



Research Paper

Tremella polysaccharides-coated zein nanoparticles for enhancing stability and bioaccessibility of curcumin

Duoduo Li^a, Zihao Wei^{a,*}, Jialin Sun^a, Changhu Xue^{a,b}^a College of Food Science and Engineering, Ocean University of China, Qingdao, 266003, China^b Qingdao National Laboratory for Marine Science and Technology, Qingdao, 266235, China

ARTICLE INFO

Keywords:

Zein
Tremella polysaccharide
Nanoparticles
Curcumin
Bioaccessibility

ABSTRACT

The purpose of the present research was to examine the ability of Tremella polysaccharide (TP) to stabilize zein nanoparticles (zein NPs) and appraise the performance of zein/Tremella polysaccharide nanoparticles (zein/TP NPs) in terms of encapsulating and delivering curcumin. In this study, the zein/TP NPs were fabricated based on the anti-solvent precipitation method, which were used to protect and deliver curcumin. The results suggested that TP could be deposited on the surface of zein NPs by virtue of electrostatic interaction, so as to improve the hydrophilicity of zein, provide better protection for curcumin and assemble more stable nanoparticles. Compared with zein NPs (54.73%), the zein/TP NPs exhibited higher encapsulation efficiency of curcumin (93.34%) and excellent re-dispersibility. Furthermore, the retention rate of curcumin encapsulated in zein/TP NPs reached 80.78% and 90.74% after UV irradiation and 80 °C heat treatment for 2 h, respectively, which proved that the addition of TP significantly improved the stability of curcumin. Meanwhile, *in vitro* digestion study demonstrated that the bioaccessibility of curcumin encapsulated in zein/TP NPs increased by 37.36% compared with in zein NPs. Therefore, the zein/TP NPs may be served as an effective and potential carrier for the delivery of nutraceuticals.

1. Introduction

Zein is the major protein in maize, which possesses superior biodegradability and biocompatibility (Xu et al., 2022). It has more than 50% non-polar amino acids, which signifies its dissolubility in ethanol solution and indissolubility in water (Chen et al., 2020; Sun et al., 2017; Zhang et al., 2020). Due to the natural hydrophobicity of zein, it can be easily fabricated as nanoparticles via a simple anti-solvent precipitation method (Meng et al., 2021). Therefore, zein nanoparticles (zein NPs) have been utilized to protect and deliver sensitive nutraceuticals and drugs, especially some hydrophobic bioactives. It has been reported that zein NPs, as delivery system of bioactives, can significantly improve the stability and bioavailability of bioactives (Rodríguez-Félix et al., 2019). Nevertheless, the unfavorable properties of zein NPs, such as water insolubility and poor stability under certain ambient pressures (e.g., high temperature), limit the application of zein NPs as delivery systems in the food field (Li and Yu, 2020; Meng et al., 2021; Sun et al., 2017). Studies have demonstrated that the addition of surfactants (e.g., Tween 80) as stabilizers can effectively dispose of the above problems via decreasing surface hydrophobicity (Hu and McClements, 2014).

Recently, another effective strategy to stabilize zein NPs has been discovered, namely, deposition of polysaccharide layers (e.g., carrageenan (Sun et al., 2017), alginate (Khan et al., 2019) and chitosan (Pauluk et al., 2019)) on zein NPs by electrostatic interaction, which can also improve the stability and water solubility of zein NPs. Furthermore, the delivery systems fabricated through the assembly of protein–polysaccharide complexes are effective delivery carriers in improving the overall physiological efficacy of bioactives (Wei and Huang, 2019a).

The different kinds of polysaccharides can produce interfacial layers with different thicknesses, structures and charges, thus changing the spatial repulsion and electrostatic interactions between the nanoparticles (Zou et al., 2021). Recently, Tremella polysaccharide (TP), a natural polysaccharide extracted from the edible *Tremella Fuciformis* fruiting body, has attracted considerable attention due to its anticancer, antioxidant, anti-inflammatory, immune regulation and memory improving activities (Han et al., 2015; Ma et al., 2021; Zhou et al., 2018). It is an acidic polysaccharide consisting of glucuronic acid, xylose and mannose with good water solubility (Ma et al., 2021; Wang et al., 2019; Xu et al., 2020). In addition, the advantageous physicochemical

* Corresponding author.

E-mail address: weizihao@ouc.edu.cn (Z. Wei).

properties of TP, such as non-toxicity, excellent emulsification and gelation (Ma et al., 2021), signify that it can be widely applied in food, cosmetics and pharmaceutical industries. However, the study on using TP as a natural stabilizer to improve the stability and ameliorate water solubility of zein nanoparticles has not been conducted yet. Therefore, the fabrication of core-shell nanoparticles with TP as the exterior polysaccharide layers and zein as the core material may be a worthwhile research direction.

Curcumin, as a yellow-orange bioactive ingredient extracted from the spice turmeric, exhibits extensive and positive pharmacological activities, such as anti-oxidation, anti-inflammation, anticancer and preventive effects on various cardiovascular diseases (Liu et al., 2020; Wei et al., 2019a; Yu et al., 2020). However, the unfavorable properties of curcumin (e.g., poor water solubility, sensitivity to UV irradiation and heating as well as low oral bioavailability) make its efficient delivery in oral products a challenge (Liu et al., 2020). Therefore, it is of great significance to develop a delivery system that enhances the stability and bioaccessibility of curcumin.

The purpose of the present research was to examine the ability of TP to stabilize zein NPs and appraise the performance of zein/Tremella polysaccharide nanoparticles (zein/TP NPs) in terms of encapsulating and delivering curcumin. In this study, the zein/TP NPs were fabricated via the anti-solvent precipitation method and the influences of the mass ratio of zein to TP on the stability and delivery properties of curcumin-loaded nanoparticles were explored. Firstly, the particle size, zeta-potential and physical stability of the nanoparticles were measured to assess the physicochemical characteristics. Subsequently, the encapsulation efficiency, photostability and thermal stability of curcumin encapsulated in zein/TP NPs were evaluated and compared with zein NPs. Finally, the re-dispersibility and bioaccessibility *in vitro* gastrointestinal digestion of curcumin-loaded zein/TP NPs were appraised.

2. Materials and methods

2.1. Materials

Zein was purchased from the Sigma-Aldrich Company (St. Louis, MO, USA). Dried Tremella slices were supplied by Qingdao Marine Food Nutrition and Health Innovation Research Institute. Curcumin (with a purity of $\geq 95\%$) was obtained from Shanghai Ruiyong Biotechnology Co. Ltd. (Shanghai, China). Pancreatin (4000 U/g), pepsin (3000 U/mg) and Tris were purchased from Solarbio Co., Ltd (Beijing, China). Pig Bile Salt (cholic acid content $\geq 65\%$) was provided by Golden Clone Biotechnology Co., Ltd. (Beijing, China).

2.2. Extraction of tremella polysaccharide

Tremella polysaccharide (TP) was extracted from dried Tremella slices. In short, after the dried Tremella slices were soaked in water (pH 7.6) at room temperature for 3–4 h to fully absorb water, the pH of the solution was adjusted to 9–10 and soaked at 60 °C for 30 min to remove fat. Then, the Tremella was removed and soaked in an acid solution of pH 6.0–6.5 for 20–40 min. After that, the Tremella was taken out, drained, crushed and put into a filter bag for 4 h extraction at 100 °C to obtain the polysaccharide extracting solution. Subsequently, the proteins in polysaccharide extracting solution were hydrolyzed with neutral protease and papain at 50–60 °C for 3 h. Finally, TP was obtained by plate frame filter with diatomite.

2.3. Preparation of zein/TP nanoparticles

Curcumin-loaded zein/TP nanoparticles were prepared according to the anti-solvent precipitation method. In brief, zein and curcumin were dispersed together in 80% ethanol with stirring for 30 min. The concentration of zein was 20 mg/mL and the mass ratio of zein to curcumin was 10:1 or 20:1. Then, 1 mL of ethanol solution containing curcumin

and zein was slowly dropped in 20 mL of gently stirred deionized water. After that, the ethanol in the above solutions was evaporated by stirring at 1000 rpm for 30 min in a water bath at 37 °C. Finally, the curcumin-loaded zein nanoparticles (zein NPs) dispersions were obtained. For further fabrication of zein/TP NPs, 5 mL curcumin-loaded zein NPs dispersions were slowly injected into the equal volume of continuously stirred TP solution (the mass ratios of zein to TP were 1:2, 1:1, 1:0.5, 1:0.33 and 1:0.25, respectively). The zein/TP NPs dispersions were stored at 4 °C for further use. The nanoparticles dispersions were freeze-dried for 48 h to obtain the nanoparticle powder, which was used for the re-dispersibility analysis of zein/TP NPs.

2.4. Particle size and zeta-potential

The average particle size and polydispersity index (PDI) of curcumin-loaded zein NPs with and without TP as a stabilizer were measured by a Zetasizer Nano-ZS90 (Malvern, UK) based on the principle of dynamic light scattering. The intensity of light scattered was monitored at a 90° angle. All the liquid samples were equilibrated for 100 s at 25 °C inside the instrument before dynamic light back scattering. The zeta-potential measurement was conducted using the same instrument. When the zeta-potential is negative, the absolute value of zeta-potential is its opposite number. The greater absolute value of zeta-potential indicates a higher stability of the dispersion solution. All measurements were reported as the average of three measurements on independently prepared samples and recorded as mean \pm standard deviation (S.D.).

2.5. pH and salt effect of curcumin-loaded zein/TP NPs dispersions

2.5.1. pH effect

The pH of freshly prepared curcumin-loaded zein/TP NPs dispersions was adjusted to values ranging from 3.0 to 7.0 using HCl or NaOH solutions.

2.5.2. Salt effect

The newly prepared curcumin-loaded zein/TP NPs dispersions were mixed with different concentrations of NaCl solution in equal volume, so that the final concentration of sodium chloride of the sample was 0–50 mM and the pH value was adjusted to 4.0. All samples were kept at rest for 24 h at room temperature.

The mean particle size, PDI and zeta-potential of the nanoparticle dispersions were determined by a Zetasizer Nano-ZS90 (Malvern, UK).

2.6. Encapsulation efficiency and loading capacity

The encapsulation efficiency (EE) and loading capacity (LC) of curcumin was detected based on a previously published method (Meng et al. (2021)). Briefly, the freshly prepared samples were diluted with 95% (v/v) ethanol solution to a suitable concentration. The initial total content of curcumin was measured at 426 nm (Fig. S1) using the UV-2355 spectrophotometer (UNICO (Shanghai) Instrument CO. Ltd). Then, 2 mL of freshly prepared dispersions were centrifuged at 10000 rpm under 4 °C for 30 min to obtain the supernatants. The absorbance of the liquid supernatant at 426 nm was also measured by UV-2355 spectrophotometer to obtain the content of free curcumin. The calibration curve was used to calculate the content of curcumin ($y = 0.1563x - 0.0021$, $R^2 = 0.9989$). Finally, the EE and LC of curcumin in NPs was calculated as follows:

$$EE (\%) = \frac{\text{Total loaded curcumin content} - \text{Free curcumin content}}{\text{Total curcumin content}} \times 100\% \quad (1)$$

$$LC (\%) = \frac{\text{Total loaded curcumin content} - \text{Free curcumin content}}{\text{Total weight of nanoparticle}} \times 100\% \quad (2)$$

2.7. Photostability

The photostability of curcumin encapsulated in zein NPs and zein/TP NPs was evaluated using an ultraviolet light lamp on the basis of [Chen et al. \(2021\)](#) with minor modifications. In brief, 10 mL freshly prepared samples of curcumin-loaded zein/TP NPs were placed in the clear glass bottles, which were exposed to UV light (0.35 W/m^2) for 30, 60, 90 and 120 min at room temperature. The zein NPs without TP coating were used as the control. The samples were diluted trifold with 95% (v/v) ethanol solution. The residual curcumin in the samples was spectrophotometrically analyzed. The concentration of curcumin at 0 min was taken as the initial concentration. The retention rate of curcumin was calculated by Eq. (3):

$$\text{Retention rate (\%)} = \frac{\text{Mass of residual curcumin}}{\text{Mass of initial curcumin}} \times 100\% \quad (3)$$

2.8. Thermal stability

To evaluate the thermal stability of curcumin loaded in zein/TP NPs, freshly prepared samples were placed in clear glass bottles, which were heated at 60°C and 80°C in the water bath for 30, 60, 90 and 120 min, respectively. After the heat treatment, the resultant solutions were promptly cooled to room temperature in an ice bath. Subsequently, the above solutions were diluted with 95% ethanol to the suitable concentration and the residual amount of curcumin was ascertained by UV-2355 spectrophotometer at 426 nm. The retention rate of curcumin was also calculated by Eq. (3).

2.9. Re-dispersibility

20 mL of fresh zein/TP NPs dispersions were lyophilized for 24 h in a lyophilizer. Then 5 mg of the lyophilized powder was dissolved in 5 mL ultrapure water and stirred for 30 min to observe its dissolution. The appearance of the re-dispersed zein/TP NPs dispersions was recorded.

2.10. Physical stability

In this study, the Turbiscan equipment (developed by Formulacion, Smart Scientific Analysis, France) was used to evaluate the physical stability of the zein NPs and zein/TP NPs dispersions. The Turbiscan instrument, based on multiple light scattering, can calculate the stability index (TSI, Turbiscan Stability Index) of dispersions to investigate the destabilization phenomenon ([De Paola et al., 2021](#); [Nastaj et al., 2020](#)). The TSI is calculated directly from the raw data acquired by the instrument: BS (backscattering) and T (transmission) signals. The higher the TSI is, the stronger the destabilization of the sample is. In short, 10 mL of zein/TP NPs and zein/TP NPs dispersions were placed in cylindrical glass vials and characterized with the Turbiscan instrument at 37°C for 30 min. The instability of the samples can directly reflected by the variation of TSI with time.

2.11. Simulated gastrointestinal digestion

The bioaccessibility of curcumin encapsulated in the zein NPs and zein/TP NPs (1:0.5) was evaluated according to the method of [Wei and Huang \(2019b\)](#) with minor modifications. The simulated gastrointestinal digestion model consists of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Firstly, SGF was acquired by dissolving NaCl and pepsin in pH-regulated water (pH 1.2) and the final concentration of NaCl and pepsin were 2 mg/mL and 1.6 mg/mL respectively. After that, the fresh nanoparticles dispersions were mingled with the equivoluminal of SGF in a flask. Then, the resulting mixtures were adjusted to pH 1.2 and digested under continuous shake at 150 rpm for 2 h in a 37°C water bath. After a period of simulated gastric digestion,

the pH of gastric digestion dispersions was adjusted to 7.5 to inactivate pepsin and terminate gastric digestion. Subsequently, the SIF (pH 7.5, with 10 mg/mL bile salt, 3.2 mg/mL pancreatin, 1.1 mg/mL anhydrous calcium chloride and 6 mg/mL Tris) was prepared. In the intestinal phase, the resulting gastric digesta was added into the equivoluminal SIF. The sample was adjusted back to pH 7.5 and gently agitated in a water bath at 37°C for 2 h. After simulated gastrointestinal digestion, the resulting digestion dispersions were centrifuged at 10000 rpm under 10°C for 40 min. The supernatant (micelle phase) was carefully extracted for evaluation of curcumin bioaccessibility ([Yuan et al., 2021](#)). The absorbance of the supernatant (micelle phase) at 426 nm was ascertained using the UV-2355 spectrophotometer to obtain the concentration of curcumin. The bioaccessibility of curcumin was calculated by Eq. (4):

$$\text{Bioaccessibility (\%)} = \frac{C}{C_0} \times 100\% \quad (4)$$

where, C_0 and C are the curcumin content before and after simulated gastrointestinal digestion, respectively.

2.12. Statistical analysis

All measurements were obtained in at least triplicate and all the data were represented as the average of three repeated experiments. The statistical analysis of the data was conducted using Origin 2019 and the IBM SPSS Statistics 25 software. When the significant level p was less than 0.05, the difference was considered statistically significant.

3. Results and discussion

3.1. Particle size, PDI and zeta-potential

The mean particle size and PDI of zein NPs and zein/TP NPs loaded with and without curcumin were depicted in [Fig. 1A](#) and [Fig. 1B](#). Compared to individual zein NPs, the average particle size of composite zein/TP NPs significantly increased. The result could be attributed to the fact that TP was deposited on the superficies of zein NPs through electrostatic attraction, leading to the formation of core-shell structure ([Fig. S2](#)) with zein as the core and TP as the shell. With the mass ratio of zein to TP changed from 1:0.5 to 1:0.25, the mean particle size of zein/TP NPs changed from $164.0 \pm 1.4 \text{ nm}$ to $260.2 \pm 9.0 \text{ nm}$, which might indicate that the low content of TP was not enough to fully stabilize zein NPs. The increase in particle size was probably related to the reduction of electrostatic repulsion and steric resistance among nanoparticles, leading to the agglomeration of different nanoparticles. Nevertheless, when the mass ratio of zein to TP changed from 1:0.5 to 1:2, the average particle size increased from $164.0 \pm 1.4 \text{ nm}$ to $225.2 \pm 1.7 \text{ nm}$, which was the result of excessive polysaccharide (TP) deposition on the surface of zein NPs. The PDI of individual zein NPs and zein/TP NPs was all less than 0.3, indicating that they all possessed excellent homogeneity, and the results of PDI index presented the same trend as the particle size. Because zein/TP NPs exhibited a suitable nano-scale size and superior homogeneity, they might be an excellent carrier for protecting and delivering curcumin. After curcumin was added, the particle size of both zein NPs and zein/TP NPs with different mass ratios all showed an obvious increase compared with the nanoparticles without loading curcumin, manifesting that the curcumin was successfully encapsulated into nanoparticles. The PDI curcumin-loaded zein/TP NPs (from 1:2 to 1:0.33) were significantly lower than that of curcumin-loaded zein NPs, which indicated that the addition of TP could ameliorate the homogeneity and dispersibility of curcumin-loaded zein NPs. Interestingly, when zein: TP was 1:0.5, the composite curcumin-loaded zein/TP NPs presented the minimum particle size and the lowest PDI. Therefore, the mass ratio of 1:0.5 possibly might be the optimal proportion of zein: TP.

Because zeta-potential represents the surface charge of nanoparticles

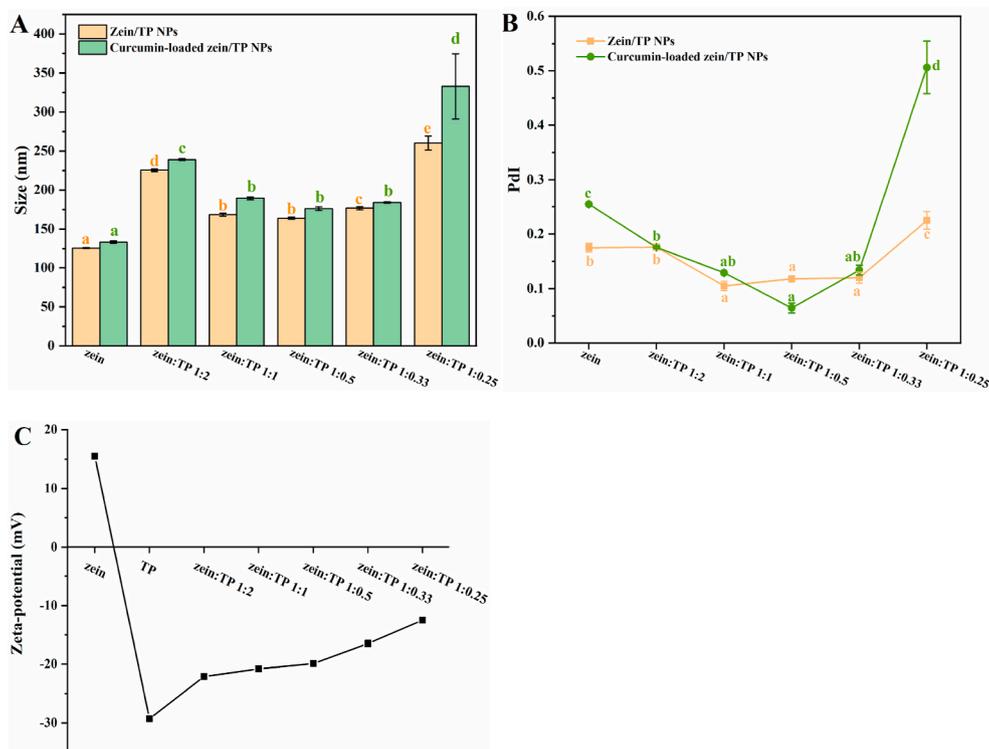


Fig. 1. The particle size (A) and PDI (B) of zein NPs and zein/TP NPs loaded with or without curcumin. The zeta-potential (C) of TP, zein NPs and zein/TP NPs at pH 4.0. (The amount of zein is fixed, the amount of TP was changed.)

and plays a crucial role in the stability of various delivery systems, the study of zeta-potential on nanoparticles is also indispensable (Fig. 1C). The zeta-potential of individual zein NPs and TP was 15.5 ± 0.38 mV and -29.3 ± 0.4 mV at pH 4.0, respectively. After adding TP, the zeta-potential of the zein NPs converted from positive value to negative value, which revealed that the anionic TP was coated on the surface of the cationic zein NPs owing to the electrostatic attraction between zein and TP. Meng et al. (2021) reported similar result that zein NPs were covered on the negatively charged carboxymethyl dextrin, making zein/carboxymethyl dextrin composite nanoparticles negatively charged. As the content of TP decreased, the zeta-potential of composite zein/TP NPs gradually increased, that is, the absolute value of zeta-potential gradually decreased. Chen et al. (2021) also found a similar phenomenon that with the increase of rhamnolipid content, the zeta-potential of gliadin-rhamnolipid nanoparticles gradually increased. Zeta-potential is a vital parameter to characterize the stability of nanoparticles suspension. The larger the absolute value of zeta-potential is, the higher the repulsive force among nanoparticles is, which inhibits

the agglomeration between nanoparticles and improves the stability of nanoparticles (Pauluk et al., 2019).

3.2. pH and salt effect on stability of curcumin-loaded zein/TP NPs dispersions

The average particle size, PDI and zeta-potential of the nanoparticles at different pH were shown in Fig. 2. When the pH value was 4.0–7.0, the particle size was within 150–200 nm and the PDI was less than 0.2, indicating that zein/TP NPs were stable. At pH 3.0, the zein/TP NPs possessed large particle size and PDI, leading to the formation of sedimentation. As depicted in Fig. 2B, the significant reduction of zeta-potential could explain these results. The charge on zein/TP NPs reduced, resulting in the weak electrostatic repulsions among zein/TP NPs. Therefore, the zein/TP NPs became unstable and easy to aggregate. It was noteworthy that zein/TP NPs was no longer plagued by isoelectric point of zein, which often led to sedimentation of zein NPs near isoelectric points.

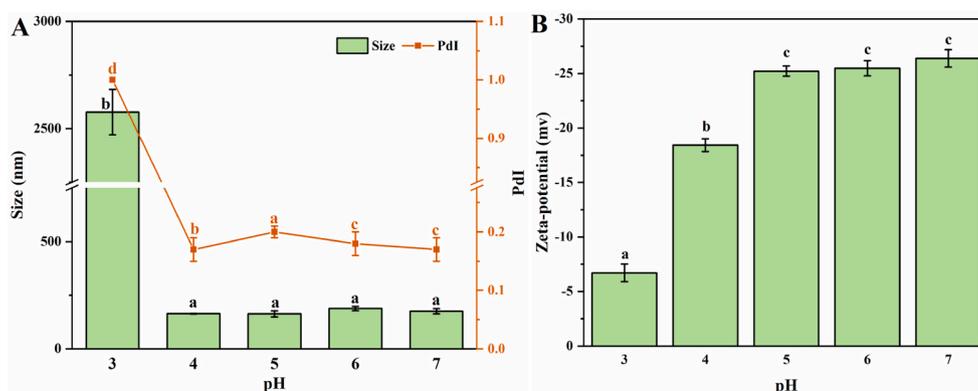


Fig. 2. Effect of pH on particle size, PDI (A) and zeta-potential (B) of curcumin-loaded zein/TP NPs.

The effect of ionic strength (NaCl addition) on the stability of curcumin-loaded zein/TP NPs dispersions was also investigated (Table 1). When the salt concentration was 0–50 mM, the average particle size and PDI of curcumin-loaded zein/TP NPs presented an increase tendency with the enhancement of ionic strength. When the salt concentration was 50 mM, the particle size and PDI were large, which might be due to the fact that the presence of salt ions weakened the electrostatic attraction between TP and zein, resulting in their inability to form stable dispersions. In the study of using chondroitin sulfate to stabilize zein NPs (Yuan et al., 2019), precipitation occurred in zein nanoparticles dispersions when salt concentration was 15 mM. Therefore, the salt resistance of the nanoparticles was improved by coating the outer layer of zein with TP.

3.3. Encapsulation efficiency and loading capacity

The encapsulation efficiency (EE) and loading capacity (LC) of curcumin in zein NPs and composite zein/TP NPs were presented in Table 2. The EE of curcumin in zein/TP NPs was significantly higher than that of zein NPs. The results demonstrated that coating TP onto zein NPs could efficaciously enhance the EE of curcumin, contributing to the increase of binding site for curcumin as well as the tighter binding between zein and TP. With the increase of TP content, the EE of curcumin gradually increased, which might be due to the fact that the interaction between zein and TP was enhanced, resulting in dense structure of the nanoparticles. This result was consistent with Sun et al. (2017), who reported that the zein-shellac composite particles exhibited a consistent increase in EE of curcumin with the increase in the levels of shellac. In order to load more curcumin, the situation when the mass ratio of zein to curcumin at 10:1 was further investigated. The result revealed that after increasing the amount of curcumin, the EE of curcumin in different mass proportions of nanoparticles all decreased, which might be due to the excessive curcumin might exceed the encapsulation capacity of nanoparticles. Similar results were presented in the work of Dai et al. (2019), where the EE of curcumin gradually decreased with the increase of curcumin quantity. Furthermore, the LC of curcumin in zein/TP NPs was also significantly higher than that of zein NPs. It was easy to notice that with the increase of mass ratio of zein to TP, the variation trend of LC was opposite to that of EE. This was due to the fact that the higher the amount of TP, the greater the total weight of nanoparticles, ultimately leading to the lower value of the LC. Taken together, coating TP on the surface of zein NPs could efficaciously improve the EE and LC of the encapsulated curcumin and the encapsulated characteristics of curcumin were related to the mass ratio of zein to TP.

3.4. Photostability

Curcumin is highly susceptible to ultraviolet light and is rapidly degraded when exposed to UV radiation (Meng et al., 2021). Studies have shown that the retention rate of free curcumin decreased to below 30% after 1 h of UV light exposure (Sun et al., 2017). In order to improve the photostability of curcumin, the degradation of curcumin captured in zein NPs and zein/TP NPs during exposure to UV light was investigated in the present work. The existence of double bonds and aromatic amino

Table 1

Effect of sodium chloride concentrations on particle size and PDI of curcumin-loaded zein/TP NPs.

NaCl (mM)	Particle size (nm)	PDI
10	257.0 ± 13.9 ^a	0.19 ± 0.02 ^a
20	347.0 ± 11.6 ^b	0.27 ± 0.03 ^{ab}
30	400.8 ± 14.7 ^{bc}	0.36 ± 0.02 ^{bc}
40	425.8 ± 41.7 ^c	0.51 ± 0.16 ^c
50	543.5 ± 66.3 ^d	0.68 ± 0.10 ^d

Different letters in the same column indicate significantly different ($p < 0.05$).

Table 2

Encapsulation efficiency and loading capacity of curcumin in zein NPs and zein/TP NPs with different mass ratios.

Sample	Encapsulation efficiency (%)		Loading capacity (%)	
	zein:Cur = 20:1	zein:Cur = 10:1	zein:Cur = 20:1	zein:Cur = 10:1
Zein	54.73 ± 0.18 ^a	31.92 ± 0.08 ^a	1.33 ± 0.01 ^a	1.52 ± 0.02 ^a
Zein:TP = 1:2	93.34 ± 0.14 ^f	91.59 ± 0.09 ^f	1.53 ± 0.04 ^b	2.95 ± 0.07 ^b
Zein:TP = 1:1	92.71 ± 0.14 ^e	89.00 ± 0.04 ^e	2.26 ± 0.01 ^c	4.24 ± 0.03 ^c
Zein:TP = 1:0.5	88.59 ± 0.07 ^d	85.10 ± 0.04 ^d	2.86 ± 0.02 ^d	5.32 ± 0.03 ^d
Zein:TP = 1:0.33	83.53 ± 0.10 ^c	77.71 ± 0.06 ^c	3.03 ± 0.02 ^e	5.43 ± 0.06 ^d
Zein:TP = 1:0.25	89.77 ± 0.12 ^b	74.61 ± 0.11 ^b	3.45 ± 0.07 ^f	5.53 ± 0.01 ^e

Different letters in the same column indicate significantly different ($p < 0.05$).

acid residues in zein molecules possessed the capability to absorb ultraviolet light, and the formation of hydrogen bonds between curcumin and TP was conducive to improving UV light resistance stability (Chen et al., 2021; Meng et al., 2021). As shown in Fig. 3A, after 2 h of UV irradiation, the retention rates of curcumin in zein/TP NPs with the mass ratio of zein to TP at 1:2, 1:0.5 and 1:0.25 were 75.49%, 80.78% and 78.81%, respectively. However, in zein NPs alone, the retention rate of curcumin was only 49.03% after 2 h UV light exposure. Curcumin encapsulated in zein/TP NPs demonstrated stronger stability against UV radiation in comparison to the curcumin in individual zein NPs. With the increasing exposure duration to UV light, the retention rates of curcumin in all zein-based NPs were reduced. It is noteworthy that the degradation rate of curcumin was significantly reduced after the addition of TP, suggesting that the existence of TP layer provided better protection from the degradation of curcumin under UV light exposure. This might be attributed to the relatively dense TP layer formed on the surface of the nanoparticles after the addition of TP. This result was consistent with that reported by Sun et al. (2017). When the mass ratio of zein to TP was 1:0.5, the retention rate of curcumin in the composite nanoparticles was the highest, which might be due to the existence of appropriate electrostatic repulsion and spatial resistance between zein and TP at this mass ratio, thus making nanoparticles most stable under this condition.

3.5. Thermal stability

As heat treatment is a common food processing process in food preparation, it is of great significance to evaluate the thermal stability of nutraceuticals. The zein/TP NPs were heated in an water bath at 60 °C (Fig. 3C) and 80 °C (Fig. 3D) for 120 min to evaluate the thermal stability of the encapsulated curcumin. It was observed that the degradation rate of curcumin in zein NPs was faster than that in composite zein/TP NPs, whether heated at 60 °C or 80 °C. After 120 min of water bath thermal treatment at 80 °C, the content of curcumin in zein NPs decreased to 74.07%, while the retention rate of curcumin in zein/TP NPs with a mass ratio of 1:0.5 was as high as 90.74%. The retention rate of curcumin in zein/TP NPs was significantly ($p < 0.05$) higher than in single zein NPs. These results indicated that encapsulation of curcumin in zein/TP NPs was an effective and feasible approach to strengthen its thermal stability. A conceivable explanation for this result was that the spatial repulsion and electrostatic repulsion among the nanoparticles were conducive to enhancing the protective ability of curcumin during thermal treatment by restraining the aggregation or disintegration of nanoparticles (Xue et al., 2018) and curcumin was efficaciously protected in the hydrophobic cavity of zein and TP (Meng et al., 2021).

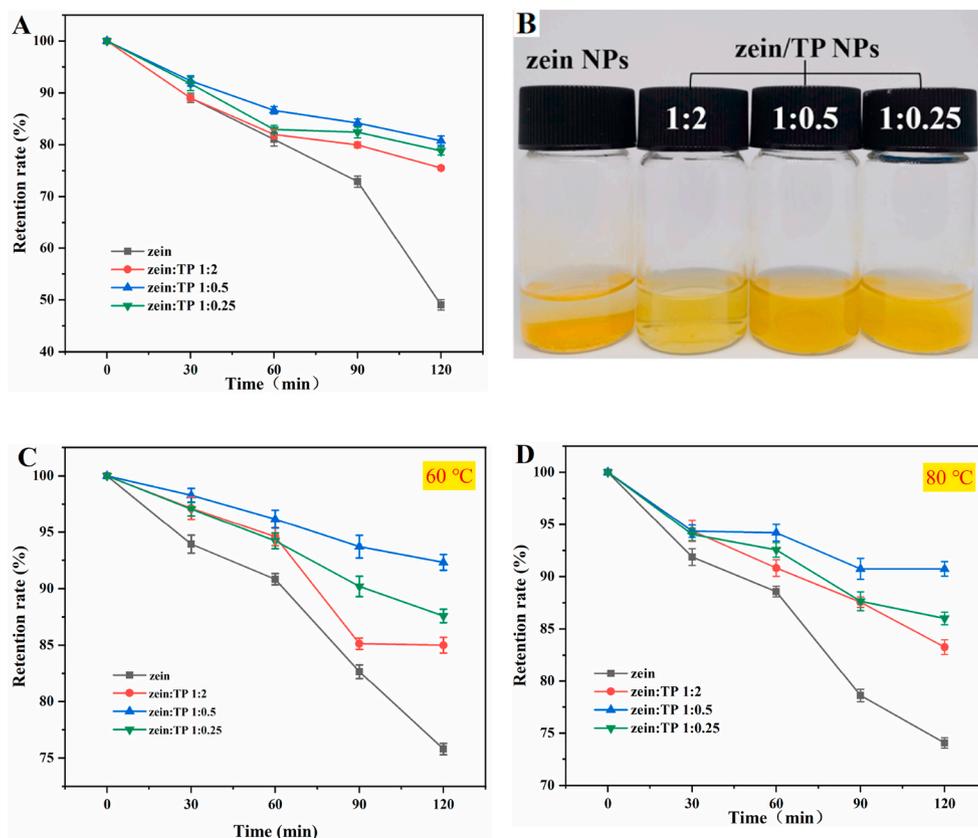


Fig. 3. (A) The retention rate (%) of curcumin loaded in zein NPs and zein/TP NPs after ultraviolet light irradiation. (B) The visual appearance of re-dispersed curcumin-loaded zein NPs and zein/TP NPs with different mass ratios. The retention rate (%) of curcumin loaded in zein NPs and zein/TP NPs with different mass ratios after thermal treatment at 60 °C (C) and 80 °C (D).

3.6. Re-dispersibility

To facilitate transportation, processing, storage and marketing, it is a feasible and promising method to freeze-dried liquid nanoparticles dispersions containing curcumin into dried powder products. The re-dispersibility of powder products is a considerable feature to retain favorable sensory quality of food. Therefore, the re-dispersibility of lyophilized curcumin-loaded nanoparticles was appraised. The freeze-dried nanoparticles were stirred in distilled water to redisperse and then their appearance was exhibited in Fig. 3B. The particle size and PDI of re-dispersed curcumin-loaded zein/TP NPs with different mass ratios were displayed in Table S1. The particle size distribution of re-dispersed curcumin-loaded zein/TP NPs with different mass ratios was shown in Fig. S3. The results showed that there was visible precipitation in the re-dispersion solution of individual zein NPs. While the lyophilized zein/TP NPs exhibited good re-dispersibility and the re-dispersed solutions showed a light-yellow appearance with no significant visible sediment. This result manifested the addition of TP could improve the solubility and re-dispersibility of zein NPs. Furthermore, when the mass ratio of zein to TP was 1:0.5, the zein/TP NPs displayed the most uniform and distinct yellow color, indicating that the zein/TP NPs with a mass ratio of 1:0.5 were more easily redispersed in water after lyophilization and possessed better stability. Meanwhile, based on the above findings, the photostability and thermal stability of curcumin in the zein/TP NPs with a mass ratio of 1:0.5 were also the most excellent. Therefore, the optimized group (zein/TP NPs with a mass ratio of 1:0.5) was selected in subsequent experiments.

3.7. Physical stability

To investigate the stability of zein NPs and zein/TP NPs dispersions,

the Turbiscan Stability Index (TSI) was employed to characterize the aggregation process of NPs (Luo et al., 2017). The rise of TSI suggested that the system became more unstable and the nanoparticles dispersions were more likely to aggregate (Gagliardi et al., 2021). The samples were incubated at body temperature (37 °C) to investigate whether their stability could be influenced by the addition of TP. It can be observed from Fig. 4, the TSI of zein/TP NPs dispersions exhibited a considerable decrease in comparison to zein NPs, which evidenced that TP had a positive impact on the stability of zein NPs. One possible reason for this result was that the anionic polysaccharides (TP) adhered tightly to the

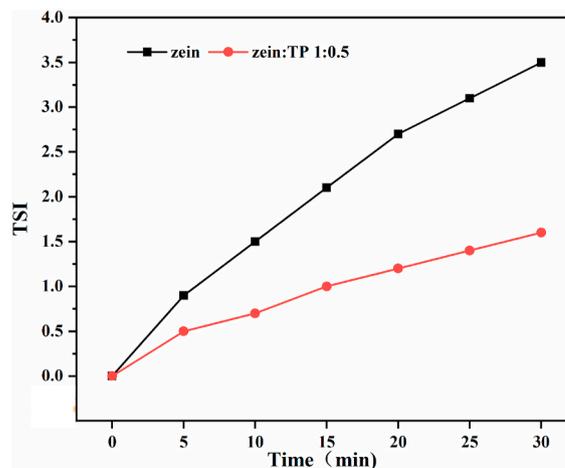


Fig. 4. The Turbiscan Stability Index (TSI) of zein NPs and zein/TP NPs with mass ratio of zein to TP at 1:0.5.

positively charged surface of zein through electrostatic attraction, leading to the stronger surface charge of zein/TP NPs compared to zein NPs. Therefore, the stronger electrostatic repulsive force among zein/TP NPs could inhibit the flocculation or precipitation of nanoparticles and confer stronger stability to the nanoparticles dispersions. A potential formation mechanism is graphically illustrated in Fig. 5. These results were in accordance with the research reported by Chen et al. (2021), the physical stability of rhamnolipid-gliadin nanoparticles was improved due to the enhancement of the absolute value of surface potential, which led to the increase of electrostatic interaction.

3.8. Simulated gastrointestinal digestion

Bioaccessibility was evaluated through an *in vitro* model of gastrointestinal digestion (Wei et al., 2019b). The bioaccessibility of curcumin can be denoted as the percentage of curcumin released into the micellar phase after *in vitro* gastrointestinal digestion (Yuan et al., 2021; Zou et al., 2021). Curcumin, a highly hydrophobic bioactive substance, is usually absorbed only after being dissolved in gastrointestinal fluids. The solubilization of curcumin can be achieved when curcumin is incorporated into the hydrophobic interiors of mixed micelles (micelles or vesicles), which are mainly formed from surfactants, bile salts, phospholipids and peptides in the gastrointestinal fluids (Yao et al., 2018; Zou et al., 2021). As depicted in Fig. 6, the bioaccessibility of curcumin encapsulated in zein NPs was 50.20%, while it could up to 87.56% when encapsulated in zein/TP NPs with the mass ratio of zein to TP at 1:0.5. The distinct improvement in the bioaccessibility of curcumin revealed that the zein/TP NPs could be regarded as an impactful delivery system of curcumin. A credible explanation for the higher bioaccessibility of curcumin in zein/TP NPs may be that the unique biosurfactant in curcumin-loaded zein/TP NPs possesses an amphiphilic structure, leading to an increase of the micelles or vesicles content in the micelle phase. The bile salts and peptides hydrolyzed from zein/TP NPs can form micelles or vesicles that can solubilize some of the curcumin and thus increase the bioaccessibility of curcumin. Another possibility is that the combination of curcumin with nonpolar patches on the surfaces of peptides hydrolyzed from proteins can also lead to increased gastrointestinal solubility of curcumin. The result is in accordance with the study carried out by Yuan et al. (2021), who demonstrated the bioaccessibility of curcumin in zein NPs coated with tea saponins was 60.83%, which was significantly higher than that of free curcumin (below 15%). However, the release rates of curcumin loaded in zein/sodium alginate and zein/sodium caseinate NPs fabricated by pH-driven method were above 90% (Li et al. (2021). In terms of the reason for these different results, in addition to the different characteristics of coating

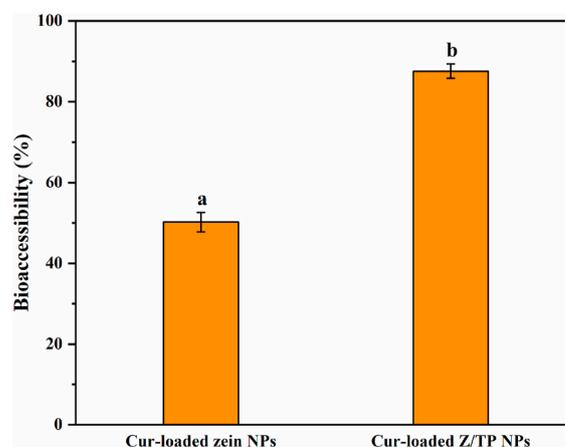


Fig. 6. The bioaccessibility of curcumin encapsulated in zein NPs and zein/TP NPs.

materials, the differences *in vitro* digestion models and preparation methods should be taken into consideration.

4. Conclusions

The composite zein/TP NPs were successfully fabricated via the anti-solvent precipitation method in this work. The TP could be deposited on the surface of zein NPs by virtue of electrostatic interaction, so as to improve the hydrophilicity of zein, provide better protection for curcumin and assemble more stable nanoparticles. Compared with individual zein NPs, the composite zein/TP NPs possessed higher encapsulation efficiency of curcumin, better re-dispersibility, greater physical stability and stronger resistance to ultraviolet light and heat. In addition, the influence of the mass ratio of zein to TP on the stability of nanoparticles was investigated in depth and the mass ratio of 1:0.5 (zein:TP) was identified as the optimum group. Therefore, the zein/TP NPs reported here were validated as a desirable carrier to deliver curcumin due to their remarkable properties, such as appropriate particle size and surface potential, high encapsulation efficiency, enhanced stability and bioaccessibility. In addition, future research is required to investigate the safety and health function effectiveness of curcumin encapsulated in zein/TP NPs in functional food, beverages and supplements. Further research should pay attention to the corroboration of the *in vivo* efficacy of bioactives via animal experiments.

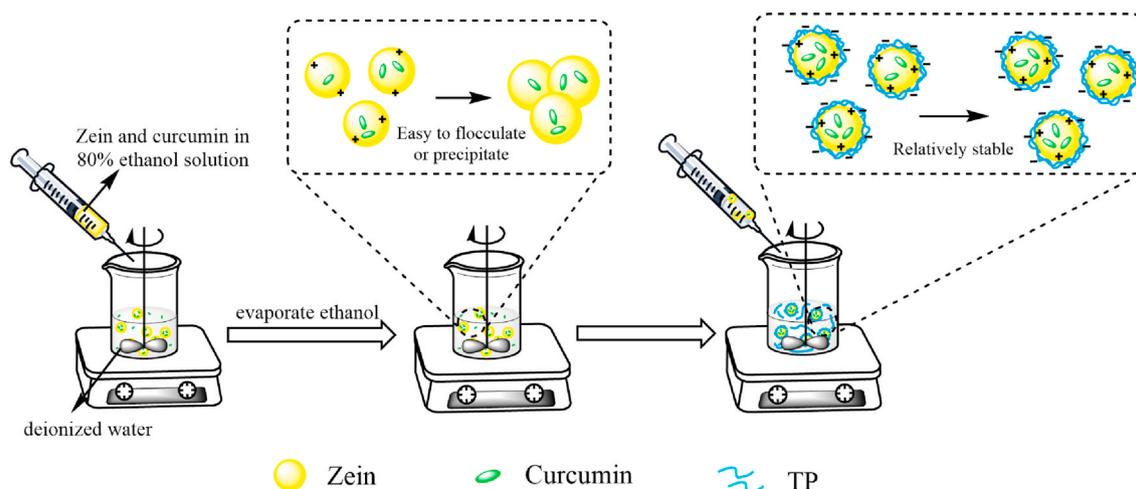


Fig. 5. Illustration for the potential formation mechanism of the curcumin-loaded zein/TP NPs.

CRedit authorship contribution statement

Duoduo Li: Investigation, Software, Data curation, Writing – original draft. **Zihao Wei:** Conceptualization, Methodology, Resources, Software, Writing – review & editing, Project administration, Funding acquisition, Supervision. **Jialin Sun:** Writing – review & editing. **Changhu Xue:** 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.

Acknowledgments

This work was supported by the start-up fund from Ocean University of China (grant No. 862001013134). This work was also funded by the National Key R&D Program of China (grant No. 2018YFC0311201). We thank Dr. Xiao Song in Ocean University of China for providing the polysaccharide samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crfs.2022.03.008>.

References

- Chen, S., Han, Y., Jian, L., Liao, W., Zhang, Y., Gao, Y., 2020. Fabrication, characterization, physicochemical stability of zein/chitosan nanocomplex for co-encapsulating curcumin and resveratrol. *Carbohydr. Polym.* 236, 116090.
- Chen, S., Ma, Y., Dai, L., Liao, W., Zhang, L., Liu, J., Gao, Y., 2021. Fabrication, characterization, stability and re-dispersibility of curcumin-loaded gliadin-rhamnolipid composite nanoparticles using pH-driven method. *Food Hydrocolloids* 118, 106758.
- Dai, L., Zhou, H., Wei, Y., Gao, Y., McClements, D.J., 2019. Curcumin encapsulation in zein-rhamnolipid composite nanoparticles using a pH-driven method. *Food Hydrocolloids* 93, 342–350.
- De Paola, M.G., Paletta, R., Lopresto, C.G., Lio, G.E., De Luca, A., Chakraborty, S., Calabrò, V., 2021. Stability of film-forming dispersions: affects the morphology and optical properties of polymeric films. *Polymers* 13, 1464.
- Gagliardi, A., Voci, S., Salvatici, M.C., Fresta, M., Cosco, D., 2021. Brij-stabilized zein nanoparticles as potential drug carriers. *Colloids Surf. B Biointerfaces* 201, 111647.
- Han, C.K., Chiang, H.C., Lin, C.Y., Tang, C.H., Lee, H., Huang, D.D., Zeng, Y.R., Chuang, T.N., Huang, Y.L., 2015. Comparison of immunomodulatory and anticancer activities in different strains of *Tremella fuciformis* berk. *Am. J. Chin. Med.* 43 (8), 1637–1655.
- Hu, K., McClements, D.J., 2014. Fabrication of surfactant-stabilized zein nanoparticles: a pH modulated antisolvent precipitation method. *Food Res. Int.* 64, 329–335.
- Khan, M.A., Yue, C., Fang, Z., Hu, S., Cheng, H., Bakry, A.M., Liang, L., 2019. Alginate/chitosan-coated zein nanoparticles for the delivery of resveratrol. *J. Food Eng.* 258, 45–53.
- Li, M., Yu, M., 2020. Development of a nanoparticle delivery system based on zein/polysaccharide complexes. *J. Food Sci.* 85, 4108–4117.
- Li, Z., Lin, Q., McClements, D.J., Fu, Y., Xie, H., Li, T., Chen, G., 2021. curcumin-loaded core-shell biopolymer nanoparticles produced by the pH-driven method: physicochemical and release properties. *Food Chem.* 355, 129686.
- Liu, Z., Smart, J.D., Pannala, A.S., 2020. Recent developments in formulation design for improving oral bioavailability of curcumin: a review. *J. Drug Deliv. Sci. Technol.* 60, 102082.
- Luo, M., Qi, X., Ren, T., Huang, Y., Keller, A.A., Wang, H., Wu, B., Jin, H., Li, F., 2017. Heteroaggregation of CeO₂ and TiO₂ engineered nanoparticles in the aqueous phase: application of turbiscan stability index and fluorescence excitation-emission matrix (EEM) spectra. *Colloids Surf. A Physicochem. Eng. Asp.* 533, 9–19.
- Ma, X., Yang, M., He, Y., Zhai, C., Li, C., 2021. A review on the production, structure, bioactivities and applications of Tremella polysaccharides. *Int. J. Immunopathol. Pharmacol.* 35, 1–14.
- Meng, R., Wu, Z., Xie, Q.T., Cheng, J.S., Zhang, B., 2021. Preparation and characterization of zein/carboxymethyl dextrin nanoparticles to encapsulate curcumin: physicochemical stability, antioxidant activity and controlled release properties. *Food Chem.* 340, 127893.
- Nastaj, M., Terpilowski, K., Solowiej, B.G., 2020. The effect of native and polymerised whey protein isolate addition on surface and microstructural properties of processed cheeses and their meltability determined by Turbiscan. *Int. J. Food Sci. Technol.* 55, 2179–2187.
- Pauluk, D., Padilha, A.K., Khalil, N.M., Mainardes, R.M., 2019. Chitosan-coated zein nanoparticles for oral delivery of resveratrol: formation, characterization, stability, mucoadhesive properties and antioxidant activity. *Food Hydrocolloids* 94, 411–417.
- Rodríguez-Félix, F., Del-Toro-Sánchez, C.L., Javier Cinco-Moroyocui, F., Juárez, J., Ruiz-Cruz, S., López-Ahumada, G.A., Carvajal-Millan, E., Castro-Enríquez, D.D., Barreras-Urbina, C.G., Tapia-Hernández, J.A., 2019. Preparation and characterization of quercetin-loaded zein nanoparticles by electrospraying and study of *in vitro* bioavailability. *J. Food Sci.* 84, 2883–2897.
- Sun, C., Xu, C., Mao, L., Wang, D., Yang, J., Gao, Y., 2017. Preparation, characterization and stability of curcumin-loaded zein/shellac composite colloidal particles. *Food Chem.* 228, 656–667.
- Wang, D., Wang, D., Yan, T., Jiang, W., Han, X., Yan, J., Guo, Y., 2019. Nanostructures assembly and the property of polysaccharide extracted from *Tremella fuciformis* fruiting body. *Int. J. Biol. Macromol.* 137, 751–760.
- Wei, Z., Zhu, J., Cheng, Y., Huang, Q., 2019a. Ovotransferrin fibril-stabilized Pickering emulsions improve protection and bioaccessibility of curcumin. *Food Res. Int.* 125, 108602.
- Wei, Z., Cheng, Y., Huang, Q., 2019b. Heteroprotein complex formation of ovotransferrin and lysozyme: fabrication of food-grade particles to stabilize Pickering emulsions. *Food Hydrocolloids* 96, 190–200.
- Wei, Z., Huang, Q., 2019a. Assembly of protein-polysaccharide complexes for delivery of bioactive ingredients: a perspective paper. *J. Agric. Food Chem.* 67 (5), 1344–1352.
- Wei, Z., Huang, Q., 2019b. Developing organogel-based Pickering emulsions with improved freeze-thaw stability and hesperidin bioaccessibility. *Food Hydrocolloids* 93, 68–77.
- Xu, X., Chen, A., Ge, X., Li, S., Zhang, T., Xu, H., 2020. Chain conformation and physicochemical properties of polysaccharide (glucuronoxylomannan) from Fruit bodies of *Tremella fuciformis*. *Carbohydr. Polym.* 245, 116354.
- Xu, Y., Wei, Z., Xue, C., Huang, Q., 2022. Assembly of zein–polyphenol conjugates via carbodiimide method: evaluation of physicochemical and functional properties. *LWT* 154, 112708.
- Xue, J., Zhang, Y., Huang, G., Liu, J., Slavin, M., Yu, L., 2018. zein/caseinate composite nanoparticles for bioactive delivery using curcumin as a probe compound. *Food Hydrocolloids* 83, 25–35.
- Yao, K., Chen, W., Song, F., McClements, D.J., Hu, K., 2018. Tailoring zein nanoparticle functionality using biopolymer coatings: impact on curcumin bioaccessibility and antioxidant capacity under simulated gastrointestinal conditions. *Food Hydrocolloids* 79, 262–272.
- Yu, Y.B., Wu, M.Y., Wang, C., Wang, Z.W., Chen, T.T., Yan, J.K., 2020. Constructing biocompatible carboxylic curdlan-coated zein nanoparticles for curcumin encapsulation. *Food Hydrocolloids* 108, 106028.
- Yuan, Y., Li, H., Liu, C., Zhu, J., Xu, Y., Zhang, S., Fan, M., Zhang, D., Zhang, Y., Zhang, Z., Wang, D., 2019. Fabrication of stable zein nanoparticles by chondroitin sulfate deposition based on antisolvent precipitation method. *Int. J. Biol. Macromol.* 139, 30–39.
- Yuan, Y., Xiao, J., Zhang, P., Ma, M., Wang, D., Xu, Y., 2021. Development of pH-driven zein/tea saponin composite nanoparticles for encapsulation and oral delivery of curcumin. *Food Chem.* 364, 130401.
- Zhang, Q., Wang, J., Liu, D., Zhu, W., Guan, S., Fan, L., Cai, D., 2020. Targeted delivery of honokiol by zein/hyaluronic acid core-shell nanoparticles to suppress breast cancer growth and metastasis. *Carbohydr. Polym.* 240, 116325.
- Zhou, Y., Chen, X., Yi, R., Li, G., Sun, P., Qian, Y., Zhao, X., 2018. Immunomodulatory effect of Tremella polysaccharides against cyclophosphamide-induced immunosuppression in mice. *Molecules* 23, 1–12.
- Zou, Y., Qian, Y., Rong, X., Cao, K., McClements, D.J., Hu, K., 2021. Encapsulation of quercetin in biopolymer-coated zein nanoparticles: formation, stability, antioxidant capacity, and bioaccessibility. *Food Hydrocolloids* 120, 106980.