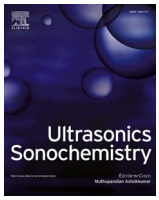
Ultrasonics Sonochemistry 95 (2023) 106367

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

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



Effect of polyphenolic structure and mass ratio on the emulsifying performance and stability of emulsions stabilized by polyphenol-corn amylose complexes

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ARTICLE INFO

Keywords:
Amylose
Polyphenols
Polyphenolic structure
Emulsifying properties
Emulsion stability

ABSTRACT

O/W emulsions stabilized by polyphenol/amylose (AM) complexes with several polyphenol/AM mass ratios and different polyphenols (gallic acid (GA), epigallocatechin gallate (EGCG) and tannic acid (TA)) were prepared by a high-intensity ultrasound emulsification technique. The effect of the pyrogallol group number of polyphenols and the mass ratio of polyphenols/AM on polyphenol/AM complexes and emulsions was studied. The soluble and/or insoluble complexes gradually formed upon adding polyphenols into the AM system. However, insoluble complexes were not formed in the GA/AM systems because GA has only one pyrogallol group. In addition, the hydrophobicity of AM could also be improved by forming polyphenol/AM complexes. The emulsion size decreased with increasing pyrogallol group number on the polyphenol molecules at a fixed ratio, and the size could also be controlled by the polyphenol/AM ratio. Moreover, all emulsions presented various degrees of creaming, which was restrained by decreasing emulsion size or the formation of a thick complex network. The complex network was enhanced by increasing the ratio or pyrogallol group number on the polyphenol molecules, which was because the increasing number of complexes was adsorbed onto the interface. Altogether, compared to GA/AM and EGCG/AM, the TA/AM complex emulsifier had the best hydrophobicity and emulsifying properties, and the TA/AM emulsion had the best emulsion stability.

1. Introduction

Emulsions are stabilized by emulsifiers (inorganic or organic) irreversibly absorbed on the oil–water interface and are widely applied in cosmetics, medicine, food and so on [1]. Although inorganic emulsifiers usually have favorable emulsifying performance, their disadvantage of low accessibility and potential toxicity has restricted their development in food emulsions [1,2]. In recent years, a growing number of organic biomacromolecule emulsifiers have been employed to stabilize food emulsions, such as proteins, starch, cellulose, polyphenols or their compound complexes [3–6].

Nevertheless, among biomacromolecule emulsifiers, only a few of them have excellent emulsifying ability, and most of them have poor emulsification performance, especially polysaccharides [7]. Thus, it is necessary to take some measures to improve the emulsifying performance of biomacromolecules, for instance, physical and chemical modification, compound emulsifiers or noncovalent interactions [5,8–10]. Compared to other modification methods, noncovalent interactions could not only enhance the emulsifying performance of biomacromolecules but also endow complexes with fresh properties, such as improvement of hydrophobicity, antioxidative and rheology [1,5]. Meanwhile, noncovalent interactions are also a green and environmental modification method, deeply loved by researchers and food consumers.

Starch is a natural and renewable biological resource and has the features of wide distribution and low price. It has been widely used in food, medicine, papermaking and other industries due to these advantages. However, as an emulsifier, the existence of many hydroxyl groups

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https://doi.org/10.1016/j.ultsonch.2023.106367
Received 8 January 2023; Received in revised form 24 February 2023; Accepted 11 March 2023
Available online 15 March 2023

on the structure leads to high hydrophilicity and terrible emulsification, which restricts starch in the development of food emulsions [5,11]. Therefore, to improve the stability of starch-based emulsions, food scientists have taken many steps to modify starch to endow it with outstanding emulsifying performance. To date, most starch modification methods have been employed, including the formation of smaller starch particles by physical modification and the improvement of starch hydrophobicity through acid hydrolysis, octenyl succinate anhydride (OSA) grafting and so on [8,12,13]. However, there have been few reports on the modification of starch by noncovalent interactions in emulsions [5,14].

Plant polyphenols, good antioxidants, can bond biomacromolecules to form complexes by covalent or noncovalent interactions, which would improve the emulsifying performance of biomacromolecules, endow complexes with antioxidant activity, excellent hydrophobicity and so on [15,16]. Gallic acid (GA), naturally distributed in palm-leaf rhubarb, Eucalyptus grandis, dogwood and other plants. It has been reported that complexes formed through noncovalent interactions between GA and biomacromolecules have excellent emulsifying and antioxidative properties. For example, emulsions stabilized by GA/zein complexes showed superior oxidative stability [17]. In addition, epigallocatechin gallate (EGCG) is the main catechin in tea. Similarly, EGCG could also interact with biomacromolecules to produce complexes with good emulsifying and antioxidative characteristics. Feng, Wang, Wang, Zhang, Gu, Xia and Huang [18] discovered that EGCG could bind with pea protein isolate/high methoxyl pectin by hydrogen bonding, and the complexes had extraordinary hydrophobicity ($\theta_{ow} = 81.60 \pm 0.4^{\circ}$) and better interfacial viscoelasticity. Tannic acid (TA) is found in the bark and fruits of many trees, such as oak and sumac. It also has the capacity to crosslink or bind biomacromolecules through noncovalent interactions [16,19]. For example, it has been reported that TA/chitin complexes are formed by intermolecular hydrogen bonds between TA and chitin with good oxidation resistance and surface potential [20]. Simultaneously, the emulsions stabilized by TA/chitin complexes possessed better viscoelasticity and physicochemical stability.

In summary, a simple mixture of plant polyphenols with biomacromolecules to form complexes to improve the emulsifying performance and oxidative stability of emulsions was successfully employed. However, there have been few studies on improving the emulsifying performance and structural properties of starch-based emulsions through noncovalent interactions between plant polyphenols and starch. At present, the simple mixture of plant polyphenols and biomacromolecules is limited to the interaction between a single polyphenol and biomacromolecule [5,14]. However, the difference in complex combinations between biomacromolecules and polyphenols with different molecular structures and the further influence on emulsions stabilized by these complexes have not been reported. In addition, although the emulsifying performance and oxidative stability of starch-based emulsions had been improved by the simple mixture of plant polyphenol and starch, starch was a mixture consisting of amylose and amylopectin. Meanwhile, most of the starch used in interacting with plant polyphenols was not further purified and separated into amylose and amylopectin. Therefore, the effect of amylose and amylopectin on the emulsification and stability mechanism of polyphenols/starch complexes and emulsions was unclear.

Therefore, to elucidate the effect of polyphenolic structure on polyphenols/starch complexes and emulsions and exclude the interference factor of the discrepancy in the molecular structure of amylose and amylopectin, we chose corn AM and polyphenols with different pyrogallol group numbers (GA, EGCG and TA) to build steady and esculent polyphenol/AM complexes and subsequently produce soybean oil-based emulsions by a high-intensity ultrasound emulsification technique. Based on the abovementioned aims, the AM dispersion was prepared, and then three polyphenols (GA, EGCG or TA) were added separately to obtain modified polyphenol/AM complexes. Next, the particle size, contact angle and emulsifying performance of the complexes were characterized. Then, optical microscopy, creaming stability, confocal laser microscopy (CLSM) and rheology were used to represent those emulsions. Furthermore, the surface loads of polyphenols and AM at the oil–water interface were also investigated. The noncovalent interactions between polyphenols and AM could regulate the emulsifying, stability and rheological properties of complexes, indicating that these complexes could act as new functional food components to extend their application in the food industry. Moreover, the interaction and emulsification mechanism of different structural polyphenols and AM would provide a reference for the interaction between polyphenols and other biomacromolecules (proteins, polysaccharides, etc.).

2. Materials and methods

2.1. Materials

Corn and soybean oil were purchased from a local supermarket (Guangzhou, China). GA (≥99%), TA (ACS grade) and fluorescent dyes (Nile Red and Nile Blue A) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). EGCG (≥98%) was purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). All other chemical reagents were of analytical grade.

2.2. Preparation of AM and polyphenol dispersions

Corn starch was separated and purified from corn grains according to the method of Lu [21] with minor modifications. The obtained starch content was 89.68%. The AM was further purified from corn starch according to the method of Klucinec and Thompson [22]. The final apparent amylose content was 95.90%. AM (1.0 g) was moistened with ethanol, and then hot acetate buffer solution (10 mM, pH 4.0, 95 °C) was added. It was then heated to 95 °C, stirred for 60 min, cooled to 25 °C, and centrifuged at 25 °C, 10000 × g for 5 min to obtain the AM dispersion (1.0%, w/v). A solution of GA (5.0%, w/v) was prepared using acetate buffer at 60 °C with continuous stirring. A solution of EGCG (5.0%, w/v) was prepared using acetate buffer at 50 °C with continuous stirring. A solution of TA (5.0%, w/v) was prepared using acetate at normal temperature with continuous stirring.

2.3. Preparation of polyphenol/AM complexes and emulsions

The polyphenol/AM complexes were produced by simple stirring at 25 °C. In brief, different concentrations of polyphenol solutions (2 mL) were added to the AM dispersion (1.0%, 5 mL) and stirred for 10 min to obtain polyphenol/AM complex solutions with mass ratios of 0.01, 0.025, 0.05, 0.125, 0.25 and 0.50. A 90Plus PALS (Brookhaven Instruments Co., USA) was used to measure the size of the polyphenol/AM complexes. To determine the contact angle, polyphenol/AM complex solutions were frozen directly in liquid nitrogen and then freeze-dried using a lyophilizer (LC-18 N, Shanghai, China).

Soybean oil-based emulsions (the oil/water ratio was 30:70 (v/v)) stabilized by polyphenol/AM complexes were prepared according to our published method [5]. In brief, 7 mL of polyphenol/AM complex solutions and 3 mL of soybean oil were successively added into a glass vial, and the mixed solutions were placed into an ultrasonic microwave cooperative reaction workstation (XO-SM100, Nanjing, China) for ultrasonic treatment in an ice bath to produce emulsions. The diameter of the ultrasonic probe was 10 mm. The ultrasound frequency and pulse duration were set at 25 kHz and on-time 2 s + off-time 8 s, respectively. Samples were effectively sonicated for 1 min at 665 W. Each emulsion was stored at room temperature for 30 days to determine the creaming index. Emulsions stabilized by AM and blank polyphenols were also formulated as a control group.

2.4. Characterization of polyphenol/AM complexes

2.4.1. Contact angle measurement

The three-phase contact angles (θ_{ow}) of the polyphenol/AM complexes were characterized through a Theat (Biolin Instrument Co., Ltd. Swiss). In brief, the lyophilized complexes were used to make pellets with a diameter of 10 mm and a thickness of 1 mm, and then the pellets were immersed in a euphotic quartz dish containing soybean oil. Then, 2 μL Mill-Q water was injected on the pellet surface. After balancing for 300 s, the appearance of droplets was captured by a video camera. Then, the outline of the water droplet was analyzed and matched to the Laplace-Young equation to obtain the contact angle of the complexes. Moreover, AM, GA, EGCG and TA were also made into pellets to determine the contact angle, which acted as the control group.

2.4.2. Emulsifying properties

The emulsifying properties of polyphenol/AM complexes were measured through the emulsifying active index (EAI) and emulsifying stability index (ESI) [23]. In detail, emulsions (50 μL) were mixed with 5 mL SDS solution (0.1%, w/v), and then the absorbance of A_0 was measured immediately at 500 nm. After 30 min of storage, the absorbance of A_{30} was obtained using the same method as A_0. The EAI and ESI were calculated using the following equation (Eqs. (1) and (2)):

$$EAI\left(m^2/g\right) = 2 \times 2.303 \times \frac{A_0 \times N}{C \times \varnothing \times 10000} \tag{1}$$

$$ESI_{30}(\%) = \frac{A_{30}}{A_0} \times 100 \tag{2}$$

where N is the dilution factor, C is the amylose mass concentration (mg/mL), and ∅ is the oil volume fraction (0.3).

2.5. Characterization of polyphenol/AM complex emulsions

2.5.1. Light microscopy

The droplets and appearance of emulsion samples were directly observed by an optical microscope (XploRA PLUS, HORIBA, France) equipped with a video camera and a 50 × magnification lens.

2.5.2. Creaming index (CI) and appearance

To assess the comparative stability of emulsions, creaming stability was measured. The fresh emulsion samples (10 mL) were stored at 25 °C for 30 days. The total altitudes of the emulsions (H_E, mm) and serum layer (H_S, mm) were measured during storage, and then the CI was determined by Eq. (3)).

$$CI(\%) = \frac{H_S}{H_E} \times 100 \tag{3}$$

The semblance of emulsions (after 30 days of storage) was captured with a camera.

2.5.3. CLSM

The emulsion microstructure was observed by confocal microscopy (Nikon, A1 HD25, Tokyo, Japan). The emulsions were first stained and then observed according to a previous method [5]. In brief, 0.2 mL of emulsion, 10 μL of Nile Red (1 mg/mL) and 10 μL of Nile Blue A (1 mg/mL) were physically mixed, and then the mixture (7 μL) was directly dropped on a glass slide and enveloped with a cover slip for CLSM observation. The oil phase was stained with Nile Red and excited at 488 nm, and the polyphenol/AM complexes were stained with Nile Blue A and excited at 640 nm.

2.5.4. Rheological measurements

The dynamic viscoelastic performance of emulsion samples was measured by a rotational rheometer (HAAKE MARS60, Thermo-Scientific, Germany) equipped with a 60 mm diameter parallel plate geometry at a gap of 1 mm. First, fresh emulsions were placed into parallel plate geometry and kept for 5 min to reach thermal equilibrium. Afterward, 0.5% strain within the linear viscoelastic region was employed for frequency sweep tests, in which the frequency was set in the range of 0.02–10 rad/s. Moreover, the flow characteristics of emulsions were also tested at shear rates from 0.1 to 300 s^{-1}. All measurements were performed in triplicate at 25 °C.

2.5.5. Surface loads and actual mass ratio of the polyphenol/AM emulsion oil–water interface

The oil–water surface loads of polyphenols and AM were measured according to the method of Zhu, Zhao, Yi, Liu, Cao, Decker and McClements [24], with slight changes. First, 20 mL of emulsion was centrifuged at 10,000 g and 25 °C for 40 min. Then, a 5 mL syringe was used to extract the bottom aqueous phase containing non-absorbed AM and polyphenols. Next, a 0.45 μm aqueous membrane was used to filter the aqueous phase to remove any residual cream phase. Finally, the weight and UV–vis spectrophotometer method was used to determine the concentrations of non-absorbed AM and polyphenols (the absorbances of GA, EGCG and TA were 250 nm, 272 nm and 274 nm, respectively). The following equation (Eq. (4)) was used to calculate the oil–water surface loads of polyphenols and AM:

$$\Gamma\left(mg/m^2\right) = \frac{(1-\Phi)d_{[3,2]}}{6\Phi}\left(C_{initial} - C_{serum}\right) \tag{4}$$

where $C_{initial}$ is the total concentration of AM, GA, EGCG or TA in the aqueous phase prior to emulsification (mg/L), C_{serum} is the non-absorbed concentration of AM, GA, EGCG or TA in the aqueous phase after emulsification (mg/L), d[3,2] is the emulsion droplet size (μm), and Φ is the oil phase volume ratio (0.3).

The actual mass ratio of polyphenols/AM on the oil–water interface was calculated by Eq. (5)):

$$Polyphenols/AM = \frac{\Gamma_{Polyphenols}}{\Gamma_{AM}} \tag{5}$$

where $\Gamma_{Polyphenols}$ and Γ_{AM} are the surface loads of polyphenols and AM, respectively.

2.6. Statistical analysis

All of the above results were represented as the average ± standard deviation based on three replications, and the significant difference ($p < 0.05$) was also determined by one-way analysis of variance. SPSS 26 and Origin 9.8.0 software were employed in the statistical analysis.

3. Results and discussion

3.1. Sizes and three-phase contact angles of the polyphenol/AM complexes

For emulsion systems, the particle characteristics of the emulsifier are conducive to elucidating the specific properties of the prepared emulsion [25]. It has been reported that polyphenols can interact with macromolecules (proteins, polysaccharides, starch, etc.) to spontaneously form soluble and insoluble complexes by the three-step binding process [19,26]. Based on this conclusion, Rt was defined as the transition mass ratio from soluble complexes to insoluble complexes. Fig. 1A shows the effective diameter of polyphenol/AM complexes with several mass ratios of polyphenols/AM (0.01–0.50) and different structures of polyphenols (GA, EGCG and TA). The effective diameter of AM (329 nm (data not shown)) was the same as that of the polyphenol/AM complexes at a ratio of 0.01. As represented in Fig. 1A, the diameter of GA/AM complexes remained constant, while the diameter of EGCG/AM and TA/AM complexes first remained unchanged and then increased with increasing mass ratio. This indicated that the interaction between GA

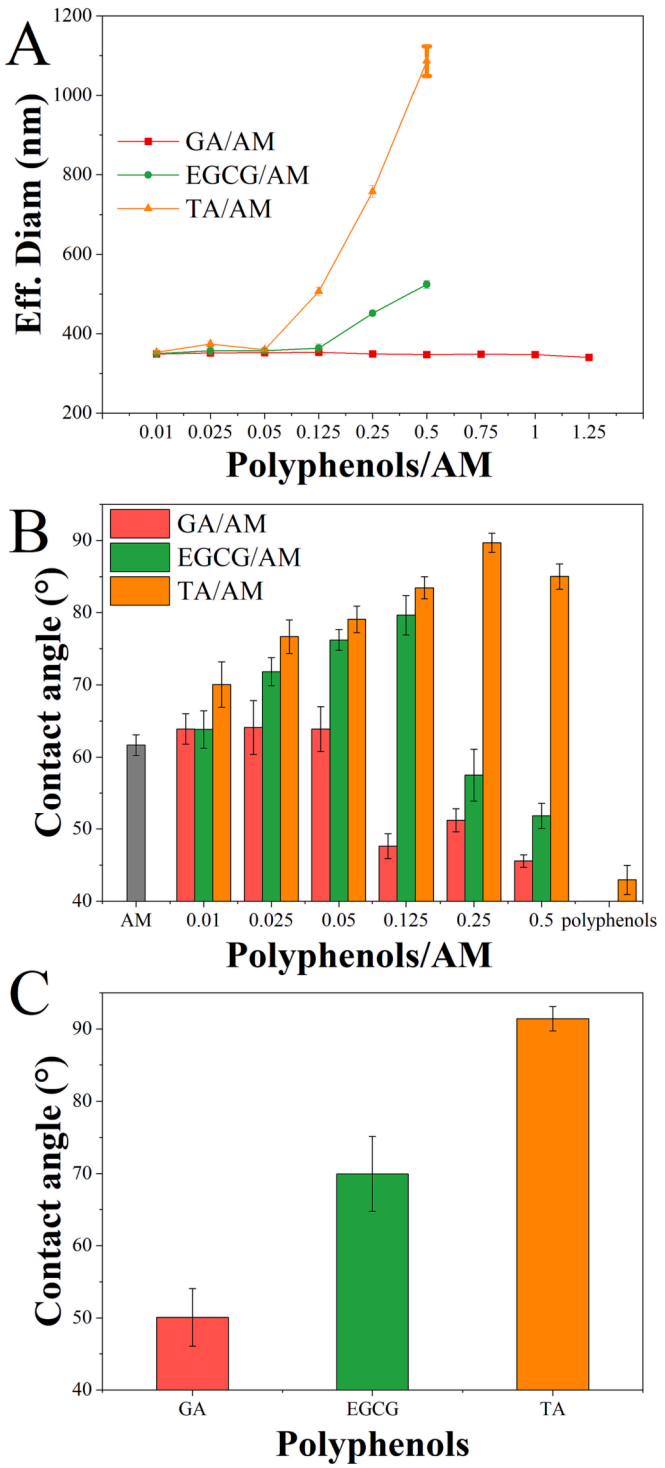

Fig. 1. Particle size (A) and contact angle (B) of polyphenol/AM complexes with several mass ratios of polyphenols/AM (0.01–0.50) and different polyphenolic structures (GA, EGCG and TA). The contact angle of blank polyphenols after 1 min of equilibration (C).

and AM did not produce any insoluble complexes (mainly soluble GA/AM complexes), while the interaction between EGCG and AM, TA and AM yielded large insoluble complexes via the performance of cross-linking EGCG and TA with AM at a high mass ratio. This result suggested that the GA/AM system had no Rt, and the Rt of the EGCG/AM system (Rt ranged between 0.125 and 0.25) was larger than that of the TA/AM system (Rt ranged between 0.05 and 0.125). It has been reported that the pyrogallol group of polyphenols is the key group for crosslinking macromolecules [27,28]. As shown in Fig. S1, GA has only one pyrogallol group, while EGCG and TA have two and five pyrogallol groups, respectively. Thus, we concluded that macromolecular cross-linking by polyphenols exists under conditions in which the polyphenols have at least two pyrogallol groups, prompting the polyphenols to cross-link AM with pyrogallol groups. At the same time, the Rt of EGCG/AM was between 0.125 and 0.25, and the Rt of TA/AM was between 0.05 and 0.125, indicating that the Rt decreased with increasing number of pyrogallol groups. This result manifested that the performance of polyphenols in cross-linking macromolecules was enhanced with increasing number of pyrogallol groups (TA > EGCG > GA). Additionally, for the EGCG/AM and TA/AM systems, soluble complexes were formed at low ratios (<Rt), and insoluble complexes began to form through cross-linking AM and soluble complexes at high ratios (>Rt).

The hydrophobicity of polyphenol/AM complexes, AM and polyphenols was measured by three-phase contact angle (θ_{ow}). As shown in Fig. 1B, the θ_{ow} of AM, GA, EGCG and TA was 62°, 0°, 0° and 43° after 5 min of equilibration, indicating that AM and the three polyphenols were all hydrophilic ($\theta_{ow} < 90°$), which was the reason that they all had poor emulsification (Fig. S2). It has been reported that θ_{ow} decreases with increasing equilibration time [29]. Therefore, to study the influence of polyphenolic structure on the hydrophobicity of polyphenols, Fig. 1C represents the θ_{ow} of polyphenols after 1 min of equilibration, and the θ_{ow} of GA, EGCG and TA was 50°, 70° and 91°. This may be due to the lowest hydroxy group content of TA when the total mass was the same. Another reason may be that the intermolecular force of TA was strongest, making the lowest number of hydrophilic hydroxy groups exposed, resulting in the higher hydrophobicity of TA. Compared with AM, the θ_{ow} of polyphenol/AM complexes first increased and then decreased with increasing mass ratio, suggesting that the hydrophobicity of polyphenol/AM complexes first improved and then decreased, which was consistent with our previous results in TA and wheat starch systems [5]. This was due to the interaction of polyphenols (GA, EGCG or TA) and AM reducing the exposed amount of hydroxyl groups via noncovalent interactions at low mass ratios, improving the hydrophobicity, while the overdose of polyphenols resulted in a decrease in hydrophobicity at high mass ratios. However, the θ_{ow} of GA/AM complex system not showed this change. This may be due to the weak interaction between AM and GA [30], resulting in much free of GA masking the real hydrophobicity of the GA/AM complexes. Additionally, it should be noted that the θ_{ow} of polyphenol/AM complexes was TA/AM > EGCG/AM > GA/AM at any mass ratio, indicating that TA/AM complexes had the highest hydrophobicity, and TA had the strongest ability to cover up the free hydroxyl groups between polyphenols and AM (TA > EGCG≫GA).

3.2. The effect of the mass ratio of polyphenols/AM and the structure of polyphenols on the emulsifying performance of polyphenol/AM complexes

The emulsifying properties of polyphenol/AM complexes were measured by EAI and ESI_{30}. The EAI represents the ability of the polyphenol/AM complexes to be adsorbed at the oil–water interface during the formation process of emulsions, and a higher EAI corresponds to stronger emulsifying performance [31]. The ESI reflects the ability of complexes to impart strength to emulsions for resistance against aggregation and coalescence [32]. Fig. 2A and B present the EAI and ESI_{30} of polyphenol/AM complexes with various mass ratios of polyphenols/AM (0.01–1.25) and polyphenolic structures (GA, EGCG and TA). For the GA/AM complex system, the EAI remained constant at a mass ratio between 0.01 and 0.05, increased (started at a ratio of 0.125) and then reached a subsequent plateau with increasing mass ratio (0.50–1.25), indicating that the emulsifying performance of GA/AM complexes improved gradually with increasing mass ratio and then reached a maximum. For the EGCG/AM or TA/AM complex system, the EAI increased first and then decreased with increasing mass ratio from 0.01 to 0.5, suggesting that the emulsifying performance of EGCG/AM and

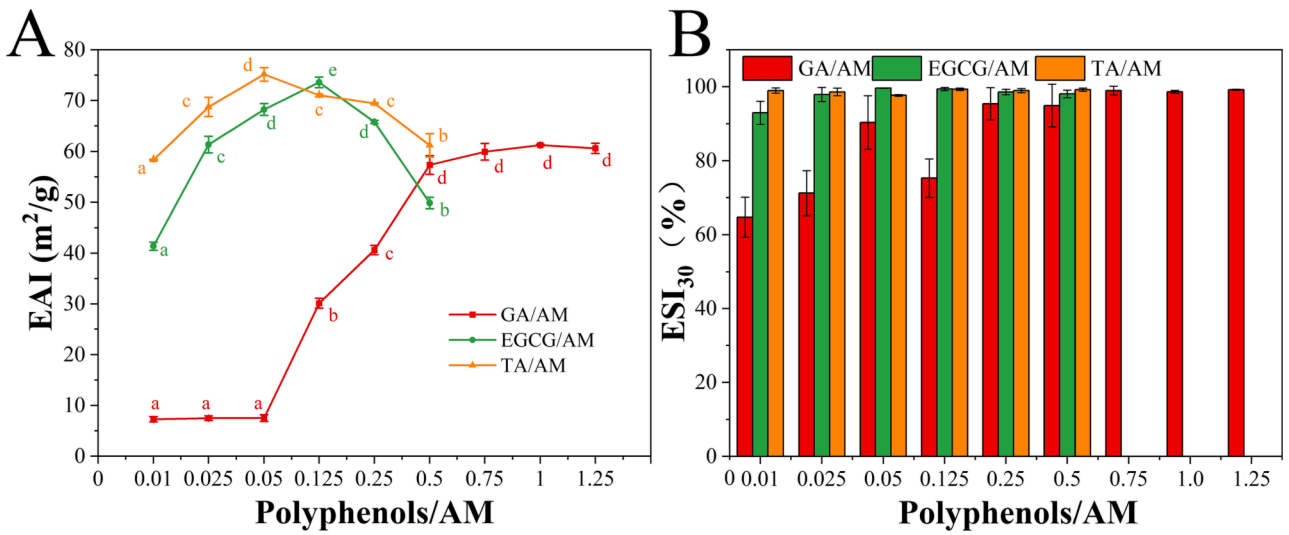

Fig. 2. The EAI (A) and ESI_{30}(%) (B) of polyphenol/AM complexes with several mass ratios of polyphenols/AM (0.01–1.25) and different polyphenolic structures (GA, EGCG and TA).

TA/AM complexes ameliorated first and then declined. The emulsifying performance of emulsifiers is determined by the particle size, content and hydrophobicity of the emulsifier. Therefore, the unchanged particle size (Fig. 1A), the gradually increasing content and the improved hydrophobicity of GA/AM complexes resulted in improved emulsifying performance of GA/AM complexes. Similar results have been reported in which the emulsifying ability was improved by enhancing the content of emulsifiers [5,16,20]. For the EGCG/AM and TA/AM complexes, for the same reason as in the GA/AM system, the emulsifying performance was enhanced by increasing the content and improving the hydrophobicity of the complexes when the mass ratio increased from 0.01 to Rt (Fig. 1A and B). Nevertheless, when the ratio increased from Rt to 0.5, giant insoluble complexes were gradually formed as the cross-linking between small soluble complexes and polyphenols (Fig. 1A), suggesting that the particle size of polyphenol/AM complexes notably increased, unavoidably resulting in a significant reduction in the content of emulsifiers. Thus, when the ratio increased from Rt to 0.5, the deduction of emulsifying performance of EGCG/AM complexes was due to the increased particle size, decreased content and decreasing hydrophobicity (Fig. 1). However, in terms of the TA/AM system, although it had better hydrophobicity (Fig. 1B), the particle size of the TA/AM complexes notably increased (Fig. 1A), unavoidably resulting in the content of emulsifiers significantly reducing [5], which finally led to a deduction of the interfacial areas; as a consequence, the emulsifying performance declined. For EGCG/AM and TA/AM complexes, regardless of whether the hydrophobicity increased or decreased, the emulsifying performance declined when the ratio > Rt, which was consistent with the increasing size results, indicating that the negative effect of increasing the size of the complexes was greater than that of hydrophobicity.

Interestingly, at any fixed ratio, the emulsifying performance of polyphenol/AM complexes was TA/AM > EGCG/AM ≫ GA/AM, which must be caused by structural differences in polyphenols. To well understand the effect of the polyphenolic structure on the emulsifying performance of polyphenol/AM complexes, to exclude the effect of content and size of complexes, the contact angle and EAI as the ratio <0.05 were further investigated. First, we list the hydrophilic or hydrophobic groups of three polyphenols that may affect emulsifying performance by itself or by the interaction with starch through noncovalent interaction. As shown in Fig. S1, GA has one benzene ring group, three phenolic hydroxyl groups and one pyrogallol group; EGCG has three benzene ring groups, eight phenolic hydroxyl groups and two pyrogallol groups; TA has ten benzene ring groups, twenty-five phenolic hydroxyl groups and five pyrogallol groups. At any same mass ratio of polyphenols/AM, the mass of the polyphenols was the same, therefore the ratio of benzene ring group number of GA: EGCG: TA was 1:1.11:1; the ratio of phenolic hydroxyl group number of GA: EGCG: TA was 1.2:1.18:1; the ratio of pyrogallol group number of GA: EGCG: TA was 2:1.5:1. In our study, the hydrophilicity or hydrophobicity of polyphenol/AM complexes was the unique influencing factor on the emulsifying performance when the ratio was <0.05. In addition, AM mainly contains hydrophilic hydroxyl groups and hydrophobic backbones, and the total content of groups is equal at the same mass ratio. Thus, the difference in hydrophobicity mainly came from polyphenols or the interaction between polyphenols and AM. Second, compared to the GA/AM system, the EAI of the TA/AM complexes (75 m^2/g) was 10 times larger than that of the GA/AM system (7.5 m^2/g) when the ratio was 0.05, while the benzene ring group numbers of GA and TA were the same, suggesting that the benzene ring group was not the key factor in the EAI of the three polyphenol/AM complexes. Similarly, compared to the GA/AM system, the EAI of the EGCG/AM complexes (68 m^2/g) was approximately 9 times larger than that of the GA/AM system (7.5 m^2/g) when the ratio was 0.05, while the contents of the phenolic hydroxyl groups of GA (1.20) and EGCG (1.18) were almost the same, suggesting that the phenolic hydroxyl group was not the key factor in the EAI of the three polyphenol/AM complexes. Thus, we could conclude that the structural difference of the polyphenols was the key factor, not the total content difference of the benzene ring group and phenolic hydroxyl group of the three polyphenols (the latter differs very little < 20%). This was due to the interaction of polyphenols and AM reducing the exposed amount of hydroxyl groups via noncovalent interactions, improving the hydrophobicity and EAI. Thus, we conjectured that the exposure of the hydroxyl group was masked mainly by the interaction of the pyrogallol group with AM through hydrogen bonding [30,33,34]. The ratio of the pyrogallol group number of GA:EGCG:TA was 2:1.5:1 at the same ratio, suggesting that increasing the number of pyrogallol groups would weaken the EAI of the polyphenol/AM complexes (Contrary to the result in Fig. 2A), indicating that the EAI of the polyphenol/AM complexes was not determined by the total pyrogallol group content in the complex system. Therefore, we surmised that the EAI of polyphenol/AM complexes was determined by the number of pyrogallol groups on the polyphenol molecular structure (polyphenolic structure). To verify the above hypothesis, we measured the EAI of polyphenol/AM complexes with an equal number of pyrogallol groups. As shown in Table 1, the EAI of polyphenol/AM complexes was TA/AM > EGCG/AM > GA/AM at the same total content of pyrogallol groups, which was consistent with increasing the number of pyrogallol groups in the polyphenol molecular

Table 1
EAI of polyphenol/AM complexes with the same total content of pyrogallol group.

Polyphenols	Mass ratio	EAI(m^2/g)	Mass ratio	EAI(m^2/g)	Mass ratio	EAI(m^2/g)
GA	0.005	6.65 ± 0.02[a]	0.01257	7.35 ± 0.01[a]	0.025	7.79 ± 0.02[a]
EGCG	0.007	40.78 ± 0.05[b]	0.0187	50.91 ± 0.39[b]	0.033	59.94 ± 1.75[b]
TA	0.01	56.07 ± 0.82[c]	0.025	67.36 ± 0.29[c]	0.05	74.22 ± 1.03[c]

Different letters (a, b, c) indicate significant differences in the same column ($p < 0.05$).

structure (GA:EGCG:TA = 1:2:5). This could be attributed to the strongest interaction between TA and AM through the 5 pyrogallol groups of TA, resulting in the lowest exposed amount of hydroxyl groups and highest hydrophobicity of the TA/AM complexes. Therefore, we concluded that the EAI of polyphenol/AM complexes improved by increasing the number of pyrogallol groups in the polyphenol molecular structure. In other words, the emulsifying performance of polyphenol/AM complexes improved by increasing the number of pyrogallol groups on the polyphenol molecules. Compared to EGCG and GA, TA has more pyrogallol groups in the molecular structure to strengthen the interaction with AM, resulting in the smallest Rt and the largest size of TA/AM complexes, a greater reduction in the exposed amount of hydroxyl groups and better hydrophobicity of TA/AM complexes. In summary, we could change the size, content and/or hydrophobicity of the polyphenol/AM complex emulsifier by the mass ratio of polyphenols/AM and the number of pyrogallol groups on the polyphenol molecular structure (polyphenolic structure) to regulate the emulsifying performance.

To further characterize the microstability of the emulsions stabilized by the three polyphenol/AM complexes, the ESI was mensurated, and the results are presented in Fig. 2B. The ESI refers to the capacity of the emulsifier to remain at the oil–water interface to impart strength to emulsions for resistance against aggregation and coalescence during the storage period [32]. For GA/AM complexes, as the mass ratio increased from 0.01 to 1.25, the ESI_{30} first increased and then was maintained at a higher value, suggesting that the stability of the GA/AM complexes at the oil–water interface gradually increased. This corresponded to the increase in surface loads of GA and AM, especially the high AM adsorption load at a high ratio, which was due to the large molecular weight AM being more useful for improving the interfacial stability of the emulsion. For the EGCG/AM and TA/AM systems, ESI_{30} remained at 95%-100%, indicating that the EGCG/AM and TA/AM complexes had outstanding stability at the oil–water interface. This result was also consistent with the higher surface loads of polyphenols and AM, further suggesting that the ability to prevent aggregation and coalescence was good. In addition, at a fixed mass ratio, the ESI_{30} of polyphenol/AM complexes was TA/AM > EGCG/AM ≫ GA/AM, which revealed that the emulsifying stability of polyphenol/AM complexes was enhanced by increasing the number of pyrogallol groups on the polyphenol molecules (TA > EGCG > GA).

3.3. Emulsion microstructures

To further prop up for the consequences acquired from EAI, Fig. 3 represents the light microscopic images of emulsions. At a given oil content (30%), the size of emulsion droplets was highly dependent on the employed mass ratio of polyphenols/AM and polyphenolic structure. For the GA/AM system, at all mass ratios, the emulsion was mainly stabilized by soluble complexes. In addition, the emulsion droplet size gradually decreased as the ratio increased from 0.01 to 0.50, which was consistent with the EAI results (Fig. 2A), indicating that the emulsifying performance of GA/AM complexes was gradually improved. However, for the EGCG/AM and TA/AM systems, the emulsion was stabilized by soluble complexes before Rt, and it was then stabilized through insoluble complexes after Rt. The droplet size rapidly decreased with the ratio increasing up to Rt (Rt_{EGCG} = 0.125, Rt_{TA} = 0.05) and then increased with the ratio increasing from Rt to 0.50. This was also in line with the EAI results (Fig. 2A), indicating that the emulsifying performance of EGCG/AM and TA/AM complexes improved first with increasing mass ratio from 0.01 to Rt and then decreased with increasing mass ratio from Rt to 0.5.

Moreover, at the fixed similar ratio (ratio ≤ 0.25), the size of emulsions was GA/AM > EGCG/AM > TA/AM, indicating that emulsifying performance of complexes was TA/AM > EGCG/AM > GA/AM system, which also corresponded to the EAI results of polyphenol/AM complexes (Fig. 2A). However, when the ratio was 0.5, the droplet size of emulsions was EGCG/AM > TA/AM > GA/AM, which also corresponded to the EAI results. The decreased emulsifying performance of EGCG/AM and TA/AM complexes was due to the cross-linking between complexes by the pyrogallol group of the polyphenols (Fig. 1A). In addition, it is worth noting that the emulsion droplets dispersed well (except for $EGCG_{0.5}$, $TA_{0.25}$ and $TA_{0.5}$), indicating that interaction did not exist between the complexes of different emulsion droplet surfaces or that the interaction was so weak that it could not oppose emulsion

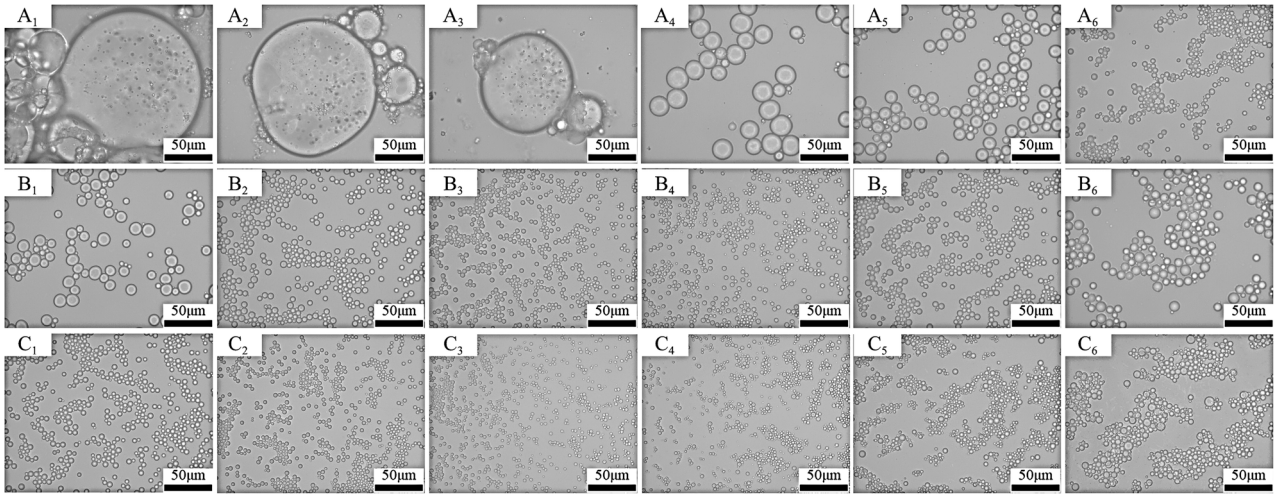

Fig. 3. Light microscopic image of emulsions stabilized by polyphenol/AM complexes with several mass ratios of polyphenols/AM (1 to 6 are for 0.01 to 0.50) and different polyphenolic structures (A:GA, B:EGCG and C:TA).

dilution. Nevertheless, for the $EGCG_{0.5}$, $TA_{0.25}$ and $TA_{0.5}$ systems, varying degrees of aggregation/flocculation took place between the emulsion droplets, which could be due to the cross-linking between polyphenols and soluble complexes absorbed at different oil droplet surfaces and then formed bridged droplets in emulsions (i.e., bridging flocculation) [5,35]. This interaction strengthened at higher mass ratios and in the TA/AM system, which could not be destroyed through dilution.

3.4. Creaming stability and appearance of emulsions

Creaming is a gravity-driven process of emulsion destabilization, which is inevitably accompanied by separation of the water and/or oil phase of the emulsion, affecting the appearance of the emulsion. The creaming index (CI) is known as a useful indicator for quantifying the extent of separation of lipids from the water phase in an emulsion [36]. Fig. 4 presents the variation of CI versus storage period of emulsions stabilized by polyphenol/AM complexes with several mass ratios of polyphenols/AM (0.01–0.50) and different polyphenolic structures (GA, EGCG and TA). Fig. 5 presents the appearance of emulsions after storage for 30 days. As shown in Fig. 4, the values of CI all first increased rapidly and then reached a plateau during the storage period, indicating that all emulsions were erratic, which could be because of emulsion aggregation and/or flocculation. This is also a common defect of micron emulsions. Generally, the creaming rate and CI are affected by the density difference between the oil phase and the water phase, the size of the droplets, and the interaction between the droplets [37]. At fixed oil and aqueous phases, creaming could be restrained by strong interaction and/or small droplet size [37,38]. For the GA/AM emulsion system (Fig. 4A), the creaming rate and CI first remained unchanged and then decreased with increasing mass ratio from 0.01 to 1.25, suggesting that the gradually dwindled droplet size (Fig. 3A) and increased EAI (Fig. 2A) could improve the emulsion stability. For the EGCG/AM emulsion system (Fig. 4B), the creaming rate and CI first decreased and then remained unchanged with increasing mass ratio from 0.01 to 0.25 and finally increased at a ratio of 0.5, suggesting that the emulsion stability first improved (0.01–0.25) and then decreased (0.5). For the same reasons as the GA/AM emulsion system, the first improvement and then decrease in emulsion stability of the EGCG/AM emulsion system was because of the gradually decreasing and then increasing droplet size (Fig. 3). However, in terms of the TA/AM emulsion system (Fig. 4C), the creaming rate and CI always decreased with increasing TA/AM mass ratio from 0.01 to 0.5, indicating that the emulsion stability gradually increased in this range. Obviously, the decreased CI with increasing ratio from 0.01 to 0.05 was in line with the gradually decreased droplet size of the emulsion. However, the declining CI was in contradiction with the increased droplet size from 0.05 to 0.5 (Fig. 3C), which could be attributed to the existence of strong interaction among droplets. The strong interaction among droplets mainly originated from the interface network of TA/AM complexes produced by cross-linking among soluble complexes by TA (Rheological and CLSM results for more details). The descending CI suggested that the interaction was strengthened in pace with increasing mass ratios of TA/AM, which was in keeping with the results of light microscopy, emulsion appearance and rheology (Figs. 3, 5 and 7). A similar result was reported in which the CI of the TA/wheat-starch emulsion decreased with increasing ratio, which could be because more bridged emulsion droplets formed and further enhanced the interaction between emulsion droplets [5]. In summary, the TA/AM emulsion could form a dense gel network under the condition of 0.5% AM concentration with a high TA additive amount.

At a fixed mass ratio of polyphenols/AM, the CI of the emulsion was GA/AM > EGCG/AM > TA/AM, decreasing with increasing number of pyrogallol groups on the molecular structure, showing that the emulsion stability was ameliorated by enhancing the number of pyrogallol groups on the structure, which could be due to the higher ESI and/or the lesser droplet size and/or the more powerful interaction between emulsion droplets (Fig. 2B, 3 and 6).

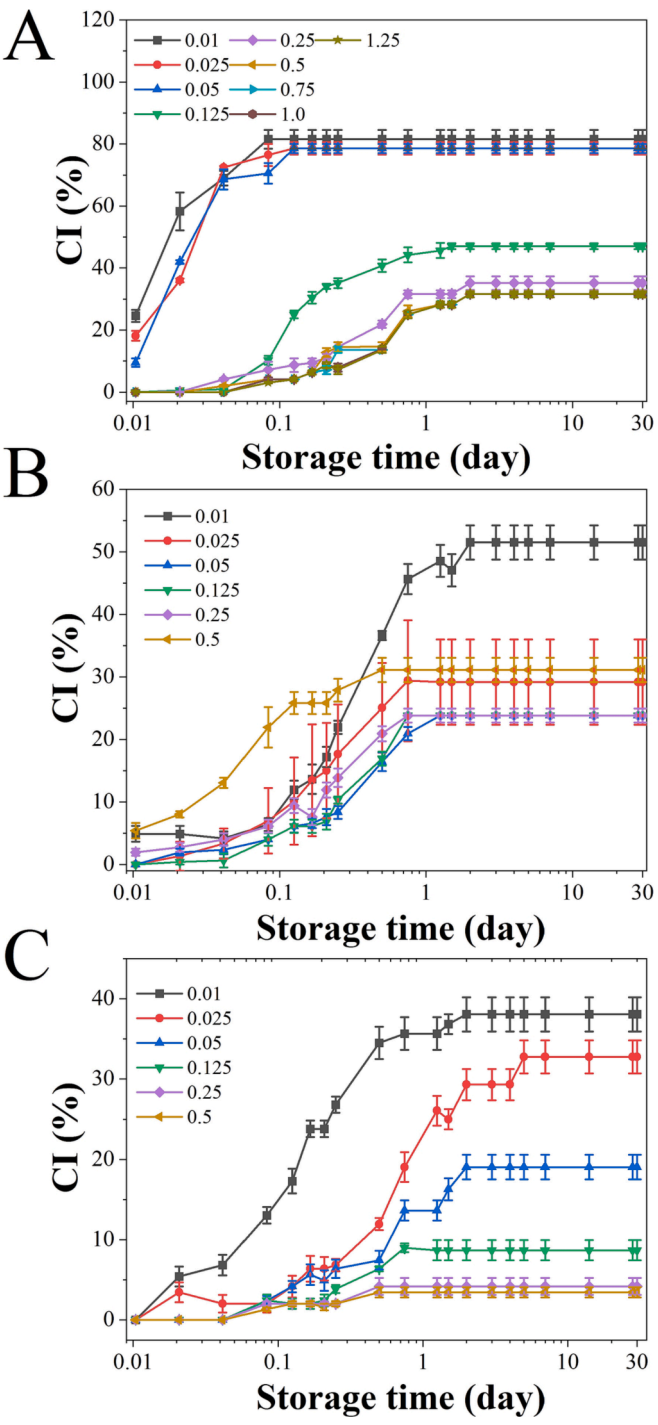

Fig. 4. Creaming index of polyphenols/AM emulsions with several mass ratios of polyphenols/AM (0.01–0.50) and different polyphenolic structures (A:GA, B: EGCG and C:TA) during storage for 30 days.

Fig. S2 presents the appearance of blank emulsions stabilized by AM, GA, EGCG or TA. As demonstrated in Fig. S2, the three polyphenols were not good emulsifiers or emulsion stabilizers, with severe oiling off of emulsions stabilized by GA, EGCG or TA. This may be due to the low hydrophobicity of polyphenols, and their small molecular weight could not prevent further coalescence of emulsion droplets [5]. Compared to polyphenols, the emulsion stabilized by AM with white creaming and disappearance of oiling off demonstrated that natural AM had some emulsifying properties, which could be attributed to the large molecular

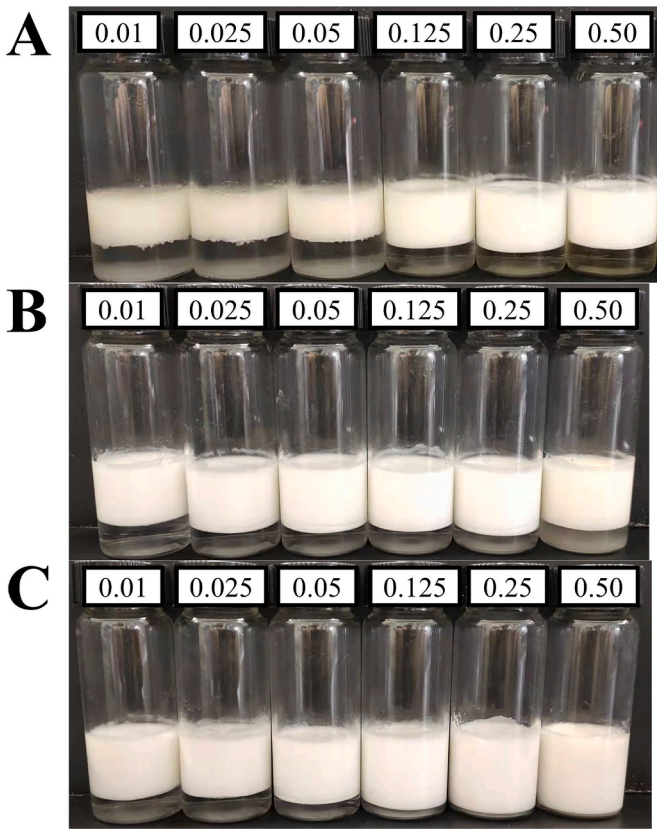

Fig. 5. Semblance of polyphenol/AM emulsions with several mass ratios of polyphenols/AM (0.01–0.50) and different polyphenolic structures (A:GA, B: EGCG and C:TA) after storage for 30 days.

weight of AM to prevent further coalescence of emulsion droplets and oiling off. Similar phenomena have also been observed in other polysaccharide emulsion systems [39]. In addition, the emulsification of AM could be further improved after adding polyphenols, which was demonstrated by enhancement of the height of the cream layer (Fig. 5). However, all samples also represented an obvious boundary between the white cream layer and the transparent serum layer. For the GA/AM system (Fig. 5A), as the mass ratio increased, the height of the cream layer first remained unchanged, then increased, and finally remained unchanged. For the EGCG/AM system (Fig. 5B), the height of the cream layer first increased and then decreased. For the TA/AM system (Fig. 5C), the height of the cream layer increased. These results were consistent with the CI results (Fig. 4). However, visible creaming was observed in all emulsion systems, but they merely showed flocculation and/or aggregation not coalescence, which was confirmed by the storage experiment for 30 days (the emulsion size remained unchanged, data not shown). This result suggested that the interfacial emulsifier layer containing polyphenols and AM could improve the stability of contiguous emulsion droplets with coalescence by producing a stronger steric barrier (Fig. 6).

3.5. CLSM

CLSM was used to characterize the microstructures of emulsions stabilized by polyphenol/AM complexes with several mass ratios (GA/AM: 0.01, 0.25, 0.5; EGCG/AM: 0.01, 0.125, 0.5; TA/AM: 0.01, 0.05, 0.5) in the 0.5% AM system (Fig. 6). The oil phase was stained green, and the polyphenol/AM complexes were stained red. The oil droplets were surrounded by polyphenol/AM complexes, confirming that an O/W emulsion had been obtained.

Regarding emulsions stabilized by different polyphenol/AM

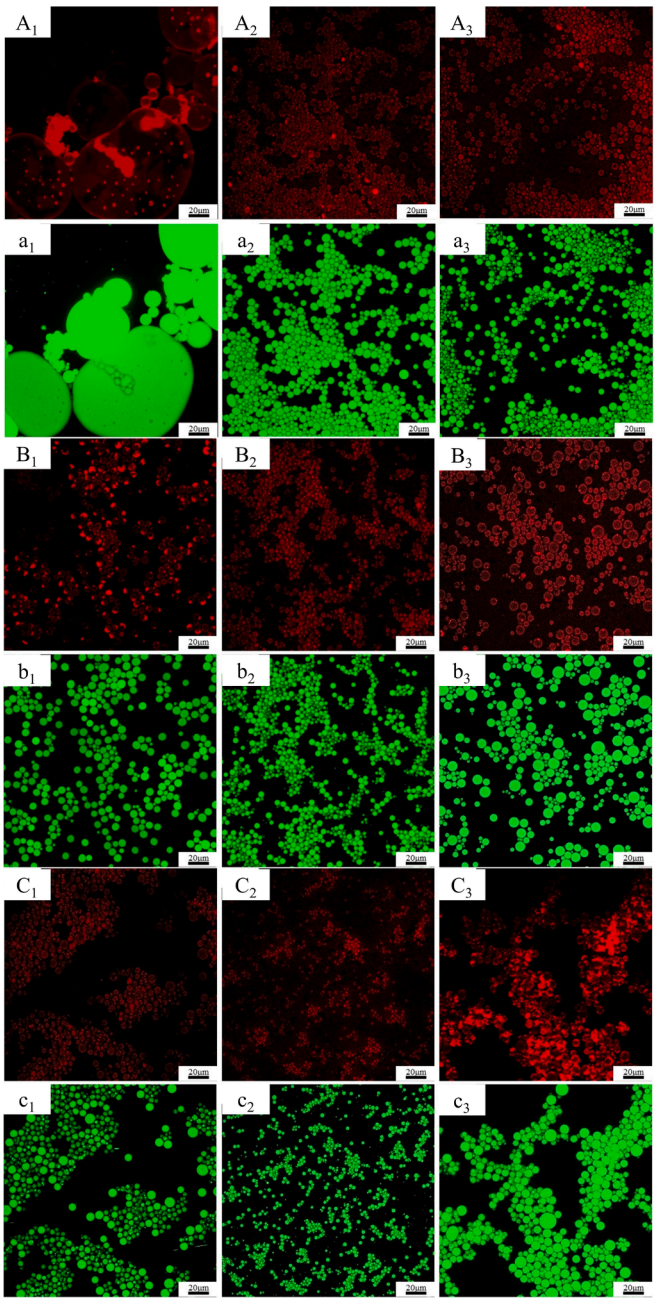

Fig. 6. CLSM images of polyphenol/AM emulsions with different ratios of polyphenols/AM (subscript 1: 0.01; subscript 2: 0.25 (GA), 0.125 (EGCG), 0.05 (TA); subscript 3: 0.50) and different polyphenolic structures (A and a: GA, B and b: EGCG, C and c: TA) in a 0.5% AM concentration.

complexes at low mass ratios (Fig. 6A1, Fig. 6B1–B2 and Fig. 6C1–C2), the surface of oil droplets had few absorbed complexes (low levels of fluorescence intensity). In contrast, at a high ratio (Fig. 6A2–A3, Fig. 6B3 and Fig. 6C3), the higher content polyphenol/AM complexes were absorbed at the oil droplet surface (high levels of fluorescence intensity), which corresponded with the greater surface load at a ratio of 0.5 (Table 2), exhibiting that the droplet surface coverage was enhanced with the ratio. It is worth noting that, compared to other polyphenols/AM emulsions, higher fluorescence intensity and interfacial complex networks were found in TA/AM emulsions at a mass ratio of 0.50 (Fig. 6C3), indicating that much more complexes surrounded oil droplets (Table 2), formed a tight interface network of complexes presenting as emulsion gels, enhanced the interaction between droplets, and

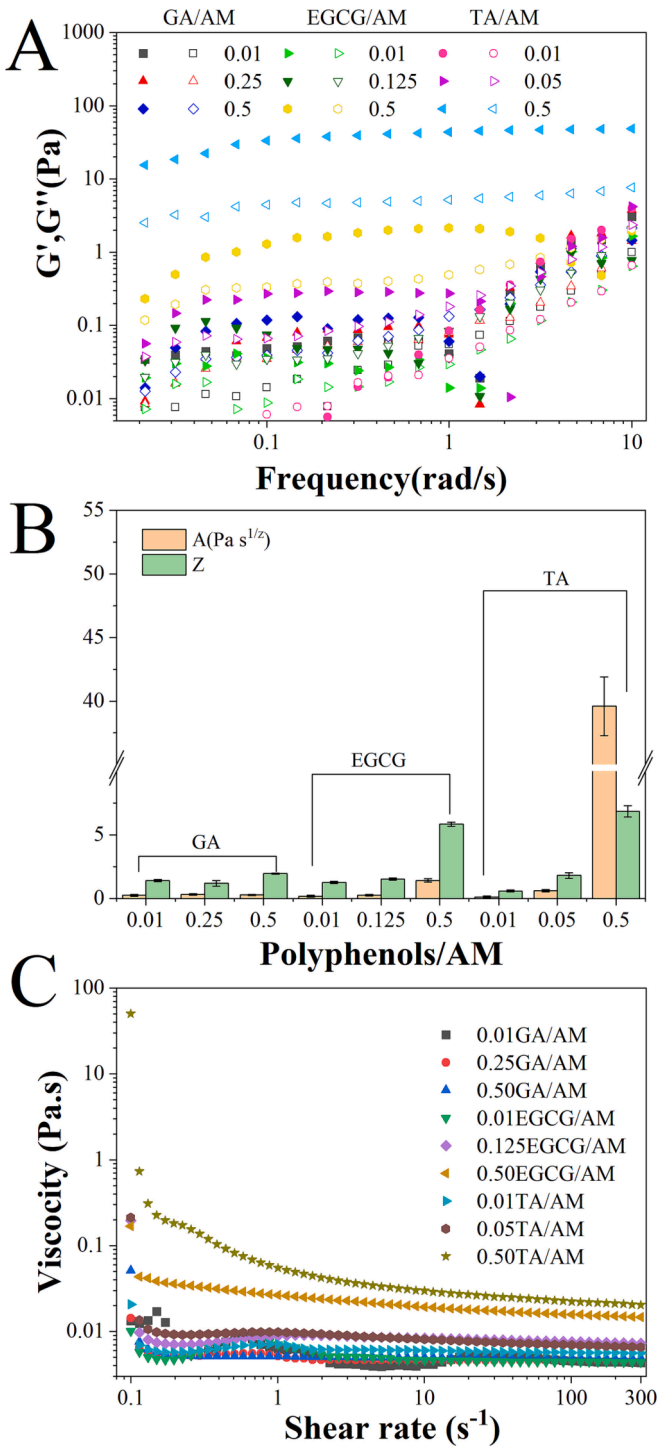

Fig. 7. The storage (G', closed symbols) and loss (G'', open symbols) modulus versus angular frequency (A), Bohlin's parameters (B) and the viscosity versus shear rate (C) of the polyphenols/AM emulsions with several mass ratios of polyphenols/AM and different polyphenolic structures (GA, EGCG and TA) in a 0.5% AM system.

resulted in a high emulsion viscoelasticity (Fig. 7). This could be attributed to the excellent performance of TA (five pyrogallol groups on the structure) in crosslinking the complexes absorbed at the oil–water interface among droplets (bridging flocculation). Similar results were reported in that TA could crosslink nanofibers to form TA/nanofiber complex nets around emulsion droplets at a high ratio [16].

Table 2
The surface load of polyphenols (GA, EGCG, TA) and AM of polyphenols/AM emulsions with various mass ratios at the oil–water interface.

	Polyphenols surface load (mg/m^2)			AM surface load (mg/m^2)		
	0.01	GA (0.25), EGCG (0.125), TA (0.05)	0.50	0.01	GA (0.25), EGCG (0.125), TA (0.05)	0.50
GA/AM	0.037 ± 0.003^a	1.175 ± 0.264	1.314 ± 0.124^a	16.521 ± 0.409^b	15.848 ± 0.813	17.236 ± 0.559^a
EGCG/AM	0.090 ± 0.004^b	1.092 ± 0.010	2.371 ± 0.209^b	14.626 ± 0.095^a	16.892 ± 0.058	19.359 ± 0.029^b
TA/AM	0.230 ± 0.000^c	1.085 ± 0.002	6.844 ± 0.204^c	16.888 ± 0.089^b	19.287 ± 0.060	21.559 ± 0.376^c

Different letters (a, b, c) indicate significant differences in the same column ($p < 0.05$).

3.6. Rheological properties of emulsions

To further study the stability mechanism of emulsions stabilized by various polyphenol/AM complexes, emulsion viscoelasticity was measured, and the results are shown in Fig. 7A. Obviously, the storage modulus (G') was larger than the loss modulus (G'') for all emulsions, indicating elastic-dominated rheological behavior [39]. In detail, when the ratio was less than Rt, the emulsion viscoelasticity primarily originated from the interaction of soluble complexes among the oil–water interface and the continuous phase, while when the ratio was more than Rt, the emulsion viscoelasticity primarily originated from the interaction of insoluble complexes among the oil–water interface. For the GA/AM emulsion system, the G' and G'' values always remained low. However, for the EGCG/AM and TA/AM emulsion systems, compared to those emulsions at ratios < Rt, G' was higher at a ratio of 0.50. Moreover, in comparison to the EGCG/AM emulsion, the TA/AM emulsion had a greater G' value at a ratio of 0.50. This result suggested that the elasticity of polyphenol/AM emulsions increased with increasing number of pyrogallol groups on the structure at a high ratio (TA/AM > EGCG/AM > GA/AM), resulting in the formation of a tighter network of emulsions (i.e., emulsion gels) (Fig. 5C and 6C3).

In light of the Bohlin model, the power law Equation $G^* = A\omega^{1/z}$ was employed to analyze all emulsions, in which G* was the dynamic modulus, ω was the frequency, Z was the coordination number to assess the amount of rheological units linked together others in the emulsion system, and A was the proportional coefficient related to the interaction intensity between rheological units [40]. The A and Z values are represented in Fig. 7B. For the GA/AM system ranging from 0.01 to 0.50, the slightly increased value of Z (i.e., increasing interaction sites) was related to the decreased droplet size and increased surface area. The constant value of A suggested that no inside structure changed in this range, which could be explained by GA having only one pyrogallol group on its structure and could not cross-link the AM to form an emulsion gel. Moreover, in terms of the EGCG/AM or TA/AM emulsion systems, the small increase in the A and Z values ranging from 0.01 to Rt was due to the same reason as the GA/AM system (the size of the emulsion decreased). However, the z value significantly increased when the ratio increased from Rt to 0.5, suggesting increasing interaction sites of polyphenol/AM complexes at the oil–water interface between different droplets. The significant increase in the A value demonstrated that the interaction between those rheological units improved significantly [5]. It is worth noting that the A value significantly and gradually increased with increasing number of pyrogallol groups on the structure at a ratio of 0.50 (TA/AM > EGCG/AM), which indicated that the interaction between those rheological units was enhanced significantly by TA. This could be due to the growing cross-linking strength with an

increasing number of pyrogallol groups on the structure, forming a continuous TA/AM complex network between emulsions and resulting in strong and compact emulsion gels (Fig. 6C3). Therefore, the increased number of pyrogallol groups on the structure led to higher values of G' and G'' of the emulsion, which reflected the harder emulsion structure, bringing on a lower CI at a high ratio (Fig. 4C).

The viscosity of polyphenol/AM emulsions is presented in Fig. 7C. All viscosities of emulsions decreased with increasing shear rate, exhibiting pseudoplastic behavior (i.e., shear-thinning phenomenon), which was a general behavior in emulsion systems and a result of the rearrangement and breakdown of emulsion aggregation and/or flocculation [35]. In contrast to a ratio < 0.5, the viscosity of EGCG/AM and TA/AM emulsions at a 0.5 ratio had more deduction with increasing shear rate. In addition, the reduced degree of viscosity of the TA/AM system was higher than that of the EGCG/AM system. It indicated that the increasing number of pyrogallol groups on the structure prompted the formation of emulsion gels at a high ratio, which caused the formation of a stronger emulsion structure that was easier to break [5,16]. In addition, it was not hard to find that the viscosity of emulsions was TA/AM > EGCG/AM > GA/AM at the same ratio of polyphenols/AM. The viscosity of emulsions is mainly influenced by the size and degree of aggregation/flocculation of oil droplets and the physical state of emulsions. When the ratio ≤ Rt, more pyrogallol groups on the structure led to smaller oil droplets, which could enhance the interaction of complexes located on different oil droplet surfaces by offering a larger surface area, resulting in an increase in emulsion viscosity. However, when the ratio > Rt, although the size of oil droplets increased with increasing ratio (EGCG/AM and TA/AM emulsion systems), the increasing amount of pyrogallol groups on the structure strengthened the interaction between oil droplets to prohibit the random flow of oil droplets by forming emulsion gels, resulting in higher viscosity, which could be demonstrated by CLSM (Fig. 6). Furthermore, the higher viscosity was associated with better emulsion stability, which could be attributed to the fact that it could inhibit the movement of oil droplets and reduce the possibility of collision and coalescence between oil droplets [5].

3.7. The surface loads and actual mass ratio of polyphenols/AM emulsions oil–water interface

The surface load is used to predict the oil–water interface complex membrane thickness in emulsions, which influences the stability and structure of emulsions [24]. The surface loads of polyphenols and AM at the oil–water interface are shown in Table 2. Obviously, the surface load of AM was greater than that of polyphenols, which confirmed that the dominant role at the oil–water interface was AM. The same result was also reported in our previous paper [5]. When the ratio < Rt, there was little increase in the polyphenols and AM surface load as the ratio increased from 0.01 to Rt, suggesting that the interfacial thickness of the polyphenol/AM complexes increased slightly, which was consistent with the lack of change in the fluorescence intensity of CLSM and emulsion structure (Figs. 6 and 7). However, when the ratio increased from Rt to 0.5, in terms of the EGCG/AM and TA/AM emulsion systems, the abrupt increase in polyphenol surface load and gradual increase in AM surface load indicated that the interfacial thickness was significantly increased, resulting in the formation of an interfacial complex network, coinciding with the improvement in emulsion viscoelasticity (Fig. 7). Moreover, the gradually increased surface load of polyphenols and AM with the ratio, especially at a high mass ratio, resulted in the high stability of the emulsion, especially contributing to the inhibition of coalescence of the emulsion. Meanwhile, the surface load of polyphenols and AM was in the order of TA/AM > EGCG/AM > GA/AM, and the increase in polyphenols was in the order of TA/AM > EGCG/AM > GA/AM as the ratio increased from 0.01 to 0.5. These findings demonstrated that the larger number of pyrogallol groups on the structure would promote the absorption of polyphenols and AM at the oil–water interface, which was due to the high hydrophobicity originating from the stronger interaction between polyphenols and AM.

As shown in Table 3, the actual mass ratios of GA/AM and EGCG/AM located in the oil–water interface were smaller than those in the continuous phase at all ratios. This was due to the weak interaction of polyphenols (GA and EGCG) and AM, resulting in the low hydrophobicity of complexes and small binding amounts of polyphenols and AM at the oil–water interface. However, the mass ratio of TA/AM absorbed on the oil–water interface was greater than that in the continuous phase at ratios < Rt, and then it was lower than that in the continuous phase at ratios > Rt for the TA/AM emulsion system. According to a similar paper reported by Wei, Li, Eid and Li [5], at the ratio of TA/starch < 0.25, the actual TA/starch ratio absorbed on the oil–water interface was higher than that in the continuous phase attributed to the larger part of TA being used to connect starch to produce excellent hydrophobicity complexes to further enhance the absorbed amount of complexes at the oil–water interface. The opposite results were observed in the ratio of TA/starch (0.50) as the amount of TA increased. Therefore, we could use the abovementioned reasons to explain why the actual TA/AM mass ratio located in the oil–water interface increased or decreased when the ratio was between 0.01 and 0.5 in this paper. At the same ratio of polyphenols/AM, the actual mass ratio of polyphenols/AM was TA/AM > EGCG/AM > GA/AM, indicating that the interface adsorption capacity was TA > EGCG > GA, which was in line with the emulsifying performance of polyphenol/AM complexes and CI of emulsions (Figs. 2 and 4). This result indicated that the emulsifying performance of polyphenol/AM complexes and stability of the emulsion enhanced by increasing the number of pyrogallol groups on the polyphenol molecule was realized by increasing the amount of polyphenol/AM complexes at the oil–water interface. Meanwhile, at a high ratio (0.5), an increased amount of soluble and insoluble complexes was formed with an increasing number of pyrogallol groups on the molecule, resulting in a gradually strengthening interfacial structure (TA > EGCG > GA), which was consistent with the CI and rheological results (Figs. 4 and 7).

3.8. The prospective mechanism of the influence of the polyphenol/AM mass ratio and polyphenolic structure on polyphenol/AM complexes and emulsions

The prospective mechanism of the influence of the polyphenol/AM mass ratio and polyphenolic structure on polyphenol/AM complexes and emulsions is represented in Fig. 8. As polyphenols were added to the AM solution, soluble and/or insoluble complexes formed gradually for the EGCG/AM and TA/AM complex systems, while soluble complexes only formed for the GA/AM system in the whole ratio range. The mass ratio of polyphenol/AM complexes increased gradually with the addition of polyphenols, indicating that an increasing amount of polyphenols were absorbed to AM and formed polyphenol/AM complexes. Moreover, the interaction between polyphenols and AM mainly formed soluble complexes with the same particle size at ratios < Rt. Nevertheless, larger insoluble complexes were gradually produced when the ratio increased from Rt to 0.5 for the EGCG/AM and TA/AM systems. The absorbed

Table 3
The actual polyphenol/AM mass ratio at the oil–water interface in emulsions containing 0.5% AM.

Samples	Polyphenols/AM mass ratio in emulsions		
	0.01	GA (0.25), EGCG (0.125), TA (0.05)	0.50
GA/AM	0.0022 ± 0.002[a]	0.0738 ± 0.0118	0.0762 ± 0.0046[a]
EGCG/AM	0.0062 ± 0.006[b]	0.0646 ± 0.0006	0.1225 ± 0.0089[b]
TA/AM	0.0137 ± 0.001[c]	0.0563 ± 0.0002	0.3176 ± 0.0121[c]

Different letters (a, b, c) indicate significant differences in the same column ($p < 0.05$).

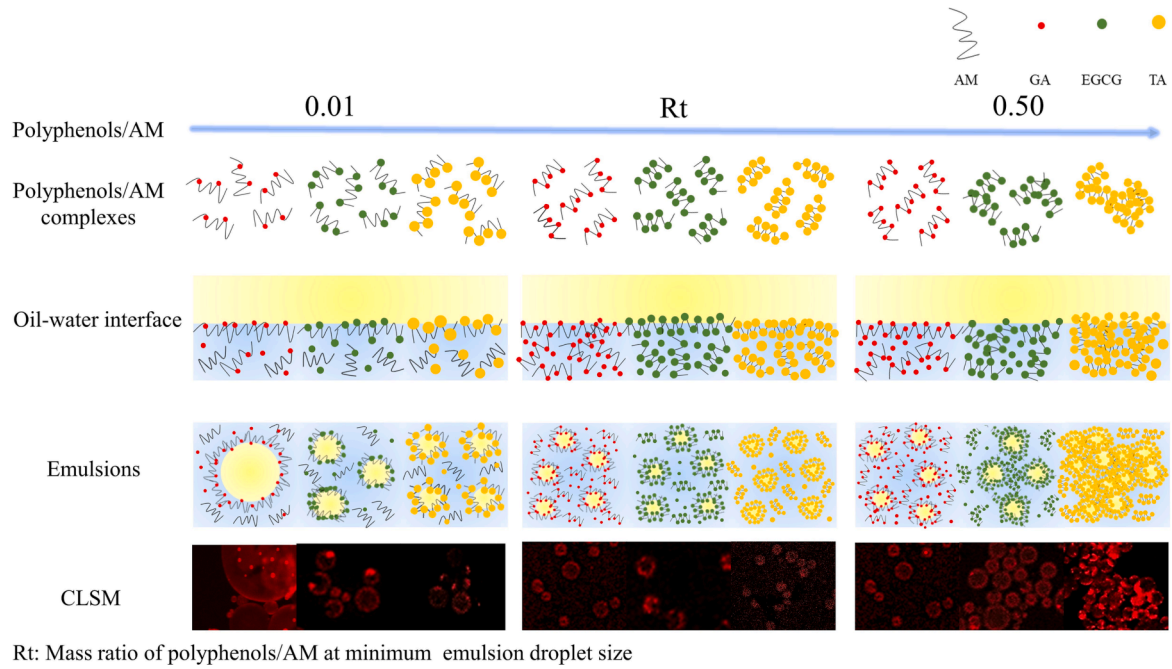

Fig. 8. Abridged general diagram of polyphenol/AM emulsions with different polyphenolic structures (GA, EGCG and TA) and various polyphenol/AM mass ratios (0.01, Rt and 0.50) in a 0.5% AM system.

number of complexes at the oil–water interface increased with increasing ratio, which was due to the improvement in surface hydrophobicity or the increased content or crosslinking of complexes. In addition, the increased number of pyrogallol groups on the polyphenol molecule would enhance the hydrophobicity and emulsifying ability of polyphenol/AM complexes (TA > EGCG > GA), resulting in the highest attached amount of TA/AM complexes at the oil–water interface. As the ratio increased from 0.01 to 0.5, the GA/AM emulsion size system gradually decreased, while the size of the EGCG/AM and TA/AM emulsion systems first decreased and then increased, which was regulated and controlled by the size, content and hydrophobicity of the polyphenol/AM complexes. When the ratio > Rt, the particle size of EGCG/AM and TA/AM complexes increased, resulting in an increase in emulsion droplet size and the formation of a strong complex network on the oil–water interface with increasing ratio from Rt to 0.5. The formation of a stronger interface complex network (i.e., emulsion gel) restrained the movement of the emulsion droplet and finally expressed favorable creaming stability (Fig. 5).

4. Conclusion

Overall, this research indicated that the polyphenol/AM mass ratio and polyphenolic structure had a fundamental influence on polyphenol/AM complexes and emulsions. Taking into account all results, we could reach some conclusions as follows. The soluble complexes and/or insoluble complexes gradually formed with the addition of polyphenols into the AM system. However, insoluble complexes only formed when the number of pyrogallol groups on the polyphenol molecule was larger than one (EGCG/AM and TA/AM systems, not the GA/AM system). Meanwhile, the Rt decreased with increasing pyrogallol group number on the molecule (TA < EGCG). In addition, the hydrophobicity of AM could also be improved by forming polyphenol/AM complexes. The emulsion size decreased with increasing pyrogallol group number at a fixed ratio (GA > EGCG > TA), and the size could also be controlled by the polyphenol/AM ratio, which was chiefly attributed to the alteration of the size, hydrophobicity and content of the polyphenol/AM complexes. In detail, the emulsion droplet size decreased with increasing mass ratio from 0.01 to Rt. The size increased with increasing mass ratio from Rt to 0.5 (EGCG/AM and TA/AM emulsions), which was due to the formation of insoluble complexes. Moreover, all emulsions presented various degrees of creaming because of flocculation/aggregation, but creaming was restrained by decreasing emulsion size (ratio < Rt) or the formation of a thick complex network (i.e., emulsion gel) at a high ratio. The complex network was enhanced by increasing the pyrogallol group number on the molecule (EGCG/AM < TA/AM emulsions) because of the increasing content of complexes attached to the interface. Therefore, compared to other complexes (GA/AM and EGCG/AM) with low pyrogallol group numbers, the TA/AM complex emulsifier had the best hydrophobicity and emulsifying properties, and the TA/AM complex emulsion had the best emulsion stability.

CRediT authorship contribution statement

Huan Xie: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. **Xianling Wei:** Funding acquisition, Writing – review & editing, Project administration. **Xiaoyan Liu:** Formal analysis, Supervision. **Weidong Bai:** Validation. **Xiaofang Zeng:** Visualization.

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.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 32101951), Guangzhou Basic and Applied Basic Research Foundation (No. 202201010186), and Guangdong Provincial Key Laboratory of Lingnan Specialty Food Science and Technology (No. 2021B1212040013).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.ultsonch.2023.106367.

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