Food Chemistry: X 18 (2023) 100751

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Fabrication and characterization of novel thermoresponsive emulsion gels and oleogels stabilizied by assembling nanofibrous from dual natural triterpenoid saponins

Yao Liu[a], Huan Zhang[a], Xiao-Wei Chen[a,*], Tao Yang[b], Cong Sun[a], Shang-De Sun[a,*]

[a] College of Food Science and Engineering, National Engineering Laboratory/Key Laboratory of Henan Province, Henan University of Technology, Zhengzhou 450001 China

[b] School of Pharmacy, Hainan Medical University, Haikou 571199, China

ARTICLE INFO

Keywords:
All-natural
High internal phase emulsion
Thermoresponsive
Emulsion gel
Oleogels
Quillaja saponin
Glycyrrhizic acid

ABSTRACT

Novel thermoresponsive emulsion gels and oleogels were fabricated by assembling nanofibrous from natural triterpenoid *Quillaja* saponin (QS) and glycyrrhizic acid (GA). The viscoelasticity of QS-coated emulsion was observed to be remarkably improved by GA and thus obtain the advantages of excellent gelatinous, thermoresponsive and reversible manner due to the viscoelastic texture from GA nanofibrous as scaffolds in continuous phase. In the gelled emulsions, the phase transition of the GA fibrosis network structure upon heating and cooling was attributed to a thermal sensitivity, whereas interface-induced fibrosis assembly of amphiphilic QS endowed the formation of stable emulsion droplets. Then these emulsion gels were further used as an effective template to fabricate soft-solid oleogels with high oil content of 96%. These findings open up new opportunities for the use of all-natural and sustainable ingredients to develop smart soft materials for replace *trans* and saturated fats in food industry and other fields.

1. Introduction

Structuring edible liquid oils into soft materials based on structural design principles has attracted much attention owing to the application of the extended functionalities in the fields of foods, medicines, and personal care cosmetics (Chelazzi, Giorgi, & Baglioni, 2018). For successful applications, outstanding emulsion gels and oleogels have enjoyed extensive interest. One of the promising strategies for oil structuring is to construct high internal phase emulsion gels, the gelatinous soft solids with a high internal dispersed phase fraction over 74% (v/v) displaysing yielding behavior (Fuhrmann et al., 2022; Gao et al., 2021). What's more high internal phase emulsion gels serves as the starting template for the preparation of soft oleogels containing>95% liquid oils (Patel et al., 2015). Traditionally, the emulsion precursors are extensively stabilized by synthetic particles, synthetic surfactants or their mixture (Martins et al., 2020). Regarding the increasing trend to the environmental sustainability, human health as well as "clean label" demands, all-natural and green ingredients to structure edible liquid oils into soft emulsion gels and oleogels have received significantly increased attention and many green emulsifiers have been investigated in recent years (Martins et al., 2020; Xu et al., 2022). The natural saponins exhibit both physical properties and interfacial activity as well as outstanding biological activities beneficial to human health, which encourage them to serve as bifunctional building blocks for colloidal soft systems (Fink & Filip, 2022; Xu, Wan, & Yang, 2021).

Among naturally occurring saponins, *Quillaja* saponin (QS), an eco-friendly plant-based biosurfactant, is extracted from the bark of *Quillaja saponaria* Molina trees. Due to it unique physicochemical properties (*e.g.*, interface assembly, high surface modulus and shear viscoelasticity) and nontrivial biological activities (*e.g.*, anti-viral, anti-inflammatory, cholesterol lowering and free radical scavenging), QS has been extensively investigated in both academic and industrial community (Fink & Filip, 2022; Reichert et al., 2019; Tsibranska et al., 2020; Vinarova et al., 2015; Zhao et al., 2021). Molecular framework, QS is a triterpenoid bidesmosidic saponin comprising two sugar moieties (hydrophilic regions) and a triterpene aglycone (hydrophobic region). It adsorbs onto oil–water interface by reducing overall free-energy and form highly viscoelastic interfacial films (Böttcher & Drusch, 2017; Reichert et al., 2019). Previous studies by Golemanov and co-workers indicates that an ordered rearrangement of the amphiphilic triterpenoid saponin at phase

---

* Corresponding authors.

E-mail addresses: fexwchen@haut.edu.cn, chenxiaowei8252@hotmail.com (X.-W. Chen), shangdesun@haut.edu.cn, sunshangde@hotmail.com (S.-D. Sun).

https://doi.org/10.1016/j.fochx.2023.100751
Received 24 February 2023; Received in revised form 27 May 2023; Accepted 9 June 2023

boundaries forms structured domains, surface nanowrinkles, and solid adsorption shells to coat oil-droplets (Pagureva et al., 2016; Tsibranska et al., 2020). Moreover, we recently also found that QS spontaneously interface-driven self-assembly (SIDSA) at the oil–water interface formed a micrometer-scale network in helical fibers and solid adsorption shells around oil-droplets (Chen et al., 2020). Thus, the unique interfacial properties of QS contributed to its application as a high-performance emulsifier in functional and engineered colloids from nanoscopic to microscopic scales. Additionally, it was also found that the high internal phase emulsion stabilized solely by QS was geared to the oil structuring during dehydration as the interface stabilization of QS enabled the formation of the emulsion offering good resistance to stress (Chen et al., 2017; Chen & Yang, 2019). However, the fragility or sol state caused by the strong electrostatic repulsion has greatly limited the wide use of the emulsion (Tsibranska et al., 2020). Therefore, widespread concerns have been focused on improving its inherent poor mechanical properties to structuring edible liquid oils into soft matters with structural design and satisfactory.

Early works demonstrate that the interactions of QS with the components of proteins, polysaccharides and inorganic nanoparticles in bulk solutions and at oil–water interfaces can strengthen its mechanical strength (Böttcher & Drusch, 2017; Reichert et al., 2018, 2019). Given the synergistic interfacial activities and biological activities, the interaction of QS with natural components has grown incessantly to enhance the mechanical strength of QS-based emulsions and oleogels. Glycyrrhizic acid (GA) a natural triterpene saponin found mainly in licorice roots, has been widely used as food sweeteners, medicinal ingredients and pharmaceutical carrier (Schmid et al., 2018; Tykarska et al., 2012). Its molecular framework consisted of a rigid a hydrophobic triterpenoid aglycon (18b-glycyrrhetinic acid) and a hydrophilic diglucuronic unit allows GA to conduct anisotropic self-assembly easily in aqueous solutions to form novel nanofibril clusters (Saha, et al., 2015). Then, a transparent supramolecular hydrogel was formed by the former, exhibiting multiple responsiveness (*e.g.*, temperature and pH), great injectable and moldable properties (Ma et al., 2019a; Zhao, Zhang, Gao, Lin, & Hu, 2019). Moreover, these self-assembled semi-flexible nanofibrils can be utilized as building blocks to fabricate the emulsions, foams and even complex colloid systems due to its high interfacial activity (Li, Wan, & Yang, 2022; Ma et al., 2019a; Xu, Wan, & Yang, 2021). Owing to the above properties, natural GA is regarded as an ideal structural promoter for the transformation of liquid oil into soft-solid structured materials.

Nowadays, research efforts in the field of emulsion science have focused on the stabilization of emulsions using a combination of all-natural surface-active species. Based on the mechanical reinforcement and interfacial stabilizing abilities of QS, the combination of GA is expected to display a synergistic effect for the stabilization emulsion gels with stimuli responsiveness ability. Such unique combined building blocks may provide an ideal template for oleogels fabrication. Here, natural glycyrrhizic acid (GA) was therefore used as structural promoter to strengthen prepare emulsion gels, and further emulsion gels precursor as template for preparing oleogels at a fixed *Quillaja* saponin (QS). The high internal phase emulsions were fabricated firstly with different concentration of GA through one-pot strategy and characterized by static light scattering, confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). Following that, oleogels were fabricated by dehydration using the resulted emulsion gels as template, and characterized by microstructure and rheological behavior. This information would provide a simplifying the fabrication process in structuring liquid oil-based gels (emulsion gels, oleogels) as extremely interested from researchers seeking for all-natural substances in potential related applications of food and others industries.

2. Materials and methods

2.1. Materials

Quillaja saponin was obtained from Ingredion Incorporated (Westchester, Illinois, USA). The glycyrrhizic acid was purchased from Macklin in the form of the ammonium salt (>98%). Commercial soybean oil was obtained at a local supermarket (Zhengzhou, Henan). Fluorescent dyes (Nile Red and Thioflavin T) were purchased from Sigma-Aldrich, Inc. (Shanghai, China). Micro-pure deionized water was used throughout this work. Other chemicals or solvents used were of analytical or better grade.

2.2. Emulsion preparation

Emulsions were first produced by emulsification in a one-pot strategy, according to the process described in our previous work (Chen, et al., 2019). Briefly, soybean oil (at a fixed of 75 wt%), *Quillaja saponin* (1.5 wt%), glycyrrhizic acid (0.5–2.0 wt%) and phosphate-buffered saline (PBS, 20 mM, pH 7.0) were mixed. Prior to emulsification, the mixture was preheated to ~80 °C in a water bath. Then, the mixture was directly emulsified by Ultra-Turrax homogenizer (IKA T10 Basic, Staufen, Germany) at 24000 rpm for 3 min. Samples of each treatment were cooled immediately at ambient temperature (25 °C) and stabilized for 24 h before being analyzed. For comparative purposes, a control emulsion consisting of 1.5 wt% *Quillaja* saponin sonly was prepared using the same emulsifying conditions as given above.

2.3. Oleogels preparation

The preparation of oleogels was performed by dehydration using the resulted emulsion gels as template. Two methods were applied to prepare oleogels by dehydration in this experiment (Chen & Yang, 2019). *Oven drying:* A certain quality of emulsion samples were dried in constant temperature oven and stored at 70 °C until a constant weight was prepared. *Vacuum drying:* The same quality of emulsions as above was dried in the 60 °C vacuum drying chamber until the sample reached the constant weight.

2.4. Characteristics of emulsion droplets

Size distribution of the droplets in emulsion was determined by static light scattering using a MasterSizer 3000 (Malvern Instruments Ltd., Worcestershire, UK). The refractive indexes of soybean oil and water were 1.47 and 1.3, respectively. The volume-weighted mean diameter $d_{4,3}$ was evaluated by $d_{4,3} = \Sigma n_i d_i^4 \Sigma n_i d_i^3$, where n_i is the number of particles of diameter d_i. Additionally, “span” was used to determine the polydispersity of the droplets created, and calculated by: span = $[d_{(v,90)}\text{-}d_{(v,10)}]/d_{(v,50)}$, where $d_{(v,10)}$, $d_{(v,50)}$, and $d_{(v,90)}$ is the diameters at 10%, 50,% and 90% cumulative volume, respectively.

2.5. Dynamic interface behavior analysis

Dynamic interface properties at oil–water interface were measured using an instrument K100C interfacial tensionmeter (Dataphysics Instruments GmbH, Germany) via a platinum plate method at 25 °C. Given in the bulk gelation at the aspect of concentration used in the emulsion, the concentration of GA aqueous solution was set to 0.01–0.04 wt% with or without QS saponin (0.015%).

2.6. Microstructure observations

Microstructure images of emulsions were obtained employing both confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). For CLSM visualization, aliquots (1 mL) of emulsion were stained with 40 μL of Nile-red and Thioflavine T (ThT) solution

(0.05% w/v and 0.1% w/v in ethanol, respectively). Then, the stained samples were observed using CLSM (TCS SP5, Leica, Germany) at a fluorescence excitation of 488 nm and 458 nm, respectively. For SEM, the was emulsions were prepared by replacing the soybean oil with cyclohexane and volatilized at room temperature (25 °C), accompanied by desolvaing under vacuum at 40 °C for 12 h. The dried powder was sputter-coated with a 5 nm thick gold/palladium layer and imaged with a LEO 1530 VP scanning electron microscope (SEM, Oberkochem, Germany).

2.7. Macrorheological measurements

The macrorheological measurements were conducted using a MARS 60 rheometer (Thermo Fisher, Germany) with a Julabo temperature controller. The apparent viscosity was recorded as a function of shear rate from 0 to 200 s^{-1}. Amplitude sweep was conducted by increasing the stress logarithmically from 0.1 to 1000 Pa to study the viscoelastic parameters [*e.g.*, elastic modulus G′, viscous modulus G″, linear viscoelastic region (LVR), and cross-over point stress γ_{co}] at a constant frequency of 1 Hz. Frequency sweeps were conducted from 0.1 to100 Hz at a constant shear stress of 1 Pa. Additionally, a time-dependent flow curve generated through the up/down shear rates was used to investigate the thixotropic nature of the soft samples. Furthermore, the temperature sweeps of the soft samples were further investigated by increasing the temperature from 5 to 90 °C, followed by cooling back to 5 °C at a rate of 5 °C/min.

2.8. Statistical analysis

All experiments were performed in triplicate and reported in the form of mean ± standard deviation (SD). Significant differences ($p <$ 0.05) were performed by an analysis of variance (ANOVA) procedure of the SPSS 22.0 statistical analysis program using Duncan's multiple range tests.

3. Results and discussion

3.1. Formation and characterization of emulsions

Amphiphilic saponins generally contribute positively to the emulsifying performance and widely used in the food industry to form and stabilize emulsion-based food and beverage products (Chen & Yang, 2019; Reichert et al., 2019; Xu et al., 2021). Herein, a one-pot strategy was carried out to fabricate the emulsion gels using natural glycyrrhizic acid (GA) as surfactants combining triterpenoid *Quillaja* saponin (QS). From the visual appearance as shown in Fig. 1**a**, high internal phase emulsion (HIPE) was successfully stabilized and fabricated with a high oil phase fraction of 75 wt%. However, there were obvious differences in the detailed appearance. Compared with QS sonly, the resulted emulsions were more self-supporting soft solids with predetermined shapes as a function of GA concentration by squeezing out the emulsions using a plastic injector. Furthermore, the formability was remarkable improved by structuring the emulsion with GA, in which gelation occurred may be attributable to a hydrogelation of glycyrrhizic acid in the continuous aqueous phase by fibrosis self-assembly (Ma et al., 2019b; Saha, Adamcik, Bolisetty, Handschin, & Mezzenga, 2015). Moreover, it can be seen from that, compared to emulsions stabilized by 1.5% QS sonly, the addition of GA showed a nonobvious droplet size distribution (Fig. 1**b**). In comparison, a polydisperse was observed in the emulsion stabilized at high GA concentration of 2.0% sonly. Indeed, the volume mean diameter ($d_{4,3}$) was 4.89 µm for the emulsion stabilized by QS sonly. High but nonsignificant ($p > 0.05$) $d_{4,3}$ was observed at 5.84, 6.62, 5.76, and 5.59 µm corresponding with GA concentrations of 0.5 wt%, 1.0 wt%, 1.5 wt% and 2.0 wt% respectively (seen in **Table S**). Generally, it should be noted that the key elements of preparing a stable emulsion are to form small oil droplets as well as sufficiently high viscosity to prevent coalescence of

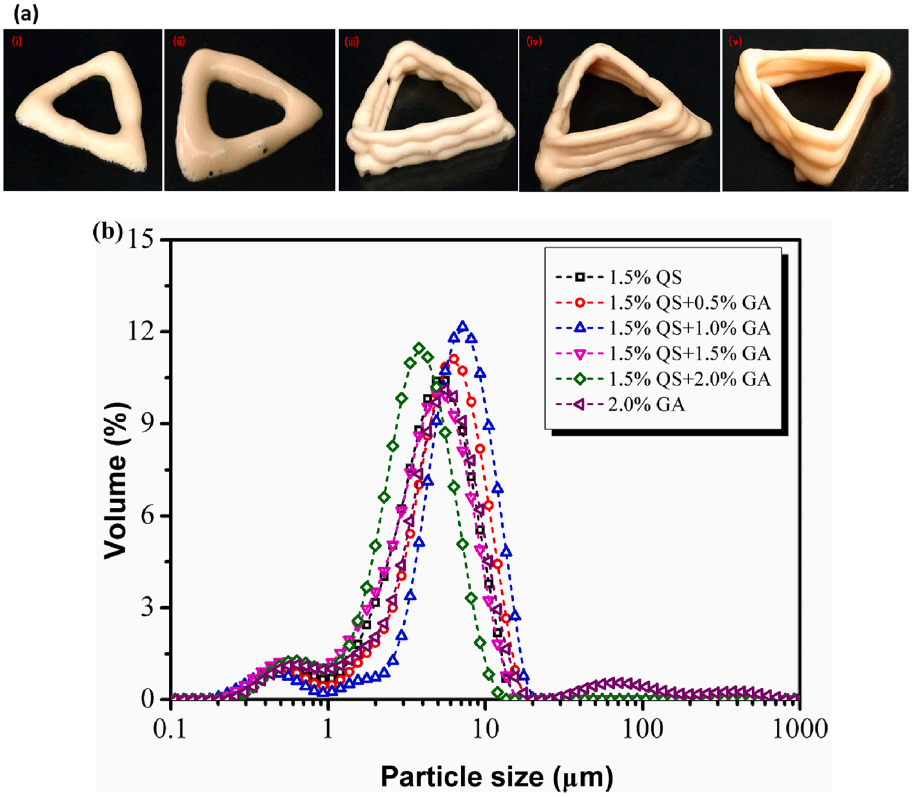

Fig. 1. Photograph (a) and particle size distribution (b) of emulsion gels prepared with different GA loadings as a fixed QS concentration of 1.5%. i) 1.5% QS, ii) 1.5% QS + 0.5 %GA, iii) 1.5% QS + 1.0% GA, iv) 1.5% QS + 1.5 %GA, v) 1.5% QS + 2.0 %GA, vi) 2.0% GA.

the oil droplets (Chen & Yang, 2019; Fuhrmann et al., 2022). Therefore, it has been claimed that GA does not contribute to the droplet size but enhance mechanical properties in this systems, which may be due to the weak interfacial activity and high hydrogenation of GA (Ma et al., 2018). In this case, vary larger droplet size of 16.8 5 μm with size distribution from tens to hundreds of micrometers (Span = 5.46) was observed in the emulsion stabilized by GA sonly. Such large droplet size prone to instability of the emulsion has an adverse effect on the development of oleogels through an emulsion-template approach (date not shown). Similarly, several authors have already reported that glycyrrhizic acid assembled nanofibrils can form a three-dimensional hydrogel network in the continuous phase, providing stronger interdroplet interactions, and thereby contribute to reinforcing the gel matrix (Ma et al., 2018, 2019b). Thereby, it was assumed that the GA could be used as efficient surfactants to co-fabricate soft high internal phase emulsion gels with QS, which can possibly replace mayonnaise and salad dressing as well as template in fabrication of oleogels.

3.2. *Interfacial properties and microstructure at oil–water interface*

Confocal laser scanning microscopy (CLSM) images were utilized to examine the network structure of the gel-like emulsions. As shown in **Fig. S1-a**, it can be seen that the amphiphilic saponins (blue) were surrounded the uniform-sized oil droplets (red), which bridged each other to form oil-in-water (O/W) emulsion system. There was no obvious change in the droplet size with increasing GA concentration (see **Fig. S1-b**), that was consistent with the results of **Table S**. Nevertheless, the close packing of emulsion droplets could allow them to interact strongly with each other, thus resulting in an increasing the strength and stability of gel network. Based on the results from Fig. 1 and **Fig. S1**, it was indicated that that the emulsion gels were probably formed by the interfacial assembly adsorption of QS at the interface as well as the interfibrillar interactions of GA in the continuous phase (Chen & Yang, 2019; Pagureva et al., 2016; Wan et al., 2018). Therefore, it can be speculated that the enhancement of the structural strength and stability of the emulsions (Fig. 1) was due to the supporting role of GA in the continuous aqueous phase.

To visualize the role of QS and GA in the emulsion systems, scanning electronic microscopy (SEM) was used to investigate the microstructure by replace the soybean oil with cyclohexane. As seen in Fig. 2, an interfacial film was formed on the inner surface (blue arrows) of the oil droplets, and the fibrous gel network (red arrows) was distributed on the surface of the interfacial film. These images further proved that the emulsion samples were oil-in-water systems, which was consistent with the results in CLSM images (**Fig. S1**). Corroboratively, it was reported that amphiphilic QS was interface self-assembled into nanofibers and firmly wrapped the oil droplets (Chen & Yang, 2019). Relatively, glycyrrhizic acid molecules were self-assemble into ultralong nanofibrils in water with uniform thickness of 2.5 nm (Ma et al., 2019b; Saha, Adamcik, Bolisetty, Handschin, & Mezzenga, 2015). Furthermore, with the increasing GA concentration the nanofibrils (red arrows) on the droplets surface gradually increased, indicating the support effect of GA on the gel network in continuous phase of QS-coated emulsions again. Combining the results of interface behavior, QS is preferentially adsorbed on the oil droplet surface, whereas GA forming a fine strands-like 3D network at the bulk.

From the dynamic interface behavior as shown in Fig. 3, both amphiphilic QS and GA were preferentially adsorbed onto the oil–water interface. Although the interface pressure was increased generally as the GA concentration changed from 0.01 to 0.04% (Fig. 3**a**), the adsorption rate was basically consistent with the final tension value once GA concentration above 0.02% (Fig. 3**c**). Compared to glycyrrhizic saponin, *Quillaja* saponin possess larger mobility and thus more rapid adsorption kinetics, essentially increasing the interfacial pressure within 1 min. Recently, it was also found that QS was mediated by oil–water interface-driven self-assembly (IDSA) into a solid “wrinkle film” (Chen et al., 2020; Pagureva et al., 2016). This interface behavior would facilitate the quick capture of QS onto the interface and prevent the desorption of QS molecules, displaying both kinetical and thermodynamical advantages in stabilizing the interface.

Compared with the interface with increasing GA at a fixed QS concentration, the interfacial pressure at the oil–water interface was increased significantly ($p < 0.05$) (Fig. 3**b**), which may be ascribed to the interfacial synergistic effect between QS and GA (Ma, et al., 2021). However, the interfacial pressure increased and then decreased with the increasing of GA concentration was depicted in Fig. 3**c**. Combined with these results, these observations suggest that increasing GA is conducive to the increasing of interfacial pressure within a certain concentration range. Once exceeds a certain concentration (>0.03%), it was no longer conducive to the stability of oil–water interface. The phenomenon may

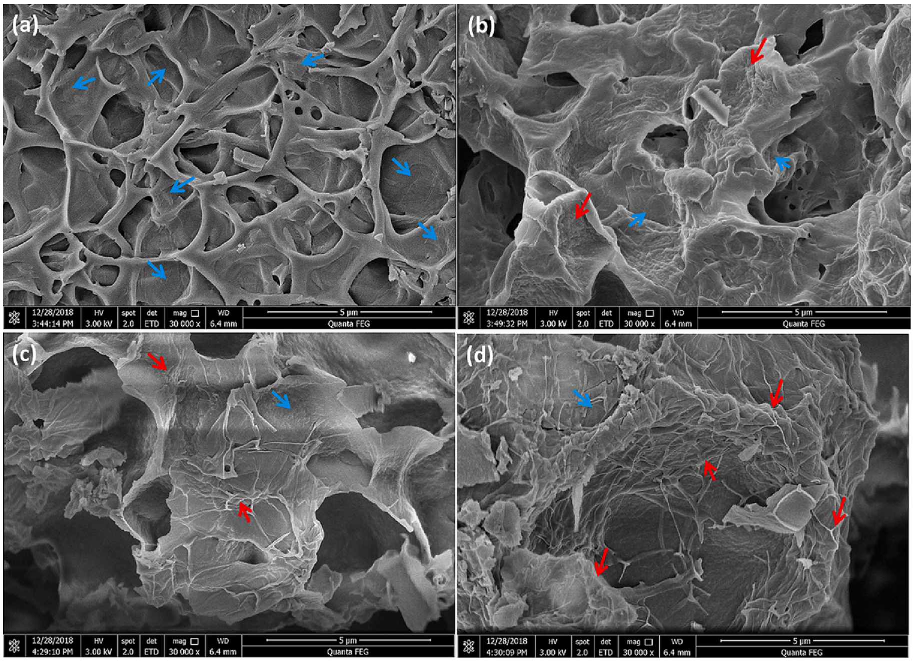

Fig. 2. SEM images of emulsion gels from freeze-dried cyclohexane-in-water emulsions stabilized by QS (1.5%) only and with varying GA from 0.5 to 2.0%. GA concentrations: (a): 0 wt%; (b) 0.5 wt%; (c) 1.0 wt%; (d) 2.0 wt%. Note: interface-driven self-assembly fibers of QS at the oil–water interface (marked by blue arrows) the unadsorbed GA in the continuous network (marked by red arrows) of emulsion gels in SEM images. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

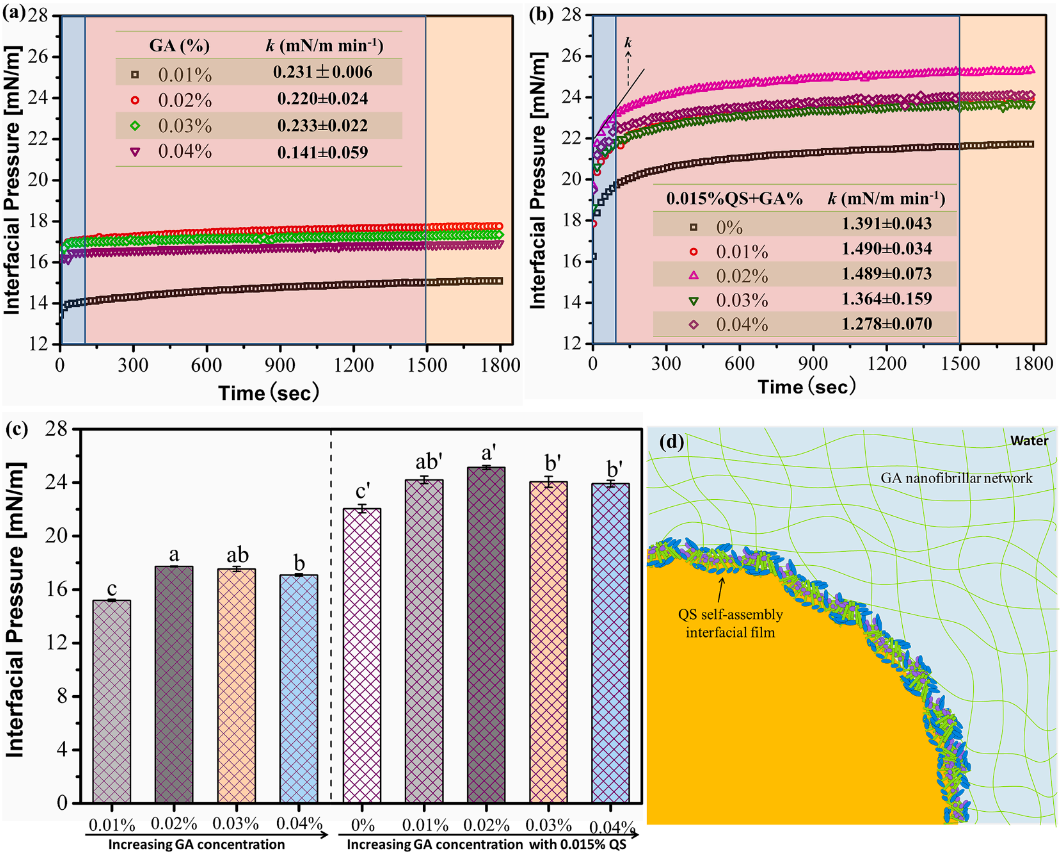

Fig. 3. The time evolution of the adsorption curves (a,b), initial adsorption rate (inset), and interfacial pressure (c) at the oil–water interface with varying GA concentrations (0.01–0.04%) and at a fixed QS of 0.015%. (d) Schematic illustration of the adsorption of QS and GA at the oil–water interface and n the aqueous phase.

due to the fibrotic self-assembly behavior of GA in aqueous phase at high concentration, thus resulting in no additional contribution to interface pressure (Ma et al., 2018; Saha et al., 2015; Wan et al., 2018). Above results show that amphiphilic QS has affinity for the hydrophobic oil phase and thus effectively increased the interfacial pressure (Fig. 3**b**) and reduced the droplet size (**Table S**). Although GA has a weak interfacial activity (Fig. 3**a**), synergistic effects between QS and GA can accelerate the whole interfacial adsorption process (Fig. 3**d**).

3.3. Effect of the GA on rheological properties of emulsion gels

As seen previously results described in Fig. 3, the oil droplets coated with QS were captured in the GA nanofibrils network, which could play a distinct structuring role on the macrostructure and microstructural morphology of emulsion gels.

3.3.1 Flow behavior. The steady-state flow behavior of emulsion gels was measured as the function of shear rate (0 to 200 s^{-1}). As depicted in Fig. 4**a**, the apparent viscosities of all emulsions were decreased with the shear rate increasing, indicating a typical shear-thinning behavior for pseudoplastic (Non-Newtonian) materials. However, the viscosities of the emulsions show a significant dependence on the GA loading as the viscosity at a low shear rate (0.1 s^{-1}) increases by 2 orders of magnitude when arrived to 2.0 wt%, compared to the one stabilized by QS sonly. This phenomenon was in line with the results of appearance (Fig. 1**a**). Moreover, these results were also confirmed by the flow parameters of emulsions stabilized with varying GA concentrations (see the inset of Fig. 4**a**). High correlation coefficient R^2 (>0.995) indicates that the nonlinear Herschel-Bulkley model fits the flow curves adequately. The values of flow behavior index (n) were<1, which further revealed a yielding behavior with a shear-thinning character (Shahbazi et al., 2022). However, the degree of shear thinning was higher for the emulsions structured by GA as compared to the control, indicating a stronger shear sensitivity. Also, the increasing in the consistency index (K) with GA concentration represented high strong network structure of the examined emulsions (Urbánková et al., 2021). Taken together the results in Fig. 1**a** and **Fig. S1** that there was nonsignificant ($p > 0.05$) effect of GA on the particle size and interfacial adsorption, the contribution of GA gelation in the continuous phase was confirmed, as schematized in Fig. 3**d**. A similar phenomenon was observed in the emulsion systems prepared by co-stabilizing of biopolymers and GA (Li et al., 2020).

3.3.2. Amplitude and frequency sweeps. Dynamic oscillatory analyses shown in Fig. 4**b** gave important information about the viscoelastic characteristics of the emulsion gels upon GA structuring. Within the linear viscoelastic region (LVR), the elastic modulus (G') of all samples was significantly larger than the viscous modulus (G"), showing that emulsions have marked elastic solid-like behavior. Moreover, increasing GA concentration the emulsion gels showed significantly broader LVR, higher G' values, and higher critical stress (crossover point of G' and G") over the applied amplitude range, suggesting high gel strength. Explicitly, Fig. 4**c** showed that both G' and G" values gradually increased with increasing GA concentration. Compared to the emulsion stabilized by QS sonly, G' in LVR increased>600-folds reached to 27755.22 ± 380.79 Pa for emulsion structured by 2% GA, suggesting an important role of GA in the viscoelasticity. In addition, the critical stress at the crossover point (G' = G") increased from about 6 Pa to 519 Pa upon increasing GA up to 2% (Fig. 4**d**). These results demonstrated GA endows elastic, soft solid-like materials.

From the frequency sweep curves as shown in Fig. 4**e**, the greater dependence of G' on frequency was observed in the emulsion stabilized

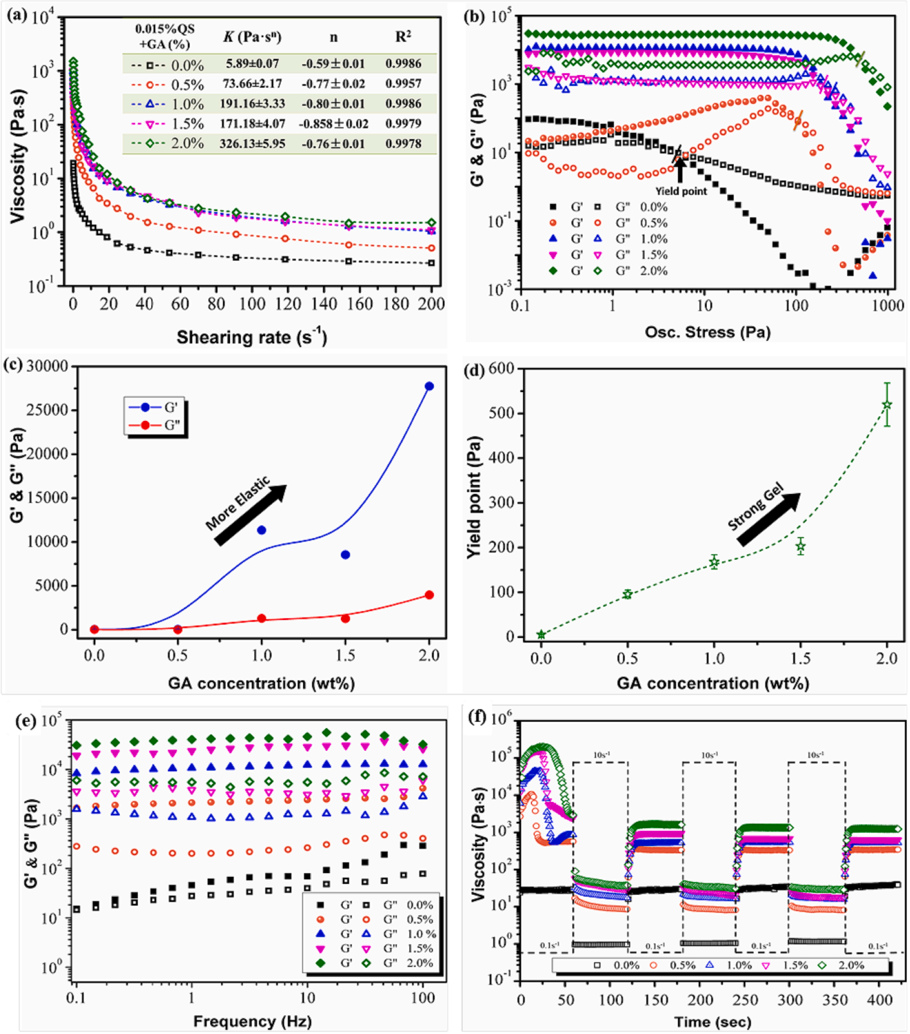

Fig. 4. (a) Viscosity curves and Herschel-Bulkley flow parameters (inset) of these emulsion gels. (b) Amplitude sweeps of emulsion gels with different GA concentration. (c) G′, G″ and (d) yield point test on emulsion gels with different GA concentration. (e) Oscillation frequency sweep test for emulsion gels with different GA concentration. G′ and G″ are shown as solid and open symbols, respectively. (f) Thixotropic properties of emulsion gels measured by plotting viscosity over time and at changing shear rates of 0.1 s^{-1} and 10 s^{-1}.

by QS only characterizing a rheological response and weak gel. However, it was noted that the emulsions structured by GA had reasonable gel strength with a slight dependence of G' and on the applied oscillation frequency. This was indicate a strengthen in gel strength especially at higher GA concentration. Furthermore, the value of G' was increased remarkably with GA increasing, which is consistent with the effect on the gel strength from the results obtained in viscosity curves (Fig. 4**a**) and amplitude sweeps (Fig. 4**b**). This can be explained by the fact that the GA nanofibrils would dominate the rheological behavior by forming hydrogel fibrillar network in the continuous phase of the emulsions, as schematized in Fig. 3**d**. This analysis can be also supported by the results of microstructure observations (Fig. 2).

3.3.3 Thixotropic properties. To insights into the structure recovery properties of these emulsion gels, the thixotropic behavior was further investigated by exposing them to a five-interval cycle of low and high shear rates (0.1 and 10 s^{-1}). As seen from Fig. 4**f**, the viscosity was dependent on time (especially at high shear rate of 10 s^{-1}), in good agreement with the data from steady-state flow measurements (Fig. 4**a**). However, the structure showed almost completely recovered even removing the high shear rate and backing to a shear rate of 0.1 s^{-1} even after five-interval cycle, illustrating a good structure recovery. This particular property of emulsion gels is interesting for application where a reversible structure breakdown and recovery is desired, especially in the food, medical, and material science industries (Chen et al., 2019; Gao et al., 2021; Li et al., 2022; Shahbazi et al., 2022; Yin et al., 2022).

3.4. *Thermoresponsive properties of emulsion gels*

Rheological evaluation was further conducted on the emulsions to monitor temperature-induced phenomena. Rheological thermoscans of the three emulsions with different GA concentrations at the temperature range of 5–90 °C are shown in Fig. 5**a**. The graphs show the dependence of the G′ with the temperature. For QS alone emulsion, the heating–cooling treatment not decreased but increased the storage moduli greatly, which may be due to the interaction among oil droplets (Du et al., 2021). It is interesting to note that the storage moduli (G') of the emulsions structured by GA experience a sudden decrease above the transition temperature (about ~ 60 °C), indicating a thermo-thinning behavior. Although continuously decrease in G′ with the rising temperature, a recoverable increase in G′ with temperature drop was observed under the combination of GA, confirming a thermoreversible property. The temperature-responsive behavior of the QS-stabilized emulsion gels by GA structuring was further confirmed to obtain the sol–gel transition under heating–cooling cycle from 25 °C to 85 °C, as illustrated in Fig. 5**b**. Moreover, a phase separation or even creaming wasn't observed after the heating–cooling cycle. Strong evidence can be found that the recycled emulsions had a quite similar droplet size distribution to the mother emulsions. The phenomenon was attributed to the hydrogel constructed by GA fibrillar network in continuous phase at low temperature, and the collapse of 3D network structure at high temperature above the transition temperature of GA hydrogel (seen in **Fig S2**). Additionally, the interface self-assembly of QS at the oil–water

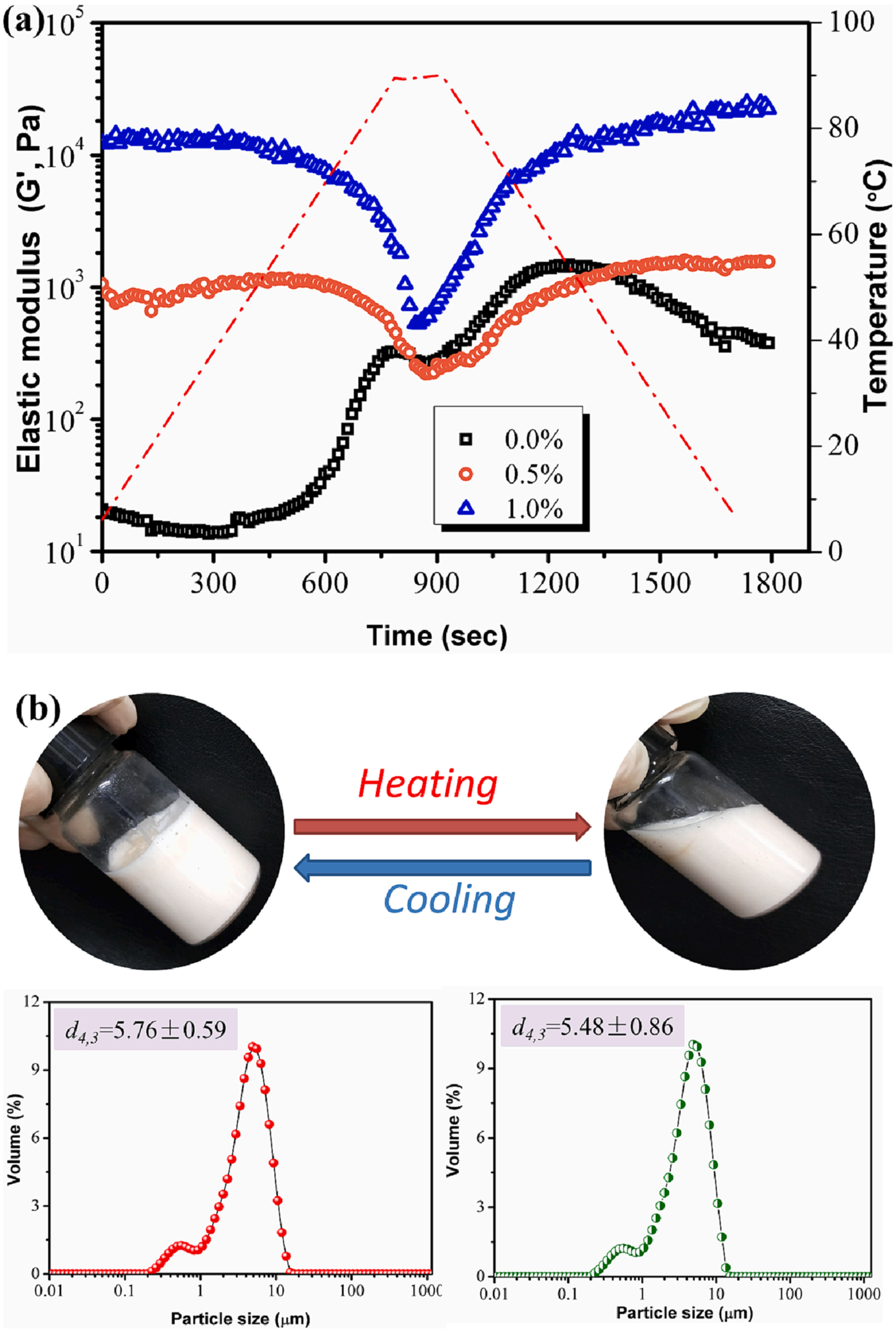

Fig. 5. (a) Gel strength as a function of temperature for heating and upon cooling cycles for emulsions prepared using 1.5% QS with varying GA concentration (0.5%, 1.5%). (b) Photographic representation and droplet size distributions of representative emulsion gel using 1.5% QS and 1.5% GA during heating at 85 °C (right) and cooling at 25 °C (left).

interface was contributed to the fabrication of stable droplets in this system (Chen, et al., 2020; Chen & Yang, 2019). In this case, the interfacial QS on droplet surfaces were also contributed to endow a high stability of emulsion. For example, structuring QS-based emulsion by GA can be to fabricate gelled emulsions with high freeze–thaw stability (seen in **Fig S3**).

3.5. Preparation and characterization of oleogels

Structuring of edible liquid oil into soft oleogels through an emulsion-template approach, dehydration (such as vacuum drying, freeze drying, and oven drying) often leads to disruption of emulsion droplets collapsing and oil-leakage (Chen & Yang, 2019; Flöter et al., 2021; Hu, et al., 2016; Patel et al., 2015). Comparatively, the time required for complete dehydration was remarkable shorter under vacuum condition than to that of oven drying (seen in **Fig S4a**). In additional, the dehydration mode (i.e., vacuum drying *vs*. oven drying) had a great effect on the oil stability with exposure to high temperature for a longer time showing more deterioration than that exposure to relatively lower temperatures for a lesser time (seen in **Fig S4b**). However, a macroscopic oil separation was observed when dehydration by freeze drying (date not shown). This phenomenon was contrary to the previous report that structured soft oleogels with high oil loading of above 97 wt % can be obtained through lyophilization from a QS-based medium internal phase emulsion (40–70 wt%) (Chen & Yang, 2019). This was attribute to a more uniform droplet size distribution with smaller droplets (0.24–0.72 μm), which enabled the system to achieve high stability against fracture during dehydration. Nonetheless, an oil-leakage was occurred in the emulsion system with a high oil internal phase (80 wt%) (Chen & Yang, 2019). These results indicating that vacuum dehydration would be a credible strategy in transforming liquid oil into soft oleogels.

Fig. 6**a** shows the appearance of as a representative example formulated with 1.5% QS and 1.5% GA. The self-supporting oleogel with an oil weight content of above 96 wt% showed a solid-like appearance and a more yellowish colour compared to the emulsion precursor. The yellowish colour would attributable to trace components in the naturally extracted saponin. Morphologically, Fig. 6**b** and its magnification (Fig. 6**c**) provided a more detailed insight into the microstructure of the oleogel. They were further evidence of GA nanofibrous (red arrows) distributed in the continuous phase and QS as self-assembled at the interface (blue arrows) forming a protective surface layer around oil droplets. As a consequence, the QS interfacial film provides a strong structural framework that trapped the oil droplets covered by fibrils and greatly limited the destabilization. These results may provide a reason to explain the good stability of the oleogels. Simultaneously, the supporting effect of GA on gel network was further confirmed (Li et al., 2020; Su et al., 2021).

To better understanding the rheological behaviors of oleogels under the external forces, the viscosity, thixotropic and rheological properties of samples were also performed. The viscosity decreased with rising shear rate in all samples, confirming the shear thinning properties of the oleogels, as displayed in Fig. 6**d**. Meanwhile, the higher in GA concentration, the faster the apparent viscosity decreased with the increasing in shear rate. That is, the behavior of emulsion pseudo plastic fluid was more obvious. At the same shear frequency, the apparent viscosity of the oleogels increased with the GA increasing, indicating that GA enhanced the flocculation of the droplets. Obviously, all samples expressed solid-like features (G'> G'') under the shear stress test (Fig. 6**e**). Besides, both G' and G'' exhibited an upward trend with increasing GA concentration, which illustrated the formation of stronger oleogels. The intersection of G' and G'' was not observed during the stress range of 10–1000 Pa, indicating a highly stable structure against external force. This result may due to the buffering of GA network structure covering around the oil droplets. Interestingly that, the oleogels emerged higher resistance to the applied stress than other previous oleogels formulated with other hydrocolloid agents such as ethylcellulose, protein, polysaccharide, protein-polysaccharide complexes (Luo et al., 2019; Meng et al., 2018; Patel et al., 2015). In the soft matters, QS-wrapped oil droplets were incorporated into the fibrillar network of GA, making stronger and tighter oleogels (Fig. 5**c**). For instance, the G' of QS-oleogels prepared in absence and presence of 2.0% GA can be mentioned; at angular stress of 100 Pa, the storage moduli were 4500 and 16,500 Pa, respectively. Similar findings were reported by Jiang, et al., who observed that an increase in the concentration of 4.6% regenerated cellulose in carboxy methylcellulose-based emulsion precursor caused an increase in G' resulting in a high gel strength of above 15,000 Pa, which is related to network formation in the bulk phase (Jiang et al., 2018). However, the increase under different GA concentrations was obvious compared to the emulsion systems (Fig. 4), that may be due to the fact that the dehydration process destructed the network structure.

To gain insight into the oleogels structural recovery, a three-interval thixotropy test (3-ITT) was performed, as shown in Fig. 6**f**. In this test, the viscosity was measured as a function of three time-intervals (600 s) under low, high and again low values of constant shear rates (10, 100, 10 s^{-1}). For all the oleogels, the viscosity was decreased with increased shear rate, representing a typical shear-thinning behavior. Once switch the shear rate from low to high, a marked reduction in viscosity was observed, probably owing to the breaking up of the high levels of interconnected structures in the oleogels. Nevertheless, once switch

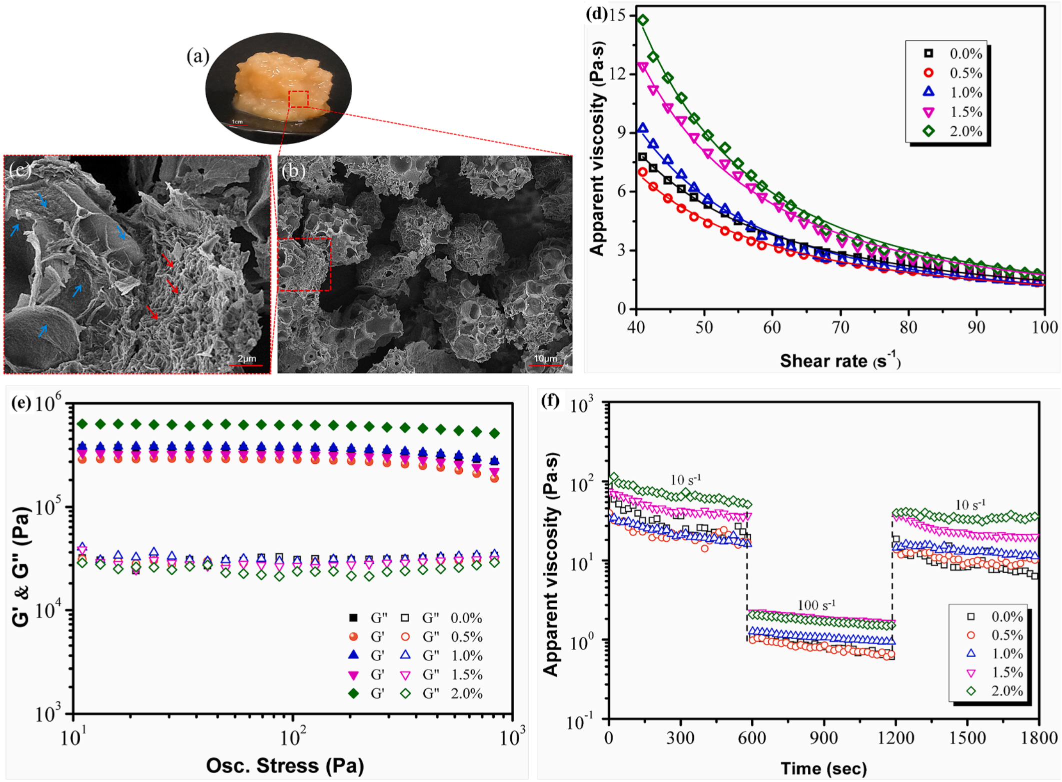

Fig. 6. Photograph (a) a n d S EM images (b,c) of oleogels prepared from a emulsion precursor containing 75 wt% soybean oil stabilized by 1.5 wt% QS and 1.5 wt% GA. (d) Viscosity as a function of the shear rate. (e) Storage modulus G′ (solid symbols) and loss modulus G″ (open symbols) as a function of amplitude sweeps. (f) Viscosity plot from a 3-interval thixotropy test of oleogels.

back to the low shear (*i.e.*, the third interval) the viscosity of oleogels almost completely recovered showing a reversible restructuration toward their initial structure. The bicomponent oleogels showed high structural recovery values from 78.5 to 85.0%, which obviously higher than that of alone QS oleogel (74.6%). The network structure formed by the interaction of oil droplet aggregation was destroyed, resulting in the viscosity decreasing (Anvari & Joyner, 2017; Fuhrmann et al., 2022).

4. Conclusions

Novel thermoresponsive emulsion gels and oleogels were fabricated by assembling nanofibrous from natural triterpenoid *Quillaja* saponin (QS) and glycyrrhizic acid (GA) with a feasible strategy. Although confirmed sole triterpenoid QS fabricated high internal phase emulsion, incorporating GA could form gelled emulsion. Moreover, the emulsions exhibited a temperature-responsive nature, which may be beneficial in mimicking the fat-like melt-in-the-mouth effect and controllable delivering bioactive components. CLSM and SEM were highlighted an interesting microstructure, where the oil droplets are tightly packed by the QS interfacial film and the GA nanofibrous gel network supported the continuous phase. Increased GA concentration caused a rise in interfacial pressure, and a decrease of droplet size. In this case, the emulsions turned from a sol-like to gel-like behaviors, soft-solid and thermos-responsive emulsion gels with enhancing mechanical strength. Following the removal of external water, soft oleogels can be further fabricated though preventing the coalescence of oil droplets. Moreover, the results also demonstrated that increasing GA concentration is conducive to the formation of stable oleogels with greater stiffness and good thixotropic recovery. This finding would provide all-natural ingredients in the structuring edible liquid oil into healthy gels for the potential application in diverse fields such as foods, pharmaceuticals, cosmetics, and materials.

CRediT authorship contribution statement

Yao Liu: Investigation, Data curation, Writing – original draft. **Huan Zhang:** Investigation, Methodology, Writing – original draft. **Xiao-Wei Chen:** Conceptualization, Project administration, Funding acquisition, Writing – review & editing. **Tao Yang:** Writing – review & editing. **Cong Sun:** Resources, Supervision. **Shang-De Sun:** Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was financially supported by, the Joint Science and Technology R&D Projects of Henan Province (222103810067), National Engineering Laboratory/Province Key Laboratory of Food Science Discipline (NL2021002), Key Research and Development Projects of Guangdong Province (2022B0202010003) and the Innovation Funds Plan of Henan University of Technology (2021ZKCJ02).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100751.

References

Anvari, M., & Joyner, H. S. (2017). Effect of formulation on structure-function relationships of concentrated emulsions: Rheological, tribological, and microstructural characterization. *Food Hydrocolloids, 72*, 11–26. https://doi.org/10.1016/j.foodhyd.2017.04.034

Böttcher, S., & Drusch, S. (2017). Saponins—Self-assembly and behavior at aqueous interfaces. *Advances in Colloid and interface Science, 243*, 105–113. https://doi.org/10.1016/j.cis.2017.02.008

Chelazzi, D., Giorgi, R., & Baglioni, P. (2018). Microemulsions, micelles, and functional gels: How colloids and soft matter preserve works of art. *Angewandte Chemie International Edition, 57*(25), 7296–7303. https://doi.org/10.1002/anie.201710711

Chen, X. W., Ning, X. Y., Zou, Y., Liu, X., & Yang, X. Q. (2019). Multicompartment emulsion droplets for programmed release of hydrophobic cargoes. *Food & function, 10*(8), 4522–4532. https://doi.org/10.1039/c9fo00558g

Chen, X. W., Sun, S. D., Ma, C.-G., & Yang, X. Q. (2020). Oil–water interfacial-directed spontaneous self-assembly of natural quillaja saponin for controlling interface permeability in colloidal emulsions. *Journal of Agricultural and Food Chemistry, 68* (47), 13854–13862. https://doi.org/10.1021/acs.jafc.0c04431

Chen, X. W., Wang, J. M., Guo, J., Wan, Z. L., Yin, S. W., & Yang, X. Q. (2017). Hierarchical high internal phase emulsions and transparent oleogels stabilized by quillaja saponin-coated nanodroplets for color performance. *Food & function, 8*(2), 823–831. https://doi.org/10.1039/c6fo01752e

Chen, X. W., & Yang, X. Q. (2019). Characterization of orange oil powders and oleogels fabricated from emulsion templates stabilized solely by a natural triterpene saponin. *Journal of Agricultural and Food Chemistry, 67*(9), 2637–2646. https://doi.org/10.1021/acs.jafc.8b04588

Du, L., Li, S., Jiang, Q., Tan, Y., Liu, Y., & Meng, Z. (2021). Interfacial interaction of small molecular emulsifiers tea saponin and monoglyceride: Relationship to the formation and stabilization of emulsion gels. *Food Hydrocolloids, 117*, Article 106737. https://doi.org/10.1016/j.foodhyd.2021.106737

Fink, R., & Filip, S. (2022). Surface-active natural saponins. Properties, safety, and efficacy. *International Journal of Environmental Health Research*, 1–10. https://doi.org/10.1080/09603123.2022.2043252

Flöter, E., Wettlaufer, T., Conty, V., & Scharfe, M. (2021). Oleogels-their applicability and methods of characterization. *Molecules, 26* (6), Article 1673. doi: 10.3390/molecules26061673.

Fuhrmann, P. L., Breunig, S., Sala, G., Sagis, L., Stieger, M., & Scholten, E. (2022). Rheological behaviour of attractive emulsions differing in droplet-droplet interaction strength. *Journal of colloid and interface science, 607*, 389–400. https://doi.org/10.1016/j.jcis.2021.08.124

Gao, H., Ma, L., Cheng, C., Liu, J., Liang, R., Zou, L., & McClements, D. J. (2021). Review of recent advances in the preparation, properties, and applications of high internal phase emulsions. *Trends in Food Science & Technology, 112*, 36–49. https://doi.org/10.1016/j.tifs.2021.03.041

Hu, Z., Marway, H. S., Kasem, H., Pelton, R., & Cranston, E. D. (2016). Dried and redispersible cellulose nanocrystal Pickering emulsions. *ACS Macro Letters, 5*(2), 185–189. https://doi.org/10.1021/acsmacrolett.5b00919

Jiang, Y., Liu, L., Wang, B., Sui, X., Zhong, Y., Zhang, L., … Xu, H. (2018). Cellulose-rich oleogels prepared with an emulsion-templated approach. *Food Hydrocolloids, 77*, 460–464. https://doi.org/10.1016/j.foodhyd.2017.10.023

Li, Q., He, Q., Xu, M., Li, J., Liu, X., Wan, Z., & Yang, X. (2020). Food-grade emulsions and emulsion gels prepared by soy protein–pectin complex nanoparticles and glycyrrhizic acid nanofibrils. *Journal of Agricultural and Food Chemistry, 68*(4), 1051–1063. https://doi.org/10.1021/acs.jafc.9b04957

Li, Q., Wan, Z., & Yang, X. (2022). Glycyrrhizic acid: Self-assembly and applications in multiphase food systems. *Current Opinion in Food Science, 43*, 107–113. https://doi.org/10.1016/j.cofs.2021.11.008

Luo, S. Z., Hu, X. F., Jia, Y. J., Pan, L. H., Zheng, Z., Zhao, Y. Y., & Jiang, S. T. (2019). Camellia oil-based oleogels structuring with tea polyphenol-palmitate particles and citrus pectin by emulsion-templated method: Preparation, characterization and potential application. *Food Hydrocolloids, 95*, 76–87. https://doi.org/10.1016/j.foodhyd.2019.04.016

Ma, L., Bertsch, P., Wan, Z., Yang, X., & Fischer, P. (2021). Synergistic effect of glycyrrhizic acid and cellulose nanocrystals for oil-water interfacial stabilization. *Food Hydrocolloids, 120*, Article 106888. https://doi.org/10.1016/j.foodhyd.2021.106888

Ma, L., Li, Q., Du, Z., Su, E., Liu, X., Wan, Z., & Yang, X. (2019b). A natural supramolecular saponin hydrogelator for creation of ultrastable and thermostimulable food-grade foams. *Advanced Materials. Interfaces, 6 (14), Article 1900417*. https://doi.org/10.1002/admi.201900417

Ma, Y., Gao, Y., Zhao, X., Zhu, Y., Du, F., & Hu, J. (2018). A Natural Triterpene Saponin-Based Pickering Emulsion. *Chemistry–A. European Journal, 24*(45), 11703–11710. https://doi.org/10.1002/chem.201801619

Ma, Y., Hao, J., Zhao, K., Ju, Y., Hu, J., Gao, Y., & Du, F. (2019a). Biobased polymeric surfactant: Natural glycyrrhizic acid-appended homopolymer with multiple pH-responsiveness. *Journal of colloid and interface science, 541*, 93–100. https://doi.org/10.1016/j.jcis.2019.01.088

Martins, A. J., Vicente, A. A., Pastrana, L. M., & Cerqueira, M. A. (2020). Oleogels for development of health-promoting food products. *Food Science and Human Wellness, 9* (1), 31–39. https://doi.org/10.1016/j.fshw.2019.12.001

Meng, Z., Qi, K., Guo, Y., Wang, Y., & Liu, Y. (2018). Macro-micro structure characterization and molecular properties of emulsion-templated polysaccharide oleogels. *Food Hydrocolloids, 77*, 17–29. https://doi.org/10.1002/ejlt.201700287

Pagureva, N., Tcholakova, S., Golemanov, K., Denkov, N., Pelan, E., & Stoyanov, S. D. (2016). Surface properties of adsorption layers formed from triterpenoid and steroid saponins. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 491*, 18–28. https://doi.org/10.1016/j.colsurfa.2015.12.001

Patel, A. R., Rajarethinem, P. S., Cludts, N., Lewille, B., De Vos, W. H., Lesaffer, A., & Dewettinck, K. (2015). Biopolymer-based structuring of liquid oil into soft solids and oleogels using water-continuous emulsions as templates. *Langmuir, 31*(7), 2065–2073. https://doi.org/10.1021/la502829u

Reichert, C. L., Salminen, H., Bönisch, G. B., Schäfer, C., & Weiss, J. (2018). Concentration effect of Quillaja saponin–Co-surfactant mixtures on emulsifying properties. *Journal of colloid and interface science, 519*, 71–80. https://doi.org/10.1016/j.jcis.2018.01.105

Reichert, C. L., Salminen, H., & Weiss, J. (2019). Quillaja saponin characteristics and functional properties. *Annual Review of Food Science and Technology, 10*, 43–73. https://doi.org/10.1146/annurev-food-032818-122010

Saha, A., Adamcik, J., Bolisetty, S., Handschin, S., & Mezzenga, R. (2015). Fibrillar networks of glycyrrhizic acid for hybrid nanomaterials with catalytic features. *Angewandte Chemie, 127*(18), 5498–5502. https://doi.org/10.1002/anie.201411875

Schmid, C., Dawid, C., Peters, V., & Hofmann, T. (2018). Saponins from European licorice roots (Glycyrrhiza glabra). *Journal of natural products, 81*(8), 1734–1744. https://doi.org/10.1021/acs.jnatprod.8b00022

Shahbazi, M., Jäger, H., & Ettelaie, R. (2022). A Promising Therapeutic Soy-Based Pickering Emulsion Gel Stabilized by a Multifunctional Microcrystalline Cellulose: Application in 3D Food Printing. *Journal of Agricultural and Food Chemistry, 70*(7), 2374–2388. https://doi.org/10.1021/acs.jafc.1c05644

Su, E., Li, Q., Xu, M., Yuan, Y., Wan, Z., Yang, X., & Binks, B. P. (2021). Highly stable and thermo-responsive gel foams by synergistically combining glycyrrhizic acid nanofibrils and cellulose nanocrystals. *Journal of colloid and interface science, 587*, 797–809. https://doi.org/10.1016/j.jcis.2020.11.039

Tsibranska, S., Tcholakova, S., Golemanov, K., Denkov, N., Pelan, E., & Stoyanov, S. D. (2020). Role of interfacial elasticity for the rheological properties of saponin-stabilized emulsions. *Journal of colloid and interface science, 564*, 264–275. https://doi.org/10.1016/j.jcis.2019.12.108

Tykarska, E., Sobiak, S., & Gdaniec, M. (2012). Supramolecular Organization of Neutral and Ionic Forms of Pharmaceutically Relevant Glycyrrhizic Acid□ Amphiphile Self-Assembly and Inclusion of Small Drug Molecules. *Crystal growth & design, 12*(4), 2133–2137. https://doi.org/10.1021/cg300160c

Urbánková, L., Sedláček, T., Kašpárková, V., & Bordes, R. (2021). Formation of oleogels based on emulsions stabilized with cellulose nanocrystals and sodium caseinate. *Journal of colloid and interface science, 596*, 245–256. https://doi.org/10.1016/j.jcis.2021.02.104

Vinarova, L., Vinarov, Z., Atanasov, V., Pantcheva, I., Tcholakova, S., Denkov, N., & Stoyanov, S. (2015). Lowering of cholesterol bioaccessibility and serum concentrations by saponins: In vitro and in vivo studies. *Food & function, 6*(2), 501–512. https://doi.org/10.1039/c4fo00785a

Wan, Z., Sun, Y., Ma, L., Zhou, F., Guo, J., Hu, S., & Yang, X. (2018). Long-lived and thermoresponsive emulsion foams stabilized by self-assembled saponin nanofibrils and fibrillar network. *Langmuir, 34*(13), 3971–3980. https://doi.org/10.1021/acs.langmuir.8b00128

Xu, M., Ma, L., Li, Q., Wu, J., Wan, Z., Ngai, T., & Yang, X. (2022). Robust and highly adaptable high internal phase gel emulsions stabilized solely by a natural saponin hydrogelator glycyrrhizic acid. *Food Function, 13*(1), 280–289. https://doi.org/10.1039/D1FO01656C

Xu, M., Wan, Z., & Yang, X. (2021). Recent Advances and Applications of Plant-Based Bioactive Saponins in Colloidal Multiphase Food Systems. *Molecules, 26 (19), Article 6075*. https://doi.org/10.3390/molecules26196075

Yin, W. J., Chen, X. W., Ma, C. G., & Wang, J. M. (2022). Fabrication and Characterization of Tunable High Internal Phase Emulsion Gels (HIPE-Gels) Formed by Natural Triterpenoid Saponin and Plant Soy Protein. *ACS Food Science & Technology., 2*, 1103–1113. https://doi.org/10.1021/acsfoodscitech.2c00082

Zhao, X., Zhang, H., Gao, Y., Lin, Y., & Hu, J. (2019). A simple injectable moldable hydrogel assembled from natural glycyrrhizic acid with inherent antibacterial activity. *ACS Applied Bio Materials, 3*(1), 648–653. https://doi.org/10.1021/acsabm.9b01007

Zhao, Z., Xiao, Y., Xu, L., Liu, Y., Jiang, G., Wang, W., & Tang, L. (2021). Glycyrrhizic acid nanoparticles as antiviral and anti-inflammatory agents for COVID-19 treatment. *ACS applied materials & interfaces, 13*(18), 20995–21006. https://doi.org/10.1021/acsami.1c02755