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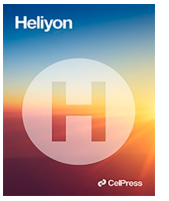

Research article

Nanoencapsulation of vitamin D_3 by ultrasonic pretreated zein hydrolysates: Stability improvement in food models

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ABSTRACT

This work is aimed to assess the effect of ultrasonic pre-treatment on the enzymatic hydrolysis of zein and the nanoencapsulation of vitamin D_3 ($VitD_3$) by zein hydrolysates (ZH). The ultrasonic pre-treatment significantly increased the degree of hydrolysis by and the α-helix, β-sheet, β-turns contents, and random coils were enhanced by ultrasonic pre-treatment. $VitD_3$ was successfully encapsulated by the developed ZH nanoparticles (NPs), with an encapsulation efficiency of 95.23 ± 1.78 %. The surface charge and particle size of the nanoparticles (NPs) were −5.45 ± 1.76 mV and 39.43 ± 7.96 nm, respectively. The detailed morphology study of NPs showed a regular spherical morphology, and the chemical structures of NPs were characterized by Fourier Transform Infrared Red spectroscopy. Additionally, the developed NPs were added to milk, which exhibited high stability after one month of storage. In conclusion, the $VitD_3$-loaded ZHNPs had considerable potential for fortifying different foodstuffs.

1. Introduction

Vitamin D_3 ($VitD_3$) as a fat-soluble vitamin has a vital role in calcium absorption and bone health [1]. Additionally, this vitamin is efficient in the prevention of chronic diseases such as cancer, hypertension, diabetes, and cardiovascular diseases [2]. $VitD_3$ can be synthesized in the skin cells under sunlight exposure or obtained from foods [3]. However, $VitD_3$ deficiency is very frequent in all age groups worldwide because of inadequate exposure to sunlight, insufficient nutritional uptake, and or the limitation of dietary sources of $VitD_3$ [4]. $VitD_3$ deficiency is caused by several chronic diseases such as osteoporosis, cardiovascular diseases, hypertension, and cancer [3]. In this regard, the fortification of food stuffs with $VitD_3$ is an efficient method to overcome this global health problem [1]. However, the fortification of food systems with $VitD_3$ is limited due to its low water solubility and high sensitivity against oxygen, light, and high temperature, which is caused by its low stability and low bioavailability [5,6]. An effective method for improving the stability and bioavailability of bioactive ingredients is nanoencapsulation [2]. The use of protein hydrolysates or peptides for the encapsulation of bioactive ingredients has garnered attention in recent years because of their many benefits, including high water solubility, low molecular weight, simple absorption, antioxidant activity, lack of adverse effects on the body, and affordability [7–9]. Zein, a

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by-product of modern maize processing, is the primary storage protein found in corn seeds [10]. This protein has a high concentration of nonpolar amino acids including leucine, proline, and alanine because it is soluble in alcohol [11]. The imbalanced amino acid composition and low water solubility of zein limit its application in encapsulation systems [6]. Zein also lacks essential nutrients because it contains less tryptophan and lysine [12]. However, zein hydrolysates (ZH) have considerable potential for application in colloidal encapsulation systems due to their vehicle superior surface activity and formation ability at the oil-water interface [12,13]. Moreover, the ZH shows health-beneficial effects that include anticarcinogenic, immunomodulatory, and angiotensin-converting enzyme (ACE) inhibitory properties [14]. According to previous literature, ZH was used for encapsulation of lutein [7], sage extract [15], curcumin [10,12], and tannic acid [11]. The traditional method of zein enzymatic hydrolysis has some drawbacks, such as a long time, a high enzyme level, and low substrate conversion rate [16]. To overcome these restrictions, ultrasonic pre-treatment is offered. The ultrasonic pre-treatment based on the cavitation phenomenon enhances the release of peptides with ACE-inhibitory activity from zein protein during enzymatic hydrolysis and improves the functionality of protein hydrolysates [17]. As the best of our studies, no research has been conducted on the utilization of ultrasonic-pretreated zein hydrolysates for nanoencapsulation of $VitD_3$. The purposes of this study are: a) assessing the effect of ultrasonic pre-treatment on the enzymatic hydrolysis of zein; and b) nanoencapsulation of $VitD_3$ by developed ZH and investigating the potential of this nanocomplex for milk enrichment as a food model.

2. Materials and methods

2.1. Materials

Sigma-Aldrich (St. Louis, USA) was the source of vitamin D3 and zein. Merck Chemicals Co. was the supplier of the remaining solvents and reagents (Darmstadt, Germany).

2.2. Ultrasound pretreatment of zein

Firstly, 200 mL of zein suspension (1 % w/v in water) was poured into a high-pressure-resistant bag. The sample was placed in an ultrasonic bath (Elma, Wetzikon, Switzerland). Then, the treatment was performed at five different fixed frequencies (22, 28, 33, 40, and 68 kHz) at 25 °C for 30 min. Then, the samples were centrifuged (Universal 320 centrifuge, Hettich, Germany) at 5000 rpm for 10 min, and the obtained zein samples were lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) at −60 °C for 48 h [16].

2.3. Enzymatic hydrolysis of zein

Phosphate buffered saline (PBS; 0.2 M, pH = 9) was used to dissolve the ultrasonic pretreated and untreated zein samples (5 % w/v). Then, the alcalase enzyme's main solution was made in PBS buffer (pH 9.0). The zein solutions were combined with the alcalase enzyme solution at a ratio of 2.5:100 g enzyme/g substrate. At 200 rpm and 50 °C, the enzyme hydrolysis was carried out with stirring for six distinct durations: 30, 60, 90, 120, 150, and 180 min. To cease the enzyme activity, the combinations were heated to 95 °C for 15 min. The hydrolysates were then allowed to cool before being centrifuged for 10 min at 5000 rpm. After being lyophilized for 48 h at −60 °C, the resulting supernatants were kept at −20 °C until needed [9].

2.4. Determination of degree of hydrolysis (DH)

Firstly, ZH suspensions were combined in an equal amount with trichloroacetic acid (TCA; 0.44 M). For 15 min, the resulting mixture was incubated at 4 °C. Subsequently, the mixture was centrifuged for 10 min at 10,000 rpm. The Bradford method was then used to ascertain the amount of protein in the supernatant (Bradford, 1976). Ultimately, the following equation was used to calculate the DH [18]:

$$\mathrm{DH}\ (\%) = \frac{\text{Protein value in the Supernatant}}{\text{Protein value in the ZH suspension}} \times 100 \quad (1)$$

2.5. Circular dichroism (CD) analysis

Using a CD spectropolarimeter (MOS-450, French Biologic Company, Grenoble, France), the secondary and tertiary structures of the zein samples were examined. A quartz cuvette with a 10-mm optical path length was used for the test, and it was run at 25 °C. The CD spectra were collected over the wavenumber range of 190–250 nm. Using the DICHROWEB process, the content of the protein secondary structure was extracted from the far-UV CD spectra [17].

2.6. Encapsulation of vitamin D_3 with ZH

Firstly, a $VitD_3$ solution (10 mg/1 mL) in absolute ethanol was prepared. The aqueous solution of the pretreated ZH with ultrasound was prepared at a concentration of 5 mg/mL. Then, 0.1 mL of $VitD_3$ solution was added dropwise into 2 mL of the prepared solutions of ZH under continuous stirring. The samples were stirred for 30 min and then centrifuged for 20 min at 10,000 rpm. The obtained supernatants as the produced nanoparticles (NPs) were lyophilized at −60 °C for 48 h [19].

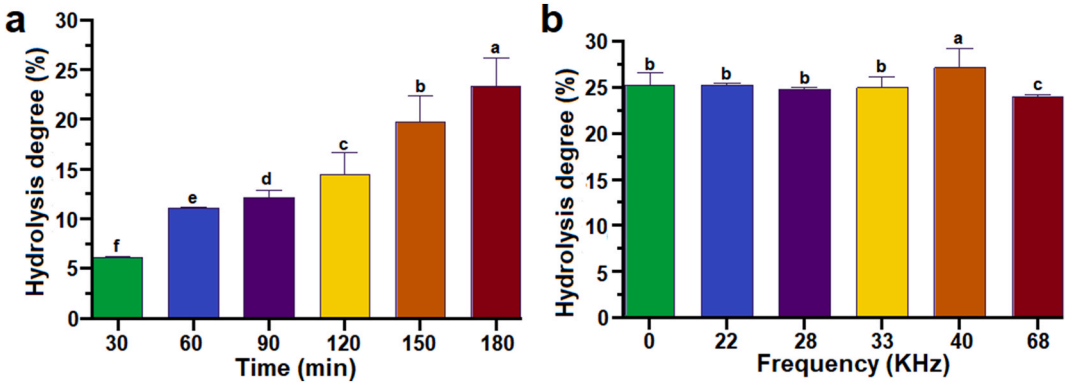

Fig. 1. The degree of hydrolysis (DH) of ZH catalyzed by alcalase at different times (a) and effect of ultrasonic frequency on degree of hydrolysis (DH). Data are expressed as mean ± standard deviation (n = 3) and different letters show significant difference at the 5 % level in Duncan's test ($p < 0.05$).

2.7. Encapsulation efficiency (EE) measurement

The EE was measured by the ultracentrifugation method. Firstly, 10 mg of NPs were dispersed in 1 mL of ethanol. After being transferred to an Amicon® filter (molecular weight cutoff of 30 kDa, Millipore, UK), the resulting dispersion was centrifuged for 10 min at 5000 rpm. The passed solution through the filter was separated and used to determine of the unloading of $VitD_3$ using UV–Vis spectroscopy (Ultrospec 2000, Biotech, UK) and the calibration curve of $VitD_3$ [6,20]. The EE was determined by the following equations:

$$EE(\%) = \frac{\text{Total VitD3 (w)} - \text{Free VitD3 (w)}}{\text{Total VitD3 (w)}} \times 100 \quad (2)$$

2.8. Particle size and zeta potential measurement

Using the photon correlation spectroscopy (PCS; Zetasizer Nano-ZS, Malvern, UK)) method, the mean particle size, polydispersity index (PDI), and zeta potential of NPs were determined.

2.9. Scanning electron microscopy (SEM)

The morphology of ZH as well as the particle shape and surface morphology of NPs were examined using the SEM (VEGA3, TESCAN, Czech Republic). The diluted samples were placed on a glass slide and allowed to dry at room temperature for this purpose. Following that, the samples were vacuum-sealed and covered in a thin coating of gold (100–150 A°) using the direct current sputtering process. Ten kV of accelerating voltage was used for the SEM.

2.10. Transmission electron microscopy (TEM)

The detailed morphology of NPs was examined using TEM (CM120, Philips, Germany). A drop of diluted samples was cast-dried on a copper grid prior to testing. A 200 kV accelerating voltage was used for the TEM.

2.11. Fourier transform Infrared red (FT-IR) spectroscopy

The chemical structures of NPs were studied by FT-IR spectroscopy (Equinox 55 LS 101, Bruker, Germany). Initially, KBr pellets were used to implant the lyophilized materials. The wavenumber range in which the spectra were obtained was 4000–400 cm^{-1}.

2.12. Stability study in simulated milk model

To evaluate the stability of NPs in the food model, the particle size, zeta potential, and EE of NPs were investigated in the simulated milk model after one month of storage.

2.13. Statistical analysis

IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, United States) was used to do a one-way analysis of variance (ANOVA) statistical analysis of the collected data. A 5 % significant threshold of *t*-test analysis was used to ascertain the differences between the

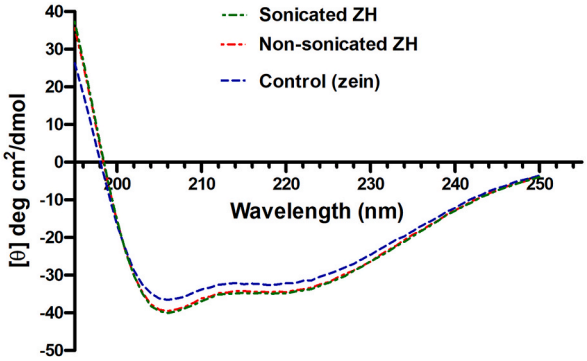

Fig. 2. Circular dichroism (CD) spectra of zein and ZH samples. ZH: zein hydrolysates.

Table 1
The contents of secondary structure of zein samples.

Samples	α-helix (%)	β-chain (β-sheet and β-turn) (%)	Unordered	Total (%)
Control	74.4 ± 1.2	8.3 ± 0.4	17.2 ± 0.7	99.9 ± 1.8
Non-sonicated ZH	75.1 ± 1.4	7.1 ± 0.6	17.2 ± 0.9	99.4 ± 1.5
Sonicated ZH	80.3 ± 1.2	10.3 ± 0.8	9.1 ± 0.8	99.7 ± 1.9

ZH: zein hydrolysates.

means.

3. Results and discussion

3.1. Characterization of zein hydrolysates

3.1.1. Determination of degree of hydrolysis (DH)

The hydrolysis process, which greatly affects the amino acid content, biological activity, and flavor of protein hydrolysates, is indicated by the DH [10]. The effect of hydrolysis time (without ultrasonic pre-treatment) on the DH was assessed, and the obtained results are shown in Fig. 1a. As a result, the DH exhibited a significant ($p < 0.05$) increasing trend with an increase in hydrolysis time. So that, the DH was enhanced from 6.11 ± 0.12 % at 30 min to 23.33 ± 3.19 % at 180 min. Similar results were observed in previous studies [12,21]. Since the DH is dependent on enzyme activity, longer alkaline catalytic activity periods result in more disruption of the native zein structure and peptide linkages, which raises the DH [22]. Furthermore, the effect of ultrasonic pre-treatment on the DH at a hydrolysis time of 180 min was investigated. The obtained results (Fig. 1b) exhibited that the ultrasound pre-treatment significantly enhanced the DH of zein. Additionally, increasing the ultrasonic frequency until 40 Hz increased the improving effect of ultrasound on the DH of zein (Fig. 1b). In this regard, the highest DH was attributed to the pre-treated sample with ultrasound at 40 Hz, whose value was 27.18 ± 2.01 %. Similarity, the improving effect of ultrasound pre-treatment on the DH of zein was reported by previous studies [16,17]. The cavitation bubbles produced by ultrasonic pre-treatment caused the rupture of protein granules and the breaking of protein chains. This phenomenon promotes the exposure of restriction sites and hydrophobic amino acids to the protein surface, resulting in an increase in the contact of the enzyme with active sites of protein and enhancing the enzymatic release of peptides [16]. The solubility and antioxidant activity of ZH have been observed to be enhanced by an increase in DH. Because peptide cleavage releases more charged groups like $NH3^+$ and COO^-, it enhances protein-water contact and electrostatic repulsion between peptides, which accounts for the increase in solubility caused by an increase in DH [22]. Furthermore, the enhancement of soluble protein fragments or peptides with antioxidant activity is responsible for the improvement of protein hydrolysates' antioxidant activity as indicated by an increase in DH. In accordance with our results, Tang et al. [21] reported that zein hydrolysates with a high DH show stronger antioxidant activities.

3.1.2. Circular dichroism (CD) analysis

The CD spectra of zein, un-pretreated ZH, and pretreated ZH with ultrasound are shown in Fig. 2. As shown in the figure, the spectra of the samples exhibited a positive peak at 195 nm and two negative peaks at 206 and 218 nm. These peaks indicate the presence of an α-helical secondary structure and the intensity of peaks reveals the α-helical content of the protein [16]. The differences among the spectra of samples are related to the intensity of the peaks. So that, the ultrasonic pretreatment and hydrolysis process increased the intensity of positive peaks but decreased the intensity of two negative peaks in comparison with pure zein. These results reflected the changes in the secondary structure of zein after the ultrasonic pre-treatment and hydrolysis processes. Table 1 presents the contents of the α-helix, β-sheet, and random coil of zein that were determined by applying the DICHROWEB procedure. As a result, the ultrasonic pre-treatment increased the percentage of α-helix, β-sheet, and β-turns. Similar results were reported in previous studies [17,23]. These

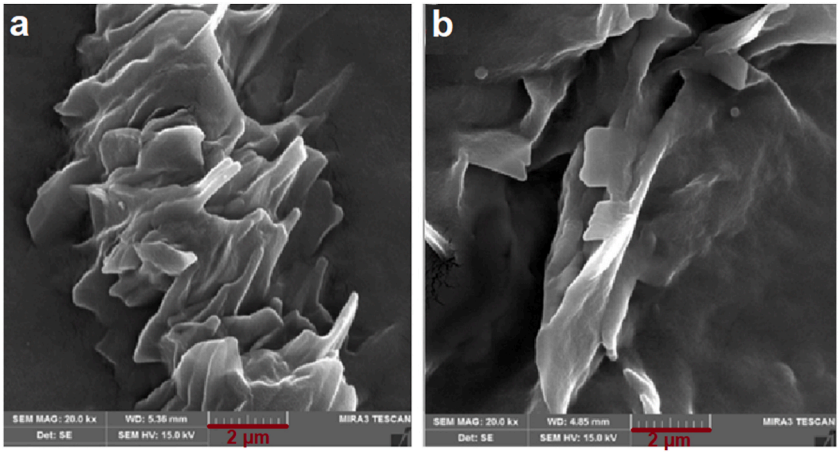

Fig. 3. The scanning electron microscopy (SEM) images of un-pretreated (a) and pretreated zein hydrolysates (b) with ultrasound.

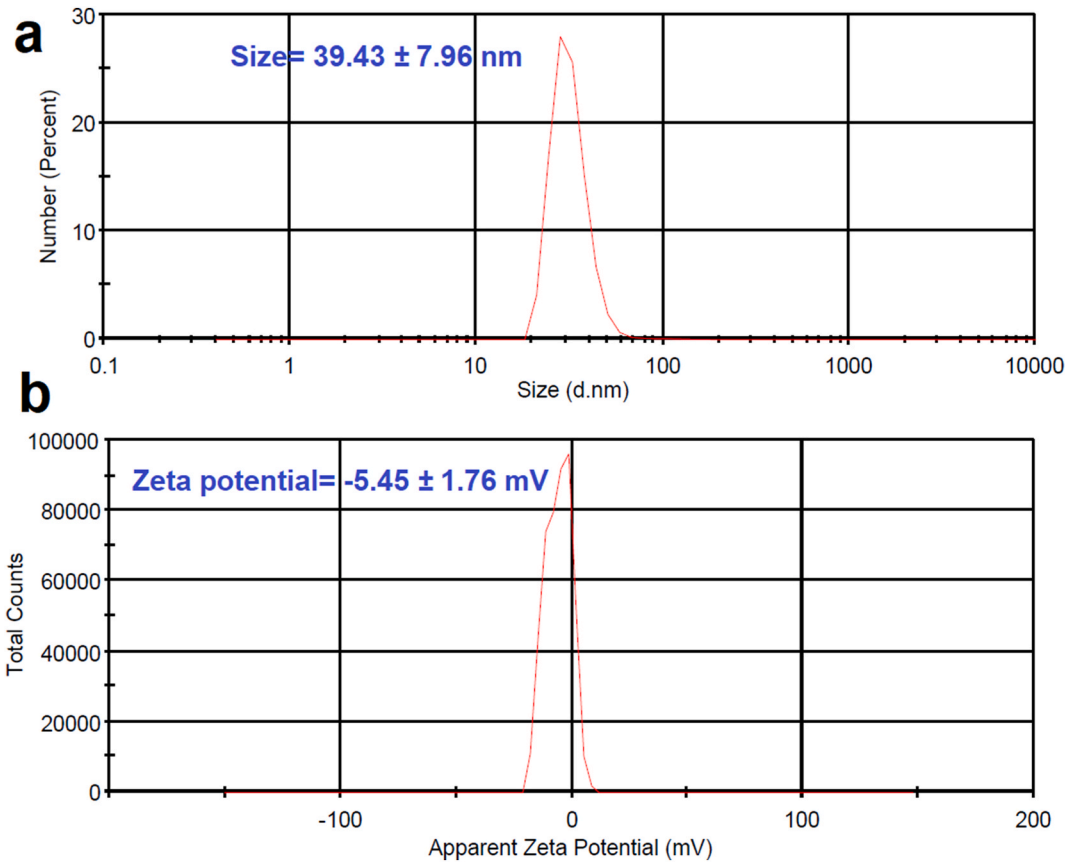

Fig. 4. Size profile (a) and zeta potential (b) of the $VitD_3$ loaded ZHNPs. Data are expressed as mean ± standard deviation (n = 3). Vit: vitamin; ZH: zein hydrolysates; NPs: nanoparticles.

findings can be explained by the exposure of hydrophobic amino acids in the core of the zein protein and the rearrangement of interactions between protein molecule chains due to the cavitation phenomenon created by ultrasonic pre-treatment.

3.1.3. Scanning electron microscopy (SEM)

Fig. 3a and b displays the SEM images of un-pretreated ZH and pretreated ZH with ultrasound, respectively. As illustrated in the figures, both ZH showed heterogeneous morphology, but the effect of ultrasonic pretreatment on the microstructure was obvious. So

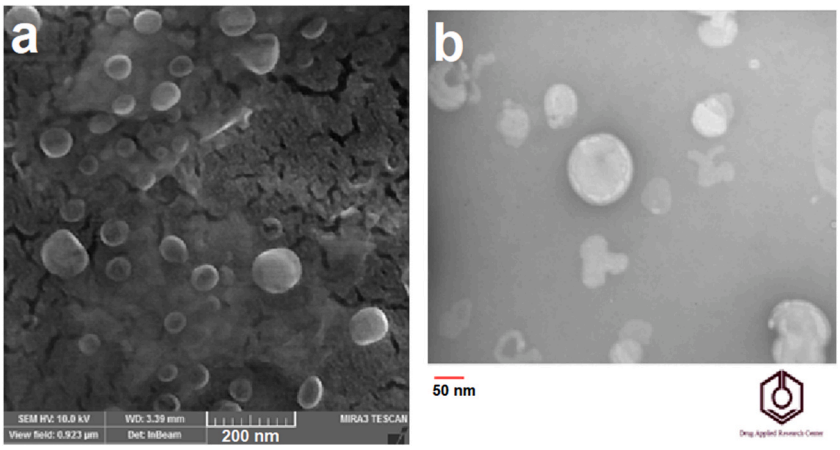

Fig. 5. The scanning electron microscopy (SEM) image (a) and the transmission electron microscopy (TEM) image (b) of the $VitD_3$ loaded ZHNPs. Vit: vitamin; ZH: zein hydrolysates; NPs: nanoparticles.

that, the pretreated ZH with ultrasound exhibited more rough structure and structural disruption. Moreover, a sponge-like texture can be seen in the SEM image of the pretreated ZH with ultrasound. The observed morphological differences correspond to the high DH of pretreated ZH and the resulted pressure by the cavitation phenomenon in this sample. The similar observations were seen by Ren et al. [16].

3.2. Characterization of nanoparticles

3.2.1. Encapsulation efficiency (EE)

The EE of biopolymer-based NPs mainly depends to the type of biopolymer and encapsulated agent, as well as preparation technique. The EE of $VitD_3$ loaded ZH NPs was obtained to be 95.23 ± 1.78 %. This value was higher than those reported for other ZHNPs and zein NPs. In line with this, it has been reported that the EE of the curcumin loaded ZHNPs [13], the encapsulated lutein with zein-derived peptides [7], and the $VitD_3$ loaded zein NPs [19] were 90, 92, and 52 %, respectively. By regarding the previous literature, encapsulation capacity of zein hydrolysates is higher than pure zein which this phenomenon can be described by the formation of more interactions between loaded compound and ZH due to the exposure of more functional groups of zein after enzymatic hydrolysis. In this case, the results of FT-IR showed the formation of interactions between $VitD_3$ and ZH which has been fully explained in Section 3.2.4. In accordance with our result, Lin et al. [6] reported that the EE of the $VitD_3$ loaded corn protein hydrolysate NPs is higher than 97 % and this high value is related to the high capability of interaction between vitamin and hydrolysates.

3.2.2. Particle size and zeta potential

The particle size of NPs is an important parameter that directly affects their stability, the release of loaded compounds, and overall bioavailability of the system as well as influence on the organoleptic properties of the fortified foodstuffs with NPs [19]. The particle size of the $VitD_3$ loaded ZHNPs was 39.43 ± 7.96 nm (Fig. 4a) that is lower than those reported by other studies for other ZHNPs and zein NPs. In this case, the particle size of the curcumin loaded ZHNPs [13], the encapsulated lutein with zein-derived peptides [7], and the $VitD_3$ loaded zein NPs [19] were higher than 50, 200 and 100 nm, respectively. Zeta potential defines the net surface charge of NPs and is another effective parameter on the colloidal stability of NPs [15]. The zeta potential of the $VitD_3$ loaded ZHNPs was −5.45 ± 1.76 mV (Fig. 4b). The previous studies reported different values for zeta potential of ZH based NPs. So that, the reported zeta potential for the curcumin loaded ZHNPs Wang et al. [12], the encapsulated lutein with zein-derived peptides [7], and the ZH based emulsions [9] are −45, −22, and −7 mV, respectively. Moreover, Lin et al. [6] reported that the zeta potential of the $VitD_3$ loaded corn protein hydrolysate NPs was lower than −25 mV and the colloidal stability of NPs was related to the electro-steric effect of corn protein hydrolysate. Similarity, the low zeta potential of the produced NPs in our study showed that the electrostatic repulsion did not play a dominant role on the colloidal stability of the produced NPs. Therefore, the stability of NPs against aggregation can be mainly attributed to the resulted steric hindrance by ZH.

3.2.3. Morphology study

Fig. 5a and b depicts the SEM and TEM images of $VitD_3$ loaded ZHNPs, respectively. The SEM image exhibited that the NPs have a regular spherical structure that the particle size of most of them was lower than 100 nm. This result is in line with the result of PCS. Moreover, no aggregation and fusion were seen in the SEM image and the NPs were good dispersed. Furthermore, the spherical shaped NPs with diameter lower than 100 nm are obvious in the TEM image. These findings indicated the successfulness of used method in the encapsulation of $VitD_3$ with ZH. Similarity, Luo et al. [19] reported that the SEM image of $VitD_3$ loaded zein NPs showed a spherical shape and smooth surfaces with particle size around 100 nm. Wang et al. [12] also reported that the curcumin loaded ZHNPs have a spherical structure with small diameter lower than 50 nm. Moreover, nano-scaled particles with spherical structure have been

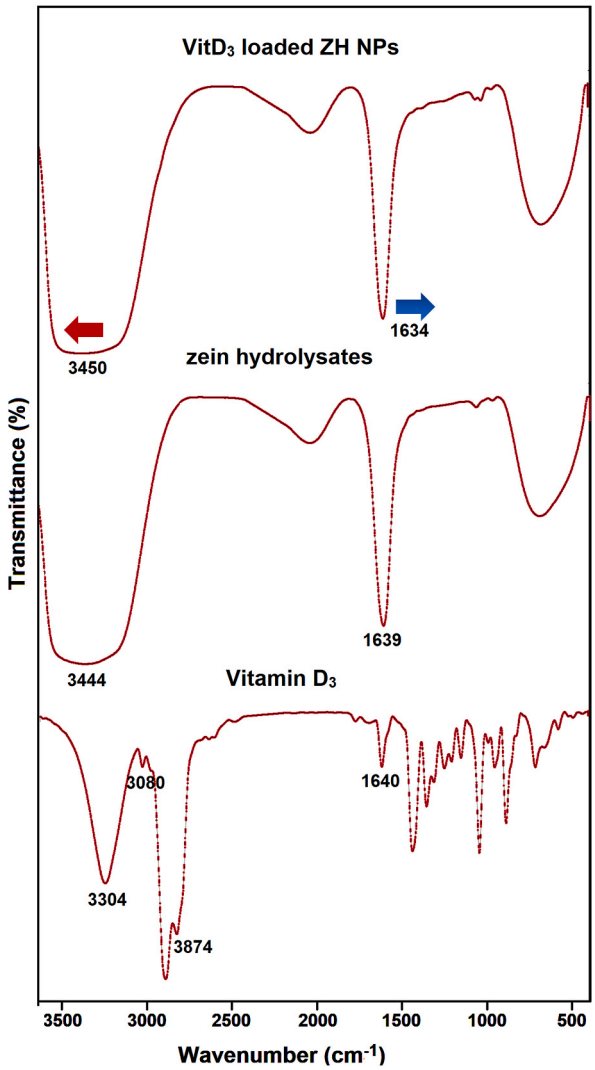

Fig. 6. Fourier transform Infrared (FT-IR) spectra of vitamin D_3, zein hydrolysates and the $VitD_3$ loaded ZHNPs. Vit: vitamin; ZH: zein hydrolysates; NPs: nanoparticles.

observed in the TEM images of $VitD_3$ loaded corn protein hydrolysate [6].

3.2.4. *Chemical structure*

Fig. 6 shows the FT-IR spectra of vitamin D_3, ZH and the $VitD_3$ loaded ZHNPs. In the spectrum of vitamin D_3, the important absorption peaks were observed at 3304 cm^{-1} (for stretching vibration of O–H bonds), 3080 and 2874 cm^{-1} (for CH_3 symmetric stretching and CH_2 asymmetric stretching), and 1640 cm^{-1} (for C=O stretching vibration) [2]. Moreover, the spectrum of the ZH exhibited several specified peaks that the important ones include: 1) The peak at 3444 cm^{-1} that attributed to the O-H stretching of the amino acids; and 2) the peak at 1639 cm^{-1} which represented the strong absorption bonds of amide I [7]. However, the spectrum of $VitD_3$ loaded ZHNPs showed spectral differences in compared to the spectrum of ZH, so that the peaks at 3444 and 1639 cm^{-1} were shifted to 3450 and 1634 cm^{-1}, respectively. Moreover, the specified peaks of $VitD_3$ were disappeared in the spectrum of $VitD_3$ loaded ZHNPs. These changes approved the formation of interactions (hydrogen bonds) between $VitD_3$ and ZH which indicated that hydrogen bonding is the main force facilitating NPs formation. However, the hydrophobic interactions also could be played an important role in the formation of $VitD_3$ loaded ZHNPs due to the hydrophobic nature of $VitD_3$ and zein. Similar spectral changes have been observed by previous studies after encapsulation of $VitD_3$ with zein [19] and corn protein hydrolysate [6].

3.2.5. *Stability study in simulated milk model*

The particle size, zeta potential and EE of the $VitD_3$ loaded ZHNPs at the common pH values of milk (pH = 6.5–6.7) after one-month storage are illustrated in Fig. 7. The particle size of NPs showed no significant difference in comparison with the initial particle size of

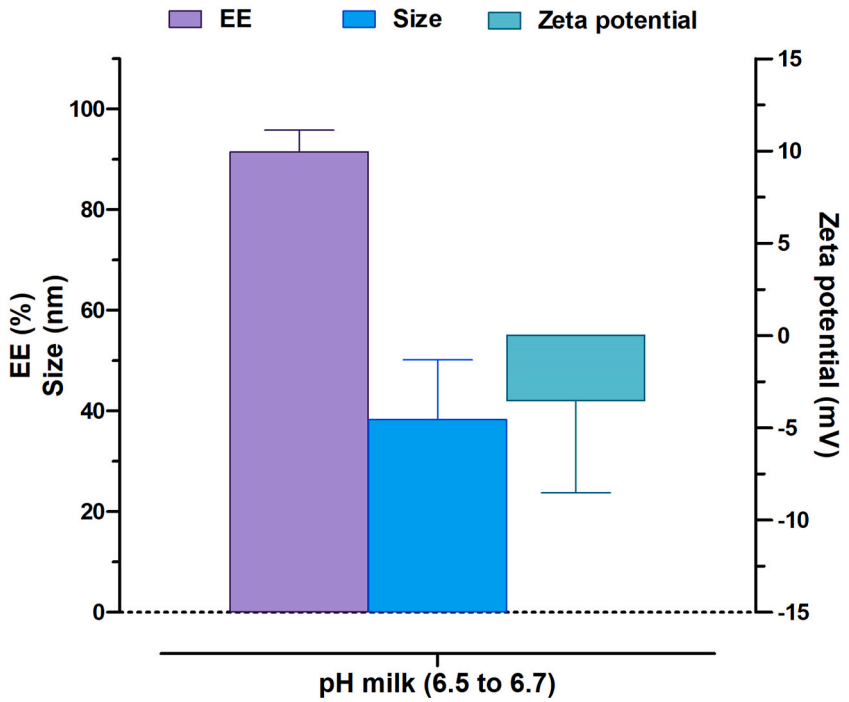

Fig. 7. The effects of pH change on the particle size, zeta potential and EE of the $VitD_3$ loaded ZH NPs. Data are expressed as mean ± standard deviation (n = 3). *p < 0.05; EE: encapsulation efficiency; Vit: vitamin; ZH: zein hydrolysates; NPs: nanoparticles.

the NPs and its value was 38.31 ± 11.85 nm. The zeta potentials of the NPs showed no significant difference after one-month storage at pH conditions of milk. Furthermore, the EE of the NPs showed no significant difference after one-month storage at pH condition of milk. In contrast with our findings, it has been reported that the particle size of ZHNPs was increased from nanoscale to microscale at milk pH [13]. However, Chuacharoen [24] reported that the addition of curcumin loaded zein nanoparticles in milk matrix improved the physical and chemical stability of particles in gastrointestinal condition.

4. Conclusion

The results of different analyses showed that the ultrasonic pre-treatment significantly improved the enzymatic hydrolysis of zein. Furthermore, $VitD_3$ was successfully encapsulated by pretreated ZH with high EE. The formation of interactions between $VitD_3$ and ZH was approved by FT-IR structural conformations. Moreover, the particle size and morphology of the developed NPs were appropriate and satisfactory. The stability study showed that the developed NPs have high stability at the common pH conditions of milk and during long storage times. Generally, the results of this study exhibited that the $VitD_3$-loaded ZHNPs have considerable potential for food fortification.

CRediT authorship contribution statement

Bahare Sadr: Writing – original draft, Methodology, Investigation. **Mahnaz Tabibiazar:** Writing – review & editing, Supervision, Investigation, Data curation, Conceptualization. **Ainaz Alizadeh:** Visualization, Validation, Supervision, Project administration, Investigation, Formal analysis. **Hamed Hamishehkar:** Validation, Methodology, Investigation. **Leila Roufegarinejad:** Validation, Investigation. **Sajed Amjadi:** Writing – review & editing, Investigation.

Data availability statement

Data will be made available on request.

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.

References

[1] Y. Tan, J. Liu, H. Zhou, J. Muriel Mundo, D.J. McClements, Impact of an indigestible oil phase (mineral oil) on the bioaccessibility of vitamin D_3 encapsulated in whey protein-stabilized nanoemulsions, Food Res. Int. 120 (2019) 264–274, https://doi.org/10.1016/j.foodres.2019.02.031.

[2] K. Liu, X.L. Kong, Q.M. Li, H.L. Zhang, X.Q. Zha, J.P. Luo, Stability and bioavailability of vitamin D_3 encapsulated in composite gels of whey protein isolate and lotus root amylopectin, Carbohydrate Polym. 227 (2020) 115337, https://doi.org/10.1016/j.carbpol.2019.115337.

[3] S. Mahdi Jafari, S. Masoudi, A. Bahrami, A Taguchi approach production of spray-dried whey powder enriched with nanoencapsulated vitamin D_3, Drying Technol 37 (2019) 2059–2071, https://doi.org/10.1080/07373937.2018.1552598.

[4] S. Jiang, G. Yildiz, J. Ding, J. Andrade, T.M. Rababahb, A. Almajwalc, M.M. Abulmeatyc, H. Feng, Pea protein nanoemulsion and nanocomplex as carriers for protection of cholecalciferol (vitamin D_3), Food Bioproc, Technol. 12 (2019) 1031–1040, https://doi.org/10.1007/s11947-019-02276-0.

[5] E. Hasanvand, M. Fathi, A. Bassiri, Production and characterization of vitamin D_3 loaded starch nanoparticles: effect of amylose to amylopectin ratio and sonication parameters, J. Food Sci. Technol. 55 (2018) 1314–1324, https://doi.org/10.1007/s13197-018-3042-0.

[6] Y. Lin, Y.H. Wang, X.Q. Yang, J. Guo, J.M. Wang, Corn protein hydrolysate as a novel nano-vehicle: enhanced physicochemical stability and *in vitro* bioaccessibility of vitamin D_3, LWT-Food Sci. Technol. 72 (2016) 510–517, https://doi.org/10.1016/j.lwt.2016.05.020.

[7] Y. Jiao, X. Zheng, Y. Chang, D. Li, X. Sun, X. Liu, Zein-derived peptides as nanocarriers to increase the water solubility and stability of lutein, Food Funct. 9 (2018) 117–123, https://doi.org/10.1039/c7fo01652b.

[8] L. Zhou, G. Xu, Z. Zhang, H. Li, P. Yao, Surface activity and safety of deamidated zein peptides, Colloids and Surfaces A: Physicochem. Eng. Aspects 540 (2018) 150–157, https://doi.org/10.1016/j.colsurfa.2017.12.070.

[9] Y. Li, B. Kong, Q. Liu, X. Xia, H. Chen, Improvement of the emulsifying and oxidative stability of myofibrillar protein prepared oil-in-water emulsions by addition of zein hydrolysates, Process Biochem 53 (2017) 116–124, https://doi.org/10.1016/j.procbio.2016.11.010.

[10] R. Pan, Y. Zou, J. Wang, Z. Wan, J. Guo, J. Yang, X. Yang, Gamma/alpha-zein hydrolysates as oral delivery vehicles: enhanced physicochemical stability and *in vitro* bioaccessibility of curcumin, International J. Food Sci. Technol. 53 (2018) 1622–1630, https://doi.org/10.1111/ijfs.13744.

[11] Y.H. Wang, Z.L. Wan, X.Q. Yang, J.M. Wang, J. Guo, Y. Lin, Colloidal complexation of zein hydrolysate with tannic acid: constructing peptides-based nanoemulsions for alga oil delivery, Food Hydrocoll 54 (2016) 40–48, https://doi.org/10.1016/j.foodhyd.2015.09.020.

[12] Y.H. Wang, J.M. Wang, X.Q. Yang, J. Guo, Y. Lin, Amphiphilic zein hydrolysate as a novel nano-delivery vehicle for curcumin, Food Funct. 6 (2015) 2636–2645, https://doi.org/10.1039/c5fo00422e.

[13] Y.H. Wang, J.M. Wang, J. Guo, Z.L. Wan, X.Q. Yang, Amphiphilic zein hydrolysate as a delivery vehicle: the role of xanthophylls, LWT-Food Sci. Technol. 79 (2017) 463–470, https://doi.org/10.1016/j.lwt.2017.01.057.

[14] Q. Liang, M. Chalamaiah, X. Ren, H. Ma, J. Wu, Identification of new anti-inflammatory peptides from zein hydrolysate after simulated gastrointestinal digestion and transport in Caco-2 cells, J. Agri. Food Chem. 66 (2018) 1114–1120, https://doi.org/10.1021/acs.jafc.7b04562.

[15] Y. Li, H. Liu, Q. Liu, B. Kong, X. Diao, Effects of zein hydrolysates coupled with sage (*salvia officinalis*) extract on the emulsifying and oxidative stability of myofibrillar protein prepared oil-in-water emulsions, Food Hydrocoll 87 (2019) 149–157, https://doi.org/10.1016/j.foodhyd.2018.07.052.

[16] X. Ren, H. Ma, S. Mao, H. Zhou, Effects of sweeping frequency ultrasound treatment on enzymatic preparations of ACE-inhibitory peptides from zein, European Food Res. Tech. 238 (2014) 435–442, https://doi.org/10.1007/s00217-013-2118-3.

[17] X. Ren, X. Zhang, Q. Liang, T. Hou, H. Zhou, Effects of different working modes of ultrasound on structural characteristics of zein and ace inhibitory activity of hydrolysates, J. Food Quality 2017 (2017), https://doi.org/10.1155/2017/7896037.

[18] K. Sarabandi, S.M. Jafari, M. Mohammadi, Z. Akbarbaglu, A. Pezeshki, M. Khakbaz Heshmati, Production of reconstitutable nanoliposomes loaded with flaxseed protein hydrolysates: stability and characterization, Food Hydrocoll 96 (2019) 442–450, https://doi.org/10.1016/j.foodhyd.2019.05.047.

[19] Y. Luo, Z. Teng, Q. Wang, Development of zein nanoparticles coated with carboxymethyl chitosan for encapsulation and controlled release of vitamin D_3, J. Agric. Food Chem. 60 (2012) 836–843, https://doi.org/10.1021/jf204194z.

[20] D. Pauluk, A.K. Padilha, N.M. Khalil, R.M. Mainardes, Chitosan-coated zein nanoparticles for oral delivery of resveratrol: formation, characterization, stability, mucoadhesive properties and antioxidant activity, Food Hydrocoll 94 (2019) 411–417, https://doi.org/10.1016/j.foodhyd.2019.03.042.

[21] N. Tang, H. Zhuang, Evaluation of antioxidant activities of zein protein fractions, J. Food Sci. 79 (2014) C2174–C2184, https://doi.org/10.1111/1750-3841.12686.

[22] B. Kong, Y.L. Xiong, Antioxidant activity of zein hydrolysates in a liposome system and the possible mode of action, J. Agric. Food Chem. 54 (2006) 6059–6068, https://doi.org/10.1021/jf060632q.

[23] J. Jin, H. Ma, K. Wang, A.E.G.A. Yagoub, J. Owusu, W. Qu, R. He, C. Zhou, X. Ye, Effects of multi-frequency power ultrasound on the enzymolysis and structural characteristics of corn gluten meal, Ultrason. Sonochem. 24 (2015) 55–64, https://doi.org/10.1016/j.ultsonch.2014.12.013.

[24] T. Chuacharoen, C.M. Sabliov, The potential of zein nanoparticles to protect entrapped β-carotene in the presence of milk under simulated gastrointestinal (GI) conditions, LWT 72 (2016) 302–309, https://doi.org/10.1016/j.lwt.2016.05.006.