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RESEARCH

Formulation and Characterization of Natural Surfactant-Stabilized Zein Nanoparticles for Encapsulation of Ergocalciferol

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Abstract

Ergocalciferol, despite its high beneficial potential in foods and pharmaceuticals, faces challenges in utilization due to its hydrophobic and sensitive properties. In this study, we developed ergocalciferol-loaded zein nanoparticles coated with modified lecithin (ML) or rhamnolipids (RL) using the anti-solvent precipitation method. Both ML- and RL-stabilized zein nanoparticles exhibited narrow particle size distribution and high encapsulation efficiency of ergocalciferol, achieving 94.54 ± 2.28% and 94.24 ± 2.35%, respectively. The ML-stabilized nanoparticles demonstrated good stability under thermal treatments (30–90 °C) and pH variations (pH 3–8). In comparison, the nanoparticles stabilized by rhamnolipid (RL) remained stable under thermal conditions but became unstable when the pH dropped below 6. Additionally, both ML- and RL-stabilized nanoparticles demonstrated an increase in particle size after the addition of salt. Furthermore, all samples displayed high bioaccessibility of ergocalciferol after in vitro digestion and excellent physicochemical stability during 30 days of storage. Therefore, the ML- and RL-stabilized zein nanoparticles present promising prospects for effectively transporting functional ingredients such as ergocalciferol.

Keywords Vitamin D · Rhamnolipids · Modified lecithin · Zein Nanoparticles · Bioaccessibility

Introduction

Vitamin D is an essential nutrient in the human body, playing a vital role in regulating calcium and phosphate absorption, and bone mineralization [1]. Beyond its involvement in skeletal health, vitamin D has been recognized for its potential to protect against non-skeletal disorders such as cardiovascular disease and glucose metabolism disorders [2, 3]. Moreover, recent investigations have shed light on the potential of vitamin D supplementation in reducing the risk of COVID-19 infections and mitigating associated symptoms, which could be attributed to the ability of vitamin D to modulate immune and oxidative responses, as well as its direct antiviral properties against coronaviruses [4, 5]. Vitamin D exists in two main forms: vitamin D2 (ergocalciferol) found in plant-based foods, and vitamin D3 (cholecalciferol) present in animal-based foods [6]. Vitamin D3 can also be synthesized in the skin upon exposure to sunlight. However, various factors such as residing in high-altitude areas, excessive use of sunscreen, and poor lifestyle and dietary habits have contributed to widespread vitamin D deficiency, emerging as a significant global public health concern [7]. Therefore, the development of vitamin D food fortification strategies becomes imperative to ensure adequate daily intake, particularly for vulnerable populations such as children, adolescents, pregnant women, and the elderly [8].

Vitamin D poses significant challenges in its application within the food industry due to its low aqueous solubility, poor chemical stability, and limited bioavailability [9]. To

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address these inherent limitations, various strategies have been developed, including nanoparticles, nanoemulsions, solid lipid nanoparticles, and nanoliposomes [10, 11]. In our previous studies, we successfully prepared vitamin D-loaded nanoemulsions using different food-grade surfactants/emulsifiers, leading to improved physical and chemical stabilities [12, 13]. Moreover, we achieved successful encapsulation of vitamin D using surfactant-based nanoparticles, enhancing its stability under specific conditions such as pH, ionic strength, and heating [14]. Interestingly, the scientific community across diverse fields, including food science, has shown great interest in lipid-free nanoparticles due to their ability to significantly enhance solubility, stability, and bioavailability of lipophilic components without the need for added lipids [15, 16]. Over the past few decades, zein has emerged as a versatile building block for food-grade nanoparticles, owing to its cost-effectiveness and unique functional properties. Zein is derived from corn as a by-product of starch and ethanol production, containing more than 50% hydrophobic amino acids. It exhibits solubility in 55–90% aqueous ethanol solutions but remains insoluble in water [17, 18]. However, zein nanoparticles tend to be unstable under conditions of moderate acidity (pH 5–7), high ionic strength, and elevated temperatures [19]. To enhance their stability against various environmental stresses, the surface of zein nanoparticles is typically modified using synthetic or natural surfactants [20]. For instance, when formulating curcumin-loaded zein nanoparticles, coating them with rhamnolipid or Tween 20 has been shown to significantly improve encapsulation efficiency and stability [20, 21]. Therefore, the application of surfactant-based surface coatings is crucial for achieving stable zein nanoparticles.

To the best of our knowledge, there is limited information available regarding the fabrication of zein nanoparticles loaded with ergocalciferol (vitamin D2) and stabilized by natural food-grade surfactants. Modified lecithin (ML), an enzymatically modified phospholipid derived from soy lecithin hydrolysis, is a natural anionic surfactant commonly used to stabilize food dispersion systems [12, 22]. Rhamnolipid (RL), a recently developed natural anionic biosurfactant (glycolipid), has also been widely employed for the formation of stable emulsions and nanoparticles [23, 24]. Although both ML and RL were successfully used to stabilize ergocalciferol loaded emulsion in our previous studies, they had different resistance to different environmental stresses [12, 24]. To further broaden the application range of these natural surfactants and provide a new stable way for ergocalciferol, we prepared ML- or RL-stabilized zein nanoparticles for the encapsulation of ergocalciferol using the anti-solvent precipitation method in this study. And then we compared the stability of these two surfactant-coated zein nanoparticles under different environmental stresses (pH 2–8, ionic strength 0-500 mM NaCl, thermal treatment 30–90 ^{o}C) and long-term storage (4 ^{o}C, 30 days). Additionally, we investigated the bioaccessibility of ergocalciferol-loaded nanoparticles using an in vitro gastrointestinal digestion model. This research offers the potential for the production of stable ergocalciferol-loaded zein nanoparticles through coating with a natural surfactant and provides valuable insights into the development of vitamin D-fortified foods.

Materials and Methods

Materials

Zein, pepsin (P7000, ≥ 250 units/mg), pancreatin (P7545, 8×USP specifications), bile extract (B8631) and rhamnolipid (R90) were purchased from Sigma Aldrich (St. Louis, MO, USA). The major enzymes in pancreatin are trypsin, amylase and lipase. The rhamnolipid consists of a mixture of di- and mono-rhamnolipids, with the purity being > 90 wt%. Ergocalciferol, ethanol, methanol, and acetonitrile were purchased from Wako Pure Chemical Industries (Osaka, Japan). The modified lecithin was obtained from Tsuji Oil Mills Co. Ltd. (Tokyo, Japan) and its chemical compositions were reported in the previous studies [13]. All chemical reagents used in the present study were of analytical grade. Ultrapure water was used in the whole study to prepare the solutions.

Preparation of Ergocalciferol-Loaded Zein Nanoparticles Stabilized by Surfactant

Ergocalciferol-loaded zein nanoparticles were prepared utilizing the anti-solvent precipitation method. In a nutshell, surfactant-containing aqueous solutions were created by dissolving 0.5% (w/w) of modified lecithin (ML) or rhamnolipid (RL) in phosphate buffer (5 mM, pH 7). Zein and ergocalciferol were dissolved in an aqueous ethanol solution (80%, v/v) at concentrations of 10 mg/mL and 1 mg/mL, respectively. The 10 mL ethanol solution, consisting of zein and ergocalciferol, was rapidly injected into a 40 mL aqueous solution with or without ML or RL using a glass syringe equipped with a 27-gauge needle. The resulting mixtures were continuously stirred at 600 rpm for 30 min to facilitate nanoparticle formation. After the stirring period, the ethanol was removed using a rotary evaporator (R100 Buchi, Switzerland) at 45 °C for 1 h under reduced pressure. The loss of ethanol was compensated by adding ultrapure water. If necessary, the resulting dispersions were adjusted back to pH 7. Finally, the obtained samples were centrifuged at 10,000 g for 30 min at 25 ^{o}C to eliminate any large particles and unencapsulated ergocalciferol. The dispersions were subsequently stored at 4 °C for further characterization.

Nanoparticles Stability Testing

Effect of pH

The freshly prepared nanodispersions were carefully adjusted to the desired pH values ranging from 2 to 8 using either 1 M HCl or 1 M NaOH. Subsequently, the resulting samples were stored at a temperature of 4 °C for a duration of 24 h before conducting particle size and ζ-potential analysis.

Effect of Ionic Strength

The freshly prepared nanodispersions were diluted with an equal volume of NaCl solutions to achieve the desired final salt concentrations ranging from 0 to 500 mM. Subsequently, the resulting samples were stored at a temperature of 4 °C for a duration of 24 h prior to conducting measurements on the particle size and ζ-potential of the nanoparticles.

Effect of Thermal Treatment

The freshly prepared nanodispersions were subjected to thermal treatment by placing it in a water bath for a duration of 1 h within the temperature range of 30–90 °C. Subsequently, the samples were cooled back to room temperature using an ice bath. This procedure was conducted to assess the impact of thermal treatment on the particle size and ζ-potential of the nanoparticles.

Storage Stability

The nanodispersion, supplemented with sodium azide (0.02%, w/w) as an antimicrobial agent, was transferred to glass vials and subsequently incubated at a temperature of 4 °C for a period of 30 days under dark conditions. Following the incubation period, measurements were conducted to determine the particle size and assess the retention of ergocalciferol in the samples.

Droplet Size and ζ-Potential Analysis

The ergocalciferol-loaded nanoparticles were characterized using the Zetasizer NanoZS (Malvern Instruments Ltd., Worcestershire, UK). The dynamic light scattering technique was employed to obtain information on the size distribution, particle size, and polydispersity index (PDI). The ζ-potential of samples was automatically determined by conducting 10–100 runs per analysis after allowing the instrument to equilibrate for 120 s at a temperature of 25 °C. The refractive indexes of zein and water were set at 1.49 and 1.33, respectively. All data for each sample were reported as the average of three readings.

Ergocalciferol Content Measurement

The content of ergocalciferol in the nanoparticles was quantified based on the methodology described in a previous study with slight modifications [14]. In brief, 0.2 mL of the nanodispersion was dissolved in 3.8 mL of ethanol and vortexed for 30 s. The resulting mixture underwent ultrasonication for 20 min and was subsequently filtered using a 0.45 µm pore size filter. The filtrate was then injected into an HPLC system (Shimadzu LC-20 A, Japan) equipped with a C18 column (4.6 × 250 mm, Anpel, China). The column temperature was maintained at 35 °C, and the wavelength was set to 265 nm. The mobile phase consisted of 75% acetonitrile and 25% methanol, with a flow rate of 1 mL/min. The quantification of ergocalciferol content in the dispersions was determined by referencing a standard curve. The encapsulation efficiency (EE) and retention (Re) of ergocalciferol were calculated according to Eqs. (1) and (2), respectively:

$$\text{Encapsulation efficiency, } EE(\%) = \frac{C_f}{C_0} \times 100 \quad (1)$$

$$\text{Ergocalciferol retention, } Re(\%) = \frac{C_1}{C_f} \times 100 \quad (2)$$

where C_f is the concentration of ergocalciferol in the fresh nanoparticles, C_1 is the concentration of ergocalciferol in nanoparticles after 30 days of storage at 4 °C, while C_o is the initially added ergocalciferol concentration for preparing nanoparticles.

In Vitro Gastrointestinal Digestion

The ergocalciferol-loaded nanoparticles stabilized by different surfactants were subjected to an in vitro digestion model that simulated the stomach and small intestinal phases according to previous studies with some modifications [14, 25]. Briefly, 15 mL of samples were mixed with 15 mL of simulated gastric fluid containing 3.2 mg/mL of pepsin and 2 mg/mL of NaCl. The resulting mixtures were adjusted to pH 2.5 using 1 M HCl or NaOH and then shaken with 100 strokes per minute for 2 h at 37 °C in a water bath. After the gastric incubation, the samples were adjusted to pH 7 using 1 M NaOH and mixed with 3.5 mL of bile extract (54 mg/mL) and 1.5 mL of salt solution (8.77 mg/mL NaCl and 1.11 mg/mL $CaCl_2$ in ultrapure water). The obtained suspension was readjusted back to pH 7 and then supplemented with 2.5 mL of pancreatin suspension (75 mg/mL in phosphate buffer, pH 7). The mixtures were maintained at pH 7 with continuous shaking at 100 strokes per minute for 2 h in a water bath (37 °C). When the digestion was complete, 10 mL of the raw digesta was centrifuged at 9,000 g

for 60 min at 10 °C. The supernatant was collected and filtered using a membrane filter with a pore size of 0.45 µm, which was considered the micellar phase. After adding 0.5 mL of the micellar phase to 4.5 mL of ethanol, ergocalciferol was extracted and quantified using the method described in "Ergocalciferol Content Measurement" section. The bioaccessibility of ergocalciferol after digestion was calculated according to the following equation:

$$\text{Bioaccessibility}(\%) = \frac{C_{Micelles}}{C_f} \times 100 \quad (3)$$

where $C_{Micelles}$ is the concentration of ergocalciferol in the micelles phase, while C_f is the concentration of ergocalciferol in the overall digesta after simulated intestinal fluid (SIF) digestion.

Statistical Analysis

All experiments were performed at least three times. The obtained data were reported as the average and standard deviation, and the statistical analysis was performed using SPSS software (version 22, IBM software, USA). The significant difference ($p < 0.05$) was shown using different letters.

Results and Discussion

Properties of Fresh Ergocalciferol-Loaded Zein Nanoparticles Stabilized by Different Surfactants

Initially, experiments were performed to determine the role of surfactant type on the formulation and properties of zein nanoparticles loaded with ergocalciferol. The zein nanoparticles were prepared by the aforementioned anti-solvent precipitation method in the absence or presence of ML or RL. In the absence of a surfactant, a huge amount of particle aggregates was observed when the ethanol solution containing zein and ergocalciferol was injected into phosphate buffer (5 mM, pH 7) (data not shown). This result was ascribed to the strong hydrophobic attraction and weak electrostatic repulsion near the isoelectric point (PI ~ 6.2) of zein [26]. However, the ergocalciferol-loaded zein nanoparticles could be successfully formulated with the addition of a surfactant (either ML or RL). The resulting zein nanoparticles stabilized by ML and RL had similar and small particle sizes around 50 nm (Table 1). These results suggest that the presence of a surfactant is important for stabilizing zein nanoparticles. During the formulation of a colloidal dispersion, surfactants can readily adsorb onto the particle surface, thereby providing stabilization through steric and electrostatic repulsive forces [12, 27]. The successful formulation of ergocalciferol-loaded zein nanoparticles by adding surfactants could be attributed to the high negative charge of ML and RL (as discussed later), which generate strong electrostatic repulsion, thereby preventing particle instability (Table 1). Dynamic light scattering measurements also revealed that the formed zein nanoparticles exhibited narrow particle size distributions, as evidenced by their low polydispersity index values (PDI < 0.2) and monomodal peaks (Fig. 1; Table 1). It is widely accepted that nanoparticles with lower PDI values exhibit improved physical stability [28, 29]. The encapsulation efficiency (EE) of ergocalciferol in ML-stabilized and RL-stabilized zein nanoparticles prepared using the anti-solvent precipitation method was found to be 94.54 ± 2.28% and 94.24 ± 2.35%, respectively (Table 1). The high EE values obtained in our study reaffirm that both ML and RL are effective natural surfactants for preparing ergocalciferol-loaded zein nanoparticles using the anti-solvent precipitation method.

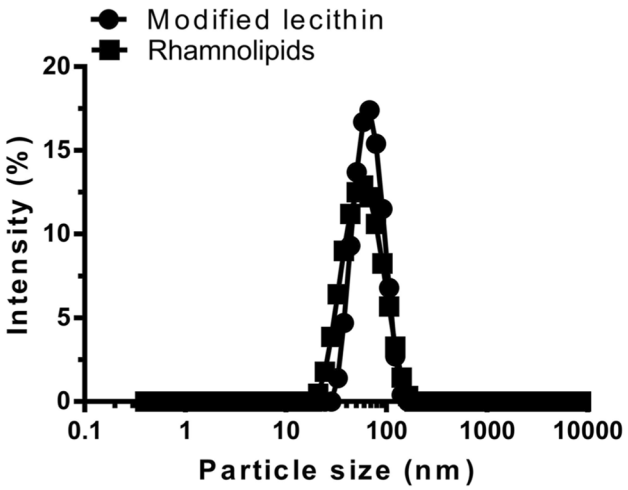

Fig. 1 The particle size distribution of modified lecithin (ML)- and rhamnolipids (RL)-stabilized zein nanoparticles encapsulating ergocalciferol

Table 1 The particle size, polydispersity index (PDI), encapsulation efficiency (*EE*) of ergocalciferol-loaded zein nanoparticles stabilized by ML and RL

	Modified lecithin (ML)	Rhamnolipids (RL)
Particle size	57.06 ± 1.71 nm [a]	51.47 ± 3.72 nm [b]
PDI	0.12 ± 0.01 [a]	0.14 ± 0.02 [a]
EE	94.54 ± 2.28% [a]	94.24 ± 2.35% [a]

Effect of pH on the Stability of Ergocalciferol-Loaded Zein Nanoparticles

As a nano-delivery system, the properties of nanoparticles can be influenced by their surrounding environment during

formation, storage, and utilization. pH adjustment is a common practice in various products to meet the requirements of manufacturers and consumers. Therefore, it is crucial to analyze the impact of pH on the stability of ergocalciferol-loaded zein nanoparticles. Figure 2 illustrates the effect of pH on the particle size, ζ-potential, and turbidity of ML- and RL-stabilized zein nanoparticles. At pH 2, the particle size of ML-stabilized zein nanoparticles was 170.14 ± 19.49 nm (Fig. 2a). However, as the pH increased to 3–8, the particle size decreased and remained relatively constant at around 60 nm. This observation can be attributed to the fact that the charge of particles at pH 2 (-26.71 ± 4.05 mV) is relatively lower than that at pH 3–8 (-39.48 ± 2.07 to -51.78 ± 2.54 mV), resulting in insufficient electrostatic repulsion at pH 2 to overcome particle aggregation (Fig. 2b). Therefore, at pH 3–8, a strong electrostatic repulsion between particles prevents aggregation and maintains a relatively small and stable particle size. Additionally, as the pH increased, the turbidity of ML-stabilized zein nanoparticles decreased, and the samples became transparent beyond pH 3 (Fig. 2c), which can be attributed to the decrease in particle size. These findings indicate that ML-stabilized zein nanoparticles exhibit excellent physical stability without sedimentation at the bottom of the container across all pH conditions (pH 2–8).

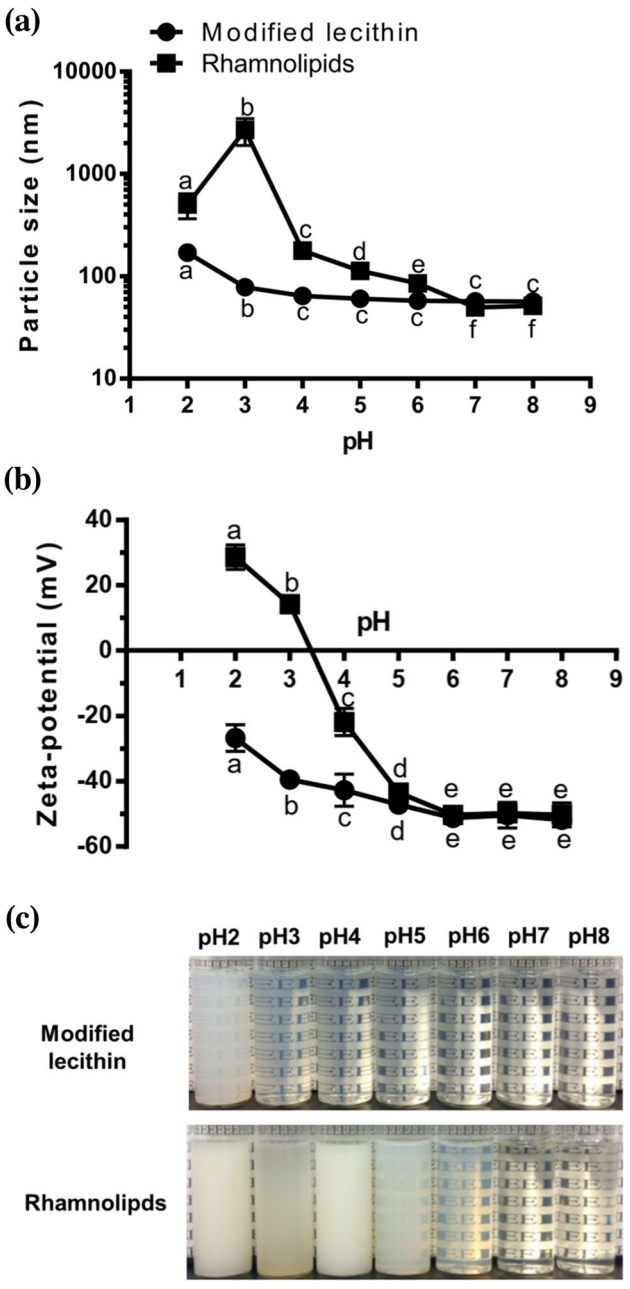

Fig. 2 The effect of pH on the particle size (**a**), ζ-potential (**b**) and turbidity (**c**) of ML- and RL-stabilized zein nanoparticles encapsulating ergocalciferol

The pH value played a critical role in determining the properties of RL-stabilized zein nanoparticles. Under acidic conditions (pH 2–6), the particle size was larger compared to neutral and alkaline conditions (pH 7–8). The particle size reached its peak at pH 3, with an average size of 2692.86 ± 787.88 nm (Fig. 2a). At pH 3, the ζ-potential of RL-stabilized zein nanoparticles was 14.24 ± 1.27 mV, leading to particle aggregation due to weak electrostatic repulsion between nanoparticles (Fig. 2b). Consequently, large clusters formed through nanoparticle aggregation and sedimented at the bottom of the container due to gravity (Fig. 2c). At pH 2 and 4, nanoparticles with large particle sizes were also formed (501.23 ± 137.14 nm and 178.26 ± 32.31 nm, respectively). Despite the high turbidity of the nanoparticle dispersion, no sedimentation occurred at the bottom of the container, indicating the formation of a relatively stable colloidal dispersion that did not undergo separation by gravity. At pH 5–6, the turbidity of the dispersions was noticeably lower compared to pH 2–4. This can be attributed to the smaller particle size of the nanoparticles and the higher ζ-potential observed at pH 5–6. The dispersions under neutral and alkaline conditions (pH 7–8) became more transparent with smaller particle sizes than those under acidic conditions, allowing for easy observation of the "E" letter behind the dispersions. The curcumin-loaded zein nanoparticles stabilized by RL and the resveratrol-loaded stabilized by RL also tended to agglomerate and precipitate at pH 3 due to the weak electrostatic repulsion between nanoparticles, but they exhibited a uniform appearance under neutral and alkaline conditions, which are similar with our findings [21, 30]. Therefore, the property of zein nanoparticles stabilized by RL was highly pH-dependent.

Effect of Salt on the Stability of Ergocalciferol-Loaded Zein Nanoparticles

Throughout product development and the passage through the human gastrointestinal tract, nanoparticles may come

into contact with various electrolytes that can influence the properties of colloidal delivery systems. Therefore, it becomes essential to investigate the effects of ionic strength on nanoparticle stability. Figure 3 illustrates the effect of NaCl concentration on the particle size, ζ-potential, and turbidity of ML- and RL-stabilized zein nanoparticles. It was found that the size of ML-stabilized nanoparticles gradually increased from 57.35 ± 1.81 to 537.86 ± 56.48 nm, revealing that ionic strength plays a negative effect on the stability of ML-coated zein nanoparticles (Fig. 3a). This result can be attributed to the gradual reduction of electrostatic repulsion between ML-coated zein nanoparticles after the addition of salt (Fig. 3b), resulting in the nanoparticles struggling to maintain their most stable state. The addition of NaCl also adversely affected the stability of RL-coated zein nanoparticles, as indicated by the observed increase in particle size and the formation of zein precipitates (Fig. 3a and c). Interestingly, the stability of RL-coated zein nanoparticles against higher NaCl concentrations was found to be higher compared to lower concentrations, as evidenced by the formation of zein precipitates within the NaCl concentration range of 100 to 200 mM (Fig. 3c). However, the reason behind this phenomenon was still unclear. We hypothesized that at high salt concentrations (300–500 mM NaCl), the conformation of rhamnolipid molecules underwent a more compact folding, resulting in a thicker interfacial layer. This structural change potentially prevented the further aggregation or coalescence of nanoparticles, thereby enhancing the stability of the system.

Effect of Temperature on the Stability of Ergocalciferol-Loaded Zein Nanoparticles

Given the crucial role of temperature in food preparation, processing, and transportation, we investigated the impact of different temperatures on the stability of ergocalciferol-loaded zein nanoparticles. Figure 4a and b illustrate the particle size and ζ-potential of nanoparticles stabilized by ML or RL when subjected to temperatures ranging from 30 to 90 °C for 1 h. The particle size of the samples slightly increased to approximately 60 nm as the treatment temperature rose. The ζ-potential of ML-stabilized nanoparticles remained stable at -50 mV, while RL-stabilized nanoparticles exhibited minor fluctuations but remained relatively stable within the range of -45 to -52.5 mV. These results suggest that ML- and RL-stabilized zein nanoparticles can resist aggregation at different temperatures during thermal treatment, owing to their high absolute ζ-potential values that provide strong electrostatic repulsion between adjacent nanoparticles. Figure 4c displays the turbidity of ML- and RL-stabilized samples subjected to various temperatures. All samples remained transparent, and the letter "E" behind the samples was easily discernible within the dispersions.

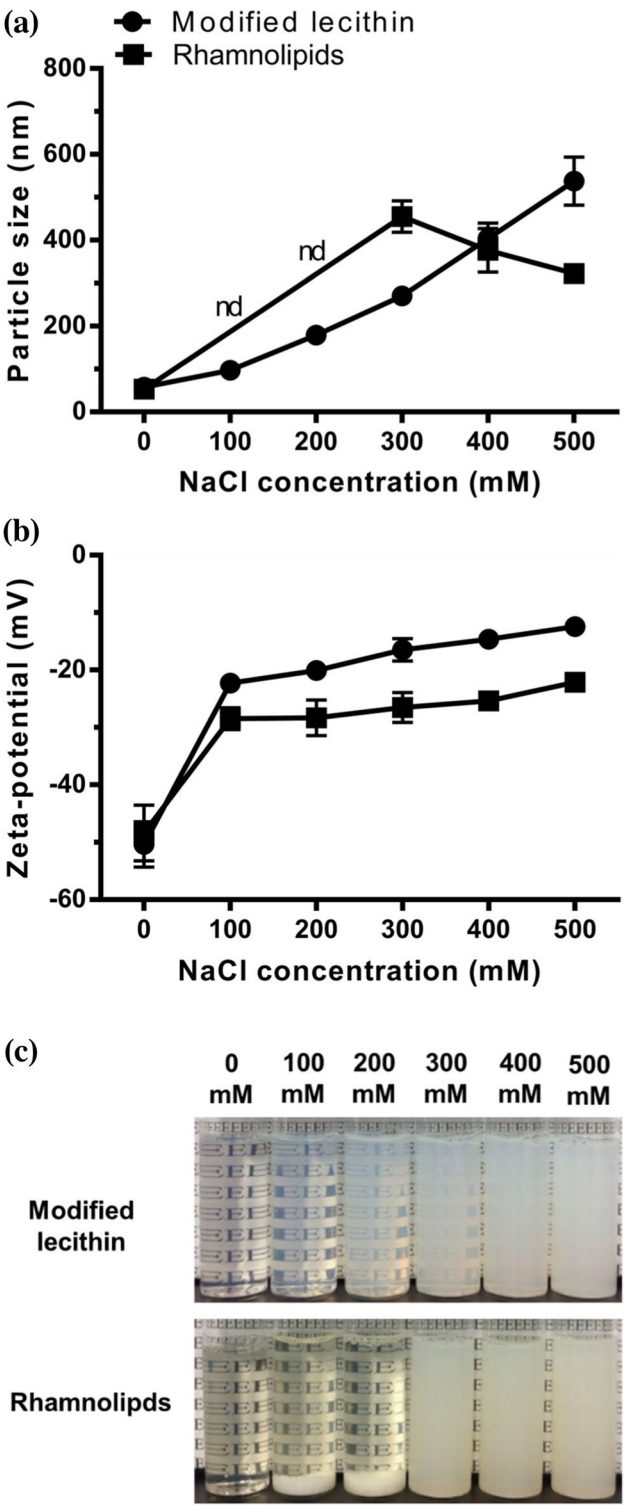

Fig. 3 The effect of ionic strength on the particle size (**a**), ζ-potential (**b**) and turbidity (**c**) of ML- and RL-stabilized zein nanoparticles encapsulating ergocalciferol

This observation indicates that no visual changes occurred in the dispersions following thermal treatment, and the dispersions exhibited low turbidity. Actually, the ML- and

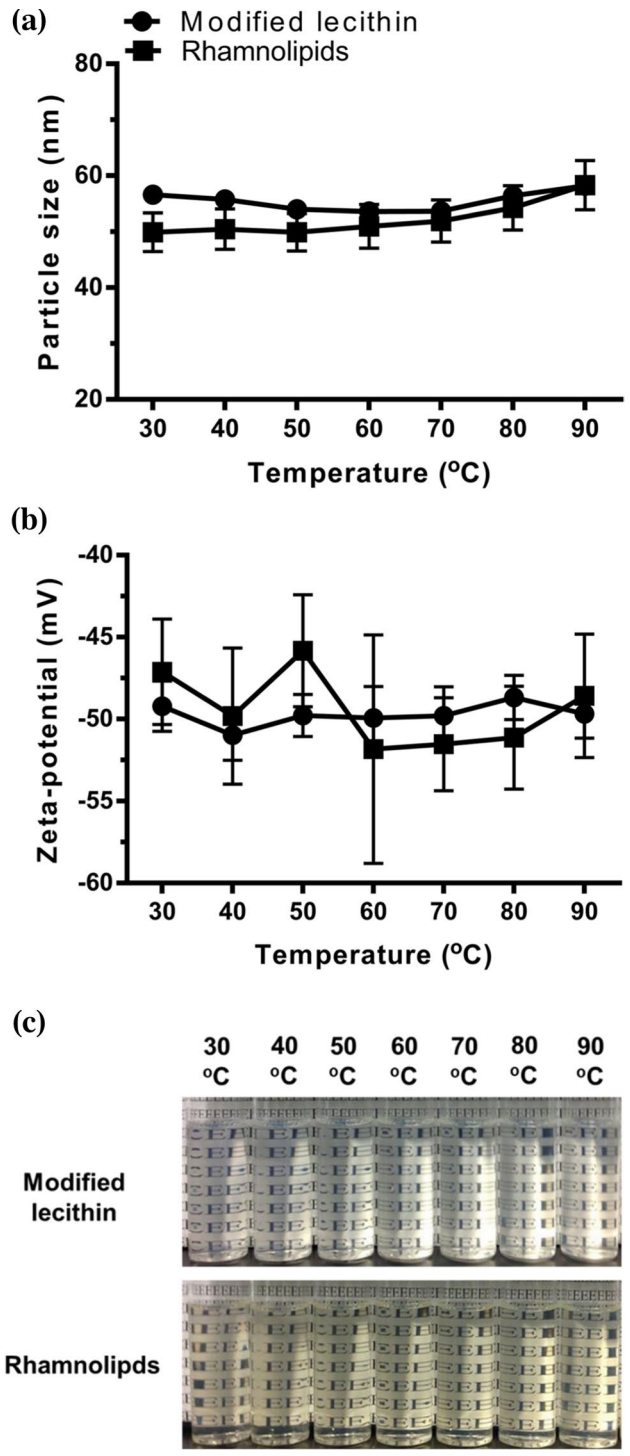

Fig. 4 The effect of thermal treatment on the particle size (**a**), ζ-potential (**b**) and turbidity (**c**) of ML- and RL-stabilized zein nanoparticles encapsulating ergocalciferol

RL- stabilized emulsions exhibited excellent stability under high temperature due to the high electrostatic repulsion, suggesting that the structure and properties of ML and RL can withstand common food processing temperatures [12, 24], which are similar with our findings. In addition, some surfactants or hydrocolloids like fucoidan, sodium caseinate, κ-carrageenan, chondroitin sulfate, and soluble soybean polysaccharide, have been used to coat on the surface of zein nanoparticles to improve their thermal stability by electrostatic repulsion and/or steric hindrance [18, 31–33]. Therefore, the thermal stability of zein nanoparticles could be improved through coating with other substance.

The Bioaccessibility and Long-Term Storage Stability of Ergocalciferol-Loaded Zein Nanoparticles

The bioaccessibility of ergocalciferol was determined by analyzing its concentration in the micellar phase after centrifugation of the raw digesta. Figure 5a presents the bioaccessibility of ergocalciferol in different zein nanoparticles stabilized by ML and RL following in vitro digestion. The ML-stabilized zein nanoparticles exhibited a relatively higher bioaccessibility of ergocalciferol (83.78 ± 3.66%) after in vitro digestion compared to those stabilized by RL (67.89 ± 10.43%). It is well documented that the hydrophobic bioactive compounds should be solubilized within the gastrointestinal fluids before absorption [34]. Solubilization typically entails the integration of bioactive molecules into the hydrophobic cores of mixed micelles, formed from bile salts, phospholipids, free fatty acids, and monoacylglycerols in gastrointestinal fluids. Moreover, the solubility of hydrophobic bioactive compounds in the gastrointestinal tract could also be enhanced if they have the ability to bind to nonpolar regions on the surfaces of proteins or peptides in the small intestine [35]. In our study, the zein nanoparticles were lipid-free, thus the most probable explanation for this phenomenon is the existence of bile salts and peptides hydrolyzed from zein in the simulated small intestinal fluids, which formed micelles or complexes that solubilized a portion of the ergocalciferol. Moreover, it has been previously reported that ergocalciferol can undergo degradation under acidic conditions and due to thermal effects during digestion, which could reduce the in vitro bioaccessibility of ergocalciferol [14].

The long-term storage stability of ergocalciferol-loaded zein nanoparticles was assessed by placing the samples at 4 °C in the dark for a period of 30 days. Figure 5b illustrates the physical and chemical stability of ML- and RL-stabilized zein nanoparticles loaded with ergocalciferol after the 30-day storage period. Both ML- and RL-stabilized zein nanoparticles maintained a consistent and similar particle size, indicating that both ML and RL are effective in formulating stable zein nanoparticles. The absence of changes in particle size can be attributed to the strong negative surface charge of ML-stabilized zein nanoparticles (-50.37 ± 3.93 mV) and RL-stabilized zein nanoparticles (-48.38 ± 4.87 mV), which inhibit particle aggregation or flocculation.

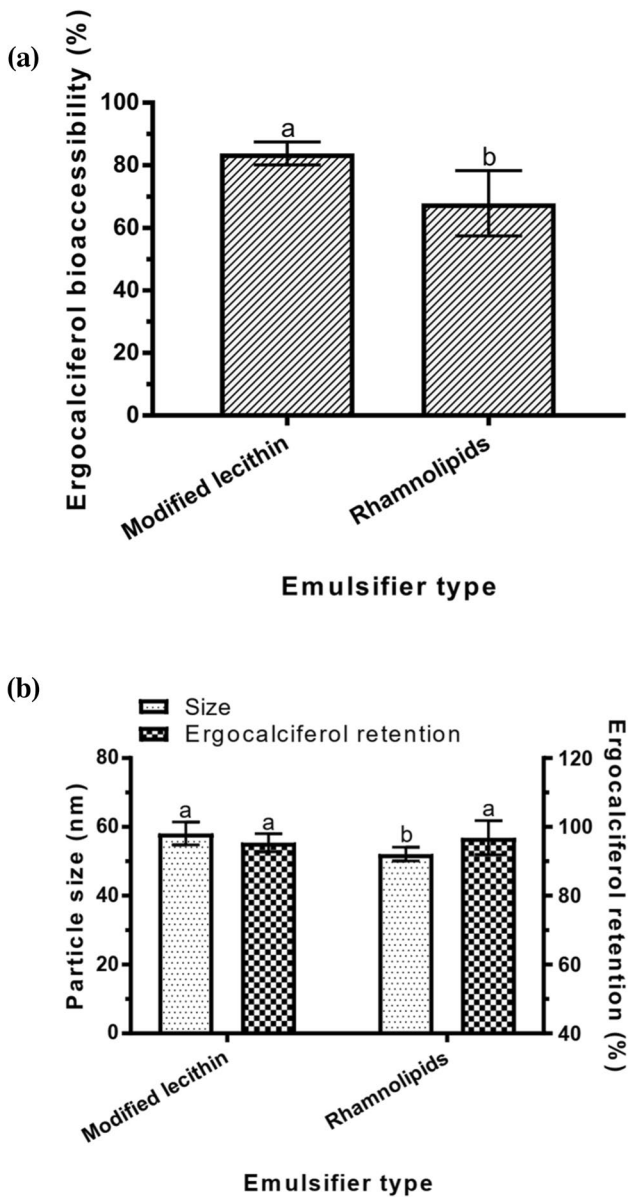

Fig. 5 The bioaccessibility of ergocalciferol after in vitro digestion (**a**) and the long-term storage stability (**b**) of ML- and RL-stabilized zein nanoparticles encapsulating ergocalciferol

Furthermore, the hydrophobic adsorption of ML and RL on the surface of zein nanoparticles provides additional steric repulsion between particles, thus preventing aggregation or flocculation. This combined effect of surface charge and hydrophobic adsorption contributes to the stability of the zein nanoparticles during storage.

The retention of ergocalciferol in ML- and RL-stabilized zein nanoparticles after long-term storage was also examined. It was observed that both ML- and RL-stabilized zein nanoparticles maintained a consistent ergocalciferol retention rate of approximately 94% after 30 days of storage at 4 °C (Fig. 5b). This finding suggests that the encapsulation of ergocalciferol in ML- and RL-stabilized zein nanoparticles effectively prevents its degradation.

Conclusion

In this study, we successfully achieved the encapsulation of ergocalciferol within zein nanoparticles stabilized by ML and RL using the anti-solvent precipitation method. The resulting nanoparticles exhibited a narrow particle size distribution and high encapsulation efficiency. Notably, the stability of ML-stabilized zein nanoparticles was hardly affected by thermal treatment and pH changes, but they became unstable with particle size increase after salt addition. The zein nanoparticles stabilized by RL demonstrated good stability when exposed to thermal treatments. However, they exhibited instability under acidic conditions as well as in the presence of NaCl. Our investigation of in vitro digestion revealed that the nanoparticles were digestible and capable of releasing ergocalciferol. Both ML- and RL-stabilized zein nanoparticles exhibited stable particle sizes and maintained high levels of ergocalciferol retention even after 30 days of storage at 4 °C. These findings contribute to the comprehensive utilization of zein nanoparticles stabilized by natural surfactants as carriers for encapsulating functional ingredients. Moreover, they provide valuable insights for the development of label-friendly commercial products fortified with ergocalciferol.

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Author Contributions Zhang Chen: Investigation, Data curation, Methodology, Writing - Original draft; Zhaoxiang Ma: Formal analysis and investigation, Methodology, Writing - Review & Editing; Jun He: Formal analysis and investigation, Data curation; Jinyi Song: Validation, Data curation; Jinyue Zhao: Visualization, Conceptualization; Yiguo Zhao: Supervision, Conceptualization, Funding acquisition, Writing - Review & Editing.

Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical Approval Not applicable, because this paper does not contain any studies with human or animal subjects.

Competing Interests The authors declare no competing interests.

Conflict of 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.

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