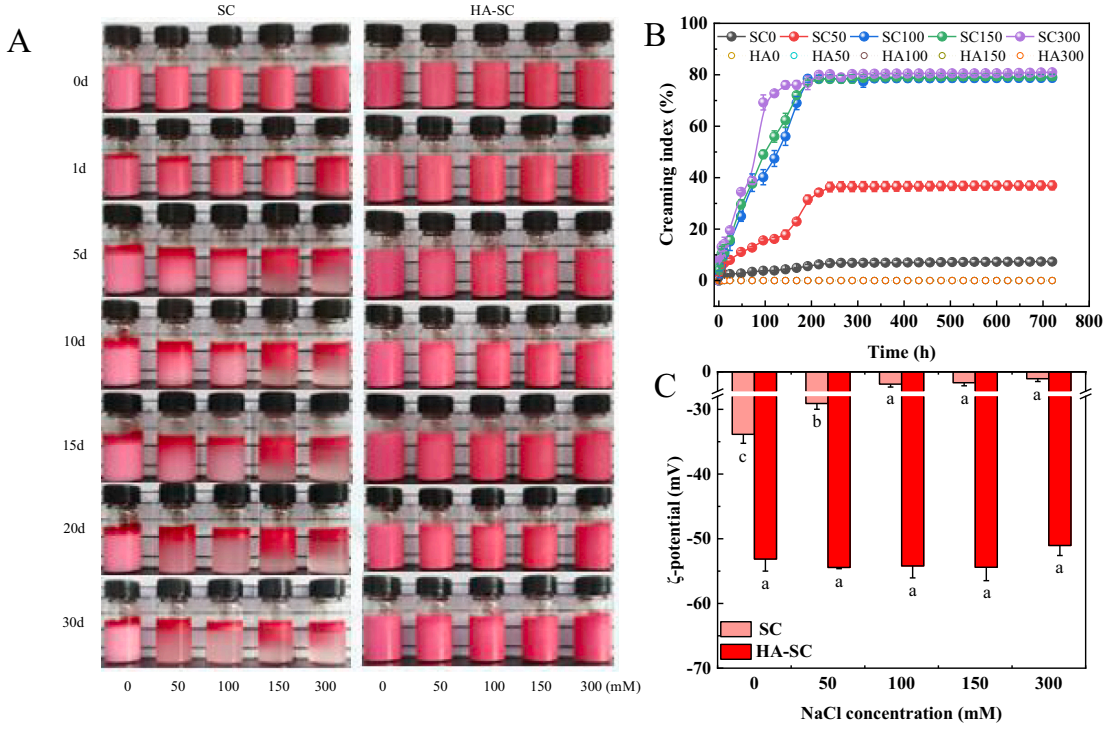


Fig. 8. Effect of NaCl on (A) morphological appearance, (B) creaming index, and (C) zeta potential of HA-SC emulsion. Bars indicate standard error. Different letters indicate significant differences ($P < 0.05$).

emulsions without NaCl had the lowest creaming index, while 300 mM NaCl had the highest creaming index. The creaming index of HA-SC emulsion remained low regardless of the concentrations of NaCl (Fig. 8B). The results demonstrated that the NaCl concentration had a significant effect on the stability of SC-stabilized emulsions, and the addition of HA mitigated this effect. The translucent phase was attributed to the rapid creaming of flocculated droplets, resulting in a serum-depleted phase of the oil [60]. Liao et al. [38] also reported that adding pectin can improve the properties of SC-stabilized emulsions against 0–200 mM ionic strengths at neutral pH; however, when the salinity was higher than 200 mM NaCl, the particle size of the SC-stabilized emulsions increased significantly. Previous studies have shown that differences in gravity and density are important factors in creaming, causing oil droplets to move upward and form a cream layer at the top of the emulsion [61]. The addition of HA can result in the formation of a weak network structure in the emulsion, impeding the movement and aggregation of droplets and thereby inhibiting the occurrence of creaming.

Furthermore, the absolute value of the zeta potential of SC-stabilized emulsions decreased gradually with the NaCl concentration, with the lowest value being 1.06 mV at a concentration of 300 mM NaCl. The absolute value of the zeta-potential of the emulsion after adding 0.125 % HA was at a high level, between 51.03 and 54.43 mV, and was not significantly affected by the NaCl concentration (Fig. 8C). The results demonstrated that the addition of HA to the emulsion maintained the high absolute value of the zeta potential. Previous studies have shown that when the absolute value of the zeta potential of the emulsion is >30 mV, emulsion droplets can be prevented from aggregating and their stability can be increased through electrostatic repulsion [62]. The SC-stabilized emulsion was destroyed by 50 mM NaCl. However, the HA-SC emulsion remained stable in the presence of high NaCl concentrations, probably because of the adsorption of Na^+ onto the negatively charged polysaccharide rather than the protein layer [63]. In summary, we believe that the addition of HA increases the stability of SC-stabilized emulsions in the presence of NaCl by inhibiting droplet movement and maintaining a high zeta potential absolute value.

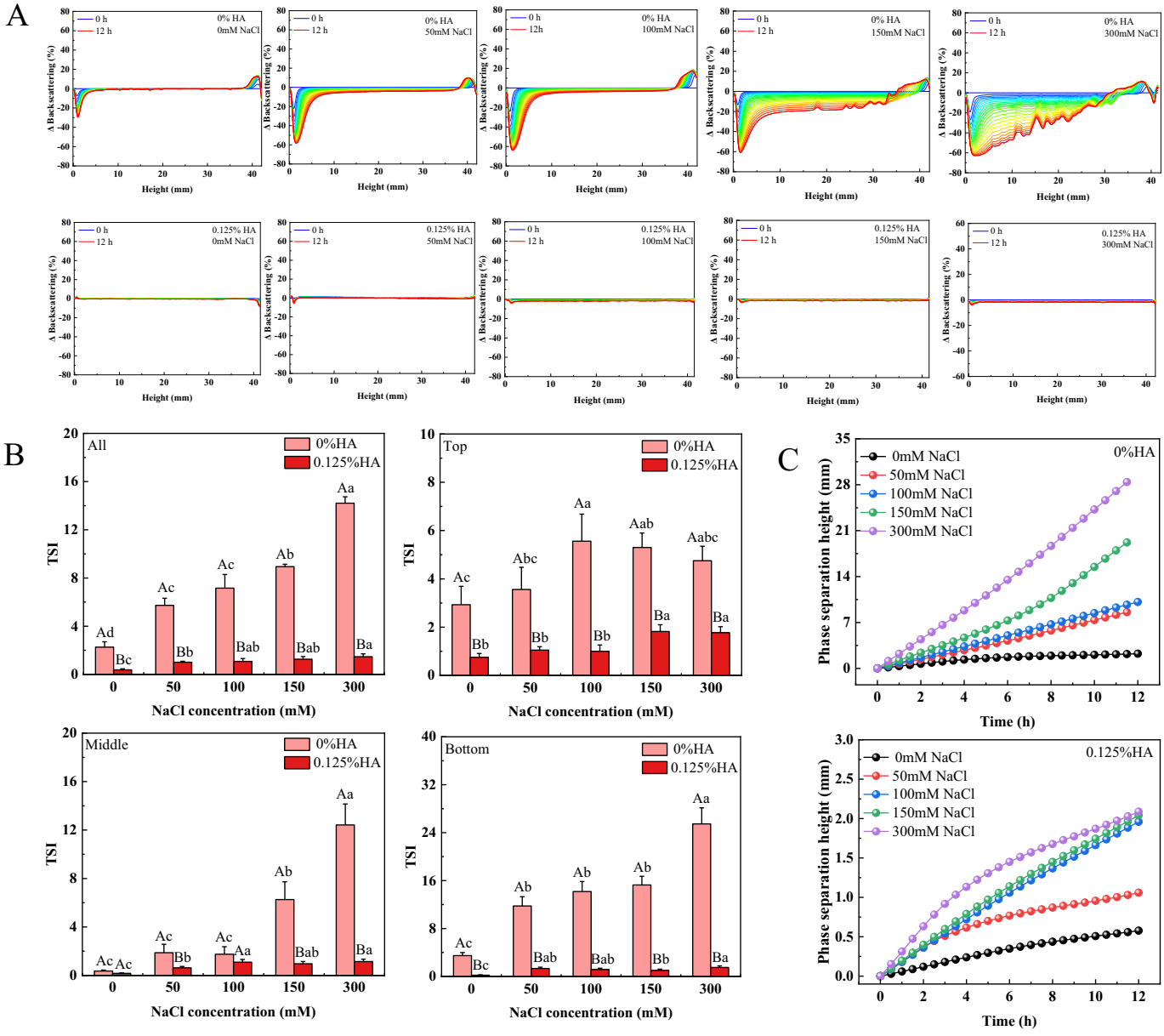


Fig. 9. Effect of NaCl on (A) ΔBS, (B) kinetic instability curves, and (C) TSI of HA-SC emulsion. Bars indicate standard error. Different letters indicate significant differences ($P < 0.05$).

3.7.2. Effects of NaCl on emulsion stability

The change in ΔBS reflects the homogeneity of the emulsion, droplet size, and concentration, and is therefore used to determine the stability of the emulsion system [64]. The ΔBS spectra of the SC-stabilized emulsions exhibited a concave peak in the bottom region and a convex peak in the top region, and the peak value increased with increasing NaCl concentration. However, there were no concave or convex peaks in the entire test area of the HA-SC emulsions, and the ΔBS curve almost overlapped and was not affected by the NaCl concentration (Fig. 9A). Moreover, the TSI values of SC-stabilized emulsions gradually increased as NaCl concentration increased. However, at the same NaCl concentration, the TSI of the HA-SC emulsion was significantly lower than that of the SC-stabilized emulsion. When NaCl concentration was 300 mM, the TSI values of all, bottom, middle, and top of the HA-SC emulsion were 1.47, 1.51, 1.14, and 1.77, respectively, which were significantly lower than those of SC-stabilized emulsions 89.66 %, 94.07 %, 90.82 %, and 62.74 % ($P < 0.05$) (Fig. 9B). Similar results were obtained for the dynamic destabilization curves (Fig. 9C). The influence of salt addition on the compatibility of macromolecules in emulsions can be explained by the effects of "salting out" events. Salting out occurs at higher salt concentrations, resulting in a decrease in protein solubility owing to protein and salt ion competition for water molecules. As a result, water in the continuous phase becomes a poor solvent for proteins, leading to protein–protein associations. Consequently, the electrostatic interactions are weakened, and steric repulsion alone is insufficient to prevent the emulsion droplets from coming into close proximity [65]. Previous studies have found that when the TSI is >3.0, emulsion creaming occurs due to emulsion precipitation [66]. At different NaCl concentrations, the TSI value of the SC-stabilized emulsions increased significantly and was always >3, but the TSI value of the HA-SC emulsion was always lower than 3. The results revealed that the concentration of NaCl had a significant effect on the ΔBS and TSI values of SC-stabilized emulsions, while the addition of HA significantly inhibited the fluctuation of ΔBS and the increase in TSI value of SC-stabilized emulsions, maintaining them at stable and low levels, respectively.

3.7.3. Effect of NaCl on the macro-rheological properties of emulsions

The rheological properties of the emulsions affected their stability, and the viscosity of the SC-stabilized emulsions remained close to zero, independent of the NaCl concentration and shear rate [67] (Fig. 10A). The HA-SC emulsion had a higher viscosity than the SC-stabilized emulsions, and the addition of NaCl increased the viscosity of the HA-SC emulsion (Fig. 10B).These results indicate that different concentrations of NaCl can improve the viscosity of the HA-SC emulsions. This finding was similar to that reported by Wei et al. [68]. Increasing the ionic strength also reduces the electrostatic repulsion and space repulsion between droplets, thereby increasing viscosity.

The modulus effectively measured the emulsifying properties of the emulsion systems [39]. At low frequencies (>1 rad/s), G′ of the SC-stabilized emulsions was always greater than G″. When the NaCl concentration was >150 mM, G′ and G″ crossed at a high frequency (Fig. 10C). The results showed that the SC-stabilized emulsions exhibited elastic behaviour when the NaCl concentration was <150 mM. When the NaCl concentration exceeded 150 mM, the SC-stabilized emulsions exhibited liquid-like properties and better fluidity. The modulus of the HA-SC emulsion increased with increasing frequency. G′ was always greater than G″ across the entire frequency range of the test, and there was no crossover point. The addition of NaCl resulted in a significant increase in the G′ of the HA-SC emulsion (Fig. 10D). These results indicated that the addition of NaCl enhanced the gel-like behaviour of the HA-SC emulsion. This may be the result of a flocculated droplet network structure forming at the emulsion interface [52]. In summary, we speculate that, in the presence of NaCl, the HA-SC emulsion forms a denser structure with a 3D network, thereby exhibiting higher viscoelasticity.

3.7.4. Effect of NaCl on micro-rheological properties of emulsions

NaCl increased the lag time of SC-stabilized emulsions, and this increased with increasing NaCl concentration (Fig. 11A). However, the

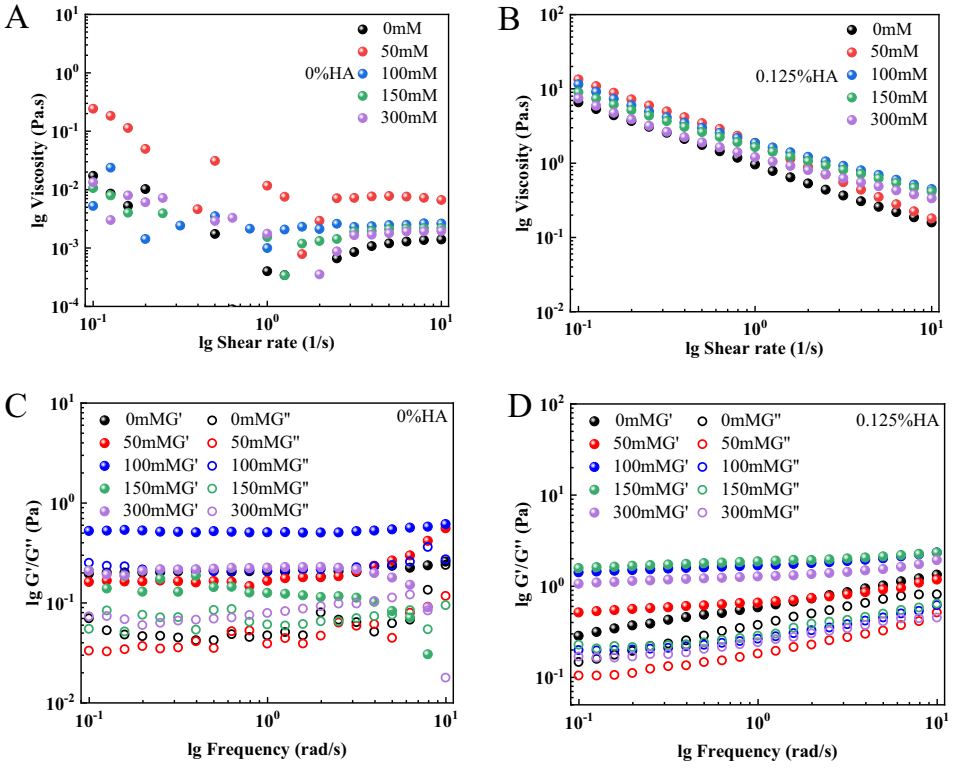


Fig. 10. Effect of NaCl on the viscosity (A, B) and modulus (C, D) of HA-SC emulsion.

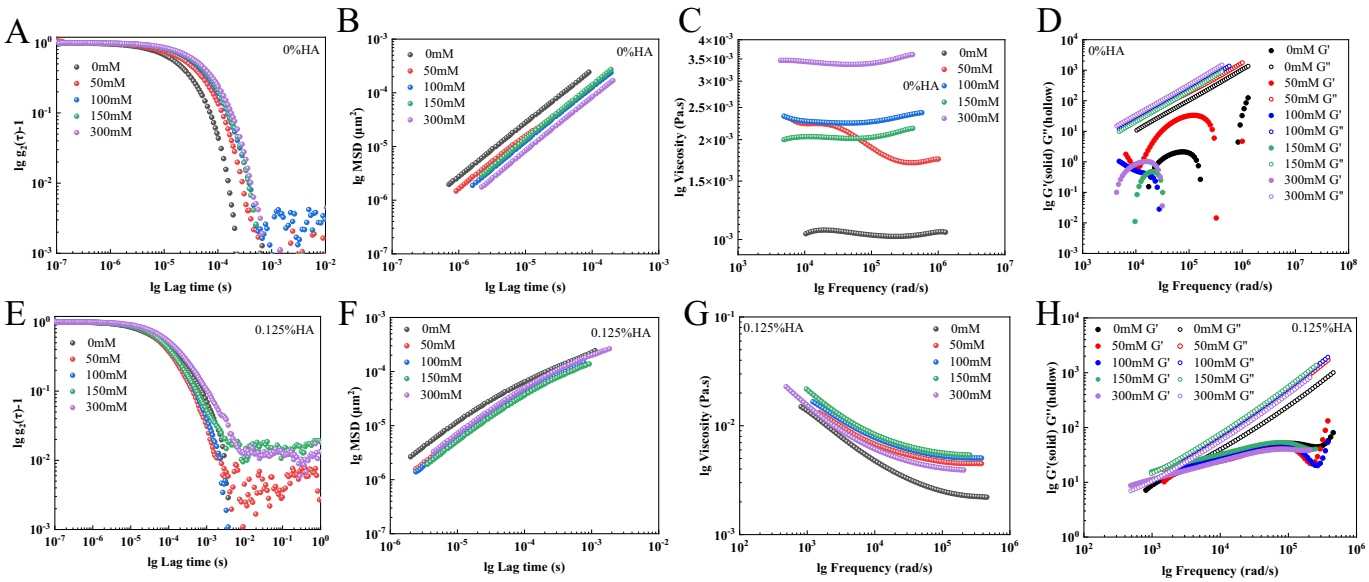


Fig. 11. Effect of NaCl on (A) intensity correlation function $g^2(\tau) - 1$ vs. time, (B) mean square displacements curve, (C) viscosity as a function of frequency, and (D) viscoelastic parameters as a function of the frequency of the SC emulsion. Effect of NaCl on (E) intensity correlation function $g^2(\tau) - 1$ vs. time, (F) mean square displacements curve, (G) viscosity as a function of frequency, and (H) viscoelastic parameters as a function of the frequency of HA-SC emulsion.

lag time of the HA-SC emulsion almost did not change with the change in NaCl concentration (Fig. 11E). This shows that NaCl had no obvious effect on droplet movement in the HA-SC emulsion [49]. The MSD of the SC-stabilized emulsions changed significantly after the addition of NaCl (Fig. 11B). However, the change and slope of the MSD of the HA-SC emulsion after NaCl addition were smaller than those of the SC-stabilized emulsions (Fig. 11F, Table S2). According to previous research, a small MSD slope improves the emulsion stability by forming an elastic interface film that allows the prepared emulsion droplets to move freely in the presence of NaCl without flocculation [51]. The viscosity of SC-stabilized emulsions almost did not change with increasing frequency, but increased with increasing NaCl concentration (Fig. 11C). The viscosity of the HA-SC emulsions decreased with increasing frequency. The addition of NaCl increased the viscosity of the emulsion; however, the concentration of NaCl had no effect on its viscosity (Fig. 11G). This demonstrates that NaCl had no effect on the HA-SC emulsion's non-Newtonian fluid properties [52]. G′ of the SC-stabilized emulsions was greater than G″ in the measuring frequency range, and the two curves did not intersect. In addition, G″ showed strong frequency and NaCl dependence (Fig. 11D). The G′ of the HA-SC emulsion was strongly frequency dependent, with a crossover point at a high frequency (>103). Before the crossover point, G′ > G″, and after the crossover point, G″ > G′ (Fig. 11H). These results indicate that the HA-SC emulsion exhibited liquid-like viscosity behaviour at high frequencies [69]. The macro-rheology of the HA-SC emulsion showed that there was no crossing point at a low frequency, and it was always G′ > G″. The results show that DWS can detect higher frequency regions and that macro-rheology and micro-rheology can work effectively together to study the viscoelasticity of a HA-SC emulsion.

4. Conclusion

The addition of HA significantly affected the stability of the SC-stabilized emulsions. Low HA concentrations reduced the emulsions stability, but >0.1 % HA significantly improved emulsion stability. Among them, 0.125 % HA had the largest effect and maintained good emulsion stability during the storage period of 30 days. HA improved the stability of SC-stabilized emulsions and reduced the creaming index by decreasing the average particle size and increasing the zeta-potential and triple-phase contact angle. Furthermore, the addition of HA formed a "3D network" structure in the emulsion, inhibiting droplet movement and increasing emulsion viscosity, improving the stability of the SC-stabilized emulsions. The emulsions used in this study were typical O/W emulsions. NaCl degraded the stability of SC-stabilized emulsions in a concentration-dependent manner. However, the effect of NaCl on the stability of SC-stabilized emulsions was significantly reduced after the addition of HA.

Our study demonstrated that the addition of HA is an important factor in improving the stability of SC-stabilized emulsions during storage and in the presence of NaCl. HA changed the rheological properties of SC-stabilized emulsions and reduced creaming and coalescence by forming a "3D network" structure between droplets, increasing the electrostatic repulsion and steric hindrance between droplets, and ultimately improving the stability of SC-stabilized emulsions. This study provides a theoretical basis for illustrating the effect of HA on the stability and rheological properties of SC-stabilized emulsions.

CRediT authorship contribution statement

Conceptualization, Xingfen He, Bin Wang, Xingwang He and Jie Chen; methodology, Xingfen He, Bin Wang and Yuhang Xue; software, Xingfen He and Bin Wang; validation, Yanhua Li, Mingxiang Hu, and Yuecheng Meng; investigation, Xingfen He, Bin Wang, and Yuhang Xue; resources, Bin Wang, Yanhua Li and Mingxiang Hu; data curation, Xingfen He and Bin Wang; writing-original draft preparation, Xingfen He, Bin Wang, Jie Chen, and Xingwang He; writing-review and editing, Xingfen He, Bin Wang, Jie Chen and Yuecheng Meng; supervision, Jie Chen and Yuecheng Meng. All authors reviewed the manuscript.

Declaration of competing interest

None.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 31871830), and the Natural Science Foundation of Zhejiang Province (Grant No. LY18C200002).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2023.123675.

References

[1] C.C. Loi, G.T. Eyres, E.J. Birch, Effect of milk protein composition on physicochemical properties, creaming stability and volatile profile of a protein-stabilized oil-in-water emulsion, Food Res. Int. 120 (2019) 83–91.
[2] D.J. McClements, S.M. Jafari, Improving emulsion formation, stability and performance using mixed emulsifiers: a review, Adv. Colloid Interf. Sci. 251 (2018) 55–79.
[3] J.A.P. Vilela, R.L.D. Cunha, High acyl gellan as an emulsion stabilizer, Carbohydr. Polym. 139 (2016) 115–124.
[4] T. Gao, X. Zhao, R. Li, A. Bassey, Y. Bai, K. Ye, S. Deng, G. Zhou, Synergistic effects of polysaccharide addition-ultrasound treatment on the emulsified properties of low-salt myofibrillar protein, Food Hydrocoll. 123 (2022), 107143.
[5] X. Ma, D.E.W. Chatterton, Strategies to improve the physical stability of sodium caseinate stabilized emulsions: a literature review, Food Hydrocoll. 119 (2021), 106853.
[6] V. Sovilj, J. Milanović, L. Petrović, Viscosimetric and tensiometric investigations of interactions between gelatin and surface active molecules of various structures, Food Hydrocoll. 32 (1) (2013) 20–27.
[7] L.C.B. Züge, H.A. Maieves, J.L.M. Silveira, V.R.D. Silva, A.D.P. Scheer, Use of avocado phospholipids as emulsifier, LWT - Food Sci. Technology 79 (2017) 42–51.
[8] D.J. McClements, L. Bai, C. Chung, Recent advances in the utilization of natural emulsifiers to form and stabilize emulsions, in: M.P. Doyle, T.R. Klaenhammer (Eds.), Annual Review of Food Science and Technology 8, 2017, pp. 205–236.
[9] C. Wang, X. Li, S. Sang, D. Julian McClements, L. Chen, J. Long, A. Jiao, J. Wang, Z. Jin, C. Qiu, Preparation, characterization and in vitro digestive behaviors of emulsions synergistically stabilized by γ-cyclodextrin/sodium caseinate/alginate, Food Res. Int. 160 (2022), 111634.
[10] J. Zhang, X. Zhang, X. Wang, Y. Huang, B. Yang, X. Pan, C. Wu, The influence of maltodextrin on the physicochemical properties and stabilization of beta-carotene emulsions, AAPS PharmSciTech 18 (3) (2017) 821–828.
[11] A. Ye, J. Lo, H. Singh, Formation of interfacial milk protein complexation to stabilize oil-in-water emulsions against calcium, J. Colloid Interface Sci. 378 (1) (2012) 184–190.
[12] D. Su, Q. Zhong, Lemon oil nanoemulsions fabricated with sodium caseinate and tween 20 using phase inversion temperature method, J. Food Eng. 171 (2016) 214–221.
[13] F.A. Perrechil, R.L. Cunha, Oil-in-water emulsions stabilized by sodium caseinate: influence of pH, high-pressure homogenization and locust bean gum addition, J. Food Eng. 97 (4) (2010) 441–448.
[14] B. Yesiltas, A.-D.M. Sørensen, P.J. García-Moreno, S. Anankanbil, Z. Guo, C. Jacobsen, Combination of sodium caseinate and succinylated alginate improved stability of high fat fish oil-in-water emulsions, Food Chem. 255 (2018) 290–299.
[15] Z. Wei, Y. Gao, Physicochemical properties of β-carotene bilayer emulsions coated by milk proteins and chitosan–EGCG conjugates, Food Hydrocoll. 52 (2016) 590–599.
[16] W. Cheng, D.J. McClements, Biopolymer-stabilized conjugated linoleic acid (CLA) oil-in-water emulsions: impact of electrostatic interactions on formation and stability of pectin-caseinate-coated lipid droplets, Colloids Surf. A Physicochem. Eng. Asp. 511 (2016) 172–179.
[17] C.K.W. Koo, C. Chung, J.-T.R. Fu, A. Sher, P. Rousset, D.J. McClements, Impact of sodium caseinate, soy lecithin and carrageenan on functionality of oil-in-water emulsions, Food Res. Int. 123 (2019) 779–789.
[18] X. Zang, J. Wang, G. Yu, J. Cheng, Addition of anionic polysaccharides to improve the stability of rice bran protein hydrolysate-stabilized emulsions, LWT Food Sci. Technol. 111 (2019) 573–581.
[19] W. Xu, Y. Xiong, Z. Li, D. Luo, Z. Wang, Y. Sun, B.R. Shah, Stability, microstructural and rheological properties of complex prebiotic emulsion stabilized by sodium caseinate with inulin and konjac glucomannan, Food Hydrocoll. 105 (2020), 105772.
[20] X.-J. Xu, S. Fang, Y.-H. Li, F. Zhang, Z.-P. Shao, Y.-T. Zeng, J. Chen, Y.-C. Meng, Effects of low acyl and high acyl gellan gum on the thermal stability of purple sweet potato anthocyanins in the presence of ascorbic acid, Food Hydrocoll. 86 (2019) 116–123.
[21] S. Moayedzadeh, A. Khosrowshahiasl, S. Gunasekaran, A. Madadlou, Spontaneous emulsification of fish oil at a substantially low surfactant-to-oil ratio: Emulsion characterization and filled hydrogel formation, Food Hydrocolloids 82 (2018) 11–18.
[22] J.A.P. Vilela, R.L.d. Cunha, Emulsions stabilized by high acyl gellan and KCl, Food Res. Int. 91 (2017) 47–54.
[23] Y. Li, L. Fan, Comparative studies on the stabilization of flos sophorae immaturus beverages by various hydrocolloids, LWT Food Sci. Technol. 123 (2020), 109117.
[24] L. Cui, H.T. Cho, D.J. McClements, E.A. Decker, Y. Park, Effects of salts on oxidative stability of lipids in Tween-20 stabilized oil-in-water emulsions, Food Chem. 197 (2016) 1130–1135.
[25] Q. Zhu, Y. Li, S. Li, W. Wang, Fabrication and characterization of acid soluble collagen stabilized Pickering emulsions, Food Hydrocoll. 106 (2020), 105875.
[26] F. Niu, Q. Zhang, J. Yu, Y. Huo, L. Zhuo, D. Niu, W. Pan, Interfacial adsorption behavior of ovalbumin/sodium carboxymethyl cellulose colloidal particles: the effects of preparation methods, Food Hydrocoll. 120 (2021), 106969.
[27] A. Sharkawy, M.F. Barreiro, A.E. Rodrigues, Preparation of chitosan/gum arabic nanoparticles and their use as novel stabilizers in oil/water Pickering emulsions, Carbohydr. Polym. 224 (2019), 115190.
[28] V. Raikos, Encapsulation of vitamin E in edible orange oil-in-water emulsion beverages: influence of heating temperature on physicochemical stability during chilled storage, Food Hydrocoll. 72 (2017) 155–162.
[29] M. Jarpa-Parra, Z. Tian, F. Temelli, H. Zeng, L. Chen, Understanding the stability mechanisms of lentil legumin-like protein and polysaccharide foams, Food Hydrocoll. 61 (2016) 903–913.
[30] Y. Jia, J. Du, K. Li, C. Li, Emulsification mechanism of persimmon pectin with promising emulsification capability and stability, Food Hydrocoll. 131 (2022), 107727.
[31] X. Xu, X. Peng, C. Huan, J. Chen, Y. Meng, S. Fang, Development of natamycin-loaded zein-casein composite nanoparticles by a pH-driven method and application to postharvest fungal control on peach against Monilinia fructicola, Food Chem. 404 (2023), 134659.
[32] S. Matsuyama, M. Kazuhiro, M. Nakauma, T. Funami, Y. Nambu, K. Matsumiya, Y. Matsumura, Stabilization of whey protein isolate-based emulsions via complexation with xanthan gum under acidic conditions, Food Hydrocoll. 111 (2021), 106365.
[33] C. Kartal, M.K. Unal, S. Otles, Production and stabilization of a flaxseed oil multilayer emulsion containing sodium caseinate and pectin, Int. J. Food Prop. 20 (4) (2016) 833–844.
[34] C. Huck-Iriart, M.S. Álvarez-Cerimedo, R.J. Candal, M.L. Herrera, Structures and stability of lipid emulsions formulated with sodium caseinate, Curr. Opin. Colloid Interface Sci. 16 (5) (2011) 412–420.
[35] J. Santos, N. Calero, L.A. Trujillo-Cayado, M.C. Garcia, J. Muñoz, Assessing differences between ostwald ripening and coalescence by rheology, laser diffraction and multiple light scattering, Colloids Surf. B: Biointerfaces 159 (2017) 405–411.
[36] Z. Lu, F. Ye, G. Zhou, R. Gao, D. Qin, G. Zhao, Micronized apple pomace as a novel emulsifier for food O/W pickering emulsion, Food Chem. 330 (2020), 127325.
[37] M.J. Martin-Piñero, M.C. García, J. Muñoz, M.-C. Alfaro-Rodriguez, Influence of the welan gum biopolymer concentration on the rheological properties, droplet size distribution and physical stability of thyme oil/W emulsions, Int. J. Biol. Macromol. 133 (2019) 270–277.
[38] W. Liao, A. Elaissari, S. Ghnimi, E. Dumas, A. Gharsallaoui, Effect of pectin on the properties of nanoemulsions stabilized by sodium caseinate at neutral pH, Int. J. Biol. Macromol. 209 (2022) 1858–1866.
[39] L. Zhang, R. Liang, L. Li, The interaction between anionic polysaccharides and legume protein and their influence mechanism on emulsion stability, Food Hydrocoll. 131 (2022), 107814.
[40] X. Xu, W. Liu, L. Luo, C. Liu, D.J. McClements, Influence of anionic polysaccharides on the physical and oxidative stability of hydrolyzed rice glutelin emulsions: impact of polysaccharide type and pH, Food Hydrocoll. 72 (2017) 185–194.
[41] L. Dai, C. Sun, Y. Wei, L. Mao, Y. Gao, Characterization of Pickering emulsion gels stabilized by zein/gum arabic complex colloidal nanoparticles, Food Hydrocoll. 74 (2018) 239–248.
[42] F.-Z. Zhou, X.-N. Huang, Z.-L. Wu, S.-W. Yin, J.-H. Zhu, C.-H. Tang, X.-Q. Yang, Fabrication of Zein/Pectin hybrid particle-stabilized Pickering high internal phase emulsions with robust and ordered Interface architecture, J. Agric. Food Chem. 66 (42) (2018) 11113–11123.
[43] P. Pocan, E. Ilhan, M.H. Oztop, Characterization of emulsion stabilization properties of gum tragacanth, xanthan gum and sucrose monopalmitate: a comparative study, J. Food Sci. 84 (5) (2019) 1087–1093.
[44] M.G. Sosa-Herrera, L.P. Martínez-Padilla, V.A. Delgado-Reyes, M. Ortega, I.P. C. Martínez, Effect of agave fructans on xanthan rheology: impact on sodium caseinate emulsion properties, J. Food Sci. 87 (7) (2022) 2858–2868.
[45] W.-Y. Liu, M.-Q. Feng, M. Wang, P. Wang, J. Sun, X.-L. Xu, G.-H. Zhou, Influence of flaxseed gum and NaCl concentrations on the stability of oil-in-water emulsions, Food Hydrocoll. 79 (2018) 371–381.
[46] Y. Hu, B. Yu, L. Wang, D.J. McClements, C. Li, Study of dextrin addition on the formation and physicochemical properties of whey protein-stabilized emulsion: effect of dextrin molecular dimension, Food Hydrocoll. 128 (2022), 107569.
[47] Y.J. Cai, X.L. Deng, T.X. Liu, M.M. Zhao, Q.Z. Zhao, S.L. Chen, Effect of xanthan gum on walnut protein/xanthan gum mixtures, interfacial adsorption, and emulsion properties, Food Hydrocoll. 79 (2018) 391–398.
[48] B. Medronho, A. Filipe, C. Costa, A. Romano, B. Lindman, H. Edlund, M. Norgren, Microrheology of novel cellulose stabilized oil-in-water emulsions, J. Colloid Interface Sci. 531 (2018) 225–232.
[49] S. Khalloufi, M. Corredig, M. Alexander, Interactions between flaxseed gums and WPI-stabilized emulsion droplets assessed in situ using diffusing wave spectroscopy, Colloids Surf. B: Biointerfaces 68 (2) (2009) 145–153.
[50] H.G.M. Ruis, K. van Gruijthuijsen, P. Venema, E. van der Linden, Transitions in structure in oil-in-water emulsions as studied by diffusing wave spectroscopy, Langmuir 23 (3) (2007) 1007–1013.
[51] F. Niu, Y. Du, Q. Zhang, B. Zhang, D. Hu, S. Ma, F. Gu, W. Pan, Ovalbumin/carboxymethylcellulose colloids: particle compactness and interfacial stability, Food Chem. 372 (2022), 131223.
[52] L.P. Martínez-Padilla, M.G. Sosa-Herrera, M. Osnaya-Becerril, Effect of the konjac glucomannan concentration on the rheological behaviour and stability of sodium caseinate oil-in-water emulsions, Int. Dairy J. 117 (2021), 104993.

[53] F. Salerni, D. Orsi, E. Santini, L. Liggieri, F. Ravera, L. Cristofolini, Diffusing wave spectroscopy for investigating emulsions: II. Characterization of a paradigmatic oil-in-water emulsion, Colloids Surf. A Physicochem. Eng. Asp. 580 (2019), 123724.
[54] Y. Zhu, X. Ren, Y. Bao, S. Li, Z. Peng, Y. Zhang, G. Zhou, Emulsification of oil-in-water emulsions with eggplant (Solarium melongena L), J. Colloid Interface Sci. 563 (2020) 17–26.
[55] M.G. Sosa-Herrera, C.L.A. Berli, L.P. Martínez-Padilla, Physicochemical and rheological properties of oil-in-water emulsions prepared with sodium caseinate/gellan gum mixtures, Food Hydrocoll. 22 (2008) 934–942.
[56] Y. Xiao, C. Chen, B. Wang, Z. Mao, H. Xu, Y. Zhong, L. Zhang, X. Sui, S. Qu, In vitro digestion of oil-in-water emulsions stabilized by regenerated chitin, J. Agric. Food Chem. 66 (46) (2018) 12344–12352.
[57] Z. Long, Q. Zhao, T. Liu, W. Kuang, J. Xu, M. Zhao, Influence of xanthan gum on physical characteristics of sodium caseinate solutions and emulsions, Food Hydrocoll. 32 (1) (2013) 123–129.
[58] T. Wang, N. Wang, N. Li, X. Ji, H. Zhang, D. Yu, L. Wang, Effect of high-intensity ultrasound on the physicochemical properties, microstructure, and stability of soy protein isolate-pectin emulsion, Ultrason. Sonochem. 82 (2022), 105871.
[59] S. Sivapratha, P. Sarkar, Multiple layers and conjugate materials for food emulsion stabilization, Crit. Rev. Food Sci. Nutr. 58 (6) (2018) 877–892.
[60] Y.S. Gu, E.A. Decker, D.J. Mcclements, Influence of pH and ι-carrageenan concentration on physicochemical properties and stability of β-lactoglobulin-stabilized oil-in-water emulsions, J. Agric. Food Chem. 52 (11) (2004) 3626–3632.
[61] S. Qamar, B. Bhandari, S. Prakash, Effect of different homogenisation methods and UHT processing on the stability of pea protein emulsion, Food Res. Int. 116 (2019) 1374–1385.
[62] Y.-T. Yao, W.-Y. Wang, H.-M. Liu, L.-X. Hou, X.-D. Wang, Emulsifying properties of chinese quince seed gum in oil-in-water emulsions, LWT Food Sci. Technol. 147 (2021), 111560.
[63] B. Wang, L.-J. Wang, D. Li, B. Adhikari, J. Shi, Effect of gum arabic on stability of oil-in-water emulsion stabilized by flaxseed and soybean protein, Carbohydr. Polym. 86 (1) (2011) 343–351.
[64] F. Yang, J. Yang, S. Qiu, W. Xu, Y. Wang, Tannic acid enhanced the physical and oxidative stability of chitin particles stabilized oil in water emulsion, Food Chem. 346 (2021), 128762.
[65] T. Schmelz, U. Lesmes, J. Weiss, D.J. McClements, Modulation of physicochemical properties of lipid droplets using β-lactoglobulin and/or lactoferrin interfacial coatings, Food Hydrocoll. 25 (5) (2011) 1181–1188.
[66] W. Jin, Y. Pan, Y. Wu, C. Chen, W. Xu, D. Peng, Q. Huang, Structural and interfacial characterization of oil bodies extracted from Camellia oleifera under the neutral and alkaline condition, LWT Food Sci. Technol. 141 (2021), 110911.
[67] M.-A. Mehrnia, S.-M. Jafari, B.S. Makhmal-Zadeh, Y. Maghsoudlou, Rheological and release properties of double nano-emulsions containing crocin prepared with angum gum, arabic gum and whey protein, Food Hydrocoll. 66 (2017) 259–267.
[68] Y. Wei, C. Sun, L. Dai, L. Mao, F. Yuan, Y. Gao, Novel bilayer emulsions costabilized by zein colloidal particles and propylene glycol alginate. 2. Influence of environmental stresses on stability and rheological properties, J. Agric. Food Chem. 67 (4) (2019) 1209–1221.
[69] K. Liu, Q.-M. Li, X.-Q. Zha, L.-H. Pan, L.-J. Bao, H.-L. Zhang, J.-P. Luo, Effects of calcium or sodium ions on the properties of whey protein isolate-lotus root amylopectin composite gel, Food Hydrocoll. 87 (2019) 629–636.