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# Zein-based Core-Shell Microcapsules for Potential Delivery of Algae Oil and Lipophilic Compounds

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**Running title:** Tunable shell thickness emulsion from zein

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## Abstract

Due to the benefits in brain development and cardiovascular health, algae oil which is rich in omega-3 fatty acids have become a popular dietary supplement in recent years. However, incompatibility with water and poor oxidative stability limits its addition in foods and beverages. In this work, formation and characterization of zein-based core-shell microcapsules with tunable shell thickness for potential delivery of algae oil were reported. They were prepared based on the *in situ* precipitation of zein from continuous phase onto the surface of oil droplets by self-assembly at a refrigerated temperature without any needs of surfactants and cross-linkers. The shell thickness could be controlled by the ratio of algae oil to zein (O/Z), which was indicated by their characterization including static light scattering, confocal laser scanning microscopy and scanning electron microscopy. Evidence from the peroxide value and hexanal level of the emulsions in a thermally accelerated storage test suggested that the oxidation degree of the loading algae oil was negatively correlated with the shell thickness of the capsules. Furthermore, the release profiles of vanillin, which was used as a model volatile lipophilic compound and masking agent for the off-flavor from algae oil, could be tuned by manipulating their shell thickness. This suggested that the all-natural edible core-shell microcapsule might further be used as a model system for delivery of lipophilic compounds. They may further promote the sustainable use of underutilized water-insoluble proteins as functional biomaterials in functional foods and pharmaceutical applications.

**Keywords:** zein, core-shell microcapsule, algae oil, tunable shell, oxidation stability

## Introduction

Emulsions generated from natural materials play an important role in the formulation, delivery and controlled release of functional materials in pharmaceuticals, biotechnology, cosmetics, food and agriculture.<sup>1-4</sup> In food industry, emulsions have been used to fortify lipophilic bioactive ingredients in foods such as docosahexaenoic acid (DHA), fat-soluble vitamins, phytosterols, carotenoids and fragrance.<sup>3-5</sup> However, an incorporation of these active compounds to foods may be restricted by their physicochemical properties, destabilization under certain conditions, unfavorable organoleptic properties (e.g. off-flavours and undesired appearance), low bioavailability, bioaccessibility and membrane permeability. Therefore, an efficient emulsion delivery system needs to focus on materials and synthesis routes with potential protection, delivery and controlled release features.

Lately, fabrication of novel carriers with natural biopolymers from sustainable sources is a fast-growing field.<sup>6-7</sup> Zein, a class of water-insoluble prolamine protein obtained from corn gluten meal, is composed of approximately equal amounts of hydrophilic and hydrophobic amino acid residues.<sup>8</sup> This amphiphilic attribute provides enormous potential for zein to self-assemble into various microstructures, including spheres, rods and films,<sup>9-10</sup> and makes it a potential candidate for prepare emulsion delivery systems for functional ingredients, such as polyphenols, essential oils and natural fragrances.<sup>8-9,11-12</sup>

Filippidi *et al.*<sup>13</sup> had recently reported an oil-filled zein-shell microcapsule. Its preparation was based on the amphipathic attribute of zein and its unique solubility profile in water-ethanol mixtures, which was finished after a 2-hour solidification for the zein-shell at 40 °C and a rotary evaporation for solvent removal. As for the thermosensitive components like algae oil, which is now a popular non-animal source of omega-3 fatty acids for dietary supplementation, these procedures might

provide an opportunity for the trigger of lipid oxidation.<sup>14</sup> Since it might lower the bioavailability of the polyunsaturated fatty acids (PUFA) and generate off-flavor that affects customer acceptance, the synthesis strategy for zein-based core-shell microcapsules need to be further developed. On the other hand, acetic acid was reported to be a better solvent to dissolve zein than aqueous ethanol solution.<sup>15,16</sup> However, it has not been widely used in the preparation of zein particles via anti-solvent precipitation till recently. In this work, acetic acid was employed to prepare the binary solution for zein. And algae oil was served as the loading oil-core and carrier for the lipophilic compounds. To minimize the oxidation of the oil phase and volatilization of the loading substance, a simple *in situ* precipitation of zein was conducted at a refrigerated temperature (4 °C) and short time after the algae oil emulsion stabilized by zein was obtained. Moreover, centrifugation rather than rotary evaporation was performed to remove the excess solvent. The shell thickness was controlled by the amount of precipitated protein and characterized by static light scattering, confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). Due to the core-shell morphology, large amounts of omega-3 fatty acids can be encapsulated in a controlled and reproducible fashion; and the effect of shell thickness on lipid oxidation was illustrated. Further, as carrier, we also evaluated the release of vanillin encapsulated in zein-based emulsion, which was set as a model of lipophilic compounds and played a part in masking the undesired flavor derived from algae oil.

## 2 Materials and methods

### 2.1 Materials

Zein (product Z 3625) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Algae oil with a peroxide value of 1.5 m equiv. O<sub>2</sub> kg<sup>-1</sup> and containing DHA ≥40% according to the

manufacturer's specifications, was supplied by Runke bioengineering Co., Ltd (China). Vanillin and the fluorescent dyes (Nile Blue A and Nile Red) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Water was purified by a Milli-Q reagent water system. All other chemicals used were of analytical grade.

## 2.2 Turbidity measurement

To determine an appropriate acetic acid-water solution concentration for emulsion preparation, turbidities of zein in different concentration of acetic acid solutions were measured using a HACH 2100N turbidimeter (Hach Co, Loveland, Colo., USA) with a range of 0-8,000 NTU (Nephelometric Turbidity Units). Briefly, different quantity of zein was dissolved in 10 mL acetic acid under constant stirring for 2 h. Then corresponding amount of deionized water was added dropwise under stirring (1,000 r/min) to make the final samples with 1% zein in different proportion of acetic acid-water solution. The turbidities were measured in triplicates and the average was reported.

## 2.3 Preparation of core-shell emulsions

The emulsions were fabricated by an *in situ* precipitation technology. Firstly, 1.0 g of zein powder was dissolved in 4.0 g acetic acid and stirred for 2 h at room temperature to complete hydration. Depending on the targeted shell thickness, 1-10 parts in weight of the algae oil were mixed with 1 part of the stock solution (5.0 g) based on the mass ratio of oil and protein. Then, the mixture was emulsified with an Ultraturax T25 homogenizer (IKA-Werke GmbH & Co., Germany) at 4,500 r/min for 30 s. Since no surfactant was added, constant stirring was necessary to prevent the oil drops from coalescing. In the meanwhile, water (20.0 g) was added drop-wise from a peristaltic pump (LSP02-1B, Longer Precision Pump Co. Ltd., China) at an approximate rate of 1.0 g/min. The temperature of the emulsion was controlled with a water bath at  $4 \pm 0.5^{\circ}\text{C}$  during the 20 min of the

109 preparation. When the weight of the added water quadrupled that of the initial weight, the point on  
110 the zein-water-acetic acid phase diagram had moved from the solution phase and deep into the  
111 precipitation phase due to the acetic acid concentration reduction. At this point, we stopped the  
112 process and centrifuge-washed excess acetic acid at 4,000 r/min and 4°C for 10 min for three times.

## 113 2.4 Chromatographic analysis of acetic acid

114 A static headspace gas chromatographic (HS-GC) procedure, which was described by Cruwys  
115 *et al.*<sup>17</sup> and Adorno *et al.*<sup>18</sup>, was applied for the measurement of residual acetic acid content in the  
116 emulsions. In specific, headspace chromatographic analysis was performed using a Combi-PAL  
117 automatic headspace sampler (CTC Analytics AG, Zwingen, Switzerland) connected to an Agilent  
118 7890B gas chromatography system (USA). 5 g of samples were added into standard 22.3 mL glass  
119 vials with PTBE septa and patented closures tightly sealed. After cultivated at room temperature for  
120 24 h, samples were measured with HS-GC analysis under the procedure as follows. 1.5 mL of the  
121 headspace vapor was directly transferred into the GC unit equipped with a HP-5 capillary column  
122 (30 m × 0.25 mm i.d.; 0.25 μm film thickness). The GC system was programmed to heat the column  
123 from 60 to 190 °C at 10 °C/min; the temperature was held at 190 °C for 1 min. Analyses were carried  
124 out using a flame ionization detector (FID) set at 250 °C.

## 125 2.5 Droplet size measurement

126 Size distribution of the droplets in emulsion was determined by static light scattering using a  
127 Malvern MasterSizer 3000 (Malvern Instruments Ltd., Worcestershire, UK). Emulsions were  
128 dropped into a water-filled measuring cell and the refractive indices of water and algae oil was set as  
129 1.33 and 1.52, respectively. All the measurements were carried out with three replicates at 25°C.

## 130 2.6 Morphology observation

*Confocal Laser Scanning Microscopy (CLSM)*. CLSM experiments were performed according to the protocol reported by Chen *et al.*,<sup>19</sup> using a TCS SP5 confocal microscopy (Leica Microsystems Inc., Germany). Emulsions were labeled by a hybrid dye solution (1 mg/mL Nile Red and 1 mg/mL Nile Blue A in isopropyl alcohol). Then the stained emulsions were placed on concave slides with coverslips covered. An argon krypton laser having an excitation line of 488 nm and a helium neon laser (He/Ne) with excitation at 633 nm were used in the observation. In the shell thickness measurement, more than 100 droplets in the range of confocal images were chosen for calculation, and their profiles with a Gaussian model were fitted according to the protocol reported by Filippidi *et al.*<sup>13</sup>

*Scanning Electron Microscopy (SEM)*. For SEM, cyclohexane, a kind of volatilized oil, was utilized as a substitution for algae oil to prepare the emulsions and dried at 70 °C afterwards. The dried powder was glued with a conductive adhesive on a stainless steel stage, and then coated with a thin conductive gold and platinum layer using a sputter coater (Hummer XP, Anatech, Union City, CA, USA). The particles were imaged with a field emission scanning electron microscope (SEM, LEO 1530 VP, Oberkochem, Germany).

## 2.7 Lipid oxidation measurement

The algae oil emulsions were prepared according to the above-mentioned process. The fresh emulsions were diluted with water to the same oil fraction and then placed in a seal screw-cap glass bottle. They were kept in an oven at 50 °C to accelerate the oxidation of algae oil for determination of the primary oxidant products (peroxide value, PV) and secondary oxidant products (headspace hexanal) according to the method described by Chen *et al.*<sup>20</sup>

*Spectrophotometric determination of peroxide value (PV)*. First, a thorough mixing of emulsion

sample (0.2 mL) and 1.5 mL of isooctane/2-propanol (3:1, v/v) was conducted. Centrifugation (1,000g, 2 min) was implemented and 200  $\mu$ L supernatant was sequentially mixed with 2.8 mL of methanol/1-butanol (2:1, v/v), 15  $\mu$ L of 3.94 mol/L ammonium thiocyanate and 15  $\mu$ L of ferrous iron solution (mixtures of 0.132 mol/L BaCl<sub>2</sub> and .0144 mol/L FeSO<sub>4</sub>). After 20 min for reaction in dark, the absorbance was measured at 510 nm by an UV-vis spectrophotometer (Genesys 10, Thermo Scientific, USA). A standard curve ( $R^2 = 0.9995$ ) of hydrogen peroxide was applied to calculate the concentrations of hydroperoxide.

*Dynamic headspace analysis of volatile secondary oxidation products.* Dynamic headspace analysis was adopted to determine the content of hexanal, a volatile secondary oxidation product. Briefly, 5 g of emulsion was tightly sealed in 20 mL headspace bottles with two silicone/PTFE seals and covered with tinfoil to keep in dark. After incubated in an oven at 50 °C for 15 days, the emulsions were sampling by dynamic headspace gas chromatography flame ionization detection (DHS-GC-FID) method with an automated Combi-PAL multipurpose autosampler (CTC Analytics AG, Zwingen, Switzerland) then equilibrated at 50°C for 10 min (stirred at 450 r/min) in autosampler heating block before collection. Subsequently, 1.5 mL of the headspace vapor was directly transferred into the GC unit (Agilent 7890B, USA) equipped with a HP-5 capillary column (30 m  $\times$  0.25 mm i.d.; 0.25  $\mu$ m film thickness) and connected with a flame ionization detector (FID). The injector, oven and FID temperature were 250, 60 and 250°C, respectively. The flow rate of hydrogen as the flame gas was controlled at 35.0 mL/min<sup>-1</sup> with air flowing at 300 mL/min<sup>-1</sup>, and nitrogen as the carrier gas flowed at 1.5 mL/min<sup>-1</sup> under split mode (10:1). Hexanal was identified by comparison of its retention time with that of an authentic standard.

## 2.8 Release of vanillin

10 mg vanillin was dissolved in 30 g of algae oil and the mixture was used to prepare the emulsion based on method mentioned above. The release behavior of vanillin from emulsions was evaluated using membrane dialysis method.<sup>21</sup> Firstly, the fresh prepared emulsions were diluted to the same oil fraction and then 20 g of the samples were filled into dialysis bags (molecular weight cutoff 6-8 kDa). Each dialysis was immersed in a 500 mL flask containing 400 mL of deionized water under magnetically stirring (500 r/min) at room temperature. After incubated for required time intervals, 5 mL of samples were taken from the flask and replaced with an equal volume of deionized water to maintain a constant volume. The concentrations of released vanillin were determined by an UV-vis spectrophotometer (Genesys 10, Thermo Scientific, USA) in the 231 nm, using a standard curve ( $R^2 = 0.9991$ ). The release percentage of vanillin was calculated using eqn (1).

$$\text{Vanillin release(\%)} = \frac{\text{amount of released vanillin (mg)}}{\text{total amount of vanillin in fresh emulsion samples (mg)}} \times 100\% \quad (1)$$

## 2.9 Statistical analysis

All experiments were performed in triplicate and the statistical analyses were performed by an analysis of variance (ANOVA) procedure of the SPSS 18.0 statistical analysis program. The results were detected by a least significant difference (LSD) test ( $P < 0.05$ ).

## 3. Results and Discussion

### 3.1. Self-assembly of zein in acetic acid-water

In addition to commonly used organic solvents e.g., aqueous ethanol solutions, thermal glycerol, propylene glycol and dimethylformamide, acetic acid is also proved to be a good solvent for zein.<sup>15,16</sup> Once the concentration of acetic acid solution changed, the solubility of zein decreased as well. As depicted in **Figure 1a**, the turbidities of zein dispersion increased with the decreasing proportion of acetic acid to water in solvent from 90% to 20%. During this process in which the solvent became

197 poor for zein, its secondary structure transformed from  $\alpha$ -helical into  $\beta$ -sheet predominantly  
198 according to the previous reports.<sup>15,16,22</sup> As a result, zein self-assembled into solid particles (**Figure**  
199 **1b**) with an average diameter of  $6.5 \pm 0.2 \mu\text{m}$  ( $d_{4,3}$ , determined by Malvern MasterSizer 3000), which  
200 were obtained by adding water to zein solution (in acetic acid) dropwise (**Figure 1b**). Large  
201 agglomerations and consequential precipitation were observed when further decreasing the  
202 proportion of acetic acid (<20%). These uncontrollable assembly and aggregation were undesirable.  
203 Therefore, zein in 20% acetic acid-water solution was the suitable termination to complete the  
204 anti-solvent process based on the above observations.

### 205 3.2 Preparation and characterization of zein microcapsules filled with algae oil

206 In the current work, algae oil-filled microcapsules were fabricated via the *in situ* deposition  
207 strategy based on the self-assembly behavior of zein in acetic acid-water mentioned above. The  
208 droplets of algae oil generated during emulsification acted as the templates for zein. And two stages,  
209 adsorption of zein molecules on the oil-water interface and their solidification during dilution of  
210 acetic acid, were involved. To minimize lipid oxidation that might be triggered by heating  
211 treatments, the latter procedure was conducted at 4°C, and centrifugation in place of rotary  
212 evaporation was adopted to remove the remaining acetic acid. As the microcapsules had been  
213 centrifuge-washed for more than twice, less than 1% acetic acid remained in the emulsion (Figure 2).  
214 Owing to the gradual reduction of acetic acid concentration, the solubility of zein decreased, which  
215 made the protein molecules adsorbed at the oil/water interface turn into the aggregation nuclei. And  
216 the rest of the zein molecules in the bulk acetic-water phase served as the building blocks for the  
217 aggregation. After this solidification accomplished, the shell around the oil-core took shape.

218 Formation of this core-shell structure depended on the algae oil to zein ratio (**O/Z**) during

219 preparation. Various parts in weight of algae oil with one part of 20wt% zein in acetic acid were  
220 mixed. And particles with various morphology, size and shell thickness were obtained, which were  
221 indicated by their CLSM images and particle size measurements (Table 1, Figure 3 and 4). In the  
222 cases that less algae oil was involved and the ratios of O/Z were 1 and 3, the average size of the  
223 resultant droplets were  $15.8 \pm 0.6 \mu\text{m}$  and  $28.0 \pm 0.6 \mu\text{m}$ , respectively. The presence of oil made the  
224 droplets grow bigger when compared to the zein particles without algae oil. However, due to the lack  
225 of adequate oil-water interface, the droplets seemed to be the mixture of oil and protein with  
226 irregular structure. As O/Z was increased to 5, 7 and 10, microcapsules with a definite core-shell  
227 structure were observed. The capsules in these cases had similar size distribution. Most of the  
228 droplets distributed at 20-90  $\mu\text{m}$ . And their average size varied from  $30.1 \pm 0.5 \mu\text{m}$  to  $33.7 \pm 0.4 \mu\text{m}$ .  
229 When the ratio of O/Z was further increased ( $>10$ ), macro-phase separation rather than  
230 emulsification was observed. Therefore, the following work focused on the microcapsules prepared  
231 by the ratio of O/Z 5 to 10. It is worth noting that this O/Z range in acetic acid-water system is more  
232 than twice that in ethanol-water system (1.75 to 4.5) reported by Filippidi *et al.*<sup>13</sup> When compared to  
233 ethanol, more oil and omega-3 fatty acids could be encapsulated with this acetic acid system.

234 As the ratio of O/Z was increased from 5 to 10, the volume fraction of algae oil significantly  
235 increased, but the resultant droplets still had similar size distribution (Table 1 and Figure 4). This  
236 suggested that O/Z affected the shell thickness of the resultant microcapsules. The CLSM images  
237 (Figure 3) confirmed that the shell thickness decreased with the increase of the O/Z ratio. And  
238 incomplete shells around the oil-core appeared when O/Z was 10. Furthermore, the calculation for  
239 shell thickness was performed based on more than 100 droplets from 5 CLSM images for each  
240 sample. As listed in Table 1, the average shell thickness decreased from  $1.58 \mu\text{m}$  to  $0.51 \mu\text{m}$ , when

the ratio of O/Z was increased from 5 to 10.

To further focus on the surface morphology of zein-shell, cyclohexane was used as the oil-core and hollow microcapsules were obtained. As shown in their SEM images (Figure 5), the shell of the capsules remained a regular spherical appearance when the O/Z of the samples were 5 and 7. And their surface seemed to be homogeneous and smooth, not composed of small zein particles. As the O/Z was 10, the resultant zein-shell became thinner by comparison. Subsequently, the shell might not be strong enough to maintain their integrity. The thickness and strength of the shell have a bearing on the protection that the capsules provide to the loading substances in the oil-core. With suitable range of O/Z, the obtained microcapsules could be candidate for loading bioactive.

Owing to the density difference, creaming is common in emulsions during storage. After rested for one month, creaming also occurred in the emulsions prepared with various O/Z (5, 7 and 10, Figure 6). Nevertheless, no visible oil leakage was detected. The creamed layer in the emulsions could be easily re-suspended with gently hand shake though they had been kept for a month. Moreover, the storage stability of the emulsions in which acetic acid had not been removed by centrifugation had also been observed (Figure 6). These emulsions seemed to be quite similar to those had be subjected to deacidification. This suggested that the centrifugation procedure did not had significant impact on the zein microcapsules.

### 3.3. Lipid oxidation in zein microcapsules

Algae oil rich in omega-3 fatty acids is highly susceptible to oxidation. This undesirable effect often resulted in lipid rancidity and off-flavor. For the algae oil delivery system, its chemical stability is an important consideration.<sup>14,20</sup> Since definite core-shell structure of the microcapsules could be prepared with an O/Z range of 5 to 10, these emulsions were subjected to an accelerated storage test

(50 °C) for 15 days. The change of peroxide value during the test and the amount of hexanal after the test were monitored (Figure 6). The two indexes were regarded as the indicators of the first and major secondary lipid oxidation products. Peroxide values of the three emulsions were on the increase during the test. Specifically, the capsules prepared with the O/Z of 5, which possessed thicker zein-shell (Table 1), showed a slow oxidation process as having  $50.37 \pm 4.72 \mu\text{M}/\mu\text{L}$  of lipid hydroperoxides on the fifth day to  $72.60 \pm 1.61 \mu\text{M}/\mu\text{L}$  on the fifteenth day. The capsules with the O/Z of 10 that were surrounded by thinner and incomplete zein-shell (Table 1 and Figure 3) showed the fastest oxidation rate and the peroxide value increased from  $38.69 \pm 3.20$  to  $99.20 \pm 1.01 \mu\text{M}/\mu\text{L}$  in the test. The development of value in the sample with the O/Z of 7 was in between these two emulsions. The higher peroxide value of the sample with O/Z of 5 at the beginning of storage (5 days) might be attributed to its higher water activity.<sup>23,24</sup>

Similarly, hexanal content of the emulsions after 15-day storage was also related to the zein-shell thickness. According to the chromatograms obtained in the HP-GC analysis (Figure 7), as the O/Z decreased from 10 to 5, the peak area of hexanal in the emulsions decreased from 10.17 to 8.08. And the mixture without core-shell structure had a peak area of 18.55. Together with the peroxide value analysis, the evidence suggested that formation of core-shell structure provided protection to algae oil. Besides, slower and less lipid oxidation occurred with thicker zein-shell. As most interactions between polar and nonpolar compounds in the system initially occur exactly at the interface, lipid oxidation is often described as an interfacial phenomenon.<sup>25</sup> The interfacial layer acts as an efficient physical barrier to block the diffusion and exchange of reactive singlet oxygen or reactive radical species. And thick and intact water-insoluble shell might play a better role, which was also reported in the fish oil encapsulation system.<sup>25,26</sup>

### 3.4. Volatile lipophilic compounds release from core-shell microcapsules

In addition to being rich in omega-3 fatty acids, algae oil can also act as carrier for lipophilic compounds. Moreover, to obtain better consumer acceptance in the practical application, the presence of fragrance can mask the flavor of algae oil. Therefore, vanillin was used as a model volatile lipophilic bioactive to assess the release behavior of the core-shell microcapsules with various shell thickness. Their release process in 20 h was recorded (**Figure 8**), and the relevant parameters were calculated (Table 2). Similar release tendencies were observed in curves. Rapid release with linear increase presented in the early 1.5 h, and the release was nearly finished in the following 3.5 h. Afterwards, a platform appeared in the rest of the time. However, vanillin encapsulation efficiency, release velocity at the early stage and release amount in the test varied due to the shell thickness of the core-shell microcapsule. For the capsules with thicker and intact zein-shell prepared with the O/Z of 5 (Table 1 and Figure 5), more vanillin was encapsulated ( $78.55 \pm 4.92\%$ ), and it was released at a velocity of  $14.53 \pm 0.25$  in the initial stage. Finally,  $45.53 \pm 0.27\%$  vanillin was released. In the case of the capsules covered by thinner and broken shell prepared with the O/Z of 10, the encapsulation efficiency remarkably decreased to  $42.62 \pm 0.36\%$ . But vanillin was released at a faster velocity ( $18.69 \pm 0.16$ ), and totally  $56.18 \pm 0.42\%$  was released. The parameters of the capsules prepared with the O/Z of 7 were in between those of the other two capsules. With a stronger and intact core-shell structure, the diffuse of vanillin loaded in the algae oil-core became more difficult. Thus, its release was relatively slow and limited. To accelerate and increase its release, higher O/Z should be used during the microcapsule preparation that led to thinner zein-shell.

Compared to other emulsions loaded with fragrance and stabilized by biopolymers,<sup>27-29</sup> volume

307 fractions of the internal phase in the emulsions of this work are relatively higher (O/Z: 5, 7 and 10).  
308 This made the microcapsules possess greater loading capacity for lipophobic substances. Moreover,  
309 the encapsulation procedure in this work conducted at low temperature and short time minimized the  
310 volatilization and oxidation of the loaded substance, which was particularly suitable for  
311 thermosensitive bioactives (e.g., algae oil, fragrances). With this method, the initial stage of  
312 oxidation during preparation, which triggers a subsequent fast oxidation during post-processing and  
313 storage, can be avoided.

314 **4. Conclusions**

315 Zein-based core-shell microcapsules with tunable shell thickness for delivery of algae oil had  
316 been prepared with an *in situ* precipitation strategy which was able to minimize the effects of lipid  
317 oxidation and improve the bioavailability of the loading lipophilic objects. During their preparation,  
318 zein was first adsorbed at the surface of oil droplets and then grew due to dilution of the solvent for  
319 zein. Microcapsules with various shell thickness could be obtained by manipulating the mass ratio of  
320 algae oil to zein. Those with thicker zein-shell had provided more protection to the algae oil, leading  
321 to slower oxidation rate and less formation of oxidation products. And the encapsulation and release  
322 profiles of vanillin a model volatile lipophilic compound from the microcapsules also depended on  
323 the shell thickness. Since acetic acid was used as the initial solvent for zein, the encapsulation for  
324 algae oil and loading bioactives could be accomplished at a refrigerated temperature and short time.  
325 As opposed to many other synthetic materials, the edible zein-coated emulsions are based on fully  
326 versatile food-grade protein and biocompatible natural renewable resources. Encapsulating the  
327 nutraceutical materials into food-grade products offers new opportunities for the use of zein-coated  
328 emulsions in functional food and pharmaceutical applications.

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## Figure Captions

**Figure 1.** Relative turbidity of zein solutions against acetic acid concentration (a) and confocal image of the zein particle without the addition of algae oil (b).

**Figure 2.** Acetic acid residual content (wt.%) in the emulsion system against centrifuge-washed times.

**Figure 3.** Confocal images of the emulsions prepared with various algae oil to zein (O/Z) ratios. Green corresponds to the oil environment, red to the zein environment. The green areas correspond to the oil-rich phase, and the red areas correspond to the protein-rich phase.

**Figure 4.** Size distribution of the algae oil droplets in the emulsions prepared with various algae oil to zein (O/Z) ratios.

**Figure 5.** SEM images of the air-dried cyclohexane-core zein-shell emulsions prepared with various oil to zein (O/Z) ratios

**Figure 6.** Storage stability observation of the emulsions prepared by various algae oil to zein (O/Z, 5, 7 and 10). Panel a, fresh prepared emulsions; panel b, emulsions after one-month storage; panel c, emulsions in panel b after gentle mixing. The upper row was samples without deacidification; the bottom row was samples that had been centrifuged to remove acetic acid and then re-dispersed to the same emulsion concentration.

431

432 **Figure 7.** Oxidative stability of the emulsions with various algae oil to zein (O/Z) ratios during an  
433 accelerated storage test (50 °C). Panel a, peroxide value of the emulsion during storage; panel b,  
434 chromatograms of the emulsions after 15-day storage, and the arrows pointed to the peak  
435 corresponded to hexenal.

436

437 **Figure 8.** Release kinetics of vanillin from the emulsions with various algae oil to zein (O/Z) ratios.

**Table 1.** Description of the microcapsule morphology, shell thickness and droplet size of the emulsions prepared by various algae oil to zein (O/Z) ratios

| O/Z ratio<br>[w/w] | Microcapsule<br>morphology <sup>a</sup>     | Shell thickness<br>$\mu\text{m}$ <sup>a</sup> | Droplet size<br>$d_{4,3}, \mu\text{m}$ <sup>b</sup> |
|--------------------|---------------------------------------------|-----------------------------------------------|-----------------------------------------------------|
| 1                  | aggregates of emulsion<br>droplets          | no shell                                      | 15.8±0.6                                            |
| 3                  | coexistence of aggregates<br>and core-shell | not full encapsulation                        | 28.0±0.6                                            |
| 5                  | core-shell                                  | 1.58±0.79                                     | 32.4±0.3                                            |
| 7                  | core-shell                                  | 1.23±0.65                                     | 30.1±0.5                                            |
| 10                 | core-shell                                  | 0.51±0.21 (incomplete<br>shell)               | 33.7±0.4                                            |
| >10                | phase separation                            | failure to encapsulate                        | - <sup>c</sup>                                      |

<sup>a</sup> Description of the microcapsule morphology and the calculation of the shell thickness were based on the confocal microscopy.

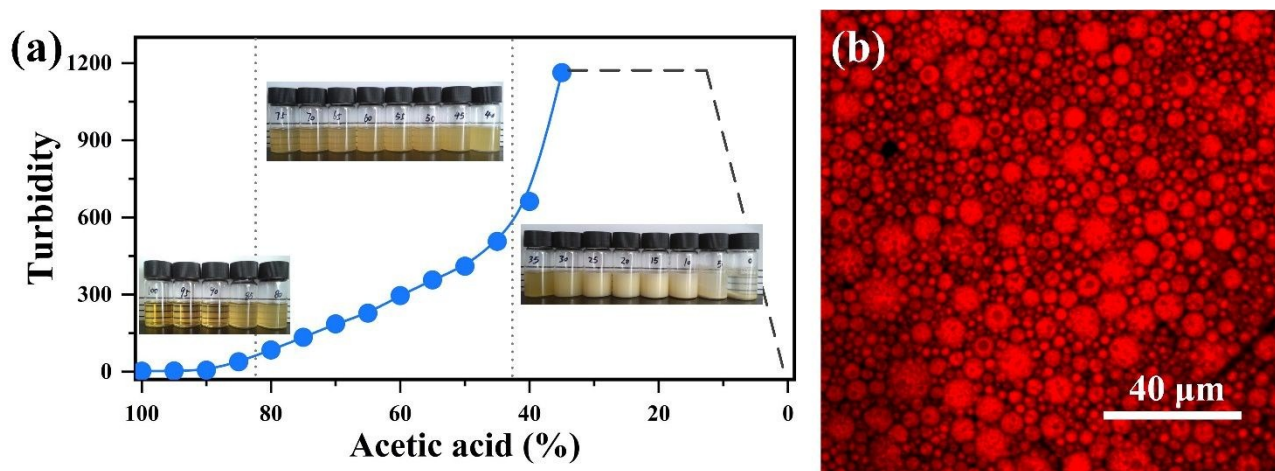
<sup>b</sup> Determined by static light scattering using a Malvern MasterSizer 3000.

<sup>c</sup> Failure in particle size measurement due to oil leakage.

**Table 2.** Summary of vanillin release profiles and encapsulation efficiency of the emulsions with various algae oil to zein ratio (O/Z).<sup>a</sup>

| O/Z ratio [w/w] | Released velocity       | P <sub>max</sub> (%) <sup>b</sup> | EE (%) <sup>c</sup>     |
|-----------------|-------------------------|-----------------------------------|-------------------------|
| 5               | 14.53±0.25 <sup>c</sup> | 45.53±0.27 <sup>c</sup>           | 78.55±4.92 <sup>a</sup> |
| 7               | 16.88±0.09 <sup>b</sup> | 52.57±0.21 <sup>b</sup>           | 60.33±3.60 <sup>b</sup> |
| 10              | 18.69±0.16 <sup>a</sup> | 56.18±0.42 <sup>a</sup>           | 42.62±0.36 <sup>c</sup> |

<sup>a</sup> Values mentioned above are the mean ± SEM (n = 3). For each column, same letters indicate no significant differences (*p* < 0.05). <sup>b</sup> P<sub>max</sub>, the maximum percentage amount of vanillin release. <sup>c</sup> EE, encapsulation efficiency.

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453 **Figure 2. Chen et al. (Color online only)**  
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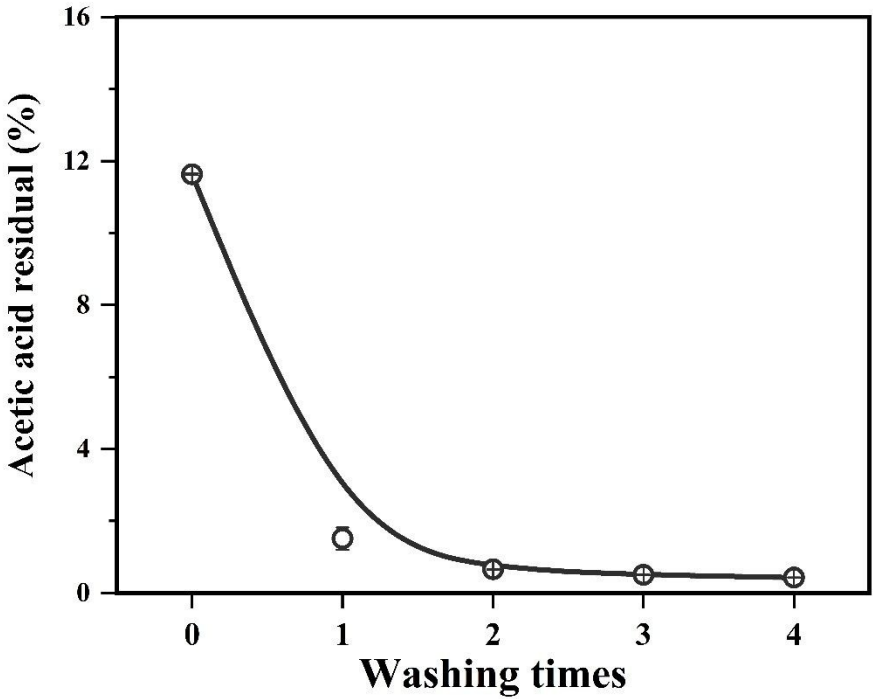
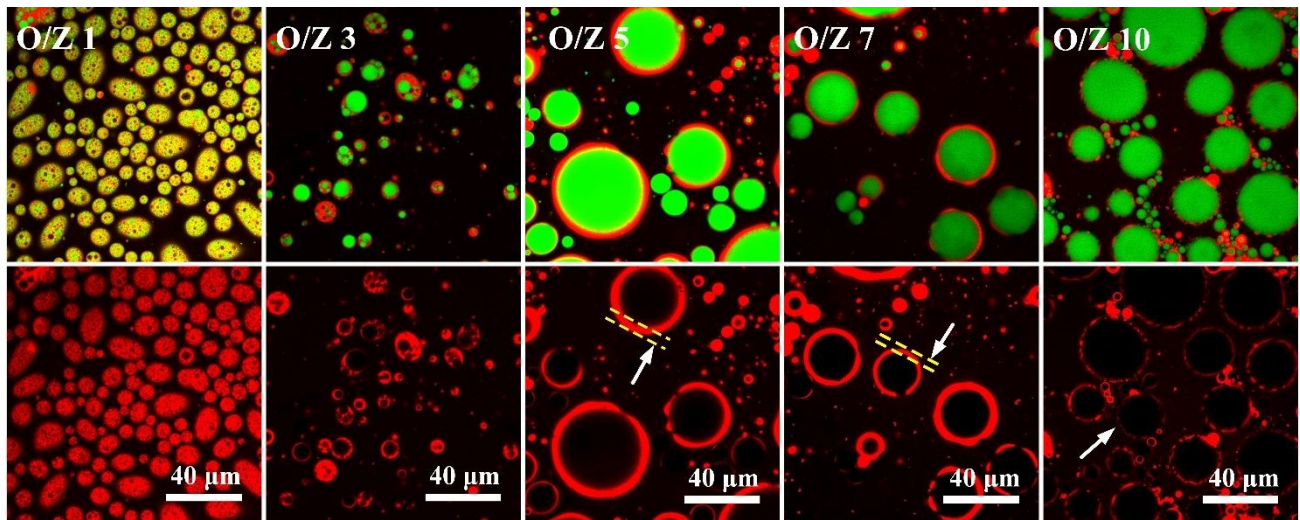


Figure 3. Chen et al. (Color online only)

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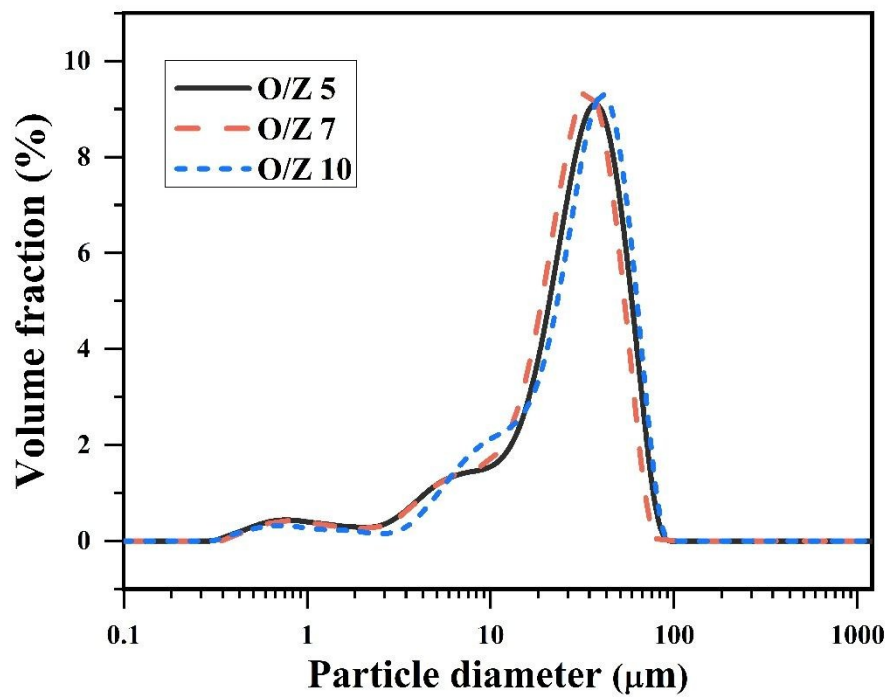
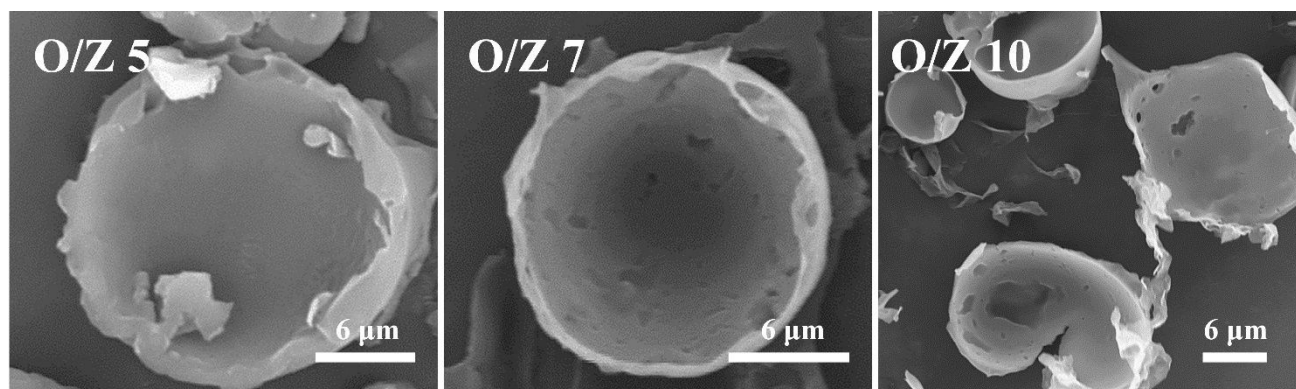


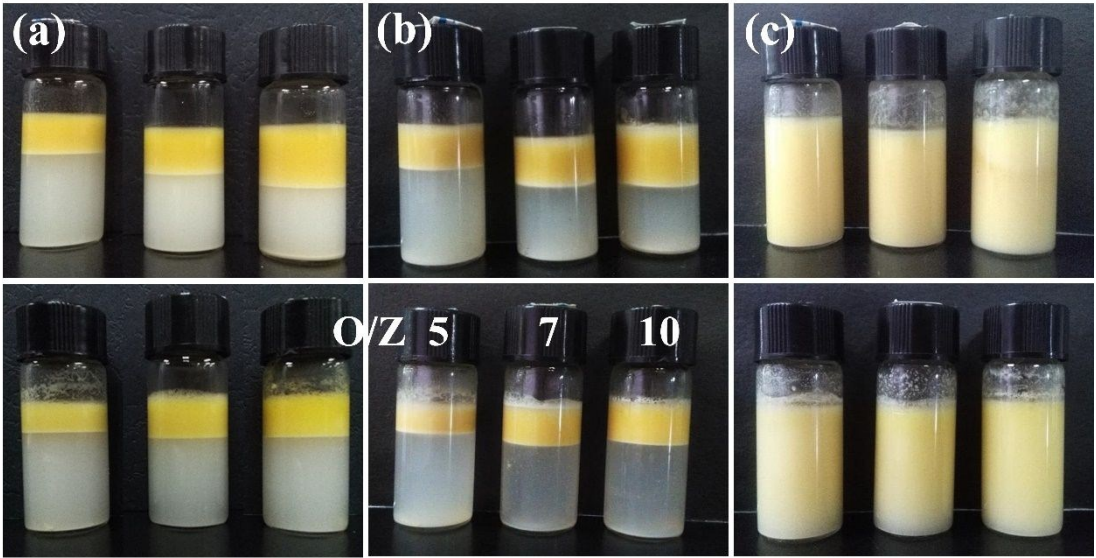
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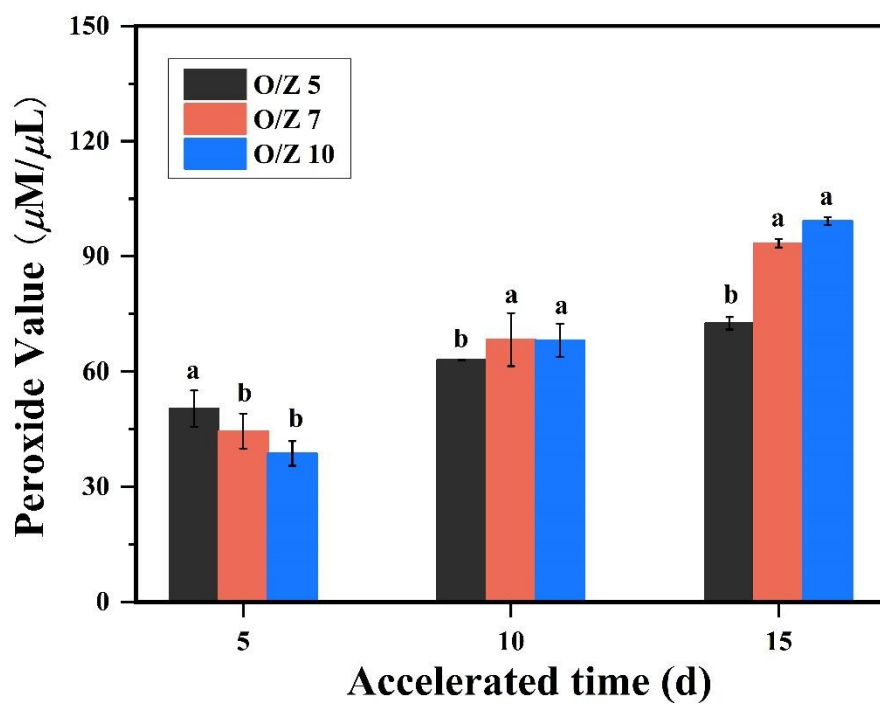
469 **Figure 6. Chen et al. (Color online only)**  
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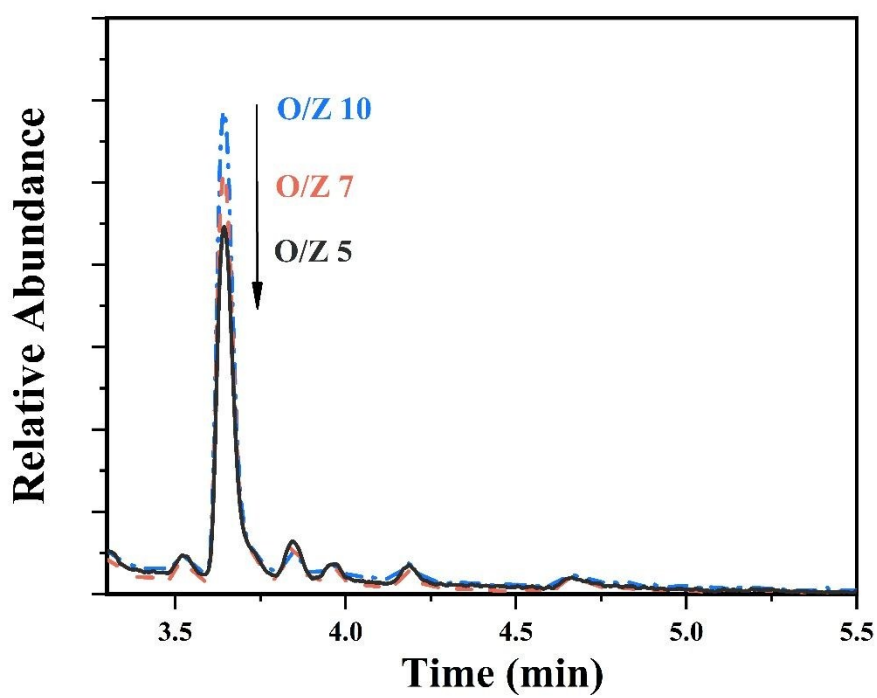
473 **Figure 7. Chen et al. (Color online only)**

474 (a)

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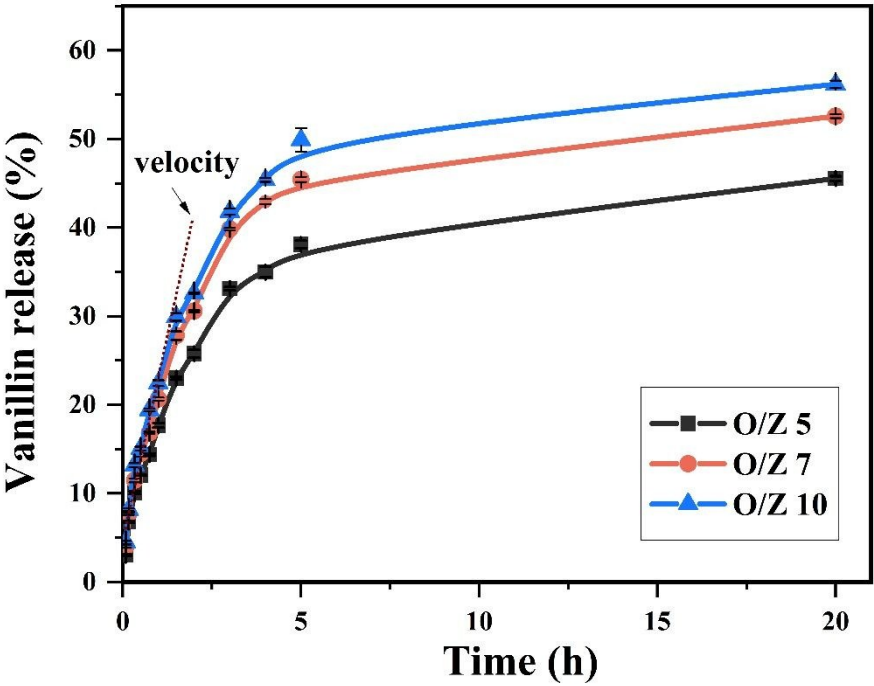
476 (b)



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479 **Figure 8. Chen et al. (Color online only)**  
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Algae oil-core zein-shell micro-capsule with tunable shell thickness was prepared at low temperature for controlling fragrance release.

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