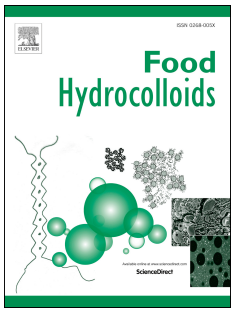

Interactions between lecithin and yolk granule and their influence on the emulsifying properties

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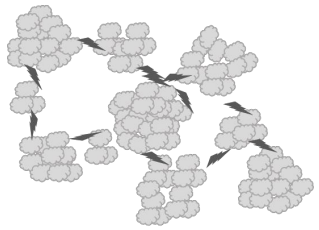
Egg yolk granular

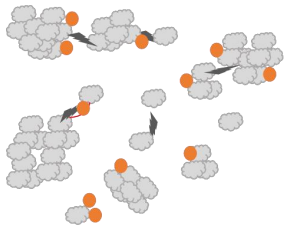
Lecithin

AFM

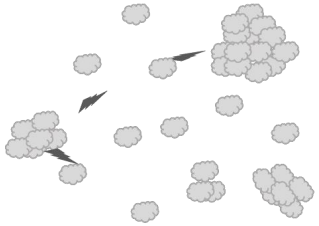
Emulsion



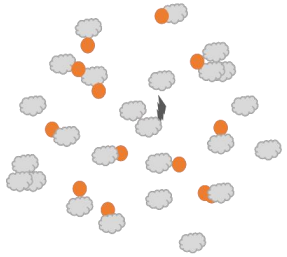
pH 7



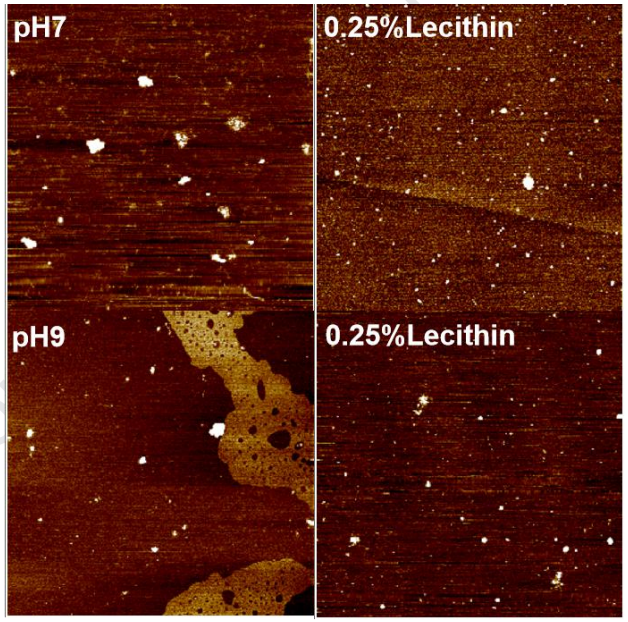
0.25%Lecithin



pH 9



0.25%Lecithin



pH 7

pH 9

Interactions between lecithin and yolk granule and their influence on the emulsifying properties

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Abstract

In this study, the interactions between egg yolk granule and soybean lecithin and their emulsion properties were investigated. For egg yolk granule, the increase of solubility and negative zeta-potential and decrease of hydrophobicity could be observed with the increase of lecithin concentrations, indicating the interactions between granule protein and lecithin. Results from the z-average particle size and the AFM image showed that the increase of solution pH and addition of lecithin could

destroy the aggregated structure of the egg yolk granule. The disrupted granule exhibited better emulsion stability than that of native granule due to the higher surface charge and lower particle size. Notably, appropriate addition of lecithin (less than 0.25%) would be conducive to the formation of high stable emulsions by modestly reducing the contact angle, while extra lecithin (more than 0.50%) would induce excessive substitution of granule protein by competitive adsorption, leading to destabilization of the O/W emulsions via surfactant-induced depletion flocculation.

Keywords: egg yolk granule; soybean lecithin; aggregated state; competitive adsorption; emulsion stability

1. Introduction

Emulsion system has received much attention in recent years as an important vehicle for delivering bioactive substances. It is a dispersion system formed by stabilizing two incompatible solutions with emulsifiers or particles, which can improve the stability and bioavailability of fat-soluble nutrients, and has a good application prospect in the health food industry (McClements, et al., 2016). Emulsion, a thermodynamic unstable system, will undergo stratification, flocculation, aggregation and Oswald maturation over time (Saberi, et al., 2014). The practical application of a single macromolecule or small molecule emulsifier is not ideal. Interfacial film formed by the small molecule emulsifier is weak and the process is reversible, and the spatial repulsion effect is weak, so that the interface film cannot effectively resist the coalescence between the emulsion droplets. Moreover,

adsorption of macromolecular emulsifier does not completely inhibit the Ostwald ripening process (Tcholakova, et al., 2008). How to prepare a highly stable emulsion embedding system is a challenging technical problem.

Hen's egg yolk is known as excellent food-derived emulsifier and plays an important role in many food products such as mayonnaise, salad dressings, baked food and ice cream (R. E. Aluko & Mine, 1997). In natural state, egg yolk is a supramolecular assembly composed of the basic units of yolk spheres and the vitelline membrane with multiple emulsifier complex characteristics, providing high stability for nutrient inside (Hsu, et al., 2009). The yolk sphere is an oil storage organelle in the egg yolk, and the vitelline membrane is a composite interface composed of lecithin (small molecule emulsifier), low density lipoprotein (biopolymer emulsifier) and egg yolk granules (interface adsorbing protein particles), which can effectively inhibit lipid oxidation. However, the process of processing and shearing will cause the natural yolk spheres to disintegrate into three phases, including yolk granule phase, plasma phase and gas-water interface adsorption layer, resulting in oxidative degradation of some fat-soluble nutrients (Marc Anton, 2013).

Hen egg yolk can be easily fractionated by simple dilution and centrifugation into two major parts, the supernatant and the granule, without any denaturation of the protein (Laca, et al., 2010). The supernatants account for about 93% of yolk lipids and 50% of yolk proteins, while the granules account for 7% of the remaining yolk lipids and 50% of yolk proteins of the remaining egg yolk (Marc Anton, 2013). Granules contain less lipids and cholesterol and more proteins than yolk and plasma. Granules

are consisted of circular complexes with diameter ranging from 0.3 to 2 μm. At low ionic strength (0.17 M NaCl), native granules take the form of non-soluble HDL-phosvitin aggregates through phosphocalcic bridges between seryl residues of HDL and phosvitin (Naderi, et al., 2017). It has been reported that HDL has good emulsifying property, and can be effectively used for constructing the delivery carrier of nutrients (Zhou, et al., 2018). Furthermore, phosvitin has a strong capacity of iron chelation that could be used for antioxidant purposes. At about 80% solubility, yolk, granules and plasma have similar emulsifying activities and granules have the best emulsion stabilization (M Anton & Gandemer, 1997). Egg yolk granules are also considered for the so-called 'Pickering' stabilization effect of emulsion droplets, because of their particle-like structure (Rayner, et al., 2014). It is known that particles at interfaces stabilize emulsions better than small molecules due to the high desorption energy upon adhesion to oil-water-interfaces. So it is meaningful for food industry to use egg yolk granules as a replacer of whole egg yolk due to its multiple positive features including low cholesterol, emulsifying ability and oxidation resistance. However, their emulsifying ability cannot be fully exerted in nature state due to its poor emulsion stability caused by large particles and poor solubility, which greatly limits the application of the egg yolk granules as emulsifier.

Lecithin is an important nutrient surfactant with excellent hypolipidemic effect (Christopher, 2015) and emulsifying, diffusing and infiltrating properties (Asomaning & Curtis, 2017). A phospholipid-protein binary complex (formed by hydrophobic interaction between a phosphatidylcholine molecule and a hydrophobic region of

protein) displayed excellent dispersing and emulsifying properties (Gao, et al., 2017). Previous study also found that the interaction between protein and lecithin affected the structure and interfacial adsorption properties of the protein, thereby enhancing its emulsifying ability and affecting the microencapsulation properties of the proteins (S. Wang, et al., 2017). Likewise, lecithin in the vitelline membrane also plays an important role in the emulsification performance of egg yolk. In the process of formulating the composite interface, lecithin can effectively reduce the oil-water interfacial tension and promote the formation of emulsion. However, the interaction mechanism between lecithin and egg yolk granules and the effects on the properties of the emulsion are still unclear.

The aim of this study was to investigate the interactions between egg yolk granule proteins of different aggregation states and lecithin. Also, the competitive adsorption and synergistic stabilization effects of granule proteins and lecithin on oil/water interface were studied. In addition, the relationship between physiochemical properties of protein-lecithin complex and the stability of emulsions were discussed. Microstructure, surface tension and interface adsorption properties of granule-lecithin complex as well as the emulsion stability index are the main parameters that were investigated.

2. Materials and methods

2.1. Materials

Fresh hen eggs were provided by the Kangde Biological Products Co., Ltd.

(Nantong, Jiangsu, China). Soy lecithin was purchased from flyyed biotech Co., Ltd. (Suzhou, Jiangsu, China). For the preparation of the emulsion, Arowana sunflower oil was bought from a local supermarket and used without further purification. The sodium 8-anilino- 1-naphthalenesulfonate (ANS) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade (Sinopharm Chemical Reagents Co., Shanghai, China).

2.2. Preparation of egg yolk granules

Egg yolk granules were prepared according to the previous method (Laca, et al., 2010) with slight modification. Egg shell was broken manually and egg yolk was separated from the albumin by carefully rolling on filter paper to ensure that no egg white proteins were mixed up. The egg yolk content was collected in a beaker after vitelline membrane was ruptured with a tweezer. Thereafter, the egg yolk material was mixed with deionized water (1:1.5 v/v). Then the pH of the diluted egg yolk was adjusted to 7.0 with NaOH (1 M) and it was kept overnight at 4 □, followed by centrifuging for 45 min at 4 □ and 8,000 g to separate into plasma (supernatant) and granule (precipitate).

2.3. Preparation of egg yolk granules emulsions

Egg yolk granules were diluted to protein concentration of 1% (w/v) with distilled water and soy lecithin was added into the granules dispersion to final concentration of 0.00%, 0.10%, 0.25%, 0.50% and 1.00%, followed by stirring at ambient temperature for 2 h using magnetic stirring. Then the pH of the aqueous

mixtures was adjusted to 7.0 and 9.0. The aqueous dispersions were prepared via two homogenization steps by pre-homogenizing firstly for 2 min at 11,000 rpm using an Ultra-Turrax blender (IKA T25 Basic, Staufen, Germany) equipped with a 12 mm diameter head and then homogenizing at 50 bar for 3 times using APV1000 homogenizer (APV Co., Crawley, U.K.). Consistent with the preparation of aqueous dispersions, the oil/water emulsions with 10% of Arowana sunflower oil and 90% (wt.) of aqueous dispersions were prepared by the same homogenization process. Furthermore, 0.02% (w/v) of sodium azide as an antimicrobial agent was added to the resulting emulsions and stored at 4 □ until analysis.

2.4. Protein solubility

Protein solubility was measured by the method previously described with a slight modification (Abugoch, et al., 2008). Each protein sample was diluted and then centrifuged at 10,000 g for 20 min at 4 □. The supernatants were collected and the protein content was assayed by the biuret method. Solubility was expressed as the ratio of protein content in supernatant to the total protein content in sample.

2.5. Particle diameter and zeta-potential

The droplet size distribution and the electrical charge (zeta-potential) of egg yolk granule dispersions and emulsions were determined using a Zetasizer Nano Brook Omni instrument (Beookhaven Instruments, USA) at 25 □. The dispersions and emulsions were diluted with deionized water and equilibrated for 60 s before measurement. Particle sizes were reported as the Z-average particle diameter

calculated from the particle size distribution. The Smoluchowsky mathematical model was used to convert the electrophoretic mobility measurements into the zeta-potential values.

2.6. Atomic force microscopy (AFM)

The microstructure of the egg yolk granule or granule-lecithin complex was observed using an atomic force microscope (Dimension Icon model, Bruker). The samples were diluted to 5 μg/mL concentration of protein with deionized water and 10 μL of the diluted sample was immediately spread onto freshly cleaved mica sheets to dry naturally. The tapping mode was chosen and at least 3 areas of each prepared sample were scanned, then a representative image was selected from at least 10 images.

2.7. Fluorescence spectra

Fluorescence spectra of samples was measured according to the procedure of Wang et al. with a slight modification, which used 8-anilo-1-naphthalenesulfonic (ANS) as a probe to interact with hydrophobic moieties on the surface of protein to give a fluorescent signal (B. Wang, et al., 1997). The sample solutions of egg yolk granule with/without soy lecithin were diluted (1:400) to avoid interference of turbidity to the test. 20 μL of ANS solution (8 mM) dissolved in phosphate buffer (50 mM, pH 7.0) was added to 4 mL of each protein dispersion. Then the mixture was vortexed for 20 s and kept in the dark for 20 min. Fluorescence scan curves were recorded at emissions from 400 to 600 nm excited at a wavelength of 390 nm using

F-7000 spectrofluorimeter (Hitachi, Japan). The emission and excitation slits were set to 5 nm, and the measurements were performed at 25 □.

2.8. Contact angle

Contact angles of samples were measured at 25 □ by a drop shape analyzer (DSA25, Kruss, Germany). 5 μL of the sample was taken and dropped on a glass slide. To eliminate interference, the sample was equilibrated for 5 min and then measured. At least six parallel measurements were taken for each sample.

2.9. Creaming index

10 mL of each emulsion was transferred into a glass vial immediately after preparation to measure the change of the creaming index of different emulsions over time. The emulsion samples were tightly sealed and then stored for 7 days at room temperature. Emulsions separated into a creamed layer at the top and a transparent serum layer at the bottom during storage. The extent of creaming was characterized by creaming index (CI, %), which was calculated by

$$CI\% = \frac{H_S}{H_E} \times 100 \quad (1)$$

Where, H_E was the total height of the emulsions and H_S was the height of the serum.

2.10. Protein adsorption fraction (AP)

Percentage of adsorbed proteins was determined according to the method

189 described by Chang et al. with some modifications (Chang, et al., 2016). Emulsions
190 (2mL) were centrifuged at 10,000 g for 30 min at 4 □. After the centrifugation, three
191 phases were observed: a cream layer at the top of the tube, an aqueous phase of the
192 emulsion, and sediment at the bottom. The cream phase was moved to collect the
193 aqueous phase and sediment. The subphase was centrifuged again to remove the
194 adsorbed proteins completed. This process was repeated 3 times and the final aqueous
195 phase and sediment were collected to measure total protein content (M_f). The weight
196 of protein added into the emulsions was recorded as MI (mg). The AP% was
197 calculated as follows:

198 $$AP\% = \frac{MI - M_f}{MI} \times 100 \quad (2)$$

199 2.11. Microstructure of emulsion droplet

200 The microstructure of emulsions was visualized using of an Axiolab A reflected
201 light-microscope (Zeiss, Berlin, Germany) with a 40 × objective lens. The emulsions
202 were diluted with deionized water at a ratio of 1:10 (v/v) before observation. About 10
203 μL of the diluted emulsion was loaded on the microscope slide and carefully covered
204 with a coverslip. The photomicrographs were captured after being equilibrated for 2
205 min. Representative images of microscopic imaging were chosen from at least four
206 similar images.

207 2.12. Statistical analysis

208 All the measurements were performed at least in triplicates, and the data were

expressed as mean ± SD. An analysis of variance (ANOVA) was carried out using the software SPSS version 17.0 for Windows (SPSS Inc., Chicago). The Duncan's multiple-range test was used to evaluate significance of difference ($p < 0.05$).

3. Results and discussion

3.1. Properties of egg yolk granule-lecithin composite dispersion

3.1.1. Protein solubility, zeta-potential and particle size

Protein solubility is an important functional property that affects the potential application of protein in food processing. As shown in Table 1, at pH 7.0, the solubility of native egg yolk granule was only 1.56%. The compact granule structure of yolk granule linked by phosphocalcic bridges made it hard to be hydrated (Naderi, et al., 2017). When the pH was elevated to 9.0, the solubility of granule protein increased to 80.18%. The increase in the number of negative charges (COO-) at alkaline condition could promote electrostatic repulsion and dissociation of granules (Causeret, et al., 2006). Regardless of the aggregated state of granule, the solubility of granule dispersion further increased as the lecithin concentration of the aqueous phase increased. The solubility of the native (pH 7.0) and disrupted (pH 9.0) granule protein increased to 72.53% and 97.45% respectively with increasing lecithin concentration up to 1.00%.

The zeta-potential of the protein can reflect the surface charge of the protein. For proteins existing in the form of colloidal particles in aqueous solution, the surface charge played an important role in the dispersion character of these particles (Chen &

Soucie, 1985). Table 1 showed that the negative value of zeta-potential in protein solution at pH 9.0 was significantly higher than that of pH 7.0. This phenomenon could be attributed to the increase in the number of negative charges (COO-). Besides, the incorporation of lecithin could enhance protein surface electronegativity, regardless of solution pH. This result may be related to the liberation of phosvitin from granules due to continual increase of solubility (Castellani, et al., 2006; Damodaran & Xu, 1996).

Particle size is another important index of particle stability and can usually affect emulsifying properties. The native granule at pH 7.0 was the largest with z-average particle size of 1905.67 nm and polydispersity index of 0.47, showing that the native granule possessed a wide distribution of particle size. At pH 9.0, the granule was disrupted, which could be observed from the decrease of z-average particle size to 126.10 nm. In addition, a significant ($p < 0.05$) decline of z-average particle size was found with the lecithin concentration increased from 0.00% to 1.00%. Our previous study proved that the changes in the particle size of granule protein dispersion were directly related with the protein aggregation state (Li, et al., 2018). It can be concluded from the above results that both increase in pH and addition of lecithin could effectively decrease the particle size of egg yolk granule by increasing solubility and surface charge.

3.1.2. Atomic force microscopy

In order to characterize the morphology of yolk granule, the microstructure of

the samples was observed by atomic force microscopy. Fig. 1 showed the 2-D AFM images of the granules (pH 7.0 and pH 9.0) with different concentrations of lecithin (0.00%, 0.25% and 1.00%). Dramatic changes in particle morphology were observed in various samples, wherein it was observed that particles at pH 7.0 without lecithin presented irregular aggregated state with greater contour sizes. At pH 7.0, the addition of lecithin gradually reduced the size of the particles, but large particles still existed and the dispersion coefficient increased. Likewise, at pH 9.0, the particle dissociated and the particle size became smaller and almost no large particle was observed. These observations were roughly in line with the changes of particle diameter of egg yolk granule (Table 1). Therefore, the disintegration of egg yolk granule aggregate at high pH and in the presence of lecithin was intuitively confirmed by the AFM pictures.

3.1.3. Fluorescence spectra

Protein hydrophobicity depends on its exposure of hydrophobic domain, which has an important influence on the emulsifying and interfacial properties of proteins. Fig. 2 showed the changes in fluorescence intensity of egg yolk granule (pH 7.0 and pH 9.0) at different concentrations of lecithin with the wavelength (λ). Compared with disrupted granule (pH 9.0), the native granule (pH 7.0) exhibited higher fluorescence intensity. Low hydrophobicity of granule protein at high pH may be ascribed to its high surface charge and solubility as shown in Table 1. The increase of solution pH contributed to the increase of surface charge, making the surface of the protein charged and became more hydrophilic, which increased solubility and decreased hydrophobicity. Our previous study has shown that salt could increase the surface

hydrophobicity of yolk proteins while improving solubility. This result seems to be related to the charge shielding effect of salt ions (Li, et al., 2018). So the surface hydrophobicity of the protein was closely related to the surface charging property of the protein. The dispersion of granule showed an obvious decrease in fluorescence intensity with lecithin concentration increased. The interactions between phosphatidylcholine and hydrophobic region of globulin easily occurred (Ohtsuru & Kito, 2014). Adding lecithin into egg yolk granular dispersion might cover and bury the surface hydrophobic amino acids of granule protein, which led to the decrease of fluorescence intensity. This might also be a reason for the increase of egg yolk granule solubility in presence of lecithin. It has been proposed that there were relatively few hydrophobic residues on the surfaces of highly soluble proteins (Venyaminov, et al., 2010). The solubility of protein depended, to a large extent, on the hydrophilicity/hydrophobicity balance of protein molecules, and was related to the amino acids composition on the surface of protein (Bigelow, 1967).

3.1.4. Contact angle

Contact angle measurement is a straightforward way to evaluate the surface tension of the particle that is related to the formation ability of interface membrane. The lower the static contact angle is, the lower the interface tension is. As shown in Fig. 3, the yolk granule in its natural state (pH 7.0) exhibited the largest contact angle among samples, and granule in the disrupted state (pH 9.0) behaved lower contact angle. This result could be ascribed to the rapid decrease in surface hydrophobicity and increase in negative charge of granule proteins at high pH. Furthermore, the

295 increase of the lecithin concentration led to a greater reduction of the contact angle of
296 granule/lecithin dispersions. Interestingly, when the lecithin concentration increased
297 to more than 0.50%, the contact angle of dispersions at pH7.0/9.0 started to decline
298 from 29.10°/25.20° to 26.80°/23.10°. This phenomenon was similar to the results of a
299 previous study, which has reported that milk proteins preferentially adsorbed to
300 oil-water interfaces at low surfactant levels due to their much higher adsorption
301 energy per molecule, but at higher levels surfactants preferentially adsorbed because
302 they pack more efficiently than proteins (Dickinson & Tanai, 1992).

303 3.2. Properties of egg yolk granule-lecithin composite emulsions

304 *3.2.1. Creaming stability*

305 The creaming index and digital images of emulsions prepared by 1% of yolk
306 granule under different lecithin concentrations were shown in Fig. 4. In the absence of
307 lecithin, emulsion stabilized by native granule (pH 7.0) start creaming after 1 day of
308 storage, while emulsion prepared with the disrupted granule (pH 9.0) began to stratify
309 on the third day. This phenomenon indicated that the dissociation of yolk granules
310 caused by pH increase was beneficial to emulsifying stability. At the same lecithin
311 concentration, emulsions formulated with disrupted granules had a significantly ($P <$
312 0.05) smaller creaming index than those prepared with native granules. Whatever the
313 state of granules (native or disrupted), the emulsifying stability of egg yolk granules
314 increased first and then decreased with the increasing lecithin proportion. Emulsions
315 containing 0.25% lecithin concentration showed the best emulsifying stability.

Notably, no droplet-free phase (serum layer) at the bottom was observed in the emulsion prepared by disrupted granules with 0.25% lecithin after 7 days of storage (as shown in Fig. 4d). This can be presumed that granule dissociation caused by appropriate lecithin could improve emulsifying stability but excessive incorporation of lecithin would result in a decrease in emulsifying activity, which may be resulted from the competitive adsorption of egg yolk granule and lecithin at interface. It could be directly seen that, the emulsion prepared by 1.00% lecithin did not exhibit high creaming stability as expected, indicating that sole lecithin was not enough to stable oil droplets.

3.2.2. Particle size and zeta-potential of fresh emulsions

The particle size and charge of the emulsion are important indicators influencing the stability of the emulsion. The mean particle diameter and zeta-potential of emulsions prepared by egg yolk granules at pH 7.0/9.0 with various concentrations of lecithin added were displayed in Fig. 5. The emulsions prepared with disrupted granules (pH 9.0) had smaller average diameter and higher negative zeta-potential value than those prepared with native granules (pH 7.0). Consequently, the emulsions prepared by disrupted granules possessed a better emulsifying ability than native granules. For both states of granules, significant ($p < 0.05$) decline of the mean particle diameter and increase of negative zeta-potential value were found as the lecithin concentration was raised from 0.00% to 0.25%. This might be due to the further dissociation of egg yolk granule aggregation state caused by addition of lecithin. The results indicated the coadsorption of yolk granule protein and lecithin on

the interface. Generally, high amount of surfactants were needed to form small droplets due to its large specific surface area (Xue & Zhong, 2014). Previous studies have reported that the interactions between protein and lecithin might lead to the reduction of interfacial free energy as a result of the protein-lecithin complex formed at interfacial films, facilitating the decrease of fat droplet size (Patino, et al., 2001). However, the mean diameter showed no significant changes when the lecithin concentration was further increased from 0.50% to 1.00%. Meanwhile the negative zeta-potential value started to decline. The decreased negative zeta-potential value could be assumed that yolk granule protein was gradually displaced by lecithin because of competitive adsorption in the oil/water interfacial layer and more lecithin were aggregated at the interface, leading to the shielding of some negative charged groups of granule proteins (Matsumiya, et al., 2014).

3.2.3. Protein adsorption fraction of fresh emulsions

The protein adsorption fraction of emulsions prepared by egg yolk granules at pH 7.0/9.0 with various concentrations of lecithin added was displayed on Fig. 6. The results showed that, at pH 7.0, the adsorbed protein content was 75.58%, possessing larger adsorption amount than that at pH 9.0 (26.74%). It indicated that granule protein was more favorable for interface adsorption when negative zeta-potential value was low, while the high electrostatic repulsion of protein molecules at higher pH was not conducive to stable adsorption of granule proteins on the interface film (Rotimi E. Aluko & Mine, 1998). For native granule, the protein adsorption fraction decreased from 75.58% to 16.27% as the lecithin concentration increased from 0.00%

to 0.50%, implying the occurrence of competitive displacement at oil-water interface. When the lecithin concentration was higher than 0.50%, the interfacial adsorption protein concentration no longer decreased, indicating that the adsorption of lecithin at the interface was saturated in the form of incomplete displacement (Yi, et al., 2019). At relatively low surfactant concentrations, surfactant molecules adsorbed to the interface and formed small islands of surfactant located within the protein network. As the surfactant concentration increased, the size of the surfactant-rich regions expanded, restricting the protein network to a smaller surface area. At relatively high surfactant concentrations, the protein region increased appreciably in thickness and eventually the protein molecules were completely displaced from the interface (McClements, 2004). Therefore, granules organized as individual aggregate separated by lecithin and these granules spread at the interface leading to the formation of a continuous protein-lecithin membrane (Destribats, et al., 2014). For disrupted granule, there was less significant ($p > 0.05$) decrease in the interfacial protein content of the emulsion as lecithin concentration ranged from 0.00% to 0.25%. With a further increase of lecithin concentrations, protein adsorption fraction declined significantly ($p < 0.05$). The result indicated that small amount of lecithin adsorbed onto the surface of emulsified oil will be conductive to the physical stability of emulsions without excessive displacement of interface proteins and confirmed the synergistic effect of granules and lecithin on the stability of emulsions. At higher lecithin concentrations, large amount of proteins were displaced from the droplet surfaces by competitive adsorption, resulting in instability of the O/W emulsions by

surfactant-induced depletion flocculation (shown in Fig. 4).

3.2.4. Microstructure of emulsion droplet

Observing the microstructure of the emulsion at different concentrations of lecithin can better understand the stabilizing effect of the protein-lecithin composite emulsion. The microstructure pictures of emulsions prepared by granules at pH 7.0/9.0 with different concentrations of lecithin added were presented in Fig. 7. In the absence of lecithin, emulsion prepared from native granule showed coarse and large oil droplets, and the emulsion droplets gather together to form larger aggregates (about 1700 nm). This might be due to the lack of sufficient electrostatic repulsion between the droplets to prevent the emulsion from flocculation. However, emulsion prepared from disrupted granules does not exhibit flocculation and the size of emulsion droplets was about 550 nm, which may be attributed to its higher surface charge. At very low concentration of lecithin (0.25%), the particle size of the fat globule decreased to 400 nm and the emulsion droplets gradually showed a uniform distribution. With further increase of lecithin concentration, some large fat globules were observed in the emulsion. The possible reason for the above phenomenon was that when the concentration of lecithin was low (0.25%), the addition of lecithin dissociated the aggregate structure of egg yolk granule and lecithin and granule protein were adsorbed to the interface and jointly reduced the interfacial tension (contact angle), thereby improving the stability of the interface membrane and reducing the size of the oil droplets (Leong, et al., 2011). However, with further increase of lecithin concentration, most of the proteins on the interface membrane

were replaced by lecithin when the concentration of lecithin was above 0.50%. At this point, continuous increase in the amount of lecithin does not change the interfacial protein content (Fig. 6). A large amount of unadsorbable lecithin may cause repulsive flocculation between oil droplets, leading to the aggregation of small oil droplets to form large oil droplets (Matsumiya, et al., 2014). As a control, emulsion prepared by 1.00% lecithin showed larger fat globules (about 1100 nm), indicating the high emulsifying efficiency of granule proteins-lecithin complex. The addition of a small amount of lecithin showed a positive effect on the formation of emulsions, but excessive lecithin triggered repulsive flocculation, thus leading to the instability of the emulsions. In a word, the microstructure of the emulsion could well reflect the creaming phenomenon of the emulsion, and the aggregation of the emulsion droplets easily led to the stratification.

4. Conclusions

This study investigated the interactions between granule proteins in different aggregation states and lecithin concentrations, and the corresponding emulsifying properties. The results demonstrated that the disrupted granule (pH 9.0) exhibited higher solubility and negative value of zeta-potential, accompanied by lower surface hydrophobicity and particle size than the native granule (pH 7.0), which contributed to the smaller surface contacting angle and emulsion stability. As the lecithin concentration increased, the protein solubility and the negative value of zeta-potential of both egg granules were further increased, in contrast to that, surface hydrophobicity, particle size and contact angle decreased. The AFM image showed that the increase of

solution pH and addition of lecithin could destroy the aggregated structure of the egg yolk granule and dissociation of aggregate structure was beneficial to the improvement of emulsifying ability. The emulsion stability of egg yolk granules showed a trend of increase first and then decrease with the increase of lecithin concentration. Appropriate addition of lecithin (less than 0.25%) could be helpful in the formation of high stable emulsions with low particle size by further dissociating the aggregate structure and slightly reducing the contact angle and increasing surface net charge. However, extra lecithin (more than 0.50%) would induce excessive substitution of granule proteins by competitive adsorption, leading to instability of the O/W emulsions by surfactant-induced depletion flocculation.

Acknowledgments

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Figures

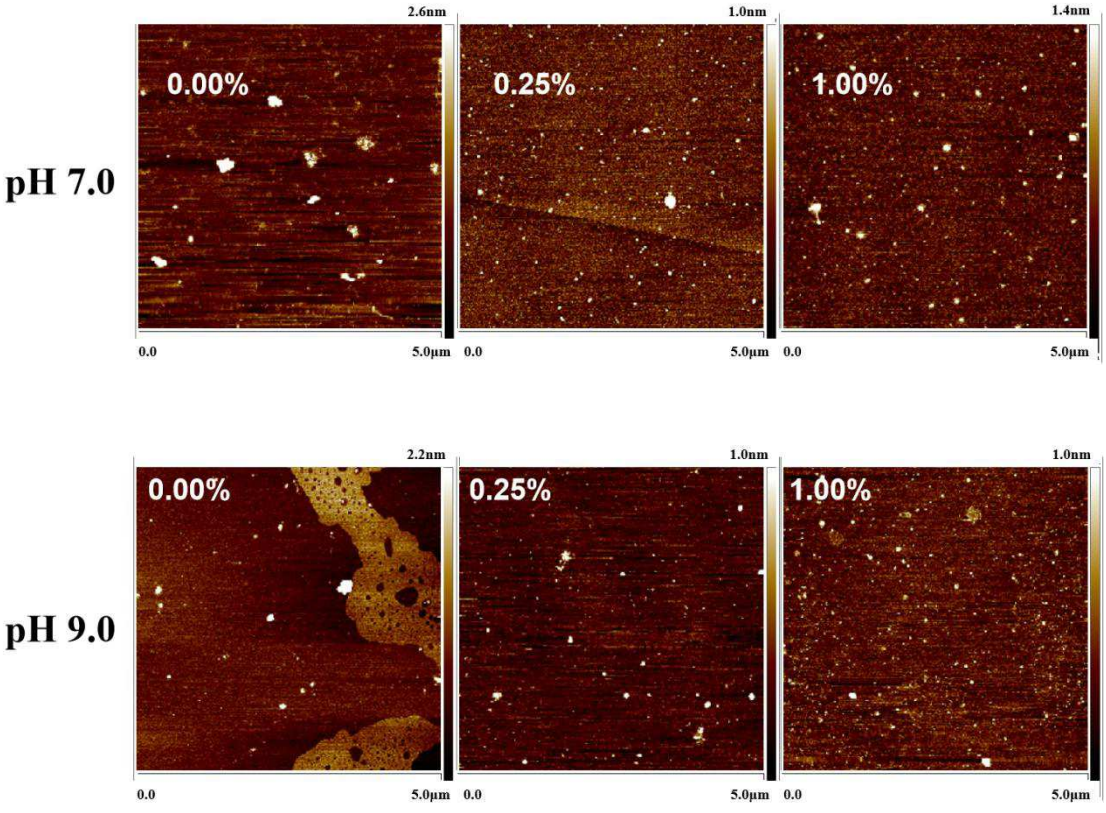

Fig. 1. 2-D AFM observations about the granules (pH 7.0 and pH 9.0) with different

555 concentrations of lecithin (0.00%, 0.25%, 1.00%)

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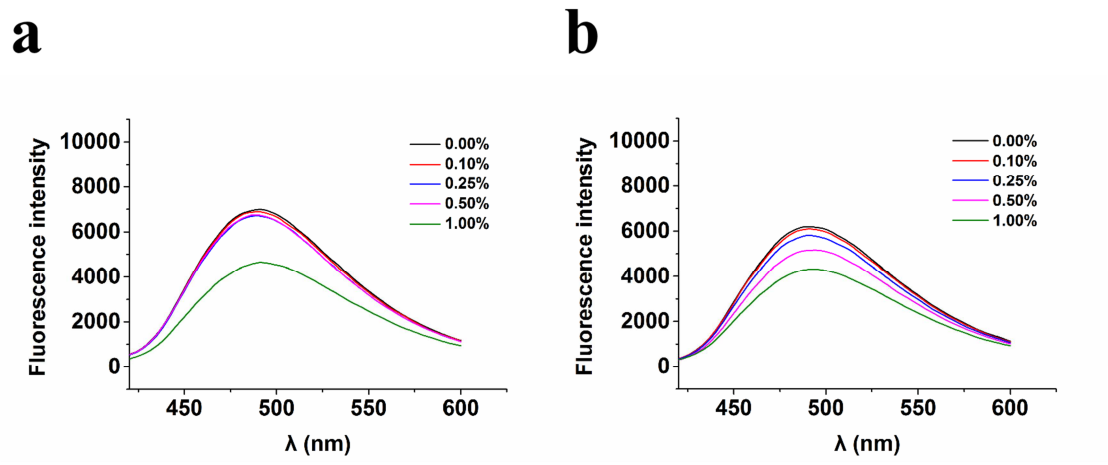

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564 Fig. 2. Fluorescence spectra about the granules (pH 7.0 (a) and pH 9.0 (b)) with

565 different concentrations of lecithin

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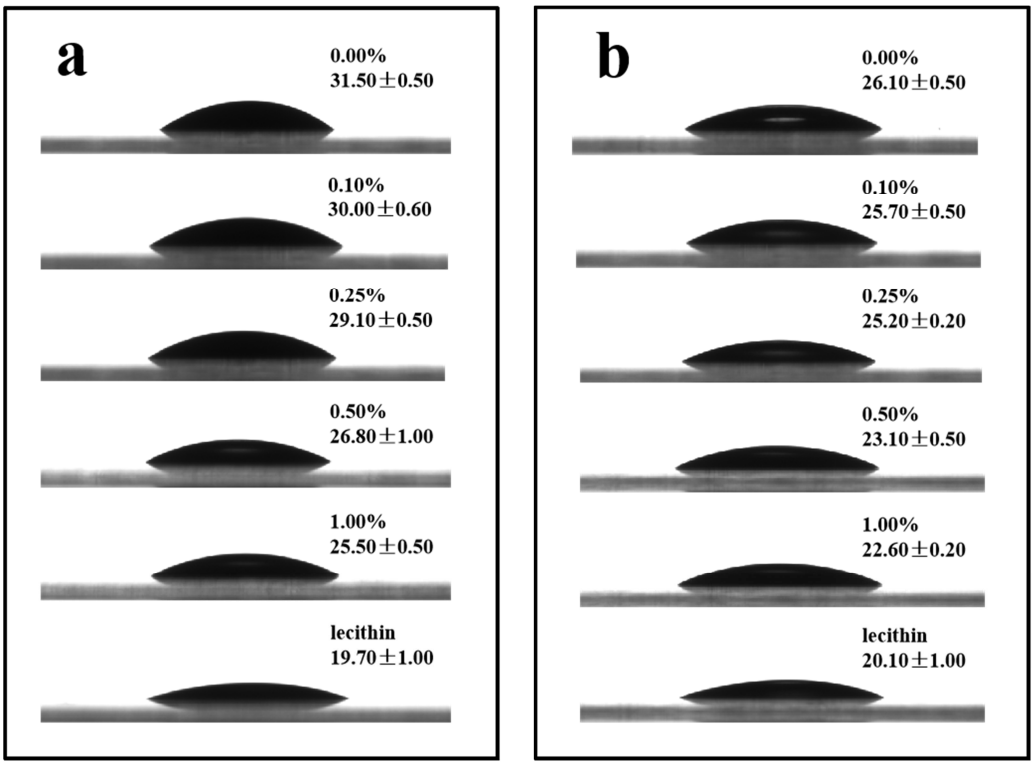

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573 Fig. 3. Contact angle about the granules (pH 7.0 (a) and pH 9.0 (b)) with different

574 concentrations of lecithin and 1.00% lecithin only

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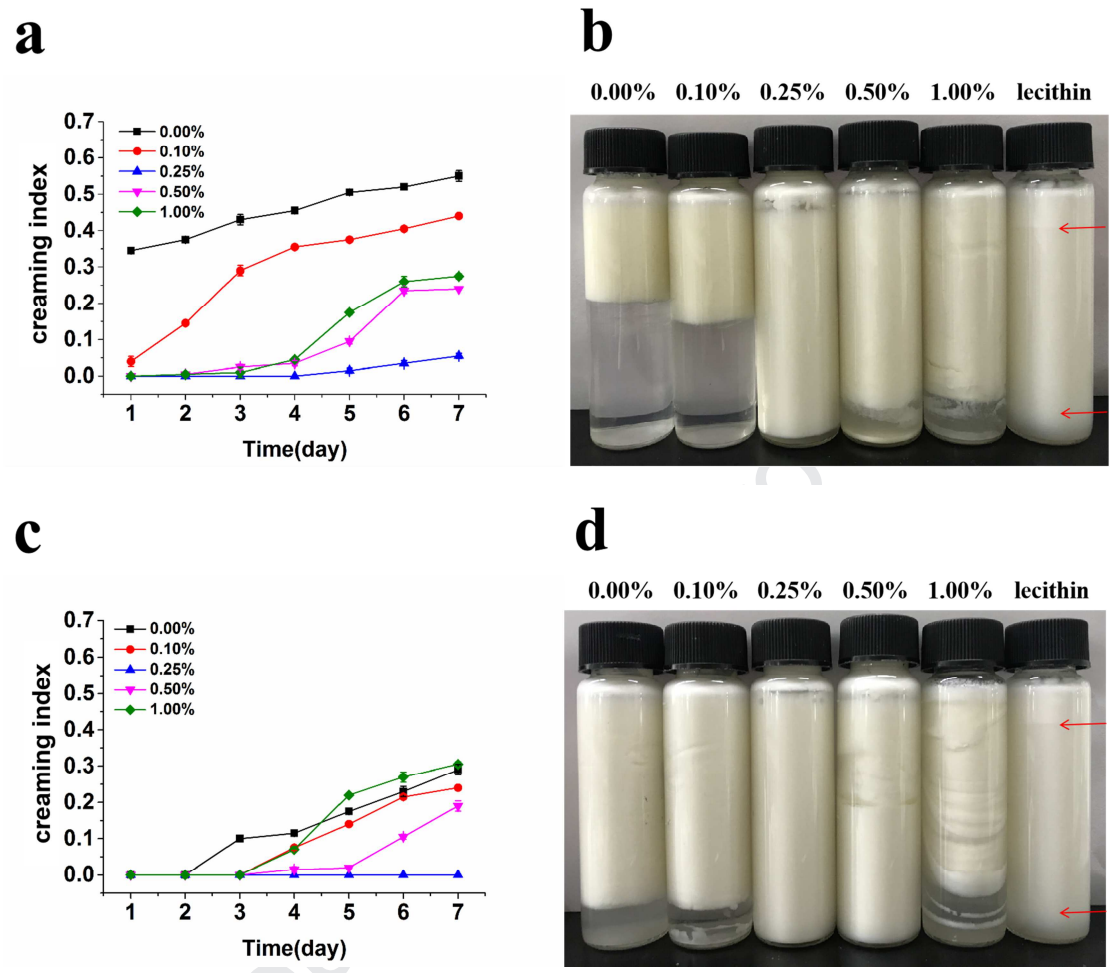

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582 Fig. 4. The creaming index (a, c) and visual appearance (one week) (b, d) of the

583 emulsions prepared by egg yolk granules (pH 7.0 (a, b) and pH 9.0 (c, d)) with

584 different concentrations of lecithin and the emulsion prepared by 1.00% lecithin only

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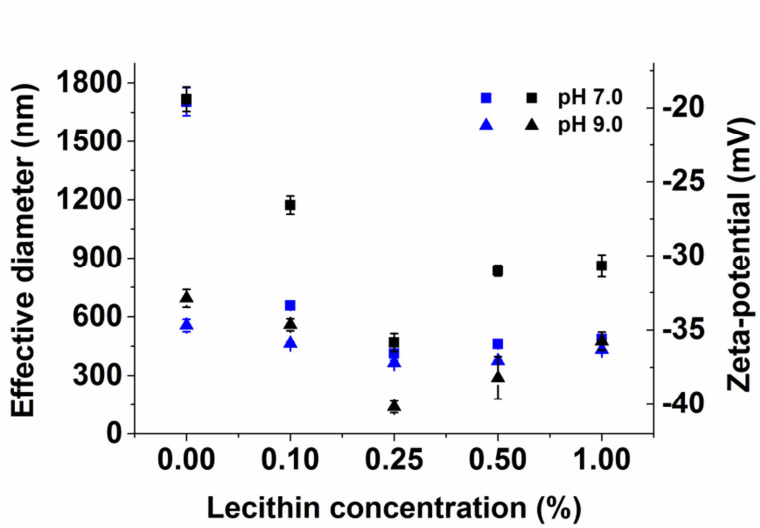

587

588 Fig. 5. The z-average particle size (nm) (blue) and zeta-potential (mV) (black) of the
589 emulsions prepared by egg yolk granules (pH 7.0 and pH 9.0) with various
590 concentrations of lecithin

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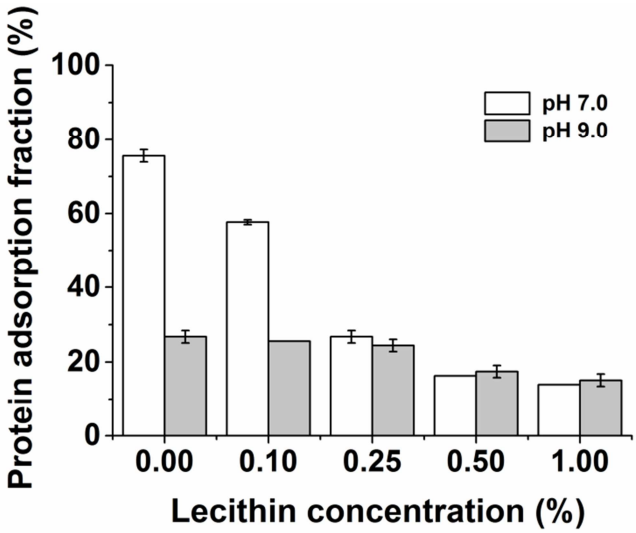

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600 Fig. 6. Protein adsorption fraction of emulsions prepared by egg yolk granules at pH

601 7.0/9.0 with various concentrations of lecithin added

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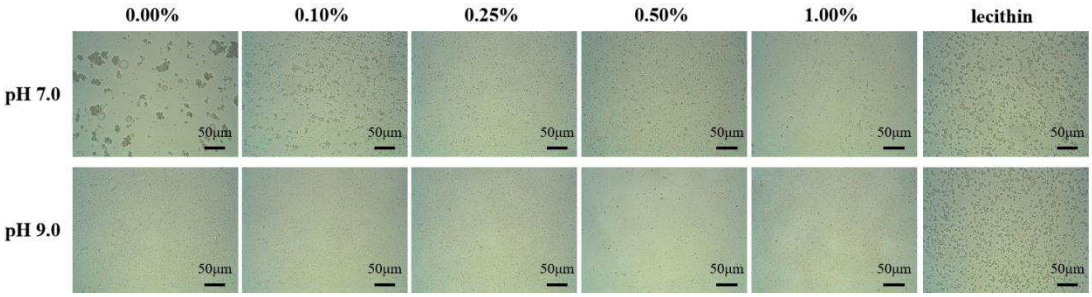

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614 Fig. 7. The microstructure pictures of emulsions prepared by granules at pH 7.0/9.0

615 with different concentrations of lecithin added and the emulsion prepared by 1.00%

616 lecithin only

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632 **Table 1**

633 Protein solubility (%), zeta-potential (mV), z-average particle size (nm) and
634 polydispersity of egg yolk granules at pH 7.0/pH 9.0 as affected by lecithin
635 concentrations (0.00-1.00% w/v).

Lecithin concentration (%)	Protein solubility (%)	Zeta-potential (mV)	Z-average particle size (nm)	Polydispersity
	pH 7.0/pH 9.0			
0.00	1.56±0.00e/ 80.18±0.35e	-20.46±0.50d/ -32.00±0.78c	1905.67±14.29a/ 126.10±3.39a	0.47±0.02d/ 0.81±0.01a
0.10	7.70±0.70d/ 85.86±0.93d	-29.15±0.25c/ -35.39±0.04b	372.73±6.81b/ 110.93±2.65b	0.55±0.01c/ 0.50±0.01b
0.25	11.56±0.81c/ 89.80±0.47c	-32.41±0.21b/ -35.96±0.07b	349.20±3.38c/ 94.57±0.24c	0.64±0.01b/ 0.44±0.01d
0.50	20.08±0.35b/ 95.23±0.23b	-33.04±0.11b/ -37.90±0.99ab	321.70±3.80d/ 83.80±3.28d	0.66±0.02b/ 0.47±0.00c
1.00	72.53±3.49a/ 97.45±0.81a	-35.29±1.12a/ -39.21±0.88a	133.90±1.95e/ 75.11±0.48e	0.79±0.02a/ 0.43±0.00d

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637 Different letters indicate significant difference ($p < 0.05$) (mean ± SD, n = 3).

Table 1

Protein solubility (%), zeta-potential (mV), z-average particle size (nm) and polydispersity of egg yolk granules at pH 7.0/pH 9.0 as affected by lecithin concentrations (0.00-1.00% w/v).

Lecithin concentration (%)	Protein solubility (%)	Zeta-potential (mV)	Z-average particle size (nm)	Polydispersity
	pH 7.0/pH 9.0			
0.00	1.56±0.00e/ 80.18±0.35e	-20.46±0.50d/ -32.00±0.78c	1905.67±14.29a/ 126.10±3.39a	0.47±0.02d/ 0.81±0.01a
0.10	7.70±0.70d/ 85.86±0.93d	-29.15±0.25c/ -35.39±0.04b	372.73±6.81b/ 110.93±2.65b	0.55±0.01c/ 0.50±0.01b
0.25	11.56±0.81c/ 89.80±0.47c	-32.41±0.21b/ -35.96±0.07b	349.20±3.38c/ 94.57±0.24c	0.64±0.01b/ 0.44±0.01d
0.50	20.08±0.35b/ 95.23±0.23b	-33.04±0.11b/ -37.90±0.99ab	321.70±3.80d/ 83.80±3.28d	0.66±0.02b/ 0.47±0.00c
1.00	72.53±3.49a/ 97.45±0.81a	-35.29±1.12a/ -39.21±0.88a	133.90±1.95e/ 75.11±0.48e	0.79±0.02a/ 0.43±0.00d

Different letters indicate significant difference ($p < 0.05$) (mean ± SD, n = 3).

Highlights

☑ The increase of solution pH and addition of lecithin could dissociate the egg yolk granule.

☑ Dissociation of egg yolk granule was beneficial to emulsion stability.

☑ Excessive displacement of interface granule proteins by lecithin resulted in stratification.

Conflict of interest statement

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.