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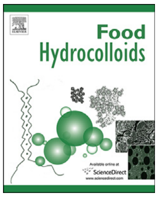

Characterization of Pickering emulsion gels stabilized by zein/gum arabic complex colloidal nanoparticles

Lei Dai, Cuixia Sun, Yang Wei, Like Mao, Yanxiang Gao*

Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Laboratory for Food Quality and Safety, Beijing Key Laboratory of Functional Food from Plant Resources, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, 100083, PR China

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ABSTRACT

Recently, Pickering emulsions have attracted extensive interests due to their advantages of "surfactant-free" and sustained delivery for bioactives. However, developing natural, biodegradable and food grade nanoparticles as Pickering emulsion stabilizers face new challenges. In this study, zein/gum arabic (GA) complex colloidal nanoparticles (ZGAPs) were prepared with a core-shell structure through hydrogen bonding and electrostatic interactions. The mean size of ZGAPs was larger than that of zein nanoparticles, and the zeta potential reversed from positive to negative, further confirming that GA molecules adsorbed onto the surface of zein nanoparticles. The three-phase contact angle ($\theta_{o/w}$) of zein nanoparticles was around 133.75°. After addition of GA, the $\theta_{o/w}$ of ZGAPs was adjusted to 88.95° closing to neutral wettability. This result indicated that ZGAPs could be developed as effective Pickering emulsifiers. Confocal laser scanning microscope images evidenced that ZGAPs formed a densely packed layer at the surface of oil droplets, which provided compact barriers of the droplets against coalescence and Ostwald ripening. At constant particle concentrations, the oil volume fraction significantly influenced the droplet sizes and rheological properties of Pickering emulsions. The droplet size and emulsified phase volume fraction of Pickering emulsions were increased with the rise of oil fraction. Consequently, Pickering emulsion gels were successfully fabricated at a higher oil fraction $\varphi \geq 0.5$, which exhibited a long-term storage stability. These findings would provide a potential way of producing Pickering emulsion gels, which showed the advantages of both emulsions and gels, and could become novel and effective delivery systems of bioactives.

1. Introduction

Emulsions based delivery systems have wide applications in the encapsulation and delivery of bioactive compounds in food industry for protecting therm against chemical degradation, enhancing bioavailability and controlled release (McClements, 2012). Conventional emulsions were stabilized by biopolymers (polysaccharides and proteins) and low molecular weight emulsifiers through forming steric elastic film or reducing the interfacial tension (Feng & Lee, 2016; Xiao, Li, & Huang, 2016). Compared to conventional emulsions, Pickering emulsions, which stabilized the oil–water interface by colloidal particles, have attracted extensive interests in recent years due to their advantages of "surfactant-free", high stability against coalescence and Ostwald ripening (Xiao, Wang, Gonzalez, & Huang, 2016). The colloidal particles could irreversibly adsorb and anchor at the oil/water interface and formed a rigid steric barrier of compact layer to stabilize Pickering emulsions (Hu et al., 2016).

Recently, studies on the Pickering emulsion stabilizers were mainly focused on the inorganic particles, such as calcium carbonate (Binks, Muijlwijk, Koman, & Poortinga, 2017), laponite clay (Ashby & Binks, 2000), silica (Zhao, Dan, Pan, Nitin, & Tikekar, 2013) and so on. However, in consideration of the environmental and food safety problems, the inorganic particles were not wise choices and had limited applicability in food industry (de Folter, van Ruijven, & Velikov, 2012). Therefore, developing natural, environmentally-friendly, biodegradable and food grade colloidal particles stabilized Pickering emulsions became a key scientific problem demanding to be promptly solved (Song et al., 2015). Certain food grade particles were testified to have better effects on stabilizing oil-in-water (O/W) emulsions, for instance, cellulose

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* Corresponding author. Box 112, No. 17 Qinghua East Road, Haidian District, Beijing 100083, China.

E-mail address: gyxcau@126.com (Y. Gao).

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nanocrystals (Cherhal, Cousin, & Capron, 2016), modified starch (Song et al., 2015), soy glycinin (Liu & Tang, 2016a, b) and prolamin (Xiao, Wang et al., 2016; Hu et al., 2016; Zou, Guo, Yin, Wang, & Yang, 2015).

Zein, the major storage protein of corn, is generally regarded as safe (GRAS) food ingredient by the US Food and Drug Administration (Patel, Bouwens, & Velikov, 2010). Due to the high surface hydrophobicity, zein can easily form colloidal nanoparticles by self-assembly. Therefore, zein particles was used as a potential food grade Pickering emulsion stabilizer. De Folter et al., (2012) reported that zein colloidal particles fabricated through anti-solvent precipitation procedure could produce surfactant-free O/W Pickering emulsions with droplet sizes in the range 10–200 μm. However, zein Pickering emulsions was unstable due to the poor wettability of zein colloidal particles (Feng & Lee, 2016; Wang et al., 2015). The colloidal particles used to fabricate Pickering emulsions should have an appropriate wettability, which was the key factor influencing the formation and stability of Pickering emulsions (Linke & Drusch, 2017). The wettability is usually expressed by oil/water three-phase contact angle ($\theta_{o/w}$). Colloidal particles with equilibrium $\theta_{o/w}$ at oil/water interface could promote effective packing of particles and form a steric barrier, thus preventing the droplet coalescence (Gao et al., 2014). In order to form stable Pickering emulsions, some feasible methods were utilized to regulate the surface wettability of zein particles. Gao et al. (2014) reported that zein and sodium stearate (SS) complexes, which formed through nonspecific hydrophobic interaction, significantly improved the diffusive mobility and equilibrated interfacial wetting properties of particles. Thus the interfacial particle was strongly favored adsorption and evidently enhanced the stability of Pickering emulsions against coalescence and creaming. However, the safety problems of SS were limited their application in food industries. Therefore, natural renewable resources (proteins and polysaccharides) were alternatively utilized to form complex particles with zein, tuning the three-phase contact angle of zein colloidal nanoparticles. Feng & Lee (2016) reported that sodium caseinate (NaCas) could adsorb onto zein colloidal nanoparticles and adjust the oil-in-water three phase contact angle $\theta_{o/w}$ close to 90°. In comparision with zein emulsions, the zein/NaCas nanocomplexes stabilized Pickering emulsions exhibited a better centrifugal stability at most pH values and ionic strengths. Highly charged zein/chitosan colloid particles (ZCCPs) with an intermediate wettability produced by a facile anti-solvent procedure were shown to be effective Pickering emulsifiers (Wang et al., 2015, 2016). Soltani and Madadlou (2016) also showed that sugar beet pectin and zein complex particles significantly improved the stability of Pickering emulsions, which was attributed to enhanced steric and electrostatic repulsion.

Gum arabic (GA), an amphiphilic polysaccharide, is widely utilized in the food industry because of its persistent stability in a wide pH range, high ionic strength, and high temperature (Bai, Huan, Li, & McClements, 2017; Chen & Zhong, 2015). Chen and Zhong (2015) presented that GA adsorbed on zein nanoparticles to stabilize nanoparticles in a wide pH range through electrostatic and hydrophobic interactions. To date, there has been no a systematic study on zein and GA complex colloidal particles as Pickering emulsion stabilizers. Interestingly, transforming Pickering emulsions to emulsion gels becoming an emerging trend. Pickering emulsion gels which possessed a gel-like network, endowed them superior stabilization (oxidative and physical stability) and better delivery for bioactives (Liu & Tang, 2016a, b; Hoffmann & Reger, 2014).

The purpose of this study was to form a novel, long-term stable, surfactant-free, and edible Pickering emulsion gels. Therefore, zein and GA complex colloidal nanoparticles ($ZGAP_S$) were fabricated by anti-solvent precipitation method as novel renewable natural particle-stabilizers of Pickering emulsion gels. The physical (particle size) and surface properties (wettability and surface charge) of complex colloidal particles were investigated. Fourier transform infrared spectroscopy (FTIR) was used to identify the driving forces for the formation of complex colloidal nanoparticles. Furthermore, the Pickering emulsion gels were characterized using particle size, confocal laser scanning microscopy (CLSM), optical microscopy and rheological measurements. The influence of oil phase ratio on the properties of Pickering emulsion gels were investigated. Moreover, the stability of Pickering emulsion gels against environmental stresses including pH and heat were also discussed. The information from this study would provide a new method for developing zein-based Pickering emulsion gels, which could be used as a novel carrier of bioactive components.

2. Materials and methods

2.1. Materials

Zein (product Z3625) and fluorescent dyes (Nile Red and Nile Blue A) were purchased from Sigma-Aldrich (St. Louis, MO, USA). GA was obtained from Shanghai Intron Food Co., Ltd. Medium chain triglyceride (MCT) oil was acquired from Lonza Inc. (Allendale, NJ, USA). All other chemical agents were analytical grade.

2.2. Fabrication of zein and GA complex colloidal nanoparticles ($ZGAP_S$)

Zein colloidal nanoparticles were prepared by anti-solvent precipitation method. Briefly, 4.0 g zein was dissolved in 100 mL 70% (v/v) aqueous ethanol solution with magnetic stirring to form stock solution. Then the stock solution was added drop-wise into 300 mL deionized water under continued stirring (600 rpm). After 30 min of constant stirring, the remained ethanol in the particle dispersions was removed by rotary evaporator (45 °C). Finally, the concentration of zein in the particle dispersions was 2% (w/v). The pH of zein particle dispersions was adjusted to 4.0 using 0.1 N HCl or NaOH.

Different concentrations of GA solutions were prepared by dissolving GA in deionized water and stirring overnight. After dissolution, GA solutions were adjusted to pH 4.0 using 0.1 N HCl or NaOH. Then zein particle dispersions (2%, w/v) were added to the different concentrations of GA solutions. The ZGAPs dispersions were formed with zein to GA mass ratios of 5:1, 3:1, 2:1, 1:1, 1:2, 1:3 and 1:5 (w/w) and termed as ZGAPs5:1, ZGAPs3:1, ZGAPs2:1, ZGAPs1:1, ZGAPs1:2, ZGAPs 1:3 and ZGAPs1:5. The ZGAPs dispersions were store at 4 °C for further analysis. Some parts of samples were lyophilized for 48 h (Alpha 1-2 D Plus freeze-drying apparatus, Marin Christ, Germany).

2.3. Particle size, zeta (ζ)-potential and polydispersity index (PDI) of ZGAPs

Dynamic light scattering (DLS) and particle electrophoresis instrument (Zetasizer Nano-ZS90, Malvern Instruments Ltd., Worcestershire, UK) were used to determine the particle size, ζ-potential and PDI of particle dispersions according to the method in our previous report (Dai, Sun, Wang, & Gao, 2016). Samples were diluted to the appropriate concentration before detecting. All measurements were carried out at 25 °C.

2.4. Wettability measurement of ZGAPs

The three-phase contact angle ($\theta_{o/w}$) of particles (zein, GA and

ZGAPs) was measured using an OCA 20 AMP (Dataphysics Instruments GmbH, Germany) following the method described by Wang et al. (2016) with some modifications. The particle powders obtained from freeze-dried were compressed to a thin tablet with thickness of 2 mm and diameter of 13 mm. Then the tablets were immersed in the MCT, which was in an optical glass cuvette. Deionized water (2 μL) was gently placed on the surface of the tablets using a high-precision injector. After equilibrium was reached, the droplets was photographed and the contour of imaged drop was simulated with LaPlace–Young equation to acquire contact angle $\theta_{o/w}$. Measurements were averaged over at least three droplets.

2.5. Fourier transform infrared spectroscopy (FTIR)

The chemical structures of zein, GA and ZGAPs were measured by Fourier transform spectrophotometer (Spectrum 100, Perkin-Elmer, Warrington, UK). Each sample (2.0 mg) was mixed with KBr (198 mg). Then the mixture was pressed into pellet and placed in the sample holder. The spectra were scanned from 4000 to 250 cm^{-1} wavenumbers in 32 scans with a 4 cm^{-1} resolution. The spectral data were processing using the software OMNIC 8.2 (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.6. Scanning electron microscope (SEM)

The micro-morphology of samples was observed by scanning electron microscope (SEM, SU8010, Hitachi) at an accelerating voltage of 5.0 kV. Tiny amount of dried powders was put on the double-sided adhesive and coated with a gold layer to avoid charging under the electron beam.

2.7. Formation of ZGAPs stabilized Pickering emulsion gels

The Pickering emulsions or emulsion gels were prepared using the same ZGAPs1:1 concentration (1%, w/v) and different oil fractions ($\varphi = 0.1, 0.3, 0.5, 0.6, 0.7$). The total volume of emulsions was set to 20 mL. Briefly, the medium-chain triglyceride (MCT) was slowly added to the particles suspension with a high-speed homogenizer at 12,000 rpm (IKA-ULTRATURRAX T25 basic, IKA® Works, Inc., Wilmington, NC, USA). After the oil completely added, the mixtures were further homogenized for another 3 min to acquire Pickering emulsions.

2.8. Emulsion type

The type of emulsions was defined by the method of drop test described by Feng & Lee (2016) with some modifications. In brief, the emulsion droplets were added to pure MCT or pure water, respectively. If the emulsion droplet dispersed rapidly in the aqueous phase and remained agglomeration in oil phase. It was regarded as an oil-in-water (o/w) emulsion. In contrast, emulsion was assessed as a water-in-oil (w/o) emulsion.

2.9. Droplet size of Pickering emulsions

The droplet size of emulsions was measured using a laser scattering particle size analyzer (LS230®, Beckman Coulter, USA). The refractive indices used for MCT and water were 1.52 and 1.33, respectively. The volume fraction-length ($D_{4,3}$) and volume-area ($D_{3,2}$) average diameters were calculated using the following equations:

$$D_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$$

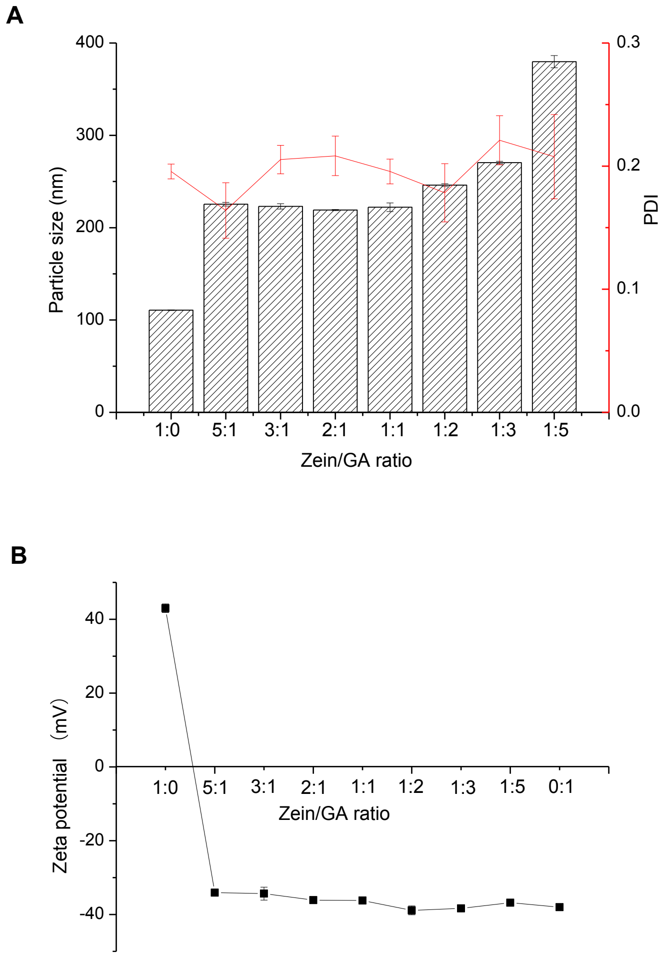

Fig. 1. Mean size (A), PDI (A) and zeta (ζ)-potential (B) of zein/GA complex nanoparticles at different zein to GA mass ratios.

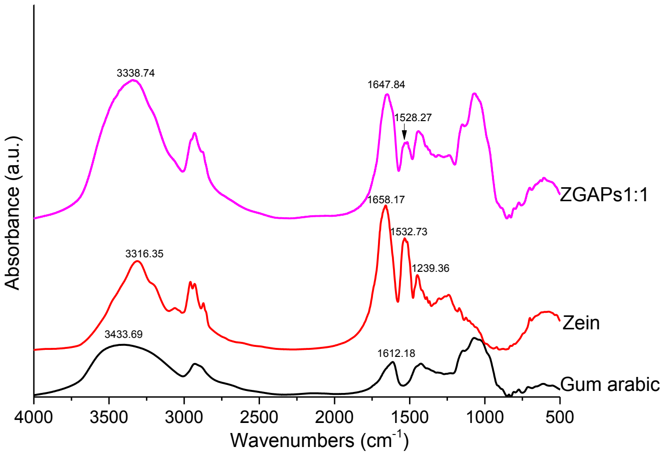

Fig. 2. FTIR spectra of zein, arabic gum (GA) and zein/GA complex nanoparticles (ZGAPs).

$$D_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}$$

The n_i is the number of particles with a diameter of d_i.

2.10. Rheology of Pickering emulsion gels

The rheological properties of Pickering emulsion gels were determined at 25 °C using an AR-1500 rheometer (TA Instruments, West Sussex, UK) with a steel parallel plate (40 mm diameter, gap 0.100 mm). For each measurement, the emulsions were deposited onto the plate and waited for 5 min to allow temperature equilibrium before measurements. For the steady-state flow measurements, the shear rate was from 0.1 to 100 s^{-1} and the apparent viscosity (η) was obtained from TA data analysis software. All the dynamic tests were performed within the linear viscoelastic region. The frequency was oscillated from 0.1 to 100 rad/s and the strain was made at 1%. The elastic modulus (G′) and loss modulus (G″) were recorded versus frequency. All measurements were performed for three times.

2.11. Optical microscopy

Microstructures of $ZGAP_S$ stabilized Pickering emulsions were observed using a optical microscope (Leica DMD 108, Leica Microsystems Inc., Heidelberg, Germany) equipped a camera. The emulsions were diluted by the continuous phase. And a drop of diluted emulsion sample was deposited on the microscope slide and covered with a cover slip. All the measurements were performed at 25 °C.

2.12. Interfacial structure

Confocal Laser Scanning Microscope (CLSM) (Leica TCS SP5, Leica Microsystems Inc., Heidelberg, Germany) was used to further visualize the interfacial structure of the Pickering emulsion droplets. The emulsions were stained with a mixed fluorescent dye solution consisting of Nile blue (0.1%) and Nile red (0.1%). Then the dyed emulsions were deposited on concave confocal microscope slides and gently covered with a cover slip. The Nile blue was used to stain the Pickering stabilizer (ZGAPs) and the Nile red was applied to dye the oil phase (MCT). The CLSM was operated using two laser excitation sources: an argon/krypton laser at 488 nm (Nile red) and a Helium Neon laser (He-Ne) at 633 nm (Nile blue).

2.13. Stability evaluation of Pickering emulsion gels

2.13.1. Effect of pH values

The effect of pH on the stability of Pickering emulsion was evaluated according to the method of Mwangi, Ho, Tey, and Chan (2016). The freshly ZGAPs stabilized emulsion gels were adjusted to pH 2, 3, 4, 7, or 9 using either 0.1 M NaOH or 0.1 M HCl. at 25 °C.

2.13.2. Effect of thermal treatment

The Pickering emulsion gels were incubated in water bath (50, 60, 70, 80 °C) for 30 min and then cooled down to 25 °C.

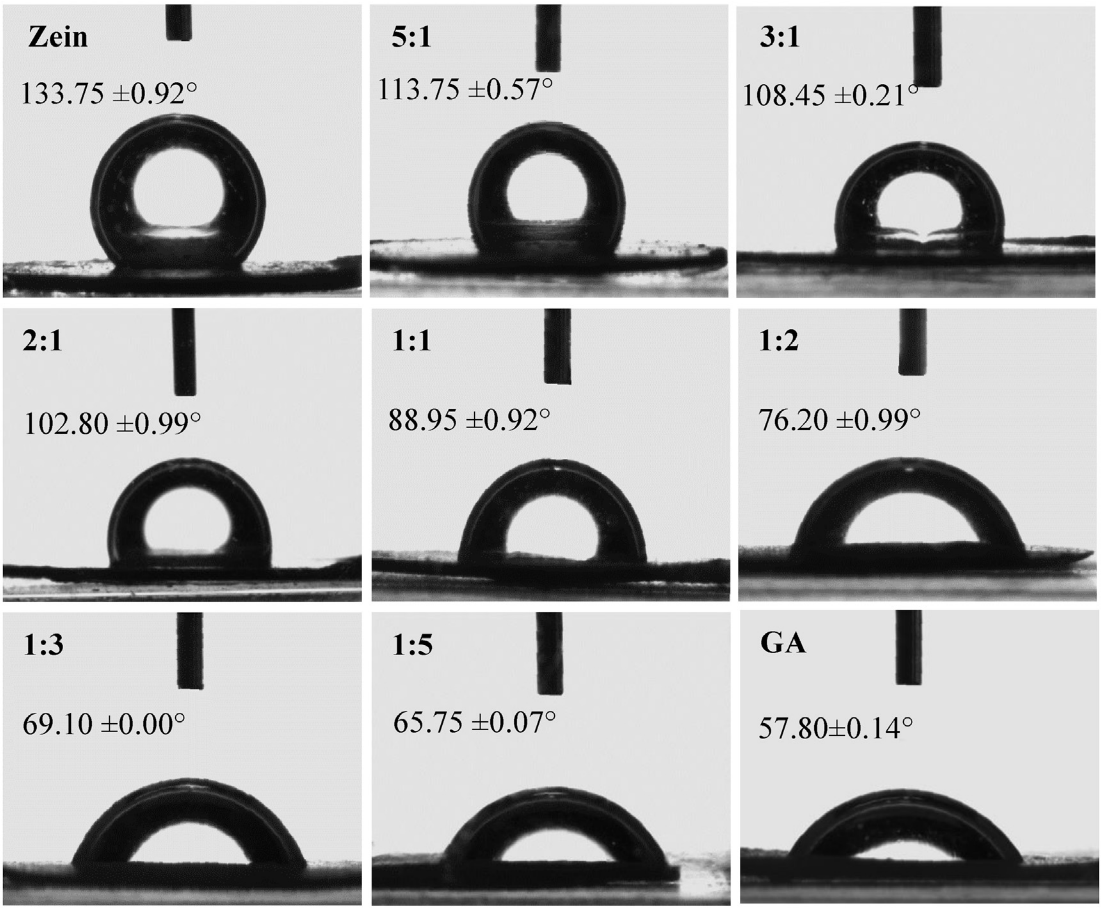

Fig. 3. Oil-in-water three-phase contact angles ($\theta_{o/w}$) of ZGAPs at different zein to arabic gum mass ratios.

After each treatment, the droplet sizes of the Pickering emulsion gels were evaluated.

2.14. Statistical analysis

All the data obtained were average values of triplicate determinations and subjected to statistical analysis of variance using SPSS 18.0 for Windows (SPSS Inc., Chicago, USA). Statistical differences were determined by one-way analysis of variance (ANOVA) with Duncan's post hoc test and differences were considered to be significant with $P < 0.05$.

3. Results and discussion

3.1. Characterization of zein and GA complex nanoparticles

3.1.1. Particle size, ζ-potential and PDI of complex nanoparticles

The mean size and PDI of zein and GA complex nanoparticles were shown in Fig. 1. In the absence of GA, the mean size of zein colloidal nanoparticles was around 110 nm with PDI = 0.2. This result was in agreement with previous study (Chen & Zhong, 2015). When zein colloidal nanoparticle dispersion was mixed with a lower level of GA solution (zein to GA mass ratio below 5:1), large aggregates were formed and rapidly precipitated (data not shown). This might be due to the anionic GA adsorbed onto the surface of cationic zein colloidal nanoparticles triggered the electrostatic deposition. Hu and McClements (2015) also reported that addition of lower level of sodium alginate (0.005% or 0.01%) could lead to extensive particle aggregations, which rapidly precipitated. The mean size of ZGAPs at a zein to GA mass ratio of 5:1 was about 225 nm, which was higher than that of zein colloidal nanoparticles ($p<0.05$). This phenomenon could be attributed to the formation of a core-shell structure of ZGAPs with a zein core and a GA shell. The mean size of ZGAPs particle was scarcely changed at a zein to GA mass ratio of more than 1:1, indicating GA was eventually saturated. Further increasing the level of GA, the mean size of ZGAPs was enhanced with the increment of GA. Addition of excessive polysaccharide cold lead to the depletion flocculation (Joye, Nelis, & McClements, 2015).

The isoelectric point (pI) of zein is around pH 6.2 (Patel et al., 2010). Therefore, zein molecules have a positive charge below their pI. Fig. 1B showed the ζ-potential of ZGAP_S at different zein to GA mass ratios. At pH 4.0, the ζ-potential values of zein colloidal nanoparticles and GA were +43 mV and −38 mV, respectively. The electrostatic attraction was attributed to opposite charges between zein and GA, leading to adsorption of GA molecules onto the surface of zein colloidal nanoparticles. The ZGAP_S at a zein to GA mass ratio 1:1 should have a negative charge about −34 mV. The potential value of ZGAP_S was slightly elevated and closed to that of GA as its level was increased. This further supported the hypothesis that GA coated on the zein colloidal nanoparticle surfaces. Similar results were also reported by other researchers (Hu & McClements, 2015; Joye et al., 2015).

3.1.2. FTIR

FTIR measurement was always used to provide information about the interactions among macromolecule polymers. The FTIR spectra of individual zein, GA and their complex nanoparticles were shown in Fig. 2. The typical peak in the range of 3100–3400 was recognized as hydrogen bonds (Chen & Zhong, 2014). In the spectra of zein and GA, the peaks of the hydrogen bonds were at 3316 and 3433 cm^{-1}, respectively. Interestingly, after GA addition, the peak of hydrogen bonds in the spectrum of ZGAPs was shifted to 3338.74 cm^{-1}, indicating the formation of hydrogen bonds between zein and GA. Liang et al. (2015) also pointed out that compared with zein nanoparticles, a peak shift of hydrogen bonds occurred in the FTIR spectrum of zein/quaternized (HTCC) chitosan nanoparticles (from 3441 to 3422 cm^{-1}), suggesting that the hydrogen bonding between zein and HTCC was a major force in the formation of complex nanoparticles. The spectrum of zein showed two prominent absorption peaks at 1658.17 and 1532.73 cm^{-1} which were represented as amide I (C=O stretching) and amide II bonds (C–N stretching), respectively (Sun, Dai, & Gao, 2017). As compared to the spectrum of zein, the bands of the amide I and amide II groups were shifted from 1658.17 toand 1532.73 cm^{-1} to 1647.84 and 1528.27 cm^{-1}, respectively. This phenomenon revealed that electrostatic interactions occurred between zein and GA. Luo, Zhang, Whent, Yu, and Wang (2011) also interpreted that compared with zein, the shifted bands of amide I and amide II groups in zein and chitosan complex was ascribed to the electrostatic interaction between zein and chitosan.

3.1.3. Wettability of complex nanoparticles

The interfacial wettability of the particles is very important in the formation of stable Pickering emulsion. The suitable wettability could promote the particles to absorb on the oil–water interface and form a steric hindrance preventing the droplet coalescence (Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011). The wetting properties of the particles were measured through investigating the three-phase contact angles $\theta_{o/w}$ of ZGAP tablets, which were immersed in MCT. Fig. 3 showed the oil/water three-phase contact angle $\theta_{o/w}$ of ZGAPs at different zein to GA mass ratios. The $\theta_{o/w}$ of zein colloidal nanoparticles without GA was around 133.75°. This result might be attributed to more than 50% of hydrophobic amino acid residues in zein molecules (Wang et al.,

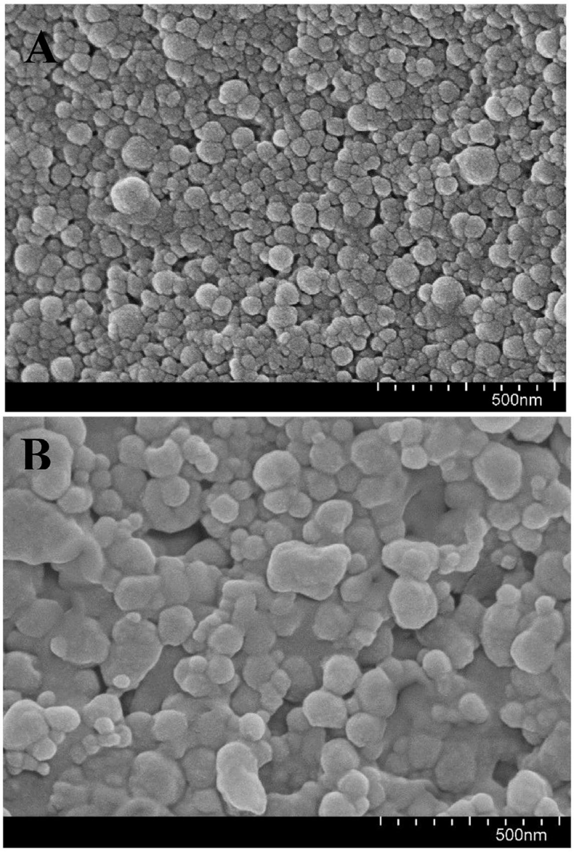

Fig. 4. SEM of zein (A) and zein/GA complex nanoparticles at a zein to GA ratio 1:1 (B).

2015). Therefore, zein nanoparticles was hardly usable for stabilizing oil-in-water Pickering emulsion. In the presence of GA, the $\theta_{o/w}$ of ZGAPs was decreased with the increased level of GA. The decreased $\theta_{o/w}$ further testified that GA molecules absorbed on the zein nanoparticle surface. Interestingly, the $\theta_{o/w}$ of ZGAPs at a zein to GA mass ratio of 1:1 was 88.95°, indicating that ZGAPs1:1 were potentially suitable for acquiring stable Pickering emulsions. The near-neutral wettability with $\theta_{o/w}$ of around 90° could not only facilitate efficient particles to absorb on the oil/water interface, but also produce a steric hindrance against the aggregation of oil droplets (Feng & Lee, 2016; Zou et al., 2015). Additionally, Wang et al. (2015) also clearly demonstrated that zein/chitosan complex particles at chitosan-to-zein ratios of 1:20, whose $\theta_{o/w}$ was about 82°, were potentially suitable materials as Pickering emulsion

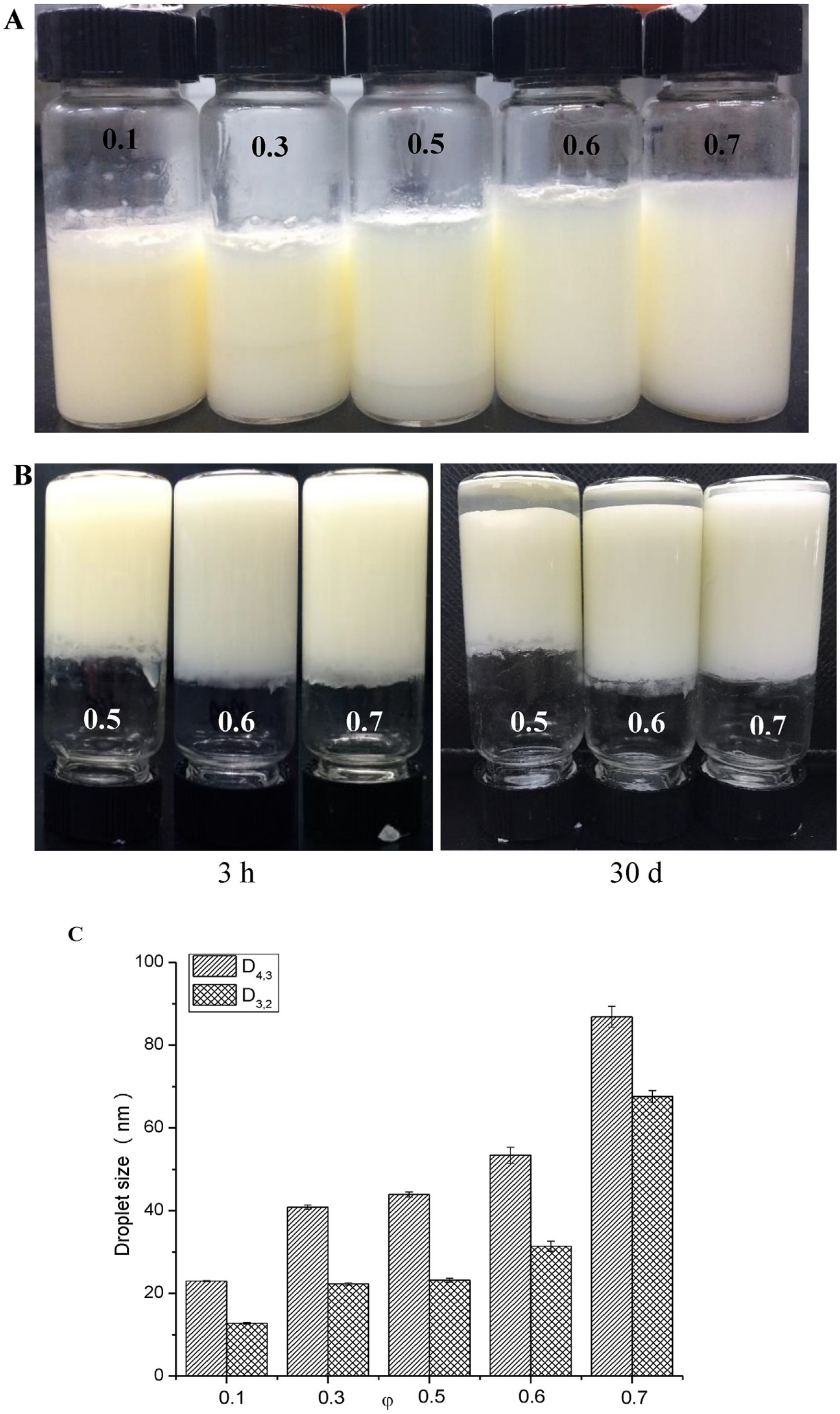

Fig. 5. Appearance photograph (A, B) and droplet size (C) of Pickering emulsions stabilized by ZGAPs1:1 with various oil fractions (φ = 0.1, 0.3, 0.5, 0.6, 0.7). A and C were measured at1 h after the preparation. B was photographed after 4 h and 30 days storage at 4 °C.

stabilizers. In contrast, the $\theta_{o/w}$ values of ZGAPs at zein to GA mass ratios of 1:2, 1:3 and 1:5 (65–76°) were obviously lower than neutral $\theta_{o/w}$ (90°), suggesting that excessive GA was not suitable to form a stable Pickering emulsion. Therefore, the ZGAPs fabricated at a zein to GA mass ratio of 1:1 were utilized to prepare Pickering emulsions.

3.1.4. Morphological observation

The surface morphology of zein and ZGAPs (zein to GA ratio at 1:1) was observed by SEM and demonstrated in Fig. 4. The zein nanoparticles exhibited a spherical shape with the uniform size (around 120 nm). Similar result was also reported by previous researchers (Luo et al., 2011). Interestingly, the presence of GA changed the surface and shape of zein nanoparticles and ZGAPs showed an irregular geometry shape and larger size. It was hardly to see individual particles clearly (Fig. 4B). Meanwhile the surface of ZGAPs was roughness, indicating that GA completely coated on the surface of zein nanoparticles. Luo, Teng, and Wang (2012) also reported that the boundary of zein and carboxymethyl chitosan complex nanoparticles was not clear, which was contributed to the highly hydrophilic polysaccharides absorbing on the surface of zein nanoparticles.

3.2. Physical properties of Pickering emulsions and gels

3.2.1. Visual appearance

A drop of the Pickering emulsions or emulsion gels in this work could disperse readily in the aqueous phase, indicating they were oil-in-water (o/w) emulsions and gels. The typical appearance of the ZGAPs stabilized Pickering emulsions or emulsion gels at different oil fractions (φ = 0.1, 0.3, 0.5, 0.6, 0.7) was presented in Fig. 5A. As previous reports, zein stabilized Pickering emulsions were not stable, which resulted in the aggregation of droplets and the release of oil on the top phase of emulsions (Zou et al., 2015).

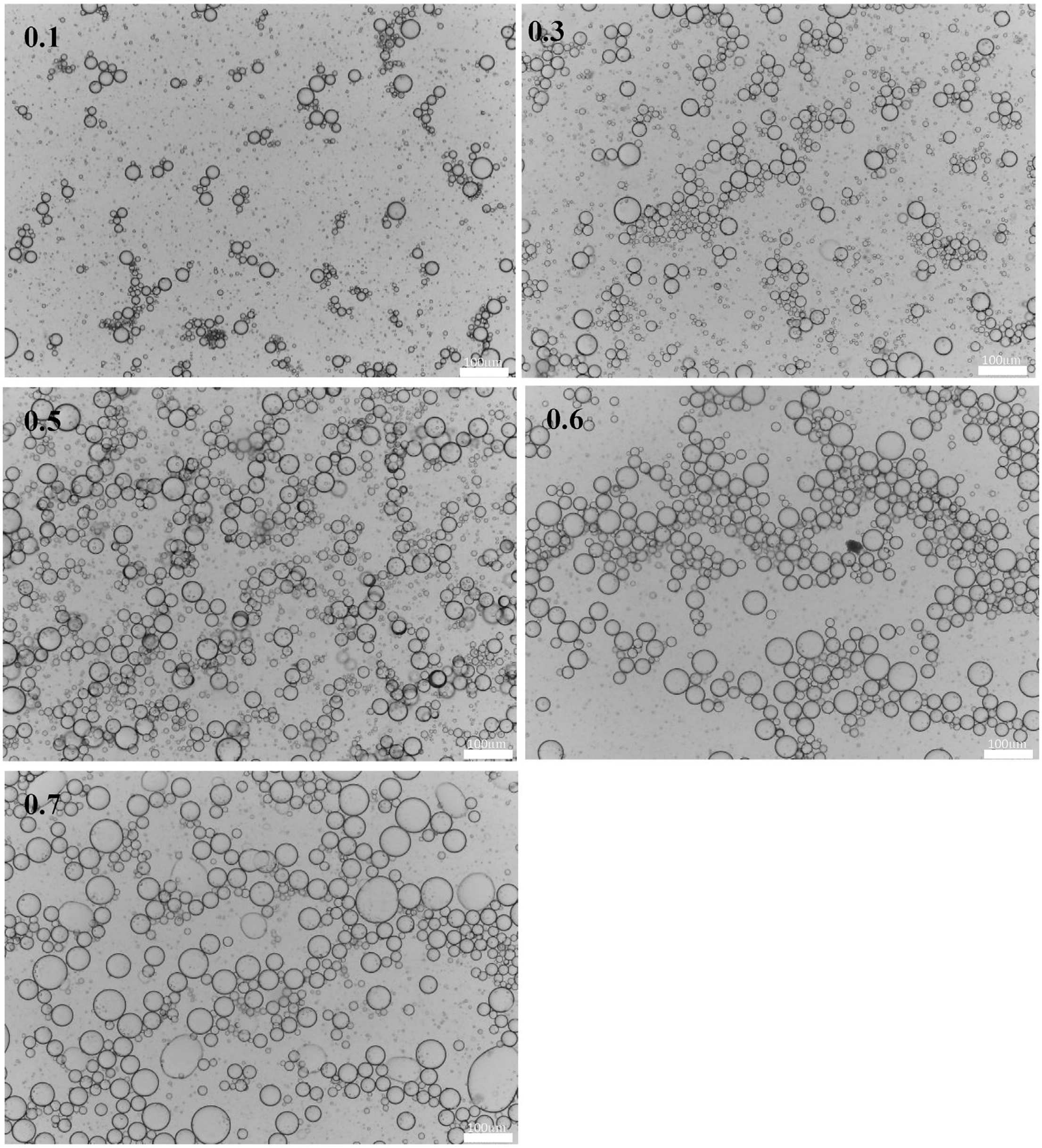

Fig. 6. Optical microscopic images of Pickering emulsions stabilized by ZGAPs 1:1 with different oil fractions (φ = 0.1, 0.3, 0.5, 0.6, 0.7).

Apparently, emulsified phase volume of Pickering emulsions stabilized by ZGAPs was increased with the oil fraction φ increasing from 0.1 to 0.7. Similar result was found by Xiao, Wang et al. (2016), who reported that increasing the oil fraction from 0.3 to 0.7 led to gradual increase in emulsified phase volume. Interestingly, Pickering emulsion gels stabilized by ZGAPs with an oil fraction ($\varphi = 0.7$) showed no creaming and phase separation, and even remained stable after 30 days of storage (Fig. 5A and B). Although the emulsions prepared with oil fractions ($\varphi = 0.5, 0.6$) exhibited creaming, no sign of oil release was observed after 30 days of storage (Fig. 5B). Nevertheless, the ZGAPs stabilized Pickering emulsions with oil fractions ($\varphi = 0.5, 0.6, 0.7$) were transformed from the emulsions to emulsion gels after 3 h of storage, which further provided the long-term storage stability of Pickering emulsions (Fig. 5B).

3.2.2. Droplet size

The mean droplet sizes ($D_{4,3}$ and $D_{3,2}$) of Pickering emulsions stabilized by ZGAPs 1:1 with different oil fractions ($\varphi = 0.1, 0.3, 0.5, 0.6, 0.7$) were shown in Fig. 5C. It can be found that all mean droplet sizes ($D_{4,3}$ and $D_{3,2}$) of Pickering emulsions were micrometer scale and increased progressively with the rise of oil fractions (φ from 0.1 to 0.7). The results could be attributed to that the total volume of emulsions was fixed (20 mL). When the oil fraction was elevated, the volume of aqueous phase was decreased, thus the amount of ZGAPs tended to be not enough to adsorb on the o/w interface, causing the formation of larger droplets. Liu and Tang (2016b) also reported that as the oil phase increased, the droplet size of Pickering emulsions basically increased, even formed coarser emulsions at higher oil fractions.

3.2.3. Morphology of Pickering emulsion gels

The morphologies of ZGAPs 1:1 stabilized Pickering emulsions or gels with different oil fractions ($\varphi = 0.1, 0.3, 0.5, 0.6, 0.7$) were observed by optical microscopy and the pictures were shown in Fig. 6. At a constant particle concentration, with the increase of oil fractions, less available particles were able to cover the surface of oil droplet. Therefore, the oil droplets became larger with increasing oil fraction. This result was consistent with that acquired by droplet size test methods. Binks and Lumsdon (2000) pointed out that there existed a phase-invert of Pickering emulsions (dispersed phase became continuous phase) in oil fractions φ 0.65–0.70. Furthermore, Wang and co-workers also showed that the dispersed droplets occurred deformation in Pickering emulsions with 70% oil phase (Wang et al., 2016). Interestingly, in the present study, the droplets of Pickering emulsions maintained circular shapes even at higher oil fraction ($\varphi = 0.7$). Therefore, zein and GA complex nanoparticles had the potential to fabricate Pickering high internal phase emulsions (HIPE), which was defined as concentrated Pickering emulsions and requested the volume fraction of internal phase larger than 0.74 (Zheng, Zheng, Wang, & Du, 2013).

The CLSM was used to provide an intuitive way to illustrate the interfacial structure of Pickering emulsions fabricated by ZGAPs and the images were shown in Fig. 7. The oil phase was stained by Nile red meanwhile the complex nanoparticles were stained with Nile blue. Therefore, the blue (Fig. 7A) and red (Fig. 7B) fluorescence fields represented oil phase and complex nanoparticles, respectively. Fig. 7C showed overlay fluorescence photos of Pickering emulsions. Apparently, the oil phase was in the interior of the droplets, while the particles were formed a densely packed layer at the boundary of droplets (Fig. 7C). This result further proclaimed ZGAPs stabilized Pickering emulsions belonged to oil in water types. The higher magnification CLSM images of a single droplet of Pickering emulsions were shown in Fig. 7A1-C1. It was found that the ZGAPs formed a compact and thick interface layer on the surface of spherical oil droplets, which generated a strict barrier for Pickering emulsions against coalescence.

3.3. Rheological properties of Pickering emulsion gels

The rheological properties of gel-like Pickering emulsions were investigated by steady-state flow and dynamic oscillatory measurements and the results were presented in Fig. 8. In all the measurements, the apparent viscosity (η) was gradually decreased

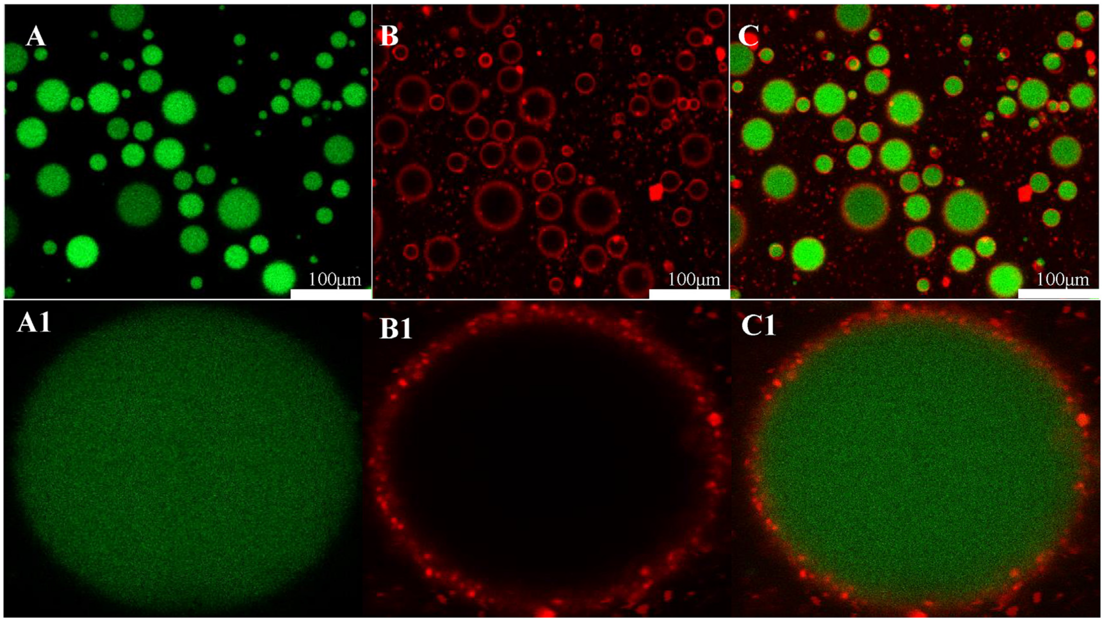

Fig. 7. CLSM images of ZGAPs 1:1 stabilized Pickering emulsions at 50% oil fraction: MCT was stained with Nile Red (blue) excited at 488 nm (A, A1); ZGAPs was stained by Nile Blue (red) excited at 633 nm (B, B1); C was combined image of A and B; C1 was combined image of A1 and B1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

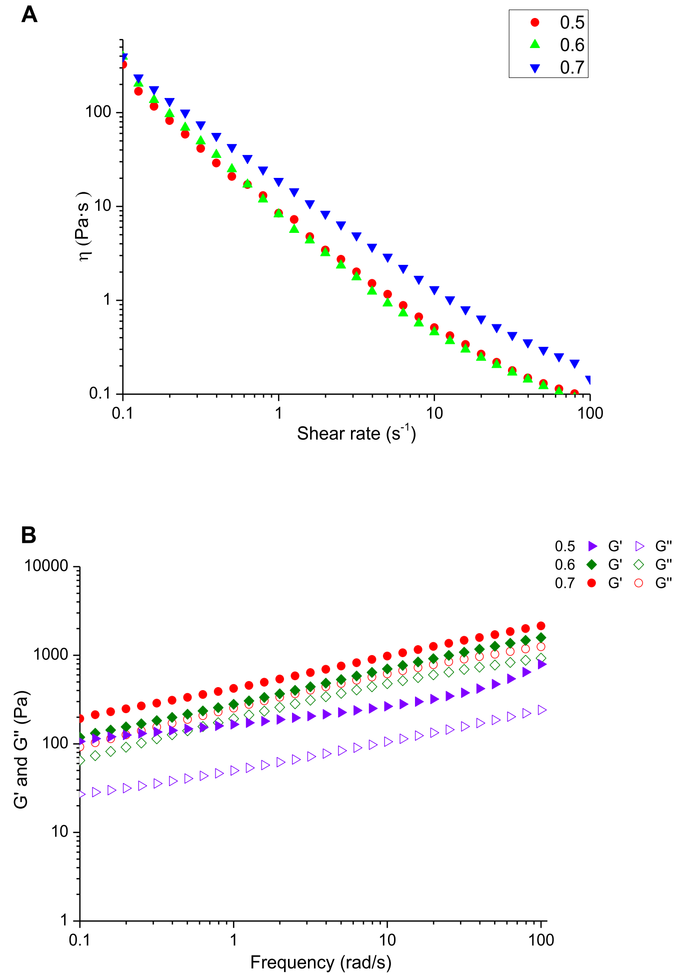

Fig. 8. Rheological properties of Pickering emulsion gels prepared by ZGAPs with various oil fractions (φ = 0.5, 0.6, 0.7). η: apparent viscosity, G′: storage moduli, G″: loss moduli.

with the shear rate increasing from 0.1 to 100 s^{-1}, indicating the shear-thinning behavior of Pickering emulsion gels (Fig. 8A). This phenomenon mainly reflected the deflocculation behavior of oil droplets in emulsion gels (Binks & Lumsdon, 2000). As expected, the η of Pickering emulsion gels was progressively increased with the increase of oil fractions (φ = 0.5–0.7). These results were consistent with previous reports by Xiao, Wang et al. (2016) and Liu & Tang (2016b).

The dynamic oscillatory measurements were applied to further illustrate the gel-structure of Pickering emulsion gels (Fig. 8B). Apparently, for all the Pickering emulsion gels, the storage modulus (G′) was obviously higher than loss modulus (G″) in the frequency range from 0.1 to 100 rad/s, indicating that the elastic gel-like structure of Pickering emulsion gels were formed. Chen et al. (2016) also reported the higher G′ than G″ confirmed the formation of elastic gel networks. The increase of oil fractions (0.5–0.7) resulted in a gradual increase in the values of the G′ and G″. A similar result was observed by Xiao, Wang et al. (2016), who reported that both G′ and G″ of Pickering emulsions stabilized by kafirin nanoparticles were elevated with increasing volume of oil, contributing to that the relative density of emulsion gel droplets was enlarged and resulted in a higher G′ at a higher oil fraction. In conclusion, when the concentration of the particles was fixed, the volume of oil had an obvious influence on the rheological properties of Pickering emulsion gels. Additionally, Zou et al. (2015) reported that the G′ of emulsion gels, which exhibited independence frequency, was the feature of particulate gels. On the contrary, the change of G′ was mainly depended on the frequency reflecting that the gel-like structure of Pickering emulsion gels was mainly due to the non-covalent physical interactions. Interestingly, the G′ of Pickering emulsion gels stabilized by ZGAPs with the oil fraction (φ = 0.5–0.7) showed the obvious frequency dependence, revealed that they were emulsion-filled protein gels.

3.4. *Stability evaluation of Pickering emulsion gels*

3.4.1. *Effect of pH*

The commercial food products experience a wide range of pH during the processing as well as in human gastrointestinal digestion. Therefore, it is necessary to evaluate the influence of pH on the stability of the droplets in Pickering emulsion based delivery system (Dai et al., 2016). The droplet sizes ($D_{3,2}$) of ZGAPs stabilized Pickering emulsion gels at various pH values were shown in Fig. 9A. Apparently, the Pickering emulsions showed highly stable against droplet aggregation at pH 2, 3, 4 and 9 and this might be due to that zein and GA complex nanoparticles exhibited a better stability at these pH values. Chen & Zhong (2015) confirmed that GA stabilized zein nanoparticles showed no noticeable differences in particle sizes at pH 5.0–8.0. However, mean diameter of the droplet at pH 7.0 was slightly larger than that at other pH values. Feng and Lee

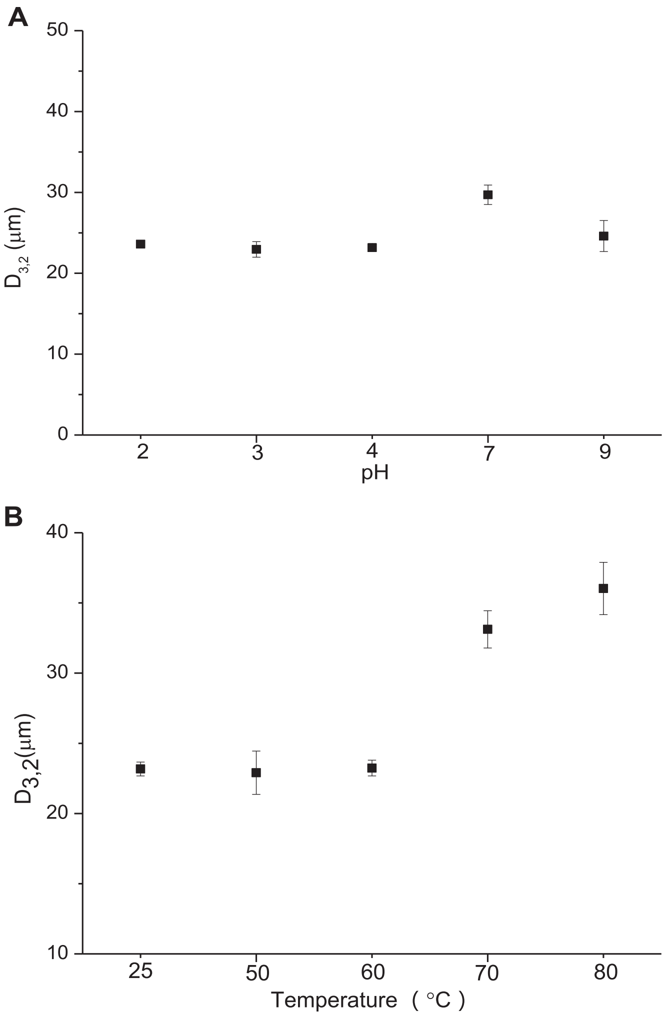

Fig. 9. Effects pH (A) and heating temperature (B) on the stability of Pickering emulsion gels.

(2016) also reported that Pickering emulsions stabilized by zein and NaCas showed a lower stability at pH 7.0 than those at pH 3.0 and 9.0 owing to the isoelectric coagulation of zein and NaCas at pH 7.0.

3.4.2. *Effect of thermal treatment*

Food products commonly undergo heat treatment during their manufacture or utilization. Therefore, the influence of temperature on the stability of Pickering emulsions was also investigated. Pickering emulsions were incubated at the temperature ranging from 50 to 80 °C (30 min) and cooled down to room temperature (25 °C). As we can see, the mean droplet sizes ($D_{3,2}$) of Pickering emulsion were remained unchanged up to 60 °C, indicating Pickering emulsion gels were kept stable against the aggregation (Fig. 9B). However, the mean diameter of droplets ($D_{3,2}$) was increased when the treatment temperature was above 60 °C. Mwangi et al. (2016) also reported that mean diameter of droplets in Pickering emulsions stabilized by chitosan particles was enhanced with the temperature increasing from 70 to 90 °C. This phenomenon was interpreted as the Brownian motion of particles increased with the rise of temperature, which might lead to redistribute particles on the oil/water interface and expose the surfaces of droplets increasing the tendency of droplet to coalesce (Mwangi et al., 2016). Thus, the higher temperature caused larger mean diameter of droplets.

4. Conclusions

In this work, zein and GA complex nanoparticles were successfully prepared through hydrogen bonding and electrostatic interactions. The wettability of the complex nanoparticles was monitored by the mass ratio of zein and GA. Interestingly, a near-neutral wettability of ZGAPs was observed at a zein to GA mass ratio of 1:1. As expected, the ZGAPs based Pickering emulsion gels were successfully fabricated at high oil volume fractions ($\varphi = 0.5$, 0.6 and 0.7). Furthermore, the CLSM images fully confirmed efficient ZGAPs absorbed at the oil/water interface forming steric barriers. The result of dynamic rheology showed that G' was higher than G'', revealing the formation of elastic gel networks in the systems. Nevertheless, the gel structure prompted a long-term storage stability (30 days) against coalescence and Ostwald ripening of Pickering emulsion gels. These findings not only develop a novel and practical Pickering emulsion gels, but also extend the application of water-insoluble proteins in delivery systems.

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