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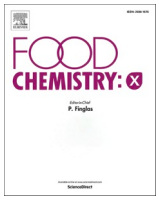

Novel Pickering emulsion stabilized by glycosylated whey protein isolate: Characterization, stability, and curcumin bioaccessibility

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ABSTRACT

Pickering emulsions prepared from protein-polysaccharide complexes have attracted increasing attention. In this study, whey protein isolates (WPI) were modified with oligochitosan using transglutaminase (TGase)-type to fabricate Pickering emulsions, and loaded with curcumin. The curcumin/protein ratio of 1:25 and oil phase fraction ($\varphi = 17$ %) are the most optimal condition for emulsions stabilization, and particle size of glycosylated WPI emulsion was 31.70 μm. Glycosylated WPI emulsion had the highest encapsulation efficiency (96.64 %) of curcumin. Microstructure analysis showed that glycosylated WPI had small droplets covered by dense interface layers. The modified WPI emulsions exhibited optimal emulsifying properties and emulsion stability, which effectively inhibited the premature water–oil stratification in emulsion. *In vitro* digestion results showed that WPI-oligochitosan complexes enhanced curcumin bioaccessibility (40.34 %). The antioxidant activity of glycosylated WPI emulsions was significantly increased. The results of this study provide helpful references for applying glycosylated WPI-stabilized Pickering emulsions, which can be used as transport carriers of curcumin.

Introduction

In recent years, Pickering emulsions have received widespread attention in various fields such as cosmetics, food, medicine, interface catalysis, and composite materials (Li et al., 2023). Pickering emulsions possess several advantages including stability, absence of surfactants, anti-agglomeration properties, low toxicity, and biodegradability. One unique feature of Pickering emulsions is the irreversible adsorption capacitiy of particles at the water–oil interface (Cui, Hossain, Wang, & Chang, 2023; Niroula, Gamot, Ooi, & Dhital, 2021). Emulsions are formed by the combination of at least two immiscible liquids, with solid particles acting as stabilizers to maintain the water–oil interface (Nimaming, Sadeghpour, Murray, & Sarkar, 2023). A rigid interface is formed through irreversible adsorption of little solid particles to achieve stability property, so that Pickering emulsion has higher environmental tolerance than emulsion stabilized by conventional surfactants (Zhang et al., 2022; Zhu, Zhang, Huang, & Xiao, 2021). The efficient adsorption of colloidal particles on the phase interface instead of the traditional low-molecular-weight surfactant helps to form the so-called Pickering emulsions (Low, Siva, Ho, Chan, & Tey, 2020). Many studies have shown that natural polymers such as proteins and polysaccharides can stabilize emulsions by reducing the interfacial tension between two liquid phases, and these methods have been widely applied (Yu, Yu, Dong, & Xia, 2022; Zhao, Wang, et al., 2023). Whey protein isolate (WPI) is a by-product of dairy production process, containing different types of globular proteins, including about 20 % milk protein, such as α-lactalbumin, β-lactoglobulin, lactoferrin, serum albumin, and immunoglobulins. Whey protein has become a promising emulsifier, stabilizer, or structuring agent in various oil-in-water emulsions. Due to its nutritional value and physical/chemical characteristics, it is an ideal material for encapsulating and delivering nutrients (Zhu et al., 2021). When environmental conditions such as pH, ionic strength, and temperature change, WPI-stabilized droplets tend to aggregation and become unstable (Kim, Kim, & Lee, 2023; Tao et al., 2023). Some polysaccharides have been used to increase the stability and function of emulsion by complexation. However, few studies have shown whether protein glycosylation can improve the stability of WPI-stabilized emulsion. Therefore, it is necessary to use glycosylated WPI as a delivery carrier to study the functional properties of the resulting emulsion.

Under the catalysis of transglutaminase (TGase, EC 2.3.2.13), amino sugars such as glucosamine and oligosaccharide chitosan can be conjugated with protein molecules, which is a safe and effective strategy in

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food processing (Shi & Zhao, 2019). The $-NH_2$ from amino-containing saccharides could be connected with the Gln residues of proteins to produced glycosylated proteins (Shi, Zhang, Zhao, & Wang, 2021). Many studies have been demonstrated that the TGase-type glycosylation could improve the functional properties of proteins. Glycosylated gluten hydrolysate significantly enhanced the solubility, emulsifying and foaming properties of proteins (Liu et al., 2022). TGase-induced soybean protein isolate-chitosan complexes improved solubility and antioxidant capacity of soybean protein isolate with the change of the structure (Gu et al., 2023). Glycosylated ovalbumin using TGase and chito-oligosaccharides changed the emulsification, foaming, gelling and antioxidant properties (Hu et al., 2023). In addition, the protein glycosylation via TGase can enhance the physicochemical properties of emulsions. The emulsions prepared by soybean protein isolate and glucosamine via TGase-type could effectively increase the freeze–thaw stability of soybean protein isolate emulsions (Zhang, Cui, Zhou, Wang, & Zhao, 2021). To our knowledge, whether TGase-type glycosylation can improve the stability of WPI-stabilized emulsions, and the interaction mechanism between amino-containing saccharides and WPI at the oil–water interface.

The aim of this study was to evaluate the influence of TGase-type glycosylation on the preparation and stability of WPI-based Pickering emulsions. Oligochitosan-modified WPI was prepared using TGase-type catalysis, and the glycosylated WPI emulsion was further fabricated. The characteristics of glycosylated WPI emulsion, including droplet size, microstructure, emulsifying performance, foaming performance, stability, antioxidant activity, encapsulation efficiency, and release behavior of curcumin during simulated *in vitro* digestion were explored.

Materials and methods

Materials

Whey protein isolate was bought from Hilmar Co., Ltd. (CA, USA). Oligochitosan with a deacetylation degree (75 %) and an average molecular weight of 1 kDa was obtained from Zhejiang Golden-Shell Biochemical Co., Ltd. (Hangzhou, Zhejiang, China). TGase was provided by Jiangsu Yiming Fine Chemical Industry Co., Ltd. (Taizhou, Jiangsu, China). Curcumin (purity $>$ 98 %) was obtained from Dalian Meilun Biological Technology Co., Ltd. (Dalian, Liaoning, China). Soybean oil was obtained from the local supermarket. Nile blue and Nile red were supplied by Sigma-Aldrich Co., Ltd. (Shanghai, China). All chemical reagents were analytical grade.

Preparation of glycosylated WPI

The preparation of glycosylated WPI was modified from previous literature with slight modifications (Zhang, Liu, Xu, & Zhao, 2016). The pH of the WPI solution was adjusted to 7.5 (8 %, w/v) and mixed with an oligochitosan solution (pH 7.5) at a molar ratio of 1:3 between the acyl donor and the oligochitosan receptor. Then, 10 U/g protein of TGase was added to the mixture and occurred glycosylation reaction for 4 h at 37 °C. The mixture solution was heated at 85 °C for 5 min to enzyme inactivation, and then quickly cooled to room temperature. Isoelectric precipitation was performed on the obtained solution at pH 4.5 to remove unreacted oligochitosan. Two isoelectric washings on the precipitate with water at pH 4.5 were conducted. Then, the precipitate was dissolved in water and neutralized to pH 7.0 to obtain glycosylated WPI with a glucosamine content of approximately 9.47 g/kg protein. The crosslinking WPI by TGase without adding oligochitosan, and other preparation steps were consistent with those of glycosylated WPI. Crosslinking WPI and untreated WPI were used as control samples.

Glycosylated WPI stabilized Pickering emulsions and curcumin encapsulation

Preparation of glycosylated WPI stabilized Pickering emulsions

The samples (WPI, crosslinking WPI, and glycosylated WPI) were adjusted to pH 3.0–6.0 prior to use. The Pickering emulsions were prepared by adding soybean oil with a volume ratio of 1:1, 1:5 and 1:9, homogenizing at 10,000 rpm for 2 min, according to the preliminary experimental results.

Curcumin encapsulation in the Pickering emulsion

The curcumin was added to the oil phase with stirred overnight, and then mixed with the protein solution at the ratio of curcumin/proteins as 1:25 to 1:45. The curcumin loaded Pickering emulsion was obtained by the method as above described.

Characterization of Pickering emulsion stabilized by glycosylated WPI

Droplets size measurements

Droplets size of Pickering emulsion stabilized by glycosylated WPI was measured using the Laser particle size analyzer (SYNC, Microtrac, Florida, USA). A small amount of emulsion was taken into the test tube. Thereafter, deionized water dilution at the volume of 100 times was added. Dilute the solution and vortex for 2 min to disperse.

Emulsion morphology observation

The microstructure of curcumin-glycosylated WPI Pickering emulsions was observed using microscopy (FL Auto2, Invitrogen, California, USA). In brief, 1 mL samples were added with 10 μL mixed fluorescent dye (including 0.1 % Nile blue and 0.1 % Nile red). The oil phase was stained with Nile red and protein signal was Nile blue. The microscopic images were obtained at excitation wavelengths of 400 nm and 510 nm by 40× objective using the dual-channel laser mode. The surface morphology and structure of the emulsions were also analyzed by scanning electron microscope (SU8010, Hitachi, Tokyo, Japan). The samples were pre-frozen and freeze-dried, sprayed with gold. The images of the samples were observed at 600× and 2000× magnification (Huang et al., 2023).

Assays of fluorescence spectroscopy

Fluorescence spectrum of the emulsions were evaluated at the F-7100 fluorescence spectrophotometer (F-7100, Hitachi, Tokyo, Japan). The excitation wavelength was set at 280 nm. The emission scan was recorded from 300 to 500 nm (Zhou et al., 2022).

Encapsulation efficiency (EE) and loading efficiency (LE) measurements of curcumin

2 mL of the samples was dispersed in 20 mL of deionized water, The content of unbound curcumin in the precipitate was determined after centrifugation at 10000× for 10 min (Liu, Sun, Cheng, & Guo, 2022). The EE was calculated by the following equation:

$$EE(\%) = \frac{1 - \text{mg of free curcumin}}{\text{mg of total curcumin}} \times 100$$

The LE was calculated using the following equation:

$$LE(\%) = \frac{\text{mg of total curcumin} - \text{mg of free curcumin}}{\text{mg of total protein}} \times 100$$

Stability of curcumin-glycosylated WPI Pickering emulsion

Stability under different pH conditions

The prepared emulsions were adjusted to pH 3.0, 5.0, 7.0, or 9.0 using 0.1 mol/L HCl or 0.1 mol/L NaOH solution, and then the particle size was determined.

Thermal stability

The emulsions were heated at 37 °C, 60 °C and 80 °C for 8 h. The retention rate of curcumin was measured every 2 h to assess thermal stability.

Stability under different ionic strength conditions

The emulsions mixed with different ionic strengths (0, 50, 100 and 200 mmol/L NaCl), and the particle size was evaluated.

Storage stability

The curcumin-glycosylated WPI Pickering emulsion and curcumin solution were stored at room temperature for 28 days. The retention rate of curcumin was measured every week to evaluate storage stability.

Determination of functional properties

Determination of foaming properties

The 35 mL samples in 50 mL beaker were foamed at 10,000 r/min for 2 min using a high-speed homogenizer (T18, IKA, Staufen, Germany). The calculation formula of foaming properties is:

$$FC(\%) = \frac{V_2}{V_1} \times 100$$

where V_1 and V_2 are the volume of solution and foam, repectively.

Determination of creaming stability

The creaming stability of the emulsions was expressed by the creaming index (CI). The CI values were calculated by the following equation:

$$CI(\%) = \frac{Hs}{Ht} \times 100$$

where Hs and Ht are the height of the upper emulsion layer and total emulsion, respectively.

Determination of emulsifying properties

50 μL of samples were added with 5 mL 0.1 % (w/v) sodium dodecyl sulfate (SDS) solution. The absorbance was measured at 500 nm using a spectrophotometer, and 0.1 % w/v SDS was set as a blank. The emulsifying activity index (EAI) and emulsifying stability index (ESI) were calculated according to the previous method (Zhou et al., 2022).

$$EAI(m^2/g) = \frac{2 \times 2.303 \times A_0 \times N}{\varphi \times L \times C \times 10000}$$

$$ESI(\%) = \frac{A_{30}}{A_0} \times 100$$

where N is dilution coefficient (N = 100), C is protein solution concentration (g/mL), L is optical path (L = 1 cm), oil phase fraction (φ = 0.25), A_0 and A_{30} are the absorbance of sample 0 min and 30 min, respectively.

Antioxidant activity of curcumin-glycosylated WPI Pickering emulsion

DPPH radical scavenging activities assay

0.2 mL of samples were mixed with 3.8 mL of DPPH solution (0.07 mmol/L in 95 % of ethanol) and kept at 30 °C for 30 min. The values were analyzed at 517 nm using a spectrophotometer. The calculation formula is as follows:

$$\text{DPPH free radical scavenging ability } (\%) = \frac{A_0 - A_s}{A_0} \times 100$$

where A_0 and A_s are the absorbance of the blank reagent and the liquid to be measured, respectively.

ABTS radical scavenging activities assay

$ABTS^+$ radicals was prepared by 7 mmol/L of ABTS solution and 2.45 mmol/L of $K_2S_2O_8$ solution mixing with a volume ratio of 2:1. $ABTS^+$ solution kept in dark at 20 °C for 16 h, and then was diluted by ethanol to reach an absorbance value of 0.70 ± 0.02 at 734 nm. The samples were mixed with $ABTS^+$ solution and kept at 20 °C for 6 min. The absorbance values were analyzed using a spectrophotometer at 734 nm. The calculation formula is as follows:

$$\text{ABTS}^+ \text{ free radical scavenging ability } (\%) = \frac{A_0 - A}{A_0} \times 100$$

where A_0 and A are the absorbance of the blank reagent and the liquid to be measured, respectively.

Digestion properties determination in vitro

In vitro digestion model

According to previous reports (Brodkorb et al., 2019), in the stomach stage, the 10 mL of samples were mixed with 10 mL of stimulated digestion fluids for the gastric phase, added with 6 μL $CaCl_2(H_2O_2)$, and then added the pepsin solution (2,000 U/mL). The solution was adjusted to pH to 3.0, incubated the samples at 37 °C for 2 h. The content of encapsulated curcumin was determined in the digestion solution every 30 min. For the small intestine stage, the above solution from the gastric phase was mixed with stimulated digestion fluids for the intestinal phase at the ratio of 1:1, added with 6 μL $CaCl_2(H_2O_2)$. The mixture was added the trypsin solution (100 U/mL), adjusted the pH to 7.0, and then incubated the samples at 37 °C for 2 h. The content of encapsulated curcumin was determined in the digestion solution at the digestion of 15 min, 30 min, 60 min, 90 min, and 120 min, respectively.

Bioaccessibility and release rate of curcumin during simulated digestion

After each phase of *in vitro* digestion, the bioaccessibility and release rate of curcumin were calculated. The equation for calculating bioaccessibility was:

$$\text{Bioaccessibility } (\%) = \frac{m_1}{M_1} \times 100$$

where m_1 and M_1 are the contents of curcumin in the digested sample and in the sample before digested, respectively.

The release rate of curcumin was calculated using the following equation:

$$\text{Releaserate } (\%) = \frac{m_2}{M_2} \times 100$$

where m_2 is the mass of the curcumin released from the digested sample at the corresponding time, M_2 is the total amount of curcumin contained in the sample for digestion.

Particle size measurements

The particle sizes of the emulsions after digestion were measured according to above method.

Statistical analysis

Data were expressed as average value and standard deviation. Differences among the tests were determined via SPSS 22.0 statistical analysis program (IBM, SPSS statistics) according to one-way ANOVA ($p < 0.05$).

Results and discussion

Characterization of glycosylated WPI complexes

The droplets size of Pickering emulsion is an important factor to

evaluate the stability of emulsion. As can be seen in Fig. 1A and B, when the pH of protein solution was 6.0, the droplets size of WPI, crosslinking WPI, and glycosylated WPI was 38.29 μm, 36.51 μm, and 31.70 μm at the oil/water ratios of 1:5, respectively. The drolpets size of glycosylated WPI emulsions is smaller than that of WPI or crosslinking WPI. After embedding curcumin, the droplets size of all emulsions ranges from 30 μm to 50 μm and evenly distributed (Fig. 1C). When the ratio of curcumin to protein was 1:25, the minimum droplet size of emulsion was 43.27 μm (WPI), 39.72 μm (crosslinking WPI) and 33.61 μm (glycosylated WPI), respectively. Compared with WPI or crosslinking WPI emulsions, Pickering emulsions stabilized by glycosylated WPI significantly reduced the droplet size of the emulsion.

The microstructure and interfacial distribution of the Pickering emulsions using an inverted microscope was observed. As displayed in Fig. 1D, the droplet size of O/W emulsions was consistent with measured by particle size analysis. Compared to the Pickering emulsions of WPI and crosslinking WPI, the Pickering emulsion of glycosylated WPI dispersed more evenly. After the oligochitosan and TGase addition, the interfacial adsorption capacity increased, indicating that the interaction between WPI and oligochitosan could form a more dense interfacial layer on the surface of oil droplets, thus stabilizing the emulsions. Moreover, glycosylation by TGase-type increased the surface charge of WPI, thereby improving the surface wettability of WPI. With the increase of electrostatic effects between particles, the adsorption of particles at the oil–water interface enhanced (Qin et al., 2022). Thus, the elastic interface layer of the emulsion formed by glycosylated protein was denser than those of WPI and crosslinking WPI, which exhibited the potential as a Pickering emulsion stabilizer.

To further characterize the droplet distribution and interfacial morphology of Pickering emulsions, the microstructure of Pickering emulsions was observed by scanning electron microscopy (Fig. 1E). All three types of emulsions exhibited multiple spherical structures. The surface of the spheres had grainy textures, which was due to electrostatic adsorption causing curcumin particles to adhere to the surface of the droplet. In addition, the emulsions stabilized by glycosylated WPI showed a denser and smoother surface, because the addition of TGase and oligochitosan improved the microstructure of WPI emulsions. With the structure was more denser, the emulsion was more stable (Zhao, Chu, et al., 2023). The similar characteristics in microstructure of glycosylated ovalbumin by TGase also were reported (Hu et al., 2023), which indicated that TGase-type glycosylation prepeared Pickering emulsion is more stable.

The interactions between proteins with aromatic amino acid residues and other molecules cause fluorescence shift or fluorescence quenching (Yuan, Zhou, Niu, Shen, & Zhao, 2023). As shown in Fig. 1F, the emission fluorescence intensity (excited at a wavelength of 280 nm) of all samples had a maximum value at approximately 335 nm, indicating that a large amount of tryptophan existed in WPI samples. In addition, the addition of TGase and oligochitosan significantly reduced the fluorescence intensity of WPI, which exhibited a shielding effect on trptophan residues. Due to the steric hindrance effect caused by TGase-type glycosylation of proteins, the fluorescence signal of amino acids is blocked, thereby weakening the fluorescence of the complexs (Zhang et al., 2023). Moreover, the maximum fluorescence emission peak of the glycosylated WPI emulsion shifted by 4–5 nm, which was due to the change of hydrophobicity around tryptophan residues in WPI or peptides, leading to chain extension (Liu, Cui, et al., 2021; Zhang et al., 2023). The maximum absorption peak of the three protein emulsions encapsulated with curcumin was significantly decreased, and weak fluorescence intensity was observed at 465 nm, which was due to the binding of curcumin with the proteins reduced the absorption of protein to fluorescent dyes (Wang et al., 2023).

Physical stability of emulsions

The mass ratio of protein to curcumin is a key factor in the preparation of Pickering emulsion, which has a graet impact on the physical properties and biological activity of Pickering emulsion. The EE and LE of Pickering emulsions loaded with curcumin prepared at different mass ratios are shown in Fig. 2A and B. When the mass ratio of curcumin to protein was 1:25, the encapsulation efficiency of WPI, crosslinking WPI, and glycosylated WPI was 95.68 %, 94.70 % and 96.64 % respectively. The loading efficiency of WPI, crosslinking WPI, and glycosylated WPI displayed the corresponding values of 3.83 %, 3.79 % and 3.87 % at curcumin/proteins ratios of 1:25. Pickering emulsion stabilized by glycosylated WPI exhibited the highest EE and LE. The higher concentration of curcumin, the higher content of protein bound curcumin (Zhou et al., 2022). The spatial structure of WPI was changed due to glycosylation, thus exposing more hydrophobic binding sites and increasing the encapsulation efficiency and loading efficiency of curcumin (Fan, Luo, & Yi, 2022).

The droplets size of Pickering emulsion with different pH is shown in Fig. 2C. As the pH ranged from 3.0 to 9.0, the droplets size first increased and then decreased. When the pH was 5.0, the droplets size significantly increased, which due to the isoelectric point of WPI near this pH (Hadian, Labbafi, Hosseini, Safari, & Vries, 2021). The electrostatic repulsion force near the isoelectric point is almost reduced to 0, resulting in protein flocculation and precipitation (Wu et al., 2015). The particle size of Pickering emulsions stabilized by glycosylated WPI remained unchanged in the pH range of 3.0–9.0, indicating that the glycosylated WPI loaded with curcumin closely adhered to the surface of the droplet, forming a dense and thick protective layer. This layer enhanced electrostatic repulsion and spatial repulsion effects, prevented droplet aggregation, and significantly improved the stability of emulsions.

As shown in Fig. 2D–F, the thermal stability of Pickering emulsions stabilized by WPI, crosslinking WPI and glycosylated WPI was investigated by measuring the retention rate of the encapsulated materials under different temperature. During sustained heating at 37 °C, 60 °C and 80 °C, the retention rate of curcumin in these emulsions gradually increased with the increase of temperature. After heating treatment, the encapsulation efficiency of curcumin in the emulsions gradually increased, which was due to the change of WPI spatial conformation caused by heating. These changes leaded to the extension of protein peptide chains, allowing curcumin to be transplanted to more exposed binding sites and promoting the effect of encapsulation.

To assess the effect of NaCl on the stability of emulsions, different concentrations of NaCl were added to the prepared emulsions. As shown in Fig. 2G, with the increase of NaCl concentration, the droplet size gradually increased. High concentrations of NaCl influenced the instability of emulsion, leaing to droplet aggregation. Especially at higher NaCl concentrations, the particle size changed more significantly. However, the glycosylated WPI-stabilized emulsion exhibited optimal ion stability. Compared with the initial stage, even at the salt concentration of 200 mM, the droplet size did not increase significantly. It indicated that the Pickering emulsion prepared by glycosylated WPI loaded with curcumin could form a solid interface between droplets and effectively enhance the salt ion stability of proteins (Chen et al., 2021; Yan et al., 2020).

The retention rate and changes in the visual appearance of Pickering emulsions during the 28 days storage at 4 °C are presented in Fig. 2H and I. All the emulsions showed obvious stratification and no oil leakage. After 28 days of storage, the retention rates of curcumin in Pickering emulsion of WPI, crosslinking WPI, and glycosylated WPI was 86.38 %, 88.10 % and 91.15 %, respectively. When WPI was combined with TGase and oligochitosan, the storage stability of emulsions considerably improved. The introduction of TGase-type glycosylation as effective stabilizers significantly enhanced the stability of emulsion compared with WPI or crosslinking WPI. The reason for the better stability of glycosylated WPI was that oligochitosan-WPI complexs effectively inhibited the structural damage and maintain the integrity of the emulsion droplets, thus endowing glycosylated WPI with ideal stability.

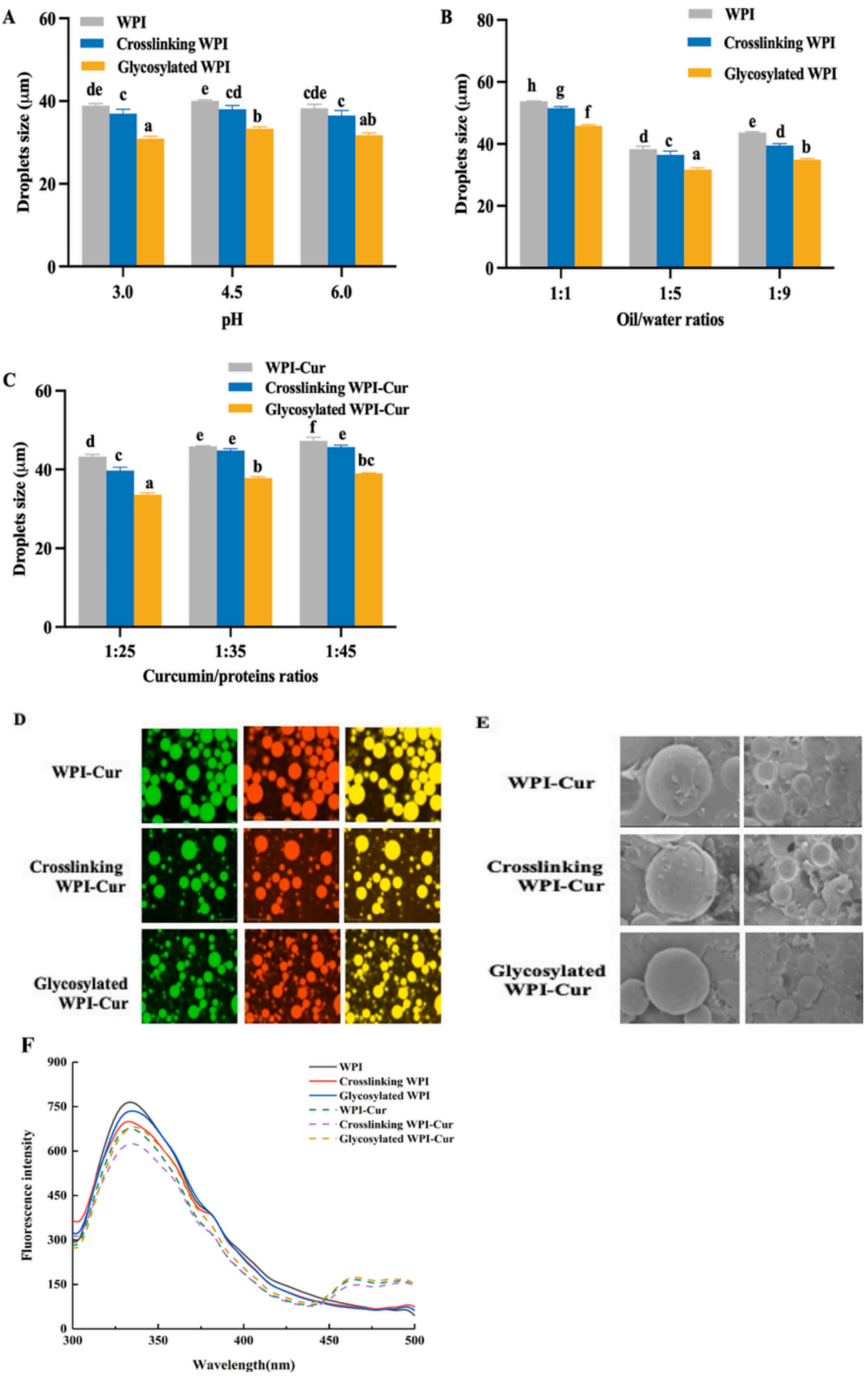

Fig. 1. Characterization of Pickering emulsions stabilized by WPI, crosslinking WPI and glycosylated WPI. Average droplets size (μm) of Pickering emulsions prepared with WPI, crosslinking WPI, glycosylated WPI at different pH of WPI (A) and oil/water ratios (B). Average droplets size (μm) (C), optical microscope images (magnification 40×) (D), scanning electron microscope (SEM) images (magnification 600× and 2000×) (E), and fuorescence intensity (F) of Pickering emulsions prepared with WPI, crosslinking WPI, glycosylated WPI embedding curcumin. Different lowercase letters indicate statistically significant differences ($p < 0.05$).

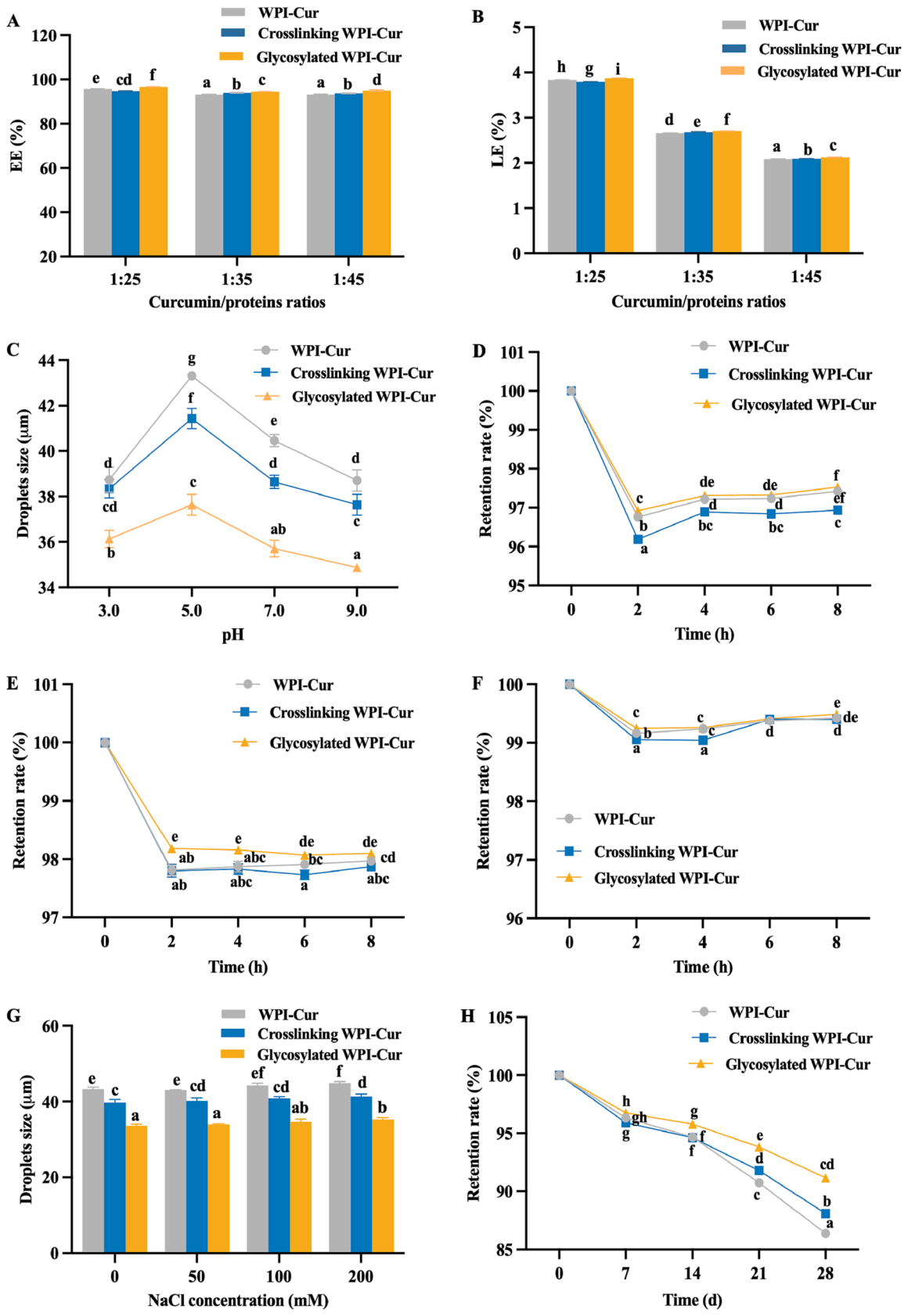

Fig. 2. The encapsulation efficiency (EE)% (A) and loading efficiency (LE)% (B) of Pickering emulsions loaded with curcumin. The droplets size measurement of the Pickering emulsions at different pH values (C). The curcumin retention rate of the Pickering emulsions under 37 °C (D), 60 °C (E) and 80 °C (F). The droplets size measurement of the Pickering emulsions under different NaCl concentrations (G). The curcumin retention rate of the Pickering emulsions at different storage time (H). The visual appearance of WPI, crosslinking WPI, and glycosylated WPI emulsions embedding curcumin under different storage time (I). Different lowercase letters indicate statistically significant differences ($p < 0.05$).

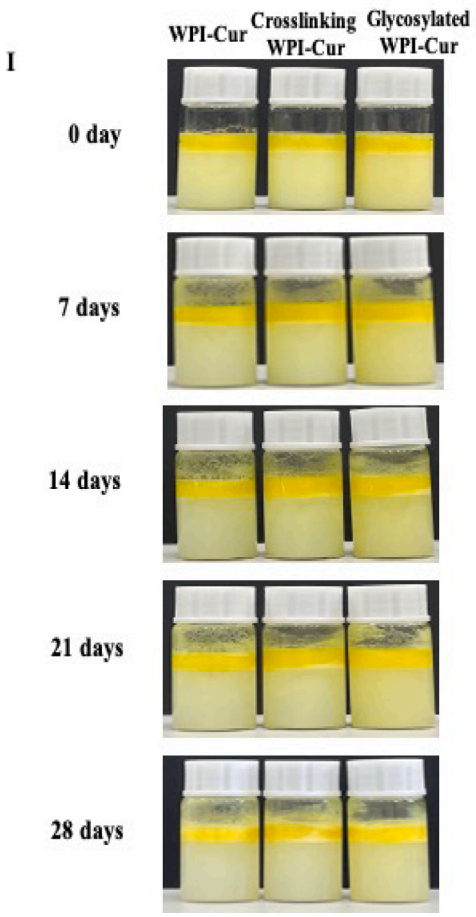

Fig. 2. (*continued*).

Functional properties of emulsions

The foaming properties of proteins are affected by many factors. Their surfactant properties enable them to adsorb at the air–water interface and form films on the surface of bubbles to stabilize foam (Cui et al., 2023). As shown in Fig. 3A, the foaming properties of WPI, crosslinking WPI, and glycosylated WPI were 34.17 %, 40.28 % and 48.61 %, respectively. Comapred with WPI, the WPI complexs modified by TGase and oligochitosan had better foaming properties. The glycosylated protein has better structure flexibility and greater foaming capacity, which can rapidly rearrange at the air water interface and genetare foam. Some studies have shown that enzymic reactions and glycosylation are effective ways to improve foaming capacity of proteins (Liang, Lin, Lu, Wu, & Gao, 2013; Zheng et al., 2015).

The creaming stability was determined by measuring the creaming index (CI). As shown in Fig. 3B, glycosylated WPI emulsion exhibited the lowest CI (78.58 %), which effectively prevented protein flocculation and exhibited best physical stability among unmodified WPI and modified WPI. The results showed that the distribution of glycosylated WPI emulsion droplets was more closely and effectively inhibited the aggregation of droplets, thereby restaining the phase separation and enhancing the stability of the emulsion. The binding of polysaccharides and proteins changing the spatial structure of WPI, allowing oil droplets to enter the network formed by polysaccharides. The smaller droplets size leads to the formation of a dense layer around the droplet interface, effectively preventing droplet aggregation (Tao et al., 2023). This indicated that the crosslinking of protein catalyzed by TGase and the grafting of protein onto oligochitosan change the steric hindrance of protein and reduce the size of emulsion droplets (Zhang et al., 2021).

The ability of proteins to form and stabilize emulsion at oil–water interface was indicated by EAI and ESI, respectively. The emulsifying capacity and stability of WPI, crosslinking WPI, and glycosylated WPI emulsions before and after loading curcumin are shown in Fig. 3C. The EAI of WPI, crosslinking WPI, and glycosylated WPI emulsions without curcumin was 32.75 m^2/g, 53.88 m^2/g, and 56.76 m^2/g, respectively. The ESI of above three emulsions without curcumin was 95.79 %, 96.76 %, and 99.64 %, respectively. Compared with WPI emulsion, crosslinking WPI and glycosylated WPI emulsion exhibited higher emulsifying activity and emulsifying stability. The increment of emulsifying capacity is related to the increased in sobulity after glycosylation of WPI. A network structure of WPI was formed under the action of TGase, effectively preventing aggregation between droplets. Moreover, the addition of oligochitosan increased –OH group and changed the oil–-water interface balance, thus improving the emulsifying properties of proteins. Glycosylation of proteins helps form a stable spatial structure, inhibit the coalescence of oil droplets, and promote their rapid diffusion at the oil–water interface, thereby contributing to the stability of emulsions (Hu et al., 2023; Liu, Zhang, et al., 2021).

Antioxidant activity of emulsions

The antioxidant activity of Pickering emulsions loaded with curcumin was evaluated by DPPH radical scavenging method (Fig. 4A). Compared with the empty Pickering emulsion, the Pickering emulsions stabilized by WPI, crosslinking WPI, and glycosylated WPI loaded with curcumin had a higher DPPH radical scavenging capacity and increased by 24.5 %, 24.5 % and 24.2 %, respectively. The Pickering emulsions of WPI, crosslinking WPI, and glycosylated WPI could maintain the bioactivity of curcumin. Glycosylated WPI emulsions exhibited a higher DPPH radical scavenging capacity, which may be due to the

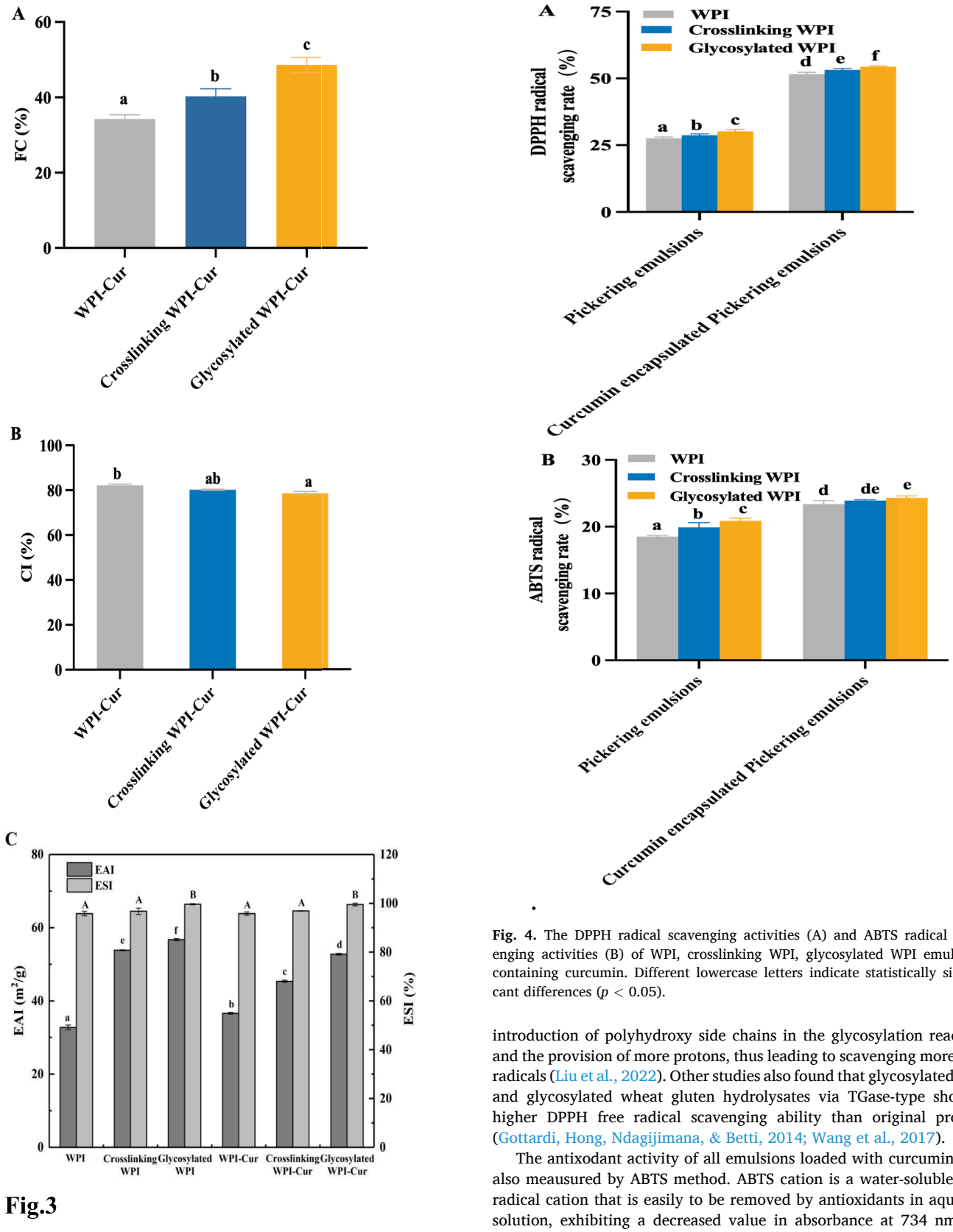

Fig.3

Fig. 3. The foaming capacity (FC)% (A), creaming index (CI)% (B), and emulsifying properties (C) including emulsifying activity index (EAI) (m^2/g) and emulsifying stability index (ESI) % of WPI, crosslinking WPI, glycosylated WPI emulsions containing curcumin. Different letters indicate statistically significant differences ($p < 0.05$).

Fig. 4. The DPPH radical scavenging activities (A) and ABTS radical scavenging activities (B) of WPI, crosslinking WPI, glycosylated WPI emulsions containing curcumin. Different lowercase letters indicate statistically significant differences ($p < 0.05$).

introduction of polyhydroxy side chains in the glycosylation reaction and the provision of more protons, thus leading to scavenging more free radicals (Liu et al., 2022). Other studies also found that glycosylated zein and glycosylated wheat gluten hydrolysates via TGase-type showed higher DPPH free radical scavenging ability than original protein (Gottardi, Hong, Ndagijimana, & Betti, 2014; Wang et al., 2017).

The antixodant activity of all emulsions loaded with curcumin was also meausured by ABTS method. ABTS cation is a water-soluble free radical cation that is easily to be removed by antioxidants in aqueous solution, exhibiting a decreased value in absorbance at 734 nm. As shown in Fig. 4B, the ABTS radical scavenging rate of WPI, crosslinking WPI, and glycosylated WPI without curcumin was 18.46 %, 19.92 % and 20.92 %, respectively. After embedding curcumin, the values of WPI, crosslinking WPI, and glycosylated WPI were 23.44 %, 23.88 % and 24.26 %, respectively. The ABTS radical scavenging capacity of

emulsion with curcumin showed more increment than the emulsions without curcumin. Moreover, Pickering emulsions stabilized glycayed WPI has the strongest ABTS radical scavenging capacity.

In vitro digestion of curcumin in emulsions

The release of nutritional and health products from food matrices in gastrointestinal fluids is a prerequisite for determining its bioaccessibility. After digestion, the bioaccessibility of Pickering emulsion stabilized by WPI, crosslinking WPI, and glycosylated WPI was 26.75 %, 30.57 % and 40.34 %, respectively (Fig. 5A). The bioaccessibility of glycosylated WPI was significantly higher than that of WPI and crosslinking WPI. Different protein treatment affected the structure and droplet size of Pickering emulsions. When WPI was combined with TGase and oligochitosan, the emulsion was more uniform and the droplet size of the emulsion was smaller, thereby increasing the surface area of the droplets, enhancing the adsorption of bile salts, thus promoting the hydrolysis of oils and improving the bioaccessibility of curcumin.

The curcumin release rates of all emulsions in gastrointestinal are illustrated in Fig. 5B and C. Pickering emulsions of WPI, crosslinking WPI, and glycosylated WPI exhibited a low release rate in stimulated

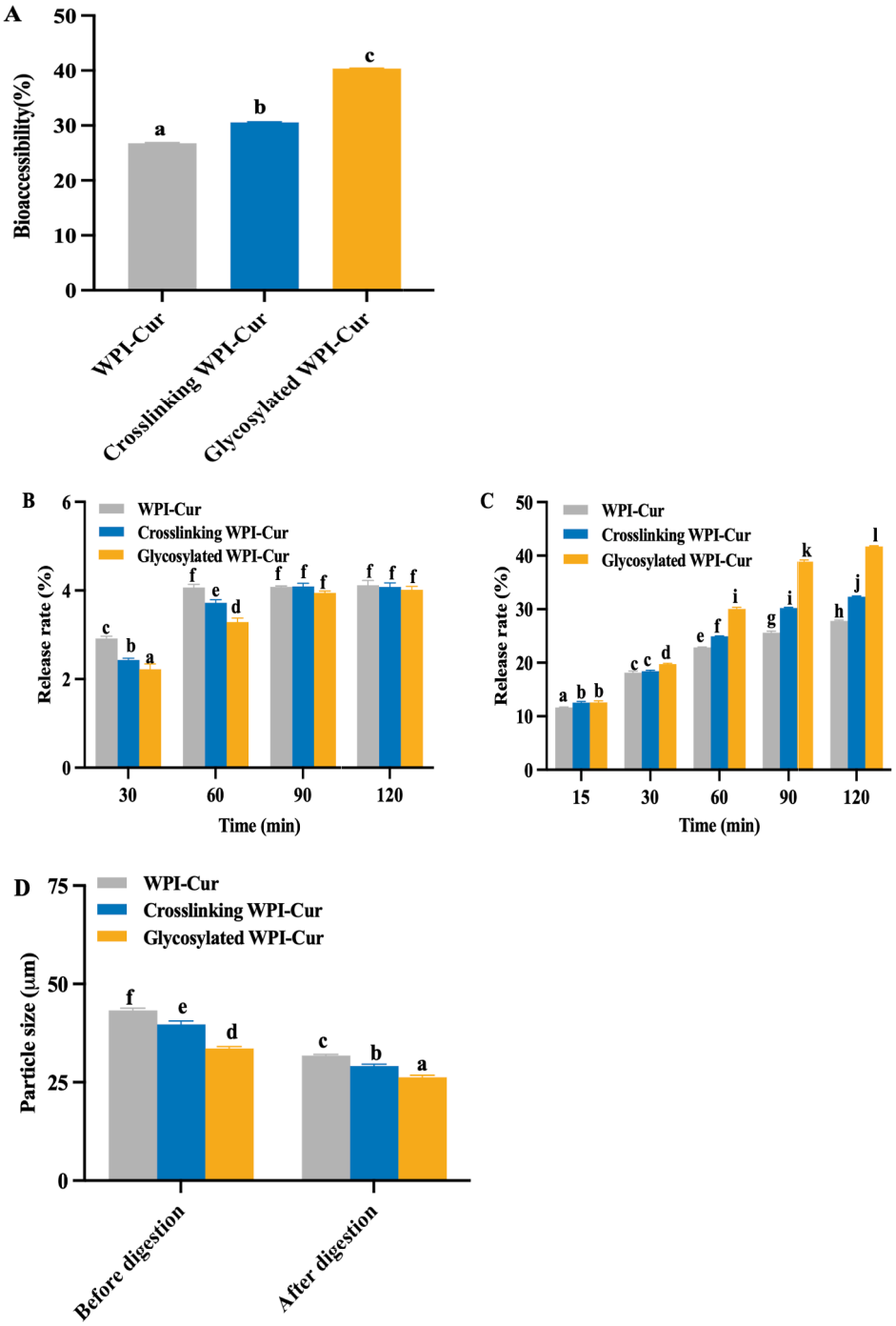

Fig. 5. The bioaccessibility (A) and release rate of curcumin instimulated gastric (B) and intestinal (C) digestion stage. The particle sizes of Pickering emulsions stabilized with WPI, crosslinking WPI, glycosylated WPI in stimulated digestion (D). Different lowercase letters indicate statistically significant differences ($p < 0.05$).

gastric fluids, which is due to the formation of large-scale electrostatic aggregates of proteins at a specific pH, thus protecting the active substances from being released prematurely. After entering the simulated intestinal digestion stage, most of protein droplets disintegrate, and the release rate of curcumin significantly increases, which is mainly due to the neutral pH environment of stimulated intestinal fluids and the presence of bile salts (Zhong, Li, Wang, & Zhang, 2023). Pickering emulsion stabilized by glycosylated WPI showed a higher curcumin release rate of 41.74 % in the stimulated gastrointestinal digestion phase than WPI (27.83 %) and crosslinking WPI (32.34 %), indicating that the Pickering emulsion prpepared with glycosylated WPI was easier to be digested and absorbed. The glycosylation of TGase-type expands the structure of peptides, exposing more protease cleavage sites of pepsin and trypsin, which can be better utilized and absorbed by the small intestine (Wang et al., 2017). Therefore, glycosylated WPI emulsion can be used as a transport carrier of curcumin, which can be released continuously during digestion, increase the stability, and enhance bioaccessibility of curcumin. In addition, after stimulation intestinal digestion, the size of droplets also significantly changed (Fig. 5D). All Pickering emulsions loaed with curcumin exhibited relatively small droplets size after digestion, which may be due to the decomposition of protein macromolecules into smaller molecules, thereby reducing the aggregation of droplets.

Conclusion

In this study, Pickering emulsions was successfully fabricated by using WPI and oligochitosan under TGase catalysis as stabilizers. The Pickering emulsions stabilized by glycosylated WPI had decreased droplets size, denser microstructure, better foaming and emulsifying property as well as creaming stability of WPI due to the modified structure of WPI. Glycosylated WPI emulsion exhibited the stronger protective effect of curcumin compared with that of WPI emulsions or crosslinking WPI emulsions. The addition of oligochitosan significantly improved the stability of WPI emulsions to resist the changes of heat treatment, pH values, ionic strength and storage time. Glycosylated WPI embedding curcumin also had enhanced antioxidant properties. In addition, after digestion, curcumin in glycosylated WPI stabilized by Pickering emulsion exhibited slow release and increased bioaccessibility of curcumin. Overall, the results exhibited that curcumin-loaded glycosylated WPI emulsion could be an effective delivery method for curcumin to improve its stability, antioxidant and bioaccessibility. This study not only provides a simple and convenient method to fabricate WPI-based Pickering emulsions, but it also expands the application potential of glycosylated WPI in food.

CRediT authorship contribution statement

Di Li: Investigation, Writing – original draft. **Yujun Jiang:** Resources, Supervision. **Jia Shi:** Supervision, Writing – review & editing.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

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