Encapsulation of indole-3-carbinol and 3,3′-diindolylmethane in zein/carboxymethyl chitosan nanoparticles with controlled release property and improved stability

Yangchao Luo, Thomas T.Y. Wang, Zi Teng, Pei Chen, Jianghao Sun, Qin Wang

1. Introduction

Cruciferous vegetables, such as broccoli and cabbage, are well-known health-promoting vegetables because they are rich in glucosinolates, the sulphur-containing compounds. Glucosinolates can be broken down through hydrolysis into different products, among which indole-3-carbinol (I3C) is a major beneficial compound that has been extensively studied using in vitro and in vivo carcinogenesis models. The results consistently indicated its potency to influence carcinogenesis during initiation and promotion phases of cancer development, including cancers of lung, breast, stomach, colon, and prostate (Kim & Milner, 2005).

However, the stability of I3C becomes the major problem for elucidating its efficacy. Under acidic conditions, I3C molecules undergo oligomerization to form a mixture of compounds, known collectively as acid condensation products (Shertzer & Senft, 2000). 3,3′-Diindolylmethane (DIM) is the dimerization product of I3C, a major oligomerization product of I3C. DIM has also been proven to have potent cancer prevention effects (Abdelrahim, Newman, Vanderlaag, Samudio, & Safe, 2006; Shorey et al., 2012; Wang, Schoene, Milner, & Kim, 2012). The in vivo bioavailability of I3C is still not clear, because the rapid acid oligomerization in the stomach after oral consumption makes it difficult to be determined and differentiated from its oligomerization products, such as DIM. Recently, the stability of I3C in neutral cell culture media has been studied, showing that more than 50% of I3C underwent dimerization into DIM in 24 h at 37 °C conditions (Bradlow & Zeleg, 2010). Some animal studies on the efficacy of I3C were based on the oral administration by gavage to the stomach (Choi, Kim, Lee, & Park, 2012; Qian, Melkamu, Upadhyaya, & Kassie, 2011), but one recent study pointed out that the clearance time for I3C was within 1 h after oral administration of I3C in mice, and many other condensation products were detected (Anderton et al., 2004). Therefore, the in vivo biological activity of I3C has been considered to be at least partially contributed by the oligomerization products during digestion in the stomach. Furthermore, I3C in vegetables or supplements degrades quickly under different processing conditions, such as heat and light (Vallejo, Tomas-Barberan, & Fang, 2004).
eralan, & Garcia-Viguera, 2002). Given the issues of instability of I3C under various conditions, it is difficult to preserve its efficacy during storage or after oral consumption; and the dimerization of I3C to DIM makes it even more complicated to be determined in terms of its individual efficacy against cancer.

Encapsulation technology, as a novel approach, has drawn increasing attention for its applications in the food industry, with the aim to protect labile compounds from harsh conditions, mask off-odor of relevant ingredients, as well as provide controlled release and target delivery of bioactives (Arvanitoyannis, 2009; de Vos, Faas, Spasojevic, & Silkema, 2010). Many delivery systems have been reported to have the effect of improving the chemical stabilities of natural bioactives (e.g. ascorbic acid, β-Carotene, caffeine acid, anthocyanins) for extending their shelf life and preserving their functionalities (Coimbra et al., 2011; Han, Guenier, Salmieri, & Lacroix, 2008; Oldtman et al., 2011). Zein, the prolamine protein from corn, and chitosan, the derivative of natural polysaccharide chitin, are both food biopolymers that have been extensively investigated for their ability to encapsulate labile food bioactives (Dudhani & Kosaraju, 2010; Elzoghby, Samy, & Elgindy, 2012; Hu, Ting, Zeng, & Huang, 2012; Patel, Hu, Tiwari, & Velikov, 2010; Xiao & Zhong, 2011). The complex nanoparticles prepared with the combination of zein and chitosan or its derivatives have been developed in our lab as versatile delivery systems for various bioactives with a wide range of hydrophobicity (Luo, Zhang, Cheng, & Wang, 2010; Luo, Zhang, Whent, Yu, & Wang, 2011). Carboxymethyl chitosan (CMCS) is one of the water soluble derivatives of chitosan and has been reported to form hydrogels and nanoparticles with calcium ions through ionic gelation for drug delivery (Luo, Teng, & Wang, 2013; Shi, Du, Yang, Zhang, & Sun, 2006; Snima, Jayakumar, Unnikrishnan, Nair, & Lakshmanan, 2012). Zein nanoparticles coated with CMCS have been proven to be a promising delivery system to improve release profiles and enhance photo-stabilities of hydrophobic nutrients, such as vitamin D3 (Luo, Teng, & Wang, 2012).

Therefore, it is of great interest to study whether the encapsulation of I3C and DIM in nanoparticles could provide a controllable release property and enhance their stabilities from harsh environmental conditions. In the current study, I3C and DIM were encapsulated into zein nanoparticles with and without CMCS coating. The comparison of the two delivery systems was studied, in terms of their physicochemical properties and their protective effects on stabilities of I3C and DIM, including thermal- and photo-stability. The effect of encapsulation on the dimerization of I3C to DIM was monitored during the stability test.

2. Materials and methods

2.1. Materials

I3C and DIM were purchased from Sigma–Aldrich Chemical Co. Ltd. (St. Louis, MO, USA). Zein with a minimum protein content of 97% was provided by Showa Sangyo (Tokyo, Japan). CMCS was purchased from Nantongxincheng Biological Product Inc. (Nantong, Jiangsu Province, China), with a deacetylation degree of 96% and a substitution degree of 65%. Dimethyl sulfoxide (DMSO), acetonitrile, and tert-butyl methyl ether were of HPLC-grade and other chemicals were of analytical grade, purchased from Sigma–Aldrich.

2.2. Preparation of nanoparticles

I3C or DIM (5 mg/ml) was dissolved in pure ethanol as a stock solution. Zein (5 mg/ml) was dissolved in 70% alcohol-aqueous solution. CMCS (1 mg/ml) was dissolved in distilled water. Zein nanoparticles were prepared by a liquid–liquid phase separation method as reported in our previous study (Luo et al., 2012). Briefly, 80 μl of I3C or DIM solution (5 mg/ml) was added into 2 ml of zein solution in a dropwise manner under mild stirring for 30 min. The prepared zein-I3C or zein-DIM solution was immediately poured into 5 ml of pure water or CMCS solution (containing 200 μl of 0.5% Tween20), under vigorous stirring until a single phase was formed. Then, 1 ml of calcium chloride solution (0.25 mg/ml) was added under stirring. The obtained opaque nanoparticles dispersion was freeze-dried for 48 h. The control nanoparticles were prepared by replacing CMCS and calcium solution with distilled water in parallel. The I3C and DIM-encapsulated zein nanoparticles were defined as Z/I and Z/D, respectively. The I3C and DIM-encapsulated zein nanoparticles with CMCS coating were defined as ZC/I and ZC/D, respectively.

2.3. Scanning electron microscopy (SEM)

Morphological structures of nanoparticles were observed by SEM (Hitachi SU-70, Pleasanton, CA, USA). Samples were first cast-dried on an aluminium pan and then cut to an appropriate size and adhered to conductive carbon tapes (Electron Microscopy Sciences, Fort Washington, PA, USA). Subsequently, they were mounted on specimen stubs and coated with a thin (<20 nm) conductive gold and platinum layer using a sputter coater (Hummer XP, Anatech, Union City, CA, USA). Representative SEM images were reported.

2.4. X-ray diffraction (XRD)

The XRD patterns of each individual ingredient, nanoparticles, as well as the physical mixture of zein and I3C/DIM were recorded on a Bruker D8-Advance Diffractometer (Bruker AXS Inc., Madison, WI, USA) with backgroundless sample holders. The physical mixture of zein and I3C/DIM was in the mass ratio of 25:1, which was the same ratio as that in the nanoparticles. The working parameters were as follows: voltage of 40 kV, current of 40 mA, and scanning rate of 3 min⁻¹.

2.5. Particle size and zeta potential

The freshly prepared nanoparticles were used for particle size and zeta potential measurements. Hydrodynamic diameters were measured by a dynamic light scattering instrument (DLS, BI-200SM, Brookhaven Instruments Corp., Holtsville, NY, USA). DLS was equipped with a 35mW HeNe laser beam at a wavelength of 637 nm. The polydispersity index (PDI) was also reported, representing the distribution of particle size. All DLS measurements were performed at 25 °C. The surface charge of different samples was measured by a Laser Doppler Velocimetry (Zetasizer Nano ZS90, Malvern, UK), using a fold capillary cuvette (Folded Capillary Cell-DTS1060, Malvern, UK). The surface charge was expressed by zeta potential, which was converted from the measured electrophoretic mobility using the Smoluchowski theory (Zhang, Luo, & Wang, 2011). All measurements were performed in triplicate.

2.6. Encapsulation efficiency (EE)

EE was measured based on our previous method with minor modifications (Luo et al., 2012). Briefly, 10 mg of lyophilized nanoparticles were flushed with 1 ml ethyl acetate three times, using No. 1 Whatman filter paper. The washed samples were dried in a vacuum oven (VWR International, Philadelphia, PA, USA). The ethyl acetate elute containing free compounds was dried in the presence of 30 μl DMSO under a stream of nitrogen gas. The residue was then suspended in 600 μl of acetonitrile. Samples were filtered through 0.2 μm membrane and the filtrate was transferred to am-
ber vials and analysed by high performance liquid chromatography (HPLC).

2.7. Release profile

The release profile measurements were carried out in a phosphate buffer saline (PBS) medium, according to our previous method (Luo et al., 2012). Briefly, freeze-dried nanoparticles (10 mg) were incubated in 30 ml PBS (pH 7.4) containing Tween 20 (0.5%) to create sink conditions for I3C and DIM, at 37°C. At designated time intervals, 2 ml of release medium was removed and replaced with fresh PBS containing Tween 20. The removed medium was then freeze dried for 24 h, and then extracted and analysed by HPLC.

2.8. Effects of encapsulation on stabilities of I3C and DIM

The photo-stability and thermal stability of encapsulated-I3C and -DIM were evaluated as a function of time. Photo-stability was carried out under exposure to ultraviolet light (UV) for 10 h. Freshly prepared samples were subjected to stability tests. The control sample was prepared by adding the free compound (I3C or DIM dissolved in ethanol) in the same amount of an alcohol-aqueous system without zein or CMCS, to obtain the final concentration equivalent to nanoparticle samples. Samples in transparent glass vials were placed in a light-proof cabinet and exposed to two 352 nm UV light bulbs (15 W) for up to 10 h. At designated time intervals, 400 μl were withdrawn from each sample and then extracted and analysed by HPLC. For the thermal stability test, freshly prepared samples were transferred to 15 ml centrifuge tubes with screw caps. The control samples were also prepared as described above. All the samples were placed in a 37°C water bath; 400 μl were withdrawn on day 1, 2, 3 and 4, and then extracted and analysed by HPLC. All measurements were performed in triplicate.

2.9. High performance liquid chromatography (HPLC)

For release and stability tests, the withdrawn samples were placed in a screw cap centrifuge tube and extracted according to a previously described method with a slight modification (Ander- ton et al., 2003). Briefly, the lyophilized powder of each sample was extracted three times with tert-buty methyl ether (TBME, 1 ml) involving 5 min sonication for the first occasion and 30 s vortexing on the following two occasions. Subsequent to each extraction, the samples were centrifuged at 6000 g for 10 min and the supernatant was transferred to a new tube. For each sample, the combined TBME layers were evaporated rapidly under nitrogen gas in the presence of 15 μl DMSO. The extracted sample in DMSO was then reconstituted in acetonitrile (300 μl) and filtered through a 0.2 μm membrane into amber vials for measurement.

HPLC analysis of extracted samples was performed on a Hewlett Packard 1100 series HPLC system (Palo Alto, CA, USA), equipped with diode array detector and an auto-sampler operated by a Chemstation. The column heater was set at 25°C. The standard/extracted sample (10 μl) was injected into the HPLC system. Chromatography was achieved using a Phenomenex C18 (250 × 2 mm, 5 μm) column in tandem with a guard column. The mobile phase consisted of water (A) and acetonitrile (B). The gradient was as follows: 15–60% B from 0 to 20 min; linear gradient to 75% B from 20 to 25 min; linear gradient to 85% B from 25 to 30 min, and kept at 85% B from 30 to 40 min, and then kept at 15% B for 5 min. Total run time was 45 min, and the flow rate was 0.25 ml/min. Samples were detected at 280 nm and quantified according to the calibration curve of I3C and DIM mixture.

Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>Particle size (nm)</th>
<th>PDI</th>
<th>Zeta potential</th>
<th>EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z/I</td>
<td>252.8 ± 7.3</td>
<td>0.11 ± 0.01</td>
<td>−11.20 ± 0.26</td>
<td>63.77 ± 1.34</td>
</tr>
<tr>
<td>Z/C/I</td>
<td>113.5 ± 2.7</td>
<td>0.19 ± 0.01</td>
<td>−19.53 ± 2.26</td>
<td>77.79 ± 1.79</td>
</tr>
<tr>
<td>Z/D</td>
<td>250.8 ± 6.4</td>
<td>0.04 ± 0.00</td>
<td>−9.98 ± 1.64</td>
<td>69.66 ± 1.52</td>
</tr>
<tr>
<td>Z/C/D</td>
<td>89.1 ± 4.3</td>
<td>0.19 ± 0.00</td>
<td>−19.80 ± 0.80</td>
<td>78.08 ± 0.69</td>
</tr>
</tbody>
</table>

Note: Z/I, I3C-encapsulated zein nanoparticles; Z/C/I, I3C-encapsulated zein nanoparticles with CMCS coating; Z/D, DIM-encapsulated zein nanoparticles; Z/C/D, DIM-encapsulated zein nanoparticles with CMCS coating. I3C, indole-3-carbinol; DIM, 3,3’-diindolylmethane. PDI, polydispersity; EE, encapsulation efficiency.
3. Results and discussion

3.1. Physicochemical evaluation

Protein is considered as a group of promising biomaterial to develop nanoparticles for encapsulation. In many cases, however, protein itself as a single encapsulant may not be enough to provide the desired protection and controlled release property for encapsulated drugs or nutrients (Chen & Subirade, 2005; Gunasekaran, Ko, & Xiao, 2007). Hence, a second layer, usually a polysaccharide is applied to coat protein nanoparticles to improve the physicochemical properties for various applications. Zein nanoparticles coated with CMCS have been developed in our lab as a promising approach to encapsulate fat-soluble bioactive compounds with improved stability and a prolonged release property (Luo et al., 2012). In the current study, zein/CMCS nanoparticles were investigated to encapsulate two labile fat-soluble phytonutrients, i.e. I3C and DIM (Fig. S1), in order to improve their stability during storage and preserve their efficacy after oral consumption. Based on our previous study (Luo et al., 2012), the mass ratios of zein/CMCS and CMCS/calcium were determined as 1:1 and 20:1, respectively. Table 1 summarises the characteristics of the nanoparticles, including particle size, PDI, zeta potential, as well as EE. Particle size of zein nanoparticles was around 250 nm after encapsulation of both bioactive compounds. As a result of being coated with CMCS, the particle size decreased to 113 nm for I3C and 89 nm for DIM. The reduction in particle size after CMCS coating may be in part due to the strong repulsive forces caused by the high surface charges of CMCS. Additionally, DIM is more hydrophobic than I3C, and therefore DIM may interact with the zein protein via stronger hydrophobic interactions upon the phase separation process resulting in nanoparticles with more compact structures. The PDI of all nanoparticle formulations were within 0.2, indicating small distribution of particle size. The zeta potential of zein nanoparticles of I3C and DIM were -11.2 mV and -9.98 mV, respectively, and dropped to around -20 mV after zein nanoparticles were coated by CMCS. The decrease of zeta potential indicated the adsorption of negatively charged CMCS to the surface of zein nanoparticles. The EE of zein nanoparticles were within 60 – 70% for both compounds, but it significantly increased to almost 80% after nanoparticles were coated with CMCS. This observation was similar to the results of our previous study that CMCS coating on zein nanoparticles improved the EE of vitamin D3 (Luo et al., 2012). Several other studies also reported the beneficial effects on EE by coating nanoparticles with a second polymer (Briones & Sato, 2010; Trapani, Sitterberg, Bakowsky, & Kissel, 2009).

3.3. XRD analysis

The XRD patterns of nanoparticles, pure ingredients as well as their physical mixtures are shown in Fig. S2. The major characteristic peaks of I3C and DIM were at 11.48°, 17.16° and 13.59°, 18.70°, respectively, indicating their highly crystalline nature, which was consistent with previous literature (Maciejewska, Niemyjska, & Żero, 2005). These characteristic peaks were also detected in the physical mixture of zein + I3C and zein + DIM samples, but the intensity of these peaks were significantly decreased due to the low mass ratio to the zein protein. In contrast, the zein protein showed two flatter humps instead of sharp peaks, indicating the amorphous nature of the protein. After encapsulation, the characteristic peaks for I3C and DIM disappeared. These results indicated that the crystal structure of I3C and DIM were converted into an amorphous state in nanoparticles, providing additional evidence of encapsulation. This phenomenon was also used as a confirmation message for encapsulation in our previous study (Teng, Luo, & Wang, 2012).

3.4. Controlled release profile

Fig. 2 shows the release profiles of I3C (A) and DIM (B) from nanoparticles in the PBS medium. Both I3C and DIM-encapsulated nanoparticles demonstrated similar trends, the burst effect occurred within 0.5 h; followed by sustained release for more than 6 h. For zein nanoparticles without CMCS coating, around 45–50% of the compounds released from nanoparticles within 0.5 h, while for CMCS coated nanoparticles the burst effect of both compounds were reduced to around 40%. The CMCS coating also helped reduce the sustained release, and this effect was more significant in I3C-
encapsulated nanoparticles. The effects of various coatings on the improvement of the release profile of compounds from nanoparticles have been well documented (Grenha, Remunan-Lopez, Carvalho, & Seijo, 2008; Luo et al., 2010, 2011). Compared with our previous study (Luo et al., 2012), however, the effect of CMCS coating on the kinetic release profiles of I3C and DIM was not as prominent as when vitamin D3 was encapsulated. The possible reason of this phenomenon might be, in part, the strong hydrophobicity of I3C and DIM and that the surfactant (Tween-20) was added during the preparation procedure of nanoparticles, resulting in the increase of the diffusion rate and the dissolution of the I3C and DIM into the release medium. This observation was also reported in a previous study pointing out that inclusion of Tween-20 into microspheres accelerated the release of encapsulated antimicrobial compounds (Xiao, Gommel, Davidson, & Zhong, 2011).

3.5. Thermal stability

The effects of encapsulation on the thermal stability of I3C and DIM were tested in the controlled incubation in 37 °C water bath. The chromatography of the standard mixture of I3C and DIM is shown in Fig. S3, with retention times of 12.5 and 28.5 min, respectively. Fig. 3 shows the result of thermal stability of I3C after incubation for 24 h. The control sample of the pure I3C compound exhibited the fastest degradation rate, followed by I3C encapsulated zein and zein/CMCS nanoparticles. From the comparison of initial chromatography of I3C control and that after incubation for 24 h (Fig. 3A and B), DIM was the major dimerization product, along with other two products whose retention times were 22.5 min (peak a) and 31.8 min (peak b), respectively. According to a previous literature (Anderton et al., 2004), it is postulated that peak a and b stand for the I3C condensation products 1-(3-hydroxymethyl)-indolyl-3-indolylmethane (HI-IM) and [2-(indol-3-ylmethyl)-indol-3-yl]indol-3-ylmethane (LTr), respectively. Zein nanoparticles provided little protection of I3C from thermal degradation (Fig. 3C and D). However, after zein nanoparticles were coated with CMCS, the protection effect was greatly improved so that only a small amount of DIM and HI-IM were formed after 24 h incubation (Fig. 3E and F). During the thermal stability measurement, the precipitation of zein nanoparticles after 24 h incubation at 37 °C was observed, due to the denaturation of zein protein under heat treatment. The denaturation of the zein protein resulted in the collapse of the nanoparticle structure and thus a loss of protection for the encapsulated I3C. CMCS, as a coating on zein nanoparticles, may decrease the denaturation rate of the zein protein and preserve the protective effects. CS nanoparticles have also been studied to encapsulate L-ascorbic acid and its stability during heat processing was greatly improved over the unencapsulated ones (Jang & Lee, 2008). Unlike I3C, DIM was much more stable under thermal treatment, as shown in Fig. 4. The DIM control maintained 80% of its original concentration even after 4 d of incubation in the 37 °C condition. The protective effects of zein and zein/CMCS nanoparticles on DIM thermal degradation showed

![Fig. 3. Effects of encapsulation on thermal stability of I3C under 37 °C. A, C, E represent I3C levels in I3C control, Z/I, ZC/I samples, respectively, at the beginning of incubation (0 h); B, D, F represent I3C levels in I3C control, Z/I, ZC/I samples, respectively, after 24 h incubation. Z/I, I3C-encapsulated zein nanoparticles; ZC/I, I3C-encapsulated zein nanoparticles with CMCS coating. I3C, indole-3-carbinol. a and b represent 1-(3-hydroxymethyl)-indolyl-3-indolylmethane (HI-IM) and [2-(indol-3-ylmethyl)-indol-3-yl]indol-3-ylmethane (LTr), respectively.](image)

![Fig. 4. Effects of encapsulation on thermal stability of DIM. Z/D, DIM-encapsulated zein nanoparticles; ZC/D, DIM-encapsulated zein nanoparticles with CMCS coating. DIM, 3,3'-diindolylmethane.](image)
a similar trend to that of I3C. The zein nanoparticles demonstrated very little protection from DIM degradation, but the CMCS coating on zein nanoparticles provided prominent protection, showing that 90% of DIM remained intact after 4 d incubation.

Although the stabilities of I3C and DIM in acidic conditions have been extensively studied, their thermal stabilities are rarely reported in the literature. From our study, I3C showed a much poorer thermal stability than DIM at 37 °C. Ciska, Verkerk, and Honke (2009) investigated the effect of boiling on the content of I3C and DIM in fermented cabbage. In their study, the content of I3C detected in both boiling water and cabbage decreased with boiling time within 30 min before reaching a plateau, due to its thermal instability; however, the DIM concentration kept increasing for 50 min. Another recent study pointed out that the I3C dimerized to DIM during cell culture experiment conditions at 37 °C (Bradlow & Zeligs, 2010). It has been well documented that I3C underwent oligomerization in an aqueous acidic medium, and the acid condensation products include seven compounds (Grose & Bjeldanes, 1992). Our study showed that I3C oligomerized to three products during the thermal treatment, with DIM as the major compound. The contents of I3C and DIM in different samples during 3 d of thermal treatment were also recorded quantitatively and summarized in Table S1. After 1 d of thermal treatment, in the control sample and Z/I nanoparticles, only 54.5% and 55.8% of original I3C was detected, respectively, and large amount of DIM (more than 10.00 μg/ml for both samples) was formed. Whereas in nanoparticles with the CMCS coating (ZC/I), 90% of I3C was intact and a minimal DIM formation was found (3.26 μg/ml). After 3 d of thermal treatment, less than 17% and 12% of I3C were detected in control and Z/I nanoparticle samples, respectively, however, 45% of I3C remained intact in ZC/I nanoparticles.

3.6. Photo-stability against UV light

In addition to temperature, light is another major factor causing oxidation, isomerization, and oligomerization of phytonutrients. Both I3C and DIM are the phytochemicals with aromatic rings possessing UV absorption ability which may result in their instability when exposed to UV light. The effects of encapsulation on their photo-stabilities are shown in Fig. 5. Both I3C and DIM were extremely susceptible to UV light exposure, and I3C degraded faster than DIM. Within 6 h of UV light exposure, there was less than 20% of I3C left in the control sample (Fig. 5A). Both nanoparticle formulations preserved I3C content to as high as 80% for the first 6 h exposure. At the end of 10 h, I3C in control sample was not detectable, but around 50% and 70% of I3C were detected in zein and zein/CMCS nanoparticles, respectively. Compared with I3C, DIM was relatively stable given that a lag phase was observed before the rapid degradation began (Fig. 5B). At 4 h of incubation, there was more than 80% of DIM in all samples but the content decreased rapidly to less than 20% in the control sample in the following incubation. The HPLC chromatograph of UV-exposed samples indicated that I3C oligomerized to DIM, HI-IIM as well as LTr but in less amount (data not shown) than those detected during the thermal treatment. Because of the hydroxyl group in the carbionil side chain connecting to indole structure, I3C is much more reactive than DIM, resulting in the fast formation of cations and consequent photo-oxidation initiated by UV light exposure (Bloch-Mechkour, Bally, & Marcinke, 2011). Encapsulation of DIM into nanoparticles prolonged the lag phase to 8 h, with more than 75% of DIM intact. At the end of 10 h incubation, more than 40% and 60% of DIM remained in zein and zein/CMCS nanoparticles, respectively, but only 20% was detected in the control sample. Therefore, encapsulation of I3C and DIM provided similar protection against UV light, and slightly better protection was observed for CMCS coated zein nanoparticles. The protection against UV light was mainly contributed by the zein protein containing aromatic side groups and double bonds which can absorb UV light. This observation was similar to that reported in our previous study (Luo et al., 2012).

3.7. Conclusions

In summary, zein and zein/CMCS nanoparticles were successfully prepared to encapsulate hydrophobic bioactives I3C and DIM. The encapsulation was evidenced by XRD. Compared with zein nanoparticles, zein/CMCS nanoparticles showed better EE and smaller particle size. Both nanoparticle formulations provided controlled release of the bioactive compounds. The stabilities of I3C and DIM were significantly improved after they were encapsulated in nanoparticles. Both zein and zein/CMCS nanoparticles provided similar protection against degradation in UV-light, however, zein/CMCS nanoparticles provided better protection of I3C against degradation and oligomerization under thermal conditions.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2013.01.113.

References


