

## Research Article

# Improvement in Entrapment Efficiency and *In Vitro* Digestion Stability of Lutein by Zein Nanocarriers with Pepsin Hydrolysis

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Zein is one of the popular bioactive carriers and play critical roles in the promotion of stability, absorption, and utilization of the nutrients and bioactive ingredients. The application of zein delivery systems for the encapsulation of bioactive ingredients has recently gained increasing interest. The aim of this work was to modify zein by pepsin and prepare the lutein-loaded zein nanoparticle (LZN) and the lutein-loaded zein hydrolysate nanoparticle (LZHN), respectively. The effects of zein hydrolysis on entrapment efficiency and *in vitro* digestion stability of lutein were also evaluated in this study. Hydrolysis of zein by the pepsin has important effects on lutein embedding. The optimal hydrolysis conditions, including the pepsin concentration (1.5%), temperature (55°C), and time (4 h), enhanced the entrapment efficiency (EE) of lutein by  $93.82 \pm 2.82\%$  as compared to  $85.18 \pm 3.28\%$  of the untreated zein, respectively. In contrast to LZN, LZHN had better structural characteristics, the average particle size decreases from  $158.40 \pm 3.22$  nm to  $112.2 \pm 1.56$  nm, and LZHN showed better dispersivity and zeta potential. The stability and release assays in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) showed that hydrolyzed zein nanocarriers by pepsin improved the digestion stability and promoted the release of lutein under gastrointestinal digestive conditions. These results suggest that hydrolyzed zein with pepsin may act as an effective carrier for lutein delivery and shows many potential advantages compared with the zein.

## 1. Introduction

Lutein is a nutritional and functional ingredient and widely exists in a variety of foods. Lutein belongs to the xanthophyll class of carotenoids; it has the peculiarity of having two hydroxyl groups in its structure, which determines its exclusive behavior, and exhibits various biologically significant activities in the body [1, 2]. It has many health benefits such as being antioxidant and preventing oxidative damage [3,4], inhibiting inflammation [5], prevention of atherosclerosis [6], and especially the unique protective actions against ocular disease [7]. For these reasons, lutein is a vital substance for promoting and maintaining good health. Unfortunately, lutein is an unstable bioactive substance; in particular, it is easy to decompose and be deactivated under gastrointestinal digestive conditions in the body. In addition, lutein has high hydrophobicity and poor absorption after

oral administration, as well as low bioavailability [8]. As a result, the application of lutein in foods and pharmaceutical industries has been greatly restricted. In order to improve the physical and chemical properties of lutein, and to effectively promote the absorption and utilization of lutein, novel edible protein delivery systems have been developed to enhance the water solubility, *in vitro* stability and *in vivo* stability, and release of lutein. They have played an important role in protecting active ingredients from degradation and inactivation [9, 10].

Zein is one of the commonly used biological active carriers and exhibits good self-assembly and delivery properties in encapsulation of bioactive components. As an edible carrier, zein has been recognized as safe as a direct human food ingredient by the Food and Drug Administration (FDA); therefore, it has been widely used for preparation of nanodelivery systems [11, 12].

Many functional and nutritional components have been successfully encapsulated into zein and synthesize zein nanoparticles to improve their physicochemical characteristics, such as tomato oleoresin, antimicrobial lysozyme, and essential oils [13]. The microstructure, chemical stability, and bioavailability of these active ingredients were significantly improved by embedding them in the zein carrier. However, as a bioactive material carrier, natural zein has some disadvantages in encapsulation and transport of lutein. Zein contains a high proportion of hydrophobic amino acids and it has low solubility which tends to aggregate in aqueous systems and easily form larger polymer particles. Additionally, the low stability and poor controlled-release performance of zein in gastrointestinal conditions have a significant impact on the delivery of the internal active substances and these defects hinder the application of zein as appropriate carriers for bioactive components [14].

To overcome these drawbacks, the recent research focuses on constructing the modified protein nanodelivery systems. In particular, the zymolytic proteins have been revealed to have many advantages as a carrier of active substances. Compared to the proteins, the protein hydrolysates or peptides possess smaller size, better polydispersity, and higher water solubility. For example, corn protein hydrolysate could be developed as a novel nanovehicle to enhance the physicochemical stability and *in vitro* bioaccessibility of vitamin D3 [15]. Zein hydrolysates could be used as oral delivery vehicles to enhance the physicochemical stability and *in vitro* bioaccessibility of curcumin [16]. Zein hydrolysate and tannic acid complex could be employed as an emulsifier in constructing a physical stable nanoemulsion delivery system and showed a remarkable increase in physical stability, high alga oil encapsulation efficiency, and antioxidative properties [17].

However, the zymolytic zein still has some drawbacks: different zein hydrolysates have different properties and their carrier capacities are affected by the conditions of enzymatic hydrolysis and characterization of enzyme. According to the research of Wang et al., the hydrolysis of zein by pepsin could improve the dispersity and stability of zein in water [18]; if zein hydrolysates could be used as nanocarriers for lutein, the solubility and digestion stability of lutein can be improved significantly, and the functional properties and *in vivo* utilization of the lutein will be obviously enhanced.

In this work, zein hydrolysates were prepared by pepsin, the effect of zein hydrolysis on entrapment efficiency of lutein was assessed, and a lutein-loaded nanocomplex system based on zein hydrolysates was first established for improving *in vitro* digestion stability and release of lutein. In particular, their structural characterization and morphology were also investigated.

## 2. Materials and Methods

**2.1. Materials.** Lutein was obtained from Sigma Aldrich (St. Louis, MO, USA). Zein was obtained from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). The pepsin was provided by Novo Nordisk (Bagsvaerd,

Denmark). Petroleum ether and ethanol were from Tianjin Damao Chemical Reagent Co., Ltd. (Tianjin, China). All other chemicals and reagents used were of analytical grade.

**2.2. Hydrolysis of Zein.** In order to investigate the effect of zein hydrolysis on the entrapment efficiency of lutein, the zein was hydrolyzed by pepsin according to the method of Jin et al. [19] with some modifications. Based on our previous researches, the hydrolysis time, pepsin dosage, and hydrolysis temperature were mainly effective factors affecting the entrapment efficiency of lutein; therefore, we optimized the three hydrolysis conditions. Briefly, 0.5 g of zein was suspended and homogenized in 25.0 mL of purified water, pepsin (0.5–3.0% w/v) was, respectively, added, and the solution was adjusted to a pH of 3.0 with hydrochloric acid. Then, the mixture was hydrolyzed, respectively, at a certain temperature (40°C–65°C) for 1–6 h. The hydrolysis reaction was finished by deactivating enzyme at 95°C for 5 min. Then, the hydrolysate was freeze-dried (LD-53, Millrock, USA) and stored at 4°C for later use.

**2.3. Preparation of LZ and LZH Nanoparticles.** The LZ and LZH nanoparticles were prepared by a liquid-liquid dispersion method, according to our previous method with a slight modification [20]. 2.5 mL of zein or hydrolysate (ZH) (2.0 mg/mL) was dissolved in 10 mL ethanol by ultrasonic treatment for 30 s, and 5 mL lutein solution (40 µg/mL) was added dropwise to the zein or ZH solution and stirred under magnetic stirring (1000 rpm) at 20°C for 30 min. In order to prevent the insoluble precipitation, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was injected into 22.5 mL of an aqueous phase and the mixed solution was transferred to a rotary evaporator and incubated in a 50°C water bath. Ethanol was removed under a reduced pressure during the evaporation reaction. The LZN and LZHN suspension were collected and kept at 20°C for further analysis.

**2.4. Entrapment Efficiency (EE) Measurement.** The encapsulation efficiency was calculated by our previously described method with a slight modification [18]. Briefly, 3.0 mL of LZH and 3 mL of petroleum ether were mixed by vortexing vigorously for 1.0 min at 20°C. The mixed sample was centrifuged at 3000 rpm for 5 min to collect the petroleum ether supernatant. The operation was repeated three times.

The concentration of lutein was determined by HPLC at 445 nm (Column: YMC-C<sub>30</sub>, 250 × 4.6 mm, 5 µm particle size, Agilent Technologies, Germany) as described by Li et al. 2015 [21]. The mobile phase was methyl tert-butyl ether and methanol at a linear gradient from 0 min (5:95, v/v) to 30 min (30:70, v/v) and a flow rate of 0.9 mL/min, the column temperature was maintained at 25°C, and 20 µL of sample was injected.

Two calibration curves were, respectively, made by dissolving the lutein standard in petroleum ether or alcohol

in a range from 0.2 to 1.0 mg/L, and the EE (%) of lutein in the nanoparticles was calculated by the following formula:

$$EE(\%) = \left( \frac{1 - W_1}{W_0} \right) \times 100\%. \quad (1)$$

$W_1$  and  $W_0$  were the weight of free amount of unloaded lutein in petroleum ether and initial weight of lutein added in the LZ or LZH nanoparticles, respectively.

**2.5. Particle Size, Polydispersity Index (PDI), and Zeta Potential Analyses.** The particle size, PDI, and zeta potential of the LZH and LZHN nanoparticles were characterized by a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). LZN and LZHN suspension were filtered with 0.45  $\mu\text{m}$  membrane before measurement. Mean particle sizes and zeta potential values were calculated as the average of triplicate measurements.

**2.6. Morphology Analysis.** The morphology of the LZN and LZHN was observed with a transmission electron microscopy (TEM) (Hitachi H-7650, Japan). One droplet of the LZN or LZHN was put on a copper grid of 400 mesh and dried in the air for 5 min. Then, the negative staining was performed with 2% phosphotungstic acid solution for 1 min. After air drying at 20°C, the samples were observed under the TEM [9].

**2.7. Stability Evaluation of Lutein in LZ and LZH Nanoparticles during In Vitro Digestion.** Simulate gastric (SGF) and simulated intestinal fluid (SIF) model were adopted to evaluate the digestion stability of lutein in LZN and LZHN by the method of Frenzel and Steffen-Heins [22] with some modifications. In order to produce the SGF, 2.0 mL of 0.8% pepsin solution (800–2000 U/mg of protein) was added to the 100 mL of distilled water, and the pH value of the solution was adjusted to 2.0 with hydrochloric acid. The SIF was prepared by dissolving 0.18 g sodium hydroxide, 0.81 g potassium dihydrogen phosphate, 0.48 g pancreatin, and 0.52 g bile salts into 100 mL purified water.

*In vitro* digestion stability of LZ and LZH nanoparticles were assessed by measuring the degradation rate of lutein in SGF and SIF separately according to the method described by Davidov-Pardo et al. [23] with a slight modification. Briefly, 10 mL of LZ or LZH solution was added to 30 mL SGF or SIF separately. The mixed solution was incubated at 37°C with continuous shaking at 100 rpm and sampled after 2–10 h, and then 0.5 mL suspensions were diluted with 10 mL ethanol, the sample was centrifuged at 6000 rpm for 10 min, and the supernatant was filtered through a membrane filter (0.45  $\mu\text{m}$ ). The lutein content was assayed based on methods described above. The degradation rate of lutein was calculated by the following equation:

$$\text{degradation rate (\%)} = \frac{M_0 - M_t}{M_0} * 100\%. \quad (2)$$

where  $M_0$  and  $M_t$  represent the lutein weight before and after *in vitro* digestion, respectively.

**2.8. In Vitro Release of Lutein in LZN and LZHN.** *In vitro* release of lutein from LZN and LZHN was evaluated by the method of our previous research, with some modifications [24]. 20 mL of each of LZN and LZHN solution was mixed with 20 mL SGF or SIF, and ten portions of mixed samples were prepared. The suspensions were incubated in a shaking water bath (100 rpm) at 37°C and sampled successively after 1–10 h, 0.5 mL suspensions were mixed well with 5 mL petroleum ether and then vortexed for 1.0 min, the sample was centrifuged at 6000 rpm for 10 min, and the supernatant was collected. The extracted lutein content was subsequently assayed as described above. All measurements were performed in triplicate. The cumulative release was calculated by the following equation:

$$\text{cumulative release (\%)} = \frac{C_n}{C} * 100\%, \quad (3)$$

where  $C_n$  and  $C$  were the lutein content of cumulative release for some time and loaded initially in the LZN and LZHN, respectively.

**2.9. Statistical Analysis.** All measurements were performed in triplicate, and the experimental results were statistically tested for significance ( $p < 0.05$ ) for analysis of variance using SPSS software. All data were reported as the mean  $\pm$  standard deviation (SD).

### 3. Results and Discussion

**3.1. Influence of Zein Hydrolysis Time on Entrapment Efficiency of Lutein.** The effect of zein hydrolysis at different times on the entrapment efficiency of lutein was studied. The zein was hydrolyzed with pepsin (1.5%) at 50°C for 1–6 h. Figure 1 indicates the variation in the entrapment efficiency of lutein in connection with hydrolysis time. It could be observed that the unhydrolyzed zein (0 h) showed lower entrapment efficiency of lutein. Instead, the entrapment efficiency of hydrolyzed zein by pepsin displayed a gradual increase with increase of hydrolysis time. When the zein was hydrolyzed for 4 h, the entrapment efficiency of lutein reached a maximum value. This is because when the zein was hydrolyzed, the pepsin hydrolysis caused structural destruction of zein, some polypeptides increased gradually, the polypeptides were combined with lutein, and the entrapment efficiency of lutein was increased. But the hydrolysis degree of zein reached the maximum at 4 h, the small molecular peptides and amino acids increased, and the entrapment efficiency of lutein was no longer increased and even showed a slight decrease after 4 h. Therefore, the entrapment efficiency of lutein was higher at 4 h hydrolysis of zein, where medium sized zein and polypeptides could be easily formed, which were beneficial to improve the encapsulation efficiency [25, 26].

**3.2. Influence of Pepsin Dosage on Entrapment Efficiency of Lutein.** The optimal pepsin dosage during the hydrolysis process is a crucial factor for encapsulation capacity of zein. In Figure 2, various pepsin dosages (0.5%, 1.0%, 1.5%, 2.0%,

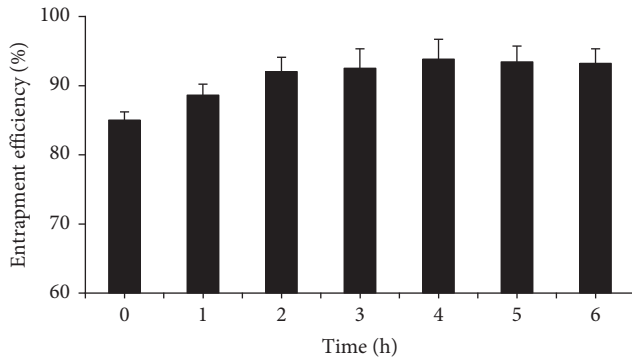


FIGURE 1: Effect of zein hydrolysis time on entrapment efficiency of lutein. The other hydrolysis conditions were kept constant: pepsin dosage 1.5%, hydrolysis temperature 50°C, and pH 3.0. Data were expressed as mean values  $\pm$  standard deviation ( $n = 3$ ).

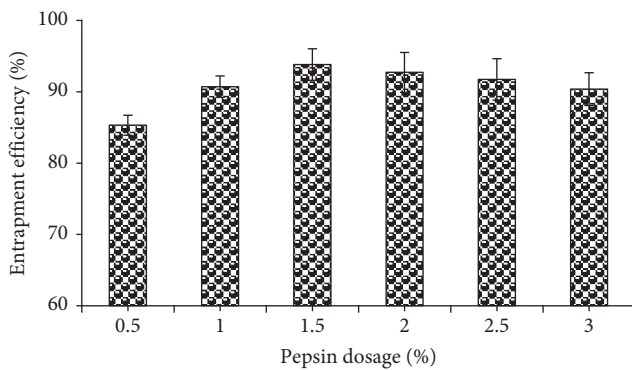


FIGURE 2: Effect of pepsin dosage on entrapment efficiency of lutein. The other hydrolysis conditions were kept constant: hydrolysis temperature 50°C, pH 3.0, and hydrolysis time 4 h. Data were expressed as mean values  $\pm$  standard deviation ( $n = 3$ ).

2.5%, and 3.0%) were used for hydrolysis of zein at 50°C for 4 h, the results showed that the entrapment efficiency of lutein increased with an increase in the pepsin dosage up to 1.5% in the hydrolysis of zein, and the higher entrapment efficiency of lutein ( $92.85 \pm 2.25\%$ ) resulted from the 1.5% pepsin dosage of hydrolysis of zein. Unfortunately, there was no homologous increase seen for higher pepsin dosage (2.0–3.0%). These results indicate that the optimal pepsin dosage is key to hydrolyze zein to obtain a high EE of lutein, the suitable pepsin dosage could cause limited enzymatic hydrolysis, they could damage the structure of zein and produced polypeptides which led to higher encapsulation capacity, and the redundant pepsin was useless for encapsulation of lutein with hydrolysate [27]. Therefore, the hydrolysis of zein with 1.5% pepsin was the most appropriate method to improve the encapsulation capacity of zein.

**3.3. Influence of Hydrolysis Temperature of Zein on Entrapment Efficiency of Lutein.** The zein was hydrolyzed with pepsin (1.5%) at different temperatures (40°C, 45°C, 50°C, 55°C, 60°C, and 65°C) for 4 h. The result is shown in Figure 3. With the increase of the hydrolysis temperature, a significant increase in EE of lutein was found to be  $93.82 \pm 2.82\%$ . The

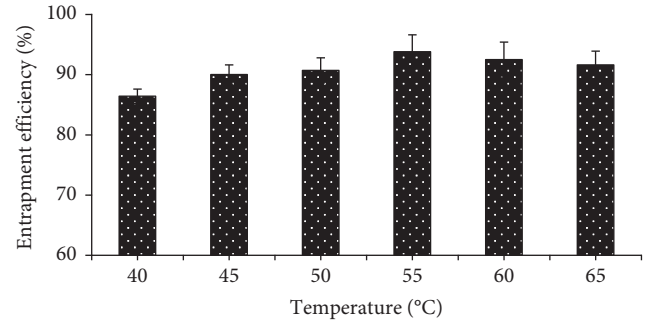


FIGURE 3: Effect of zein hydrolysis temperature on entrapment efficiency of lutein. The other hydrolysis conditions were kept constant: pepsin dosage 1.5%, hydrolysis time 4 h, and pH 3.0. Data were expressed as mean values  $\pm$  standard deviation ( $n = 3$ ).

EE decreases gradually when the temperature raises above 55°C. These results indicate that temperature is critical for enzymes hydrolysis; optimal temperature is known to be the chief reason for enzymes hydrolysis of zein and has an important influence on structure and property of zein. On one hand, the enzyme reaction has an optimal temperature and high temperatures could deactivate enzymes. On the other hand, the suitable temperature is a benefit for modification of zein [28]. Therefore, the optimal temperature is 55°C. The zein was hydrolyzed with 1.5% pepsin at 55°C for 4 h, the zein was appropriately modified, the maximum EE could be reached at  $93.82 \pm 2.82\%$ , and once the temperature reached 55°C, there would not be any further significant microstructure damage; increase of hydrolysis temperature of zein to more than 55°C cannot lead to high EE of lutein which could be due to excessive degradation of zein and pepsin loss. Therefore, the hydrolysis temperature of zein at 55°C was the most optimum temperature.

### 3.4. Physicochemical Characterizations

**3.4.1. Particle Size, Polydispersity Index (PDI), and Zeta Potential Analyses.** The lutein-loaded zein nanoparticles (LZN) and lutein-loaded zein hydrolysate nanoparticle (LZHN) were successfully prepared by liquid-liquid dispersion method [29], and the average particle size, PDI, and zeta potential of samples were measured, respectively.

The particle size analysis revealed that the average particle size of the LZN and LZHN was obviously different (Figures 4(a) and 4(b)); the average particle size of the LZN was  $158.40 \pm 3.22$  nm, while the zein was hydrolyzed; the macromolecular zein was broken down into small proteins and peptides; the size and hydrophobicity decreased [30], and when the lutein was loaded in zein hydrolysate to form LZHN, the average particle decreased to  $112.24 \pm 1.56$  nm. Therefore, the size of LZHN was smaller than the size of LZN.

The PDI values of LZN and LZHN were less than 0.5, and both of them displayed good polydispersity index. The PDI of LZN was  $0.147 \pm 0.026$ , but it was lower in LZHN ( $0.039 \pm 0.008$ ) on account of the hydrolysis of zein, and possessed better dispersion. The reason may be that the

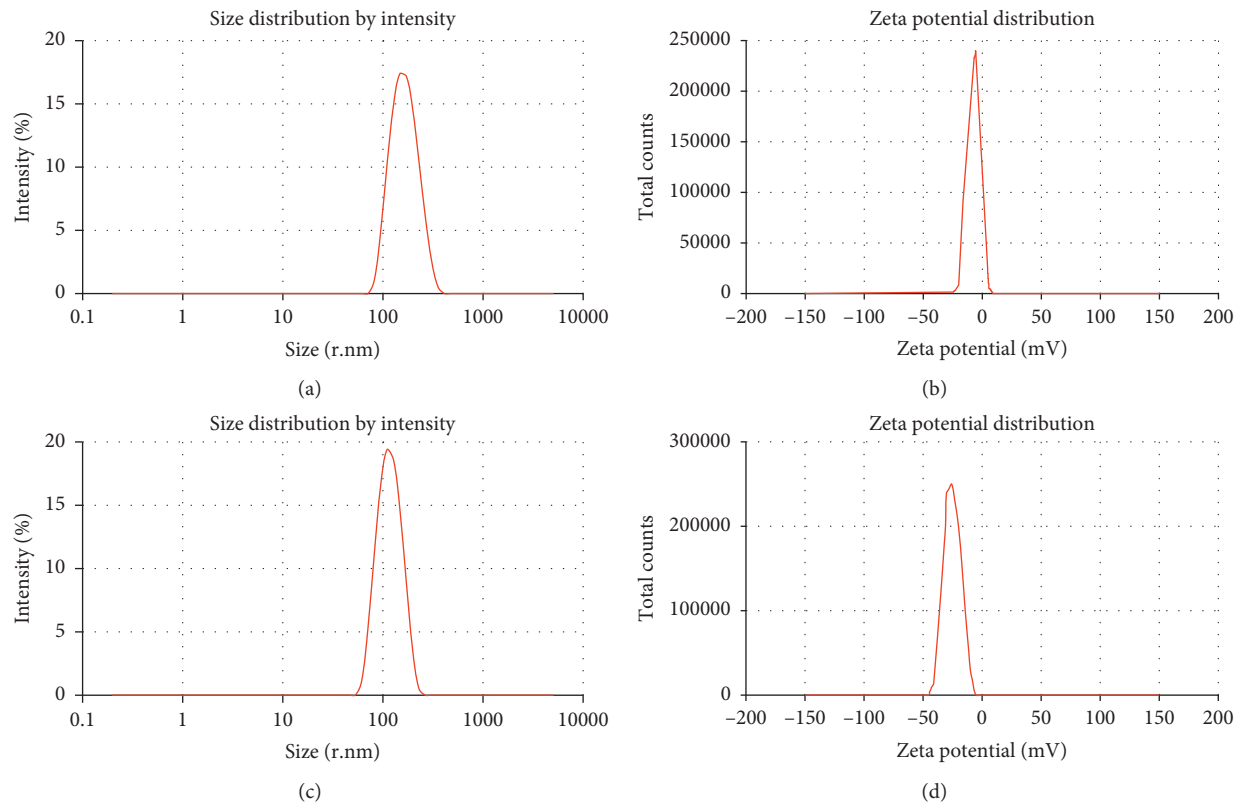


FIGURE 4: The average particle size (a, c) and zeta potential (b, d) of LZN (a) and LZHN (b).

water-soluble groups in zein increased due to proteolysis, and the solubility and dispersion of LZHN were therefore improved. It also means that zein hydrolysate is helpful to improve the stability and release of lutein-loaded zein nanoparticles and improve the solubility and dispersivity of lutein in water.

Zeta potential diagram showed that LZN possessed a lower negative charge of  $-5.8 \pm 0.06$  mV (Figure 4(b)), and this indicated that the LZN was unstable and prone to aggregation. Compared to the LZN, LZHN possessed an average negative charge of  $-25.6 \pm 1.06$  mV (Figure 4(d)), and hydrolysis resulted in a significant change in zeta potential. The obvious changes of zeta potential revealed that there were more negative charge groups in the zein hydrolysate after enzymatic hydrolysis, and they could prevent zein protein from aggregating into large particles and improve the stability and dispersibility of zein particles. Therefore, the entrapment efficiency and dispersivity of lutein were also improved.

**3.4.2. Transmission Electron Microscopy (TEM).** The microstructure of the lutein-loaded zein nanoparticle (LZN) and the lutein-loaded zein hydrolysate nanoparticle (LZHN) was examined by TEM. As shown in Figure 5, the lutein was embedded in the zein and hydrolysate through self-assembly. LZN (Figures 5(a) and 5(c)) and LZHN (Figures 5(b) and 5(d)) showed spherical shape nanoparticles of approximately 100–200 nm in diameter, consistent with the previous examined results of particle size,

and they formed an embedded core-shell structure to protect lutein. LZN displayed bigger size than LZHN and tended to assemble and constitute larger nanoscale particles, leading to an increase in particle size [31]. Instead, LZHN showed smaller nanoparticles and seemed to be well distributed in the suspension system. The hydrolysis decreased the hydrophobic interaction of zein and exhibited a regular spherical carrier structure; zein hydrolysate did not cause lutein aggregation and exhibited a higher entrapment efficiency.

**3.5. In Vitro Digestion Stability.** The digestion stability of lutein was investigated by measuring the lutein degradation rates of the uncoated lutein, LZN, and LZHN in SGF and SIF. Figure 6 indicates that the uncoated lutein decomposed rapidly in SGF and SIF, and lutein degradation rates reached  $42.93 \pm 1.78\%$  and  $34.77 \pm 2.07\%$  after 10 h of incubation, respectively.

When the lutein was coated with LZ, the lutein was found to degrade at a slower rate than the uncoated lutein. Degradation rates of lutein in SGF (Figure 6(a)) and SIF (Figure 6(b)) were  $32.32 \pm 1.36\%$  and  $27.42 \pm 1.31\%$ , respectively. The lutein stabilized by the LZHN complex showed a remarkable stability, and the lutein degradation rates decreased to  $26.81 \pm 1.53\%$  and  $25.66 \pm 0.66\%$  after 10 h of incubation, respectively.

This could be attributed to the fact that the EE of lutein in LZHN was higher than LZN, and the free lutein content decreased in the digestive juice system whereas zein and zein

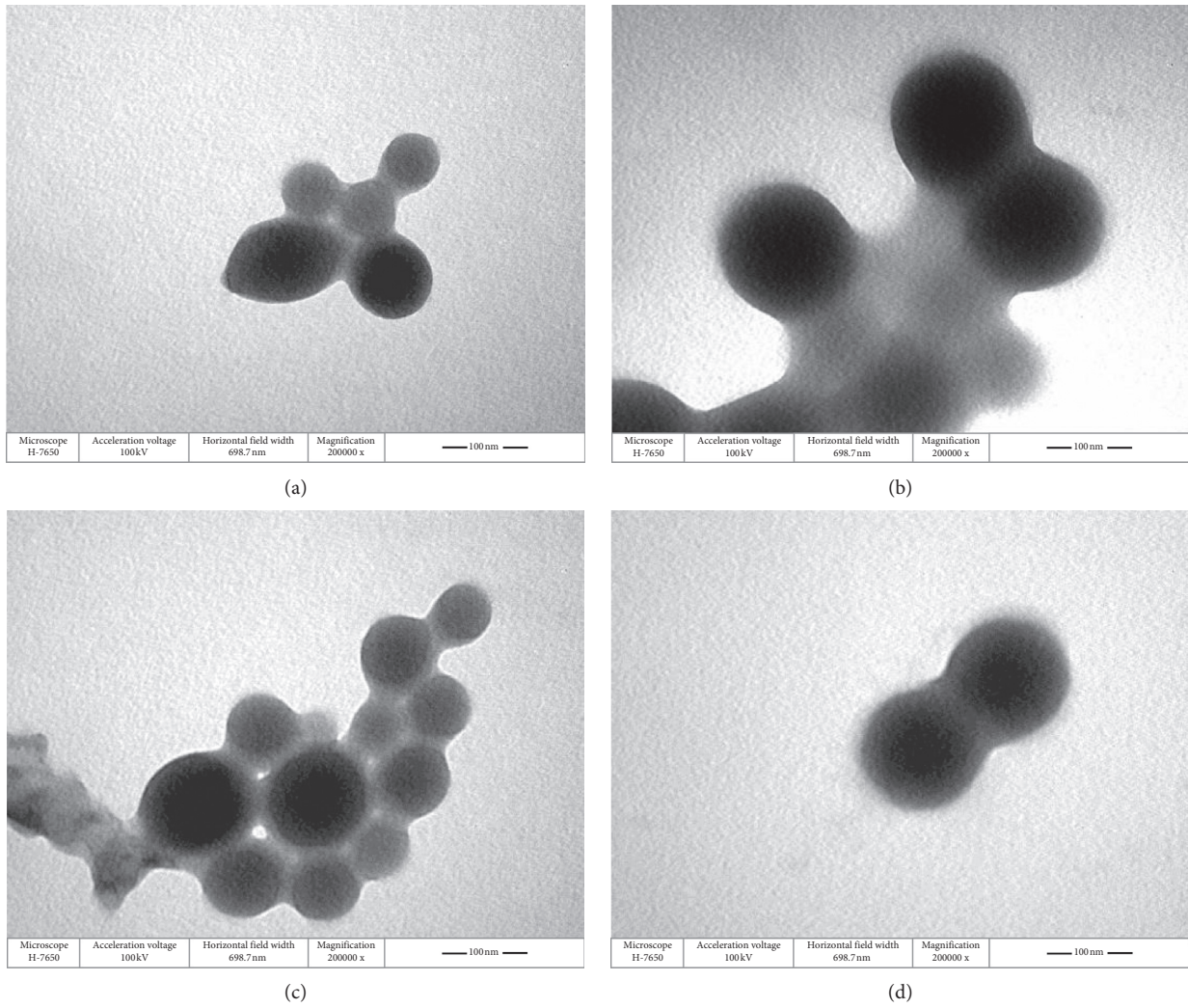


FIGURE 5: Transmission Electron Microscope (TEM) images of zein and lutein-loaded zein nanoparticle (LZN) (a, c), and zein hydrolysate nanoparticle and lutein-loaded zein hydrolysate nanoparticles (LZHN) (b, d).

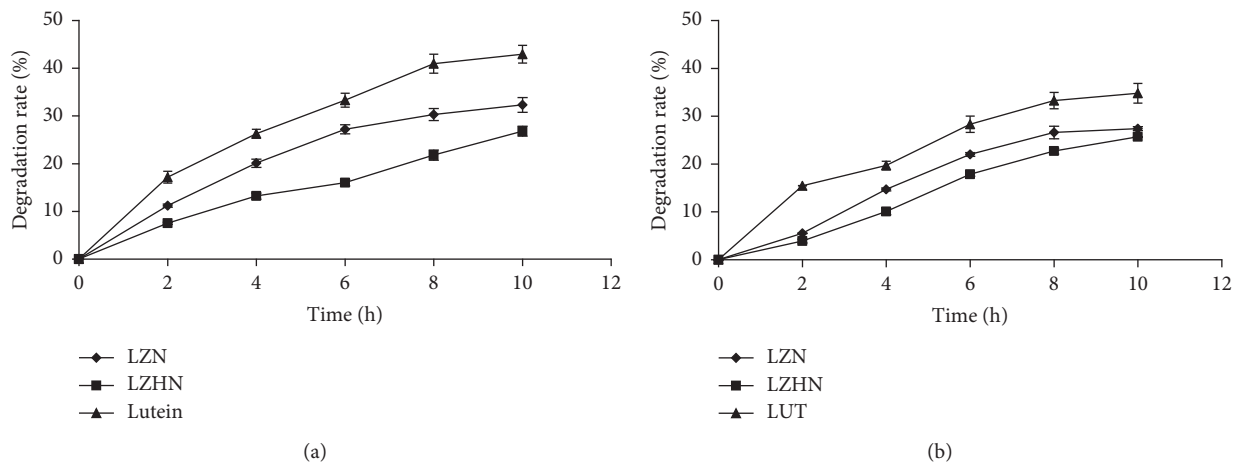


FIGURE 6: Lutein degradation rate of free lutein, LZN, and LZHN during digestion in SGF (a) and in SIF (b) for 10 h.

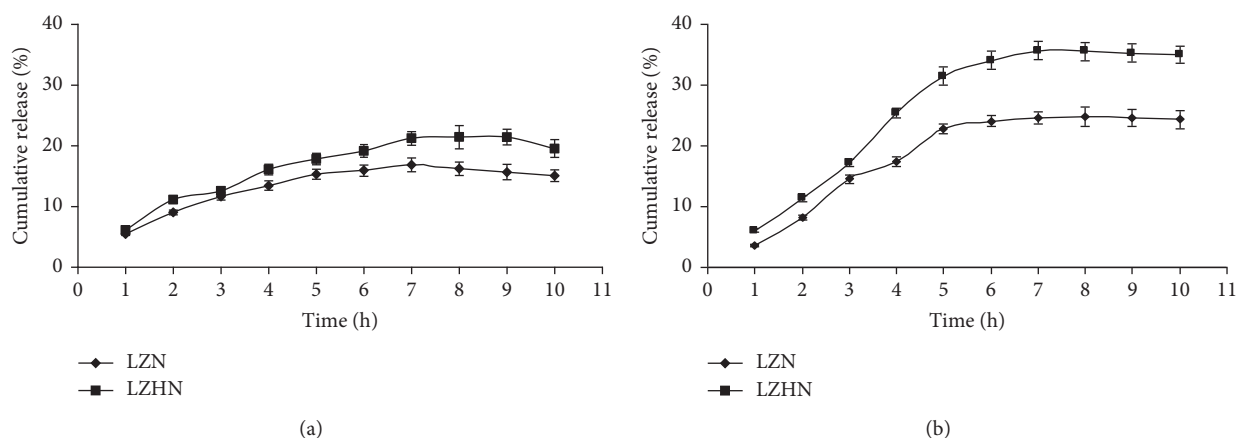


FIGURE 7: Lutein cumulative release of LZN and LZHN during digestion in SGF (a) and in SIF (b) for 10 h.

hydrolysate could protect embedded lutein from degradation [32]. Furthermore, the zein hydrolysate could decrease the following hydrolysis by pepsin and therefore protect loaded lutein from degradation. The results revealed that zein hydrolysates displayed better stability and they could prevent degradation of lutein in SGF and SIF. For this reason, LZN and LZHN could decrease the degradation of lutein in acidic and alkaline conditions and increased the digestion stability of lutein.

The result verified that free lutein was more susceptible to degradation than its encapsulation in nanoparticles, and the stability of lutein could be increased in digestive process by embedding lutein in LZN and LZHN. Particularly, the digestion stability of the lutein loaded in zein hydrolysate was much higher than the lutein loaded in zein.

**3.6. In Vitro Release Characteristics.** The lutein release from LZN and LZHN was performed for 10 h in SGF and SIF, respectively. Figure 7 showed that lutein cumulative release of LZN and LZHN was  $16.86 \pm 0.96\%$  and  $21.22 \pm 0.84\%$  after 7 h of incubation in SGF, respectively (Figure 7(a)). And the cumulative release decreased slightly due to the degradation of lutein. But the lutein cumulative release of LZN and LZHN increased to  $24.03 \pm 0.92\%$  and  $34.08 \pm 1.48\%$  after 6 h of incubation in SIF (Figure 7(b)), respectively. And then the cumulative release reached the sustained release after 6 h of incubation. The results showed that LZHN released much more encapsulated lutein in SIF than in SGF, and the lutein in LZHN was released faster than in LZN in the same time under the SGF and SIF traditions.

The results revealed that lutein cumulative release in LZHN was higher than that of zein in SGF and SIF, and the release of lutein was better in alkaline intestinal solutions than acidic gastric juice. The possible reasons are that zein hydrolysate has stronger hydrophilicity than zein, it displayed good solubility and dispersibility in SGF and SIF, and the lutein loaded in the zein hydrolysate could be easily released from nanoparticles. For this reason, the cumulative release of lutein was higher, especially in intestinal fluids. In conclusion, zein hydrolysate is a suitable carrier for lutein delivery.

## 4. Conclusion

In this work, lutein-loaded zein and its hydrolysate nanoparticles were successfully synthesized as a novel nanocarrier for lutein. The hydrolysis of zein was found to significantly affect the entrapment efficiency of lutein. Zein hydrolysate nanoparticle exhibited excellent physicochemical characterizations, and lutein-loaded hydrolytic zein had a smaller nanoscale size and exhibited good dispersibility for the micromolecular zein and polypeptide. Meanwhile, the digestive stability and release of lutein in zein hydrolysate nanoparticles in SGF and SIF were significantly enhanced. Nonetheless, the molecular weight of the zein hydrolysate used for embedding lutein is still uncertain, and the *in vivo* absorption and bioavailability of lutein in zein hydrolysate nanoparticle is not clear.

In conclusion, enzymatic hydrolysis of zein by pepsin was a simple and effective means for improving the coating performance and load capacity, and it seems to be a superior carrier for improving the *in vitro* digestion stability of lutein. This may be useful for potential applications for development of active ingredients carrier.

Our future studies mainly focus on the development of zein hydrolysate by different sources of enzymes, the effect of molecular weight and amino acid distribution to the encapsulation and transport capacity will be studied, and the stability and *in vivo* absorption of lutein will be further investigated. The novel and effective modified zein delivery system of lutein will be the emphasis of our research.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Additional Points

**Practical Application.** The application of pepsin hydrolysis has potential for increasing the encapsulation and transport capacity of the zein. Zein is a common and important carrier of lutein, while natural zein has poor solubility and easily

aggregates in water, which displayed low embedding rate and poor release property for lutein. Pepsin hydrolysis could significantly increase the entrapment efficiency and *in vitro* digestion stability of lutein. Pepsin hydrolysis is a simple and effective method to modify the properties of zein, which can be beneficial in food industries for the load and delivery of active substance.

## Conflicts of Interest

The authors declare that this article content has no conflicts of interest.

## Acknowledgments

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