**Enhancement of Curcumin Water Dispersibility and Antioxidant
Activity using Core-shell Protein-polysaccharide Nanoparticles**

**Xiaoxia Huang^a, Xulin Huang^a, Yushi Gong^a, Hang Xiao^b, David Julian McClements^b,
^{c*}, Kun Hu^{a**}**

^a Food Science School, Guangdong Pharmaceutical University, Zhongshan 528458, China

^b Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA

^c Department of Biochemistry, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah
21589, Saudi Arabia

Journal: Food Research International

Submitted: March, 2016

* Corresponding author at: Department of Food Science, University of Massachusetts, Amherst, MA
01003, USA. Tel.: +1 (413) 545 1019; fax: +1 (413) 545 1262. E-mail address:
mcclements@foodsci.umass.edu (D.J. McClements).

**Corresponding author at: Food Science School, Guangdong Pharmaceutical University, Zhongshan,
528458, China. Tel.: +86 (760) 8820 7022; Fax: +86 (760) 8820 7056. E-mail address:
huk88@126.com (Kun Hu)

Abstract: Curcumin has strong antioxidant activity, but poor water-solubility and chemical stability, which limits its utilization as a nutraceutical in many applications. Previously, we developed a core-shell (zein-pectin) nanoparticle delivery system with high curcumin loading efficiency, high particle yield, and good water dispersibility. However, this system was unstable to aggregation around neutral pH and moderate ionic strengths due to weakening of electrostatic repulsion between nanoparticles. In the current study, we used a combination of alginate (high charge density) and pectin (low charge density) to form the shell around zein nanoparticles. Replacement of 30% of pectin with alginate greatly improved aggregation stability at pH 5 to 7 and at high ionic strengths (2000 mM NaCl). Curcumin encapsulated within these core-shell nanoparticles exhibited higher antioxidant and radical scavenging activities than curcumin solubilized in ethanol solutions as determined by Fe (III) reducing power, 1, 1-Diphenyl-2-picrylhydrazyl free radical (DPPH·), and 2, 2'-azinobis-(3-ethylbenzothiazoline)-6- sulfonic acid radical cation (ABTS·⁺) scavenging analysis. These core-shell nanoparticles may be useful for incorporating chemically unstable hydrophobic nutraceuticals such as curcumin into functional foods, dietary supplements, and pharmaceuticals.

Keywords: curcumin; zein; pectin; alginate; nanoparticles; antioxidant; core-shell; nutraceuticals

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of normal cellular metabolism, and can be either harmful or beneficial to living organisms depending on their locations and concentrations within living organisms (Valko et al., 2007). High levels of ROS and RNS are deleterious to human health, and play an important role in numerous diseases, such as cancer (Valko, Rhodes, Moncol, & Mazur, 2006), aging, arthritis, inflammation (Moskovitz, Yim, & Chock, 2002; Valko et al., 2007), and heart disease (Heitzer, Schlinzig, Krohn, Meinertz, & Münzel, 2001). Hydrogen peroxide, hydroxyl free radical, superoxide radical, nitric oxide, and singlet oxygen are the most common forms of ROS, and they can damage cellular proteins, and DNA, which can lead to alterations in gene expression (Gentile, Allegra, Livrea, & D'Alessio, 2004) and promotion of degenerative diseases (Hensley, Robinson, Gabbita, Salsman, & Floyd, 2000; Valko et al., 2007). Scavenging ROS is one of the most effective means of decreasing the level of oxidative stress, which may be achieved using antioxidant compounds extracted from natural plants (Chen et al., 2011; Singh & Kumari, 2015; Wootton-Beard & Ryan, 2011; Zou, Lu, & Wei, 2004).

Polyphenols from plant-sources (such as curcumin from turmeric) exhibit strong antioxidant, anti-inflammatory, and anticancer effects (Anand et al., 2008; Kou et al., 2013; Naksuriya, Okonogi, Schiffelers, & Hennink, 2014; Yen, Wu, Tzeng, Lin, & 2010). Despite its potential health benefits, curcumin has found limited use as a pharmacological or nutraceutical agent, which can be partly attributed to its low water solubility, chemical instability, and poor oral bioavailability (Anand, Kunnumakkara, Newman, & Aggarwal, 2007). Recently, there has been interest in using zein nanoparticles to encapsulate curcumin to increase its water dispersibility, chemical stability, and bioavailability (Gomez-Estaca, Balaguer, Gavara, & Hernandez-Munoz, 2012; Hu et al., 2015; Liu, Wei, Wang, Hu, & Jiang, 2016; Patel, Hu, Tiwari, & 2010a; Zou et al., 2016a; Zou et al., 2016b). More than 50% of the amino acids in are hydrophobic, which makes it one of the few natural proteins that can be readily solubilized in aqueous ethanol solutions (60~90%) but not in water (Dong, Padua, &

Wang, 2013; Zhang, Luo, & Wang, 2011). This property makes zein a suitable for development of nanoparticle delivery systems to encapsulate lipophilic curcumin molecules (Patel, Heussen, Dorst, Hazekamp, & Velikov, 2013). However, zein nanoparticles have poor aggregation stability when exposed to certain environmental conditions, such as pH, salt, and temperature. This problem can be partly overcome coating the zein nanoparticles with a layer of polysaccharide molecules, which was attributed to their ability to modulate the electrostatic and steric repulsion between the particles (Hu & McClements, 2015; Luo, Teng, & Wang, 2012; Luo et al., 2013).

In a previous study, our group fabricated core-shell nanoparticles that consisted a hydrophobic zein core coated by a hydrophilic pectin shell using an electrostatic deposition method (Hu et al., 2015). These nanoparticles were relatively small ($d < 100$ nm) and had a relatively high curcumin loading efficiency (>86%) (Hu et al., 2015). The encapsulated curcumin was in an amorphous form and interacted with zein through hydrophobic interactions. The curcumin-enriched nanoparticles could be converted into a powdered form that had good water-dispersibility. However, these nanoparticles became highly unstable to aggregation around pH 6, which can be attributed to a weakening of the electrostatic attraction between pectin and zein (since the isoelectric point of zein is around pH 6.2) (Shukla & Cheryan, 2001). A decrease in the magnitude of the attachment energy of the polysaccharide molecules for the protein nanoparticle surfaces may lead to bridging flocculation. Moreover, the nanoparticles were also highly sensitive to ionic strength at both neutral and acidic pH due to a reduction in the electrostatic repulsion between the pectin and the zein nanoparticles, as well as between different nanoparticles.

The aim of the current study was to determine whether alginate, a more highly charged anionic polysaccharide than pectin, could be used to modulate the and steric interactions in the system, and thereby increase nanoparticle stability to pH and ionic strength changes. It was hypothesized that alginate molecules would be strongly attached to the zein nanoparticle surfaces, and that there would be a stronger repulsion between different zein nanoparticles. The impact of the nature of the polysaccharide coating on the antioxidant activity of encapsulated curcumin was also

studied, as it is important to show that a strategy used to improve the water dispersibility and physical stability of an encapsulated active ingredient does not adversely affect its beneficial functional properties.

2. Materials and methods

2.1. Materials

Zein (Lot SLBD5665V), pectin (pectin from citrus peel, Lot SLBH9128V), L-Ascorbic acid (LotSTBF1107V), and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS, Lot SLBL5589V) were obtained from Sigma–Aldrich (St. Louis, USA). Curcumin (>98%) was obtained from Acros Organics (New Jersey, USA); Sodium alginate was from Bright Moon Seaweed Group (Qingdao, China); 1, 1-Diphenyl-2-picrylhydrazyl free radical (DPPH·) and Trolox (6-hydroxy-2, 5, 7, 8- tetramethylchroman-2- carboxylic acid) were purchased from Tokyo Chemical Industry (Tokyo, Japan); HPLC grade ethanol alcohol and dimethyl sulfoxide (DMSO), trichloroacetic acid ($\geq 99.0\%$), potassium peroxydisulfate ($\geq 99.5\%$), iron(III) chloride hexahydrate ($\geq 99.0\%$), and potassium hexacyanoferrate (III) ($\geq 99.5\%$) were obtained from Hwei Co., Ltd. (Guangzhou, China). All other chemicals were of analytical grade.

2.2 Solution preparation

2.2.1. Curcumin-zein stock solution

2.0 g of zein was dissolved in 100.0 mL of 85% (v/v) aqueous ethanol solution using magnetic stirring at 500 rpm (IKA R05, USA) for 1 h, and then 0.24 g curcumin powder was added and stirred for another 30 min in the dark. The solution was centrifuged at $1006\times g$ for 15 min to separate any insoluble material.

2.2.2. Pectin /sodium alginate solution

Pectin and sodium alginate powder were dispersed in distilled water at a concentration of 0.2% (w/v) respectively, and stirred at 700 rpm for 30 min at 70 °C

ensure good solubility. The test tubes containing the solutions were then cooled with tap water to ambient temperature, and the solutions were stirred for another hour. A series of pectin / alginate solutions (0%, 30%, 50%, 70%, and 100% pectin) were prepared by mixing different volume ratios of pectin and alginate solutions. The solutions were filtered to remove any insoluble particles, and adjusted to pH 4.0 with mol/L HCl.

2.3. Preparation of curcumin loaded zein-pectin/alginate nanoparticles

Curcumin-loaded nanoparticles were prepared using a previously reported method (Hu et al., 2015) with some modification. 4.0 mL of curcumin-zein stock solution was rapidly injected into 16.0 mL acidified water (pH 4.0) using a syringe with continuous magnetic stirring at 900 rpm, and then stirred for another 3 min in the dark. The in the resulting nanoparticle dispersion was then removed by evaporation using a evaporator (RE 2000A, Yarong, China). Acidified water (pH 4.0) was then added to bring the final volume back to 20.0 mL. Curcumin-loaded zein-polysaccharide nanoparticles were made by pouring an aliquot (20.0 mL) of the zein-nanoparticle dispersion into a series of 25.0 mL polysaccharide solutions with different pectin/alginate ratios (see section 2.2.2) with continuous stirring at 700 rpm for 30

2.4. Nanoparticle characterization

2.4.1. Field emission scanning electron microscopy

Freshly prepared fluid nanoparticle dispersions were converted into a powder using spray-drying (Mini spray-dryer B-290, Buchi, Swiss) with inlet temperature 135°C, outlet temperature 78°C, nitrogen flow 40 ml/min, and pump speed scale 30%. The dried nanoparticles were analyzed using a field emission scanning electron microscope (Nova NanoSEM 430, FEI, Netherlands) with an acceleration potential of 10 kV using high vacuum mode. A layer of gold coating (15 nm) was applied to the mounted samples prior to analysis.

2.4.2. Particle size and charge measurements

The particle size distribution of the colloidal dispersions was measured by dynamic light scattering (DLS) using a commercial instrument (Nano-ZS, Malvern Instruments, UK). The Z-average diameter and polydispersity index (PDI) were calculated from the light scattering measurements. Each sample was analyzed in duplicate. The electrical properties (ζ -potential) of the particles in the colloidal dispersions were characterized using a micro-electrophoresis device (Nano-ZS, Malvern Instruments, UK). Colloidal dispersions were diluted with 5 mM phosphate-citric acid buffers at the same pH as the samples prior to analysis to avoid multiple scattering effects. Each sample was analyzed in duplicate.

2.5. Determination of curcumin content in nanoparticles

10 mg of freeze-dried nanoparticles were solubilized in 10 mL DMSO and then stirred overnight in the dark. The resulting solution was centrifuged at 16,099 $\times$ g for 30 min. The supernatant was diluted 10 times with DMSO, and the absorbance at a wavelength of 435 nm was measured using a UV-visible spectrophotometer (TU-1901, Persee, China). The curcumin concentration in the samples was then determined using a calibration curve that had been previously established using standard solutions (0–10 mg/mL free curcumin in DMSO).

2.6. Stability to environmental conditions

2.6.1. Effect of pH

Freshly prepared dispersions were mixed with equal volumes of 10 mM citric acid-phosphate buffers with pH values ranging from 3.0 to 7.0. If necessary, the samples were then adjusted to the target pH values using 1 M NaOH or HCl. The experiment was performed in triplicate.

2.6.2. Effect of salt

Curcumin-loaded dispersions were mixed with equal volumes of 10 mM citric acid-phosphate buffers with pH values at pH 5.0 or 7.0. Different amounts of sodium

chloride were then added to these dispersions. The final salt concentrations of the samples ranged from 0 to 2.0 M. Each experiment was performed in triplicate.

2.7. Antioxidant activity of curcumin loaded nanoparticles

2.7.1. Determination of reducing power capacity

The reducing potential of the curcumin-loaded nanoparticles was evaluated according to a method described previously (Zou et al., 2004) with some In brief, 1 mL of samples containing different curcumin concentrations (50–200 µg/mL), either dissolved in ethanol or encapsulated in nanoparticles, were mixed with mL of phosphate buffer solution (PBS, 0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide solution (1%, w/v). The above reaction mixture was incubated for 20 min at 50°C. After this step, trichloroacetic acid (TCA, 2.5 mL, 10%) was mixed with the reaction mixture to stop the reaction and then the system was centrifuged for 10 min 3000 rpm. Supernatant (2.5 mL) was taken out and mixed with 2.5 mL double water and 0.5 mL ferric chloride solution. The absorbance was measured at 700 nm using a UV-visible spectrophotometer. Ascorbic acid was used as a positive control. The experiment was performed in triplicate.

2.7.2. DPPH· scavenging activity

The DPPH· scavenging capacity of the samples was determined using a method described previously (Yen et al., 2010) with some modifications. Encapsulated curcumin and ethanol-dissolved curcumin were prepared at multiple concentrations. Briefly, 2 mL of a 100 µM DPPH· ethanol solution was mixed with equivalent of encapsulated curcumin and ethanol-dissolved curcumin and subsequently incubated at room temperature in the dark. After 30 min, the absorbance of the reaction solution was measured at 517 nm. The free radical scavenging activity from the sample was calculated using equation 1:

$$\text{DPPH} \cdot \text{ Scavenging (\%)} = \frac{(A_c - A_s)}{A_c} \times 100\% \quad (1)$$

Where A_s and A_c are the absorbance of sample and control (ethanol) solutions,

respectively. The scavenging activity (SC_{50}) was used to compare the levels of free radical scavenging activity of different samples, which was taken to be the concentration of sample required to reduce the scavenging by 50%. The experiment was performed in triplicate.

2.7.3. $ABTS^{\cdot+}$ scavenging capacity

The $ABTS^{\cdot+}$ scavenging experiment was based on one described previously (Re et al., 1999). Briefly, ABTS was dissolved in deionized water to reach a concentration of 7 mM. Then, potassium persulfate was dissolved at an overall concentration of 2.45 mM in the ABTS solution and the mixture was allowed to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\cdot+}$ solution was diluted with 10 mM PBS (pH 7.4) to an absorbance of $0.70 (\pm 0.02) \text{ cm}^{-1}$ at 734 nm and equilibrated at 30 °C. Powdered curcumin was dissolved in absolute ethanol, and encapsulated curcumin was dispersed in deionized water. The concentrations of curcumin in ethanol and the nanoparticle dispersion were adjusted to achieve a 20–80% inhibition of the blank absorbance by pretesting. Ten microliter samples or Trolox standards (at 0.5–2.0 mM concentrations) were dissolved in 1 mL of $ABTS^{\cdot+}$ solution, and the absorbance reading was taken at 30 °C exactly 1 min after initial mixing for up to 6 min. Phosphate buffer solution and absolute ethanol were used as blanks respectively. The scavenging capability of the samples was determined using equation 2 and converting to Trolox equivalent antioxidant capacity (TEAC, mg/mg antioxidant) using a standard curve prepared using Trolox solutions:

$$ABTS^{\cdot+} \text{scavenging (\%)} = \left(1 - \frac{A_s}{A_c}\right) \times 100\% \quad (2)$$

Here, A_s and A_c are the absorbance of sample and control solutions (PBS) respectively. Each experiment was performed in triplicate.

2.8. Data analysis

Statistical tests were performed using the SPSS 20.0 computer program (SPSS Statistical Software, Inc., Chicago, IL, USA). The results are reported as averages and standard deviations of duplicate or triplicate measurements. Means were subjected to

243 Duncan's test and a p-value of <0.05 is considered statistically significant.

244 3. Results and discussion

245 3.1. Morphology of curcumin encapsulated nanoparticles

246 The morphology of the freeze dried nanoparticles analyzed by scanning electron
247 microscopy (SEM) is shown in Figure 1. The particles appeared spherical with
248 smooth surfaces. Most of the particles had diameters in the range 100 to 200 nm, but
249 there were also some smaller particles. The particle size observed by SEM was in
250 agreement with the mean particle diameter determined by dynamic light scattering,
251 which was around 160 nm.

252 3.2. Effect of alginate on colloid stability at different pH

253 Delivery systems must function over a range of pH conditions in commercial
254 products, and they may be exposed to considerable variations in pH as they pass
255 through the gastrointestinal tract after ingestion. We therefore compared the influence
256 of pH on the size, charge, and stability of the polysaccharide-coated protein
257 nanoparticles prepared in this study. The mean diameter of zein nanoparticles coated
258 with only pectin were relatively small (188 to 220 nm) from pH 3.0 to 5.0, increased
259 somewhat in the range from pH 6.0 to 6.5 (302 and 360 nm), and then decreased
260 again at pH 7.0 (158 nm) (Fig. 1). The larger particle diameter measured at pH 3 than
261 at pH 7 can be attributed to the fact that the adsorbed pectin molecules contributed to
262 the effective size of the core-shell particles. The approximate thickness of the shell
263 can be calculated from the formula: $\delta = (d_{CS} - d_C)/2$, where d_{CS} is the diameter of the
264 core-shell particle and d_C is the diameter of the core only. These results suggest that
265 the thickness of the pectin shell was about 15 to 30 nm depending on the pH, which is
266 consistent with the known radius of hydration of pectin molecules in solution (Villay
267 et al., 2012).

268 The pH stability of the zein nanoparticles clearly depended on the composition of
269 the polysaccharide shell (Fig. 2). When 30% of the pectin was replaced with

alginate, the nanoparticles were relatively stable to aggregation from pH 4.0 to 7.0 (d = 159 to 230 nm), but exhibited some aggregation at pH 3.0 (d = 400 nm). When pectin was replaced with 50% or 70% of alginate, the particles become more unstable to aggregation at pH 3.0 and 6.5, and they totally aggregated when pectin was completely replaced by sodium alginate (data not shown).

Measurements of the pH-dependence of the electrical characteristics of the different core-shell nanoparticles were performed to help explain the differences in their aggregation stability. Initially, however, the ζ -potential *versus* pH profile of pure pectin and alginate solutions were measured to determine the differences in their electrical characteristics (**Fig. 3a**). Although both pectin and alginate are anionic polysaccharides with carboxyl groups, the ζ -potential of alginate was more negative than that of pectin at the same pH, which suggests that alginate had a higher charge density than pectin. This may have partly been because the alginate is a linear anionic molecule, whereas the pectin has a linear anionic backbone with neutral side branches. The ζ -potential of the core-shell nanoparticles was negative across the whole pH range studied, and fairly similar to that of the polysaccharide molecules in solution (**Fig. 3b**). Conversely, the ζ -potential-pH profile of the zein-polysaccharide nanoparticles was quite different from that of the uncoated zein nanoparticles, which were positively charged below pH 6. The ζ -potential of the core-shell nanoparticles became more highly negative as the alginate-to-pectin ratio increased, which would be expected given the higher negative charge on the alginate molecules (**Fig. 3a**).

The influence of the alginate-to-pectin ratio on the pH-stability of the nanoparticles may be explained by changes in the electrostatic interactions between the zein nanoparticles and polysaccharides. The charge on the zein-nanoparticles goes from positive at low pH to negative at high pH with a point of zero charge around pH 6.2 (**Fig 3a**). On the other hand, the charge on the pectin and alginate molecules goes from highly negative at neutral pH, to less negative at acid pH because of protonation of the carboxyl groups (-COOH) on the polysaccharide chains at pH values around their $pK_a \approx 3.5$ (Harnsilawat, Pongsawatmanit, & McClements,

2006). At pH 4 to 5, the highly negatively charged polysaccharides strongly bound to the surfaces of the positively charged zein nanoparticles through electrostatic attraction. At pH 3.0, the zein nanoparticles were still positively charged, but the negative charge on the pectin and alginate molecules was appreciably reduced (Fig. 2) because the carboxyl groups became partly protonated. As a result, the polysaccharide molecules may have been less strongly attached to the zein nanoparticle surfaces, and so they could partially detach from one particle and stick to another particle leading to bridging flocculation. Similarly, at pH 6.5, the zein nanoparticles lost much of their positive charge and so the anionic polysaccharides were less strongly attached to their surfaces, again leading to bridging flocculation due to sharing of polysaccharides between different nanoparticles. Interestingly, it appeared that 100% pectin led to the best stability to aggregation at pH 3, but 30% alginate/70% pectin led to the best stability at pH 6.5. These results clearly show that the pH stability of the core-shell nanoparticles can be tailored by altering the composition of the polysaccharide shell. The precise molecular origin of this effect is currently unknown.

These results have important implications for the application of the coated zein nanoparticles in different food and beverage applications. In particular, it will be important to optimize the nature of the coating used for specific pH environments.

3.3 Effect of ionic strength on particle stability

There are appreciable variations in ionic strength in different food and beverage products, as well as in different regions of the human gastrointestinal tract, and so it is important to understand how the coated nanoparticles behave in different ionic environments. In this section, we therefore examined the influence of salt addition upon the aggregation stability of core-shell nanoparticles with different polysaccharide compositions: 100% pectin or 30% alginate/70% pectin. These experiments were carried out at pH 5 where the polysaccharides should adsorb to the zein nanoparticle surfaces due to electrostatic attraction, and at pH 7 where the majority of polysaccharides should be in the aqueous phase.

At pH 7, the system containing 100% pectin was stable in the absence of salt, but

became highly aggregated in the presence of 50 mM or higher NaCl. The particle size of the samples could not be reliably measured because the aggregates formed were too large for analysis by the DLS instrument, but the photographs of the samples clearly showed the presence of a yellow precipitate at the bottom of the tubes at higher salt levels (**Fig. 4b**). Conversely, the system containing 30% alginate/70% pectin only showed a moderate increase in mean particle size from 0 to 300 mM NaCl (**Fig. 4a**) and the systems appeared more homogeneous with only a thin layer of sediment visible at the bottom of the tubes (**Fig. 4b**). This suggests alginate was more effective at protecting the droplets from aggregation in this pH range. We hypothesize that some of the anionic polysaccharide molecules remained attached to cationic patches on the surfaces of the zein nanoparticles at neutral pH, even under conditions where the net charge on both the polysaccharides and the zein nanoparticles was negative. However, the addition of salt to the system should weaken these attractive interactions due to electrostatic screening, thereby causing partial displacement of the polysaccharide molecules from the particle surfaces. A higher level of salt would be required to displace the alginate molecules from the nanoparticle surfaces because they had a higher charge density and should therefore be attached more strongly.

At pH 5, the zein-nanoparticles coated with 100% pectin were relatively stable to aggregation from 0 to 200 mM NaCl, but underwent extensive aggregation at higher levels (**Fig. 5**). Conversely, the zein-nanoparticles coated with 30% alginate/70% pectin were relatively stable up to 2000 mM NaCl. This suggested that replacement of some of the pectin with alginate increased the aggregation stability of the core-shell nanoparticles, which may have been because alginate molecules were more effective at increasing the electrostatic or steric repulsion between the coated nanoparticles. Alternatively, the alginate molecules may have been attached to the nanoparticle surfaces more strongly, and therefore were more resistant to displacement due to weakening of the electrostatic attraction at high salt levels (Hu & McClements, 2014). There results have important implications for optimizing the composition of the polysaccharide shell used to coat the zein nanoparticles for specific applications in the

food industry where salt levels and pH may vary considerably between products.

3.4 Antioxidant activity of curcumin loaded nanoparticles

Finally, the impact of encapsulation of curcumin within core-shell nanoparticles on its antioxidant activity was determined. This kind of analysis is important because any strategy used to improve the water-dispersibility and physical stability of curcumin should not adversely affect its functional attributes. In these experiments core-shell nanoparticles that had a 30% alginate/70% pectin coating were prepared since this system gave the best physical stability.

3.4.1. Reducing power capacity

The reducing power of curcumin encapsulated in coated nanoparticles or dissolved in ethanol solution was compared with that of ascorbic acid (a strong reducing agent) using a modified iron (III) to iron (II) reduction assay. In addition, the reducing power of the empty (curcumin-free) nanoparticles was also tested as a negative control. The empty nanoparticles had little reducing power, whereas the curcumin-loaded nanoparticles had a reducing power that was just lower than that of ascorbic acid (**Fig. 5**). In addition, the reducing power of curcumin encapsulated in the coated nanoparticles was appreciably higher than that of curcumin solubilized in ethanol at the same curcumin concentration.

3.4.2. DPPH· scavenging capacity

The DPPH· scavenging capacity of curcumin-loaded nanoparticles, curcumin solubilized in ethanol, and ascorbic acid are compared in Figure 7. A high free radical scavenging capacity is given by a low SC_{50} value, *i.e.*, a lower concentration of substance is required to reduce the activity of the probe by 50%. **Curcumin solubilized in ethanol had the poorest scavenging capacity ($SC_{50} = 16.7 \mu\text{g/mL}$) followed by curcumin encapsulated in nanoparticles ($13.9 \mu\text{g/mL}$) and then ascorbic acid ($5.0 \mu\text{g/mL}$), with the differences being statistically significant ($P < 0.05$).** These results indicate that encapsulated curcumin is more efficient at scavenging free radicals than curcumin solubilized in ethanol. Previous studies have reported that the

DPPH $\cdot$ scavenging ability of curcumin encapsulated in polyvinyl pyrrolidone nanoparticles was similar to that of curcumin solubilized in DMSO, and much stronger than that of curcumin dispersed in water (Yen et al., 2010). The DPPH $\cdot$ scavenging ability of empty nanoparticles was also measured, but this was found to be very weak and did not change with increasing particle concentration, so the SC₅₀ value of the empty nanoparticles could not be determined. This result suggests that the antioxidant activity of the curcumin-loaded zein nanoparticles is primarily due to the curcumin rather than the zein.

3.4.3. ABTS $\cdot^+$ scavenging capacity

The ABTS $\cdot^+$ scavenging capacity of curcumin encapsulated in nanoparticles or dissolved in ethanol was also measured (**Fig. 8**). Since we could not define the molecular weight of zein-polysaccharide nanoparticles, the Trolox equivalent of antioxidant capacity (TEAC) was presented as mg Trolox/mg antioxidant instead of mM Trolox/mM antioxidant. The TEAC of encapsulated curcumin was about 1.44 mg/mg, which meant that the antioxidant capacity of one milligram of encapsulated curcumin was equivalent to that of 1.44 mg of Trolox. The TEAC of curcumin solubilized in ethanol was 0.78 mg/mg, which suggested that encapsulated curcumin was about twice as efficient at scavenging ABTS $\cdot^+$ than curcumin solubilized in ethanol. Empty (curcumin-free) nanoparticles exhibited very weak ABTS $\cdot^+$ scavenging capacity (**Fig. 8**), which highlights that it was encapsulation of the curcumin, rather than the presence of zein that was responsible for the improved antioxidant activity.

4. Conclusion

In summary, curcumin-loaded zein nanoparticles that were only coated with pectin were unstable to aggregation around neutral pH and at low to moderate NaCl concentrations, which was attributed to a relatively weak electrostatic attraction between zein and pectin, and a relatively weak electrostatic repulsion between different nanoparticles. Partial replacement of some of the pectin with alginate (a

more negatively charged polysaccharide) altered the pH stability of the core-shell nanoparticles. At some pH values, the alginate/pectin-coated zein nanoparticles were more stable to aggregation than the pectin-coated ones, but at other pH values they were less stable. This suggests that the interfacial composition should be tailored for specific applications, depending on the pH of the commercial product that the nanoparticles will be utilized in. Partial replacement of the pectin with alginate also improved the salt stability of the nanoparticles, which was attributed to an increase in electrostatic and steric repulsion. The curcumin-loaded core-shell nanoparticles were shown to have superior antioxidant and radical scavenging activities than curcumin solubilized in ethanol. Thus, these curcumin loaded core-shell nanoparticles may be practically useful for incorporating curcumin into functional foods and beverages, as well as dietary supplements and pharmaceutical products.

5. Acknowledgements

This work was funded by International Cooperation Research Program of Guangdong Provincial Department of Science and Technology (No. 2015A050502051). This material was partly based upon work supported by the Cooperative State Research, Extension, Education Service, USDA, Massachusetts Agricultural Experiment Station (MAS00491) and USDA, NRI Grants (2013-03795, and 2014-67021).

6. References

- Anand, P., Kunnumakkara, A., Newman, R., & Aggarwal, B. (2007). Bioavailability of Curcumin-Problems and Promises. *Molecular Pharmaceutics*, 4, 807-819.
- Anand, P., Thomas, S. G., Kunnumakkara, A. B., Sundaram, C., Harikumar, K. B., Sung, B., . . . Aggarwal, B. B. (2008). Biological activities of curcumin and its analogues (*Congeners*) made by man and Mother Nature. *Biochemical Pharmacology*, 76, 1590-1611.
- Chen, Y., Huang, B., He, J., Han, L., Zhan, Y., & Wang, Y. (2011). In vitro and in vivo antioxidant effects of the ethanolic extract of *Swertia chirayita*. *Journal of Ethnopharmacology*, 136, 309-315.
- Dong, F., Padua, G. W., & Wang, Y. (2013). Controlled formation of hydrophobic surfaces by self-assembly of an amphiphilic natural protein from aqueous solutions. *Soft Matter*, 9, 5933.

- Gentile, C., Tesoriere, L., Allegra, M., Livrea, M. A., & D'Alessio, P. (2004). Antioxidant betalains from cactus pear (*Opuntia ficus-indica*) inhibit endothelial ICAM-1 expression. *Annals of the New York Academy of Sciences*, 1028, 481-486.
- Gomez-Estaca, J., Balaguer, M. P., Gavara, R., & Hernandez-Munoz, P. (2012). Formation of zein nanoparticles by electrohydrodynamic atomization: Effect of the main processing variables and suitability for encapsulating the food coloring and active ingredient curcumin. *Food Hydrocolloids*, 28, 82-91.
- Harnsilawat, T., Pongsawatmanit, R., & McClements, D. (2006). Characterization of β -lactoglobulin–sodium alginate interactions in aqueous solutions: A calorimetry, light scattering, electrophoretic mobility and solubility study. *Food Hydrocolloids*, 20, 577-585.
- Heitzer, T., Schlinzig, T., Krohn, K., Meinertz, T., & Münzel, T. (2001). Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation*, 10, 2673-2678.
- Hensley, K., Robinson, K., Gabbita, S., Salsman, S., & Floyd, R. (2000). Reactive oxygen species, cell signaling, and cell injury. *Free Radical Biology & Medicine*, 28, 1456-1462.
- Hu, K., Huang, X., Gao, Y., Huang, X., Xiao, H., & McClements, D. J. (2015). Core-shell biopolymer nanoparticle delivery systems: synthesis and characterization of curcumin fortified zein-pectin nanoparticles. *Food Chem*, 182, 275-281.
- Hu, K., & McClements, D. J. (2014). Fabrication of surfactant-stabilized zein nanoparticles: A pH modulated antisolvent precipitation method. *Food Research International*, 64, 329-335.
- Hu, K., & McClements, D. J. (2015). Fabrication of biopolymer nanoparticles by antisolvent precipitation and electrostatic deposition: Zein-alginate core/shell nanoparticles. *Food Hydrocolloids*, 44, 101-108.
- Kou, M. C., Chiou, S. Y., Weng, C. Y., Wang, L., Ho, C. T., & Wu, M. J. (2013). Curcuminoids distinctly exhibit antioxidant activities and regulate expression of scavenger receptors and heme oxygenase-1. *Molecular Nutrition and Food Research*, 57, 1598-1610.
- Liu, G., Wei, D., Wang, H., Hu, Y., & Jiang, Y. (2016). Self-assembly of zein microspheres with controllable particle size and narrow distribution using a novel built-in ultrasonic dialysis process. *Chemical Engineering Journal*, 284, 1094-1105.
- Luo, Y., Teng, Z., & Wang, Q. (2012). Development of zein nanoparticles coated with carboxymethyl chitosan for encapsulation and controlled release of vitamin D3. *Journal of Agricultural and Food Chemistry*, 60, 836-843.
- Luo, Y., Wang, T. T., Teng, Z., Chen, P., Sun, J., & Wang, Q. (2013). Encapsulation of indole-3-carbinol and 3,3'-diindolylmethane in zein/carboxymethyl chitosan nanoparticles with controlled release property and improved stability. *Food Chemistry*, 139, 224-230.
- Moskovitz, J., Yim, M. B., & Chock, P. B. (2002). Free radicals and disease. *Archives of Biochemistry and Biophysics*, 397, 354-359.
- Naksuriya, O., Okonogi, S., Schiffelers, R. M., & Hennink, W. E. (2014). Curcumin nanoformulations: a review of pharmaceutical properties and preclinical studies and clinical data related to cancer treatment. *Biomaterials*, 35, 3365-3383.
- Patel, A. R., Heussen, P. C., Dorst, E., Hazekamp, J., & Velikov, K. P. (2013). Colloidal approach to prepare colour blends from colourants with different solubility profiles. *Food Chem*, 141, 1466-1471.
- Patel, A. R., Hu, Y., Tiwari, J. K., & Velikov, K. P. (2010a). Synthesis and characterisation of

488 zein–curcumin colloidal particles. *Soft Matter*, 6, 6192.
 489 Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant
 490 activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology &*
 491 *Medicine*, 26, 1231-1237.
 492 Shukla, R., & Cheryan, M. (2001). Zein the industrial protein from corn. *Industrial Crops and Products*,
 493 13 171-192.
 494 Singh, R., & Kumari, N. (2015). Comparative determination of phytochemicals and antioxidant activity
 495 from leaf and fruit of *Sapindus mukorossi* Gaertn. – A valuable medicinal tree. *Industrial*
 496 *Crops and Products*, 73, 1-8.
 497 Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and
 498 antioxidants in normal physiological functions and human disease. *International Journal of*
 499 *Biochemistry and Cell Biology*, 39, 44-84.
 500 Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and
 501 antioxidants in oxidative stress-induced cancer. *Chemical and Biological Interactions*, 160,
 502 1-40.
 503 Villay, A., de Filippis, F. L., Picton, L., Le Cerf, D., Vial, C., & Michaud, P. (2012). Comparison of
 504 polysaccharide degradations by dynamic high-pressure homogenization. [Article]. *Food*
 505 *Hydrocolloids*, 27, 278-286.
 506 Wootton-Beard, P. C., & Ryan, L. (2011). A beetroot juice shot is a significant and convenient source of
 507 bioaccessible antioxidants. *Journal of Functional Foods*, 3, 329-334.
 508 Yen, F. L., Wu, T. H., Tzeng, C. W., Lin, L. T., & Lin, C. C. (2010). Curcumin nanoparticles improve the
 509 physicochemical properties of curcumin and effectively enhance its antioxidant and
 510 antihepatoma activities. *Journal of Agricultural and Food Chemistry*, 58, 7376-7382.
 511 Zhang, B., Luo, Y., & Wang, Q. (2011). Effect of acid and base treatments on structural, rheological, and
 512 antioxidant properties of α -zein. *Food Chemistry*, 124, 210-220.
 513 Zou, L., Zheng, B., Zhang, R., Zhang, Z., Liu, W., Liu, C., . . . McClements, D. J. (2016a). Enhancing the
 514 bioaccessibility of hydrophobic bioactive agents using mixed colloidal dispersions:
 515 Curcumin-loaded zein nanoparticles plus digestible lipid nanoparticles. *Food Research*
 516 *International*, 81, 74-82.
 517 Zou, L., Zheng, B., Zhang, R., Zhang, Z., Liu, W., Liu, C., . . . McClements, D. J. (2016b). Food-grade
 518 nanoparticles for encapsulation, protection and delivery of curcumin: comparison of lipid,
 519 protein, and phospholipid nanoparticles under simulated gastrointestinal conditions. *Rsc*
 520 *Advances*, 6, 3126-3136.
 521 Zou, Y., Lu, Y., & Wei, D. (2004). Antioxidant Activity of a Flavonoid-Rich Extract of *Hypericum*
 522 *perforatum* L. in Vitro. *Journal of Agricultural and Food Chemistry*, 52, 5032-5039.
 523

Figures

Fig. 1. SEM image of polysaccharide-coated zein nanoparticles loaded with curcumin. The polysaccharide coating contained 70% pectin and 30% alginate. Sample preparation involved freeze-drying the nanoparticle suspension and then coating with gold.

Fig. 2. Effect of pH on average diameters of zein-polysaccharide core/shell nanoparticles loaded with curcumin. The polysaccharide shells contained different ratios of alginate and pectin.

Fig. 3a. Effect of pH on ζ -potential of zein nanoparticles and anionic polysaccharides (alginate and pectin).

Fig. 3b. Effect of pH on ζ -potential of zein-polysaccharide core/shell nanoparticles that were loaded with curcumin. The core/shell nanoparticles were constructed using different ratios of pectin and alginate.

Figure 4a. Effect of NaCl concentration on the particle diameters at pH 7.0. The polysaccharide coating of the nanoparticles consisted of 70% pectin and 30% alginate ($p < 0.05$).

Figure 4b. Effect of NaCl concentration on the apparent stability of colloidal dispersions at pH 7.0. *Left:* particles coated with 70% pectin and 30% alginate; *Right:* particles coated with 100% pectin. Notice the yellow sediment at the bottom of some test tubes due to precipitation.

Figure 5a. Effect of NaCl concentration on the mean particle diameters of colloidal dispersions (polysaccharide-protein zein nanoparticles) at pH 5.0 ($p < 0.05$). The symbol “*” means that the particles were so highly aggregated that their dimensions were too large to measure using dynamic light scattering.

Figure 5b. Effect of NaCl concentration on the stability of colloidal dispersions (polysaccharide-protein zein nanoparticles) at pH 5.0. *Left:* nanoparticles coated with 70% pectin and 30% alginate; *Right:* nanoparticles coated with 100% pectin.

Figure 6. The reducing power capacity of ascorbic acid (control), curcumin loaded in nanoparticles, curcumin in ethanol, and empty nanoparticles. The zein nanoparticles had a coating of 70% pectin and 30% alginate.

Figure 7. Comparing the DPPH radical scavenging ability of ascorbic acid (control), curcumin loaded in nanoparticles, and curcumin in ethanol. The zein nanoparticles had a coating of 70% pectin and 30% alginate. The DPPH $\cdot$ scavenging ability of empty nanoparticles was very weak and did not change with particle concentration, so the SC₅₀ value of the empty nanoparticles could not be determined ($p < 0.05$).

Figure 8. The Trolox equivalent antioxidant capacity (TEAC) for scavenging $\text{ABTS}^{\cdot+}$ of curcumin loaded in nanoparticles, curcumin in ethanol, and empty nanoparticles ($p < 0.05$).

Fig. 1. SEM image of polysaccharide-coated zein nanoparticles loaded with curcumin. The polysaccharide coating contained 70% pectin and 30% alginate. Sample preparation involved freeze-drying the nanoparticle suspension and then coating with gold.

Fig. 2. Effect of pH on average diameters of zein-polysaccharide core/shell nanoparticles loaded with curcumin. The polysaccharide shells contained different ratios of alginate and pectin.

Fig. 3a. Effect of pH on ζ -potential of zein nanoparticles and anionic polysaccharides (alginate and pectin).

Fig. 3b. Effect of pH on ζ -potential of zein-polysaccharide core/shell nanoparticles that were loaded with curcumin. The core/shell nanoparticles were constructed using different ratios of pectin and alginate.

Figure 4a. Effect of NaCl concentration on the particle diameters at pH 7.0. The polysaccharide coating of the nanoparticles consisted of 70% pectin and 30% alginate ($p < 0.05$).

Figure 4b. Effect of NaCl concentration on the apparent stability of colloidal dispersions at pH 7.0. *Left:* particles coated with 70% pectin and 30% alginate; *Right:* particles coated with 100% pectin. Notice the yellow sediment at the bottom of some test tubes due to precipitation.

Figure 5a. Effect of NaCl concentration on the mean particle diameters of colloidal dispersions (polysaccharide-protein zein nanoparticles) at pH 5.0 ($p < 0.05$).

Figure 5b. Effect of NaCl concentration on the stability of colloidal dispersions (polysaccharide-protein zein nanoparticles) at pH 5.0. *Left:* nanoparticles coated with 70% pectin and 30% alginate; *Right:* nanoparticles coated with 100% pectin.

Figure 6. The reducing power capacity of ascorbic acid (control), curcumin loaded in nanoparticles, curcumin in ethanol, and empty nanoparticles. The zein nanoparticles had a coating of 70% pectin and 30% alginate.

Figure 7. Comparing the DPPH radical scavenging ability of ascorbic acid (control), curcumin loaded in nanoparticles, and curcumin in ethanol. The zein nanoparticles had a coating of 70% pectin and 30% alginate. The DPPH· scavenging ability of empty nanoparticles was very weak and did not change with the particle concentration, so the SC₅₀ value of the empty nanoparticles could not be determined ($p < 0.05$).

Figure 8. The Trolox equivalent antioxidant capacity (TEAC) for scavenging $\text{ABTS}^{\cdot+}$ of curcumin loaded in nanoparticles, curcumin in ethanol, and empty nanoparticles ($p < 0.05$).

Title: “Enhancement of Curcumin Water Dispersibility and Antioxidant Activity using Core-shell Protein-polysaccharide Nanoparticles” by Huang et al

Journal: *Food Research International*

Graphical abstract

Curcumin-loaded core-shell nanoparticles were fabricated by antisolvent precipitation of zein, followed by electrostatic deposition of pectin and/or alginate. The pectin-alginate ratio impacted the physical stability and antioxidant activity of the curcumin-loaded nanoparticles.