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Enhanced Dispersibility and Bioactivity of Curcumin by Encapsulation in Casein Nanocapsules

Kang Pan,[†] Qixin Zhong,*[,†] and Seung Joon Baek[‡]

[†]Department of Food Science and Technology and [‡]Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, The University of Tennessee in Knoxville, Knoxville, Tennessee 37996, United States

ABSTRACT: In this work, a novel encapsulation method was studied by spray-drying a warm aqueous ethanol solution with codissolved sodium caseinate (NaCas) and lipophilic food components, using curcumin as a model compound. The encapsulation caused the loss of crystallinity of curcumin. After hydration of spray-dried powder and centrifugation, 137 μg/mL curcumin was dispersed in the transparent dispersion, which was 4 decades higher than its water solubility. Dynamic light scattering and atomic force microscopy results showed that curcumin-loaded casein nanoparticles were bigger than those of NaCas processed at encapsulation conditions but were smaller than those of the native NaCas. The increased nanoparticle dimension, together with fluorescence and FTIR spectroscopy results, suggested that curcumin was entrapped in the nanoparticle core through hydrophobic interactions. The curcumin encapsulated in casein nanoparticles had higher biological activity, as assessed by antioxidant and cell proliferation assays, than pristine curcumin, likely due to the improved dispersibility. This simple approach may be applied to encapsulate various lipophilic bioactive compounds.

KEYWORDS: *sodium caseinate, curcumin, encapsulation, dispersibility, antioxidant activity, activity against cancer cell growth*

INTRODUCTION

Curcuminoids, including curcumin I (diferuloylmethane), curcumin II (monodemethoxycurcumin), and curcumin III (bisdemethoxycurcumin), are natural polyphenolic compounds isolated from the rhizome of turmeric (*Curcuma longa*).[1] Due to the phenolic groups and conjugated double bonds, curcumin has been observed to possess high cytotoxic activity against tumor cells, strong antioxidant properties, and anticarcinogenesis against a wide range of cell lines.[2−4] Curcumin III has a higher activity as a cytotoxic agent than curcumin I and curcumin II, which may be ascribed to the demethylation.[3] Curcumin exhibits the potential to interrupt the NF-κB and AP-1 pathways, thereby inhibiting the expression of certain genes that are critical regulators of inflammation.[5,6] When tested as a cancer prevention compound, human clinical trials showed some benefits without significant side effects when curcumin was orally administered at up to 8 g daily for 3 months.[7] The promise of curcumin as a therapeutic agent against cancer, Alzheimer's disease, and inflammatory-related diseases, however, requires strategies overcoming its limited bioavailability.[8,9] A critical factor limiting the bioavailability is its extremely low water solubility, which is estimated to be 11 ng/mL.[10]

Several techniques have been studied to increase the solubility and bioavailability of curcumin. Glucosylation of curcumin to curcumin-4′,4″-*O*-β-D-digentiobioside by *Catharanthus roseus* increased the solubility to around 240 mg/mL.[10] However, the bioactivity and toxicity of the glucosylated curcumin were not evaluated. Dissolving curcumin in the oil body of emulsions improved dispersibility, bioavailability, and anti-inflammation activity.[11−13] Nanoformulations utilizing synthesized or natural polymers such as zein nanoparticles, poly(ethylene glycol)−poly(ε-caprolactone), alginate−chitosan−pluronic composite, and Pluronic block copolymer also increased the therapeutic effectiveness.[14−16] Pharmaceutical grade cyclodextrins have been used to load curcumin to a solubility up to ∼2 mg/mL,[17] and hydrophobically modified starch was observed to disperse 18.4 μg/mL curcumin after high-pressure homogenization and centrifugation.[18] Solid lipid nanoparticles involving polysorbate 80[19] and organogel-based nanoemulsions[20] utilizing Tween-20 were also studied to enhance the bioavailability of curcumin. Despite these significant developments, much work is needed to prepare nanodispersions for transparent functional beverage applications using generally recognized as safe ingredients such as naturally occurring food biopolymers.

Caseins have unique properties for fabricating delivery systems of food and pharmaceutical compounds, in addition to being a very important source of calcium and essential amino acids.[21] As relevant to this study,[22] purified camel β-casein was mixed with curcumin in ethanol, followed by evaporation to remove the solvent and dilution with distilled water. After centrifugation, the supernatant contained 28 μg/mL curcumin. The β-casein can self-associate to form micelles that are capable of dissolving lipophilic compounds but is too costly for food applications. Casein micelles in bovine milk can be dissociated into nanoclusters by high-pressure homogenization,[23] which was used to encapsulate vitamin D2 in nanoparticles of sodium caseinate formed upon depressurization.[24] High-pressure homogenization, however, is currently expensive for food applications. Casein micelles can also be dissociated by calcium chelating agents such as citrate and ethylenediaminetetraacetate[25] or at alkaline conditions.[26] These dissociation conditions,

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however, may not be applicable to encapsulate curcumin. Conversely, dissociation of casein micelles has been reported by heating to above 60 °C in aqueous ethanol with >35% ethanol, because of the improved solvent quality and the shifting of pK_a values of phosphoserine.[27] This important property of casein micelles has not been utilized to encapsulate lipophilic bioactive compounds, such as curcumin, that have a much higher solubility in aqueous ethanol than in water. Curcumin and casein in warm aqueous ethanol may form nanoscale complexes that can be prepared in powdered form by spray-drying for the convenience of transportation, storage, and application. Spray-drying is a low-cost and scalable technique that has been extensively used for solvent removal and encapsulation.[28,29] If spray-dried powder can be hydrated to prepare transparent dispersions with encapsulated lipophilic compounds such as curcumin, a simple encapsulation method can then be developed for the production of transparent functional beverages.

The primary objective of this work was to study the possibility of preparing transparent dispersions of curcumin by hydrating spray-dried powders from warm aqueous ethanol solution with curcumin and caseins. This was supported by physicochemical properties of powders and the prepared aqueous dispersions, including nanoscale structural changes of caseins. The secondary objective was to evaluate the potential of encapsulation in improving the bioactivity of curcumin, which was tested for antioxidant properties and in vitro activity against the growth of cancer cells, as studied by Yu and Huang.[18] To simplify sample preparation, sodium caseinate (NaCas), a commercially available ingredient produced by acid precipitation of caseins from bovine milk followed by neutralization using sodium hydroxide for spray-drying, was used in this work. This enables a safe, low-cost, and scalable approach to incorporate lipophilic bioactive compounds in products such as functional beverages.

MATERIALS AND METHODS

Chemicals. Curcumin was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). The product had a purity of >90% w/w according to the vendor. NaCas was from American Casein Co. (Burlington, NJ, USA). Other chemicals were obtained from either Sigma-Aldrich or Thermo Fisher Scientific (Pittsburgh, PA, USA).

Encapsulation of Curcumin by Spray-Drying. Four grams of NaCas was hydrated in 200 mL of 40% v/v aqueous ethanol. After being heated at 60 °C in a water bath for 5 min, an excess amount (1.0085 g) of curcumin was mixed with the NaCas solution by blending at 10000 rpm for 4 min using a Cyclone I.Q. microprocessor homogenizer (VirTis, Gardiner, NY, USA). The homogenizer was equipped with a 20 mm diameter rotor−stator shaft assembly with openings of 1 mm in width and 10 mm in height. Centrifugation at 290*g* (model 4540 R, Eppendoff, Hamburg, Germany) for 5 min was carried out to remove the excess amount of curcumin. The supernatant was transferred and spray-dried at an inlet temperature of 105 °C and an outlet temperature of 68 °C using a B-290 mini spray-dryer (Büchi Labortechnik AG, Flawil, Switzerland). A NaCas sample was processed at the same conditions without curcumin, named processed NaCas hereafter.

Estimation of Curcumin Loading in Spray-Dried Powder. Five milligram of spray-dried powder was suspended in 10 mL of chloroform[18] and was stirred overnight at room temperature (21 °C). After centrifugation at 6000*g* for 10 min (Minispin plus, Eppendoff), the supernatant was transferred and filtered through a PTFE syringe filter with 0.45 μm pore size (Fisher Scientific, Pittsburgh, PA, USA). The permeate was diluted 20 times in chloroform, and the absorbance at 419 nm was measured using a UV−vis spectrophotometer (Evolution 201, Thermo Scientific, Waltham, MA, USA)[18] to determine curcumin concentration on the basis of a calibration curve previously established using standard solutions with different amounts of free curcumin dissolved in chloroform.

Preparation of Transparent Dispersions and Determination of Curcumin Concentration. To prepare transparent dispersions, 0.25 g of spray-dried powder was hydrated in 20 mL of deionized water for 6 h at room temperature (21 °C) using a stirring plate, followed by centrifugation at 12800*g* for 15 min (model 4540 R, Eppendoff) to obtain the transparent supernatant. The concentration of casein in the transparent dispersion was determined using the Commassie blue reagent from Thermo Fisher Scientific (Pittsburgh, PA, USA), with bovine serum albumin as a reference protein. To determine curcumin content using the literature method,[18] the transparent dispersion was mixed with a certain amount of chloroform by vortexing for 1 min and subsequent stirring overnight. The bottom organic phase was transferred after phase separation, and the absorbance at 419 nm was determined to obtain curcumin concentration as above. A control sample estimating the complexation ability of NaCas was prepared by simply mixing curcumin and NaCas, at the same mass ratio as in the preparation of spray-dried powder, in deionized water using a magnetic stirring plate for 4 h, followed by centrifugation and quantification of curcumin as above.

Atomic Force Microscopy (AFM). The transparent curcumin dispersion and native NaCas dispersion were diluted in deionized water to an overall solute concentration of 10 ppm. Two microliters of each diluted sample was spread evenly onto freshly cleaved mica sheets that were mounted on sample disks (Bruker Corp., Santa Barbara, CA, USA) for AFM. A rectangular cantilever having an aluminum reflective coating on the backside and a quoted force constant of 2.80 N/m (FESPA, Bruker Corp.) and a Multimode microscope (Bruker AXS, Billerica, MA, USA) operated in the trapping mode were used to scan the sample. Images were generated with a preset scan area of 2.0 × 2.0 μm at a scanning speed of 1 Hz.

Differential Scanning Calorimetry (DSC). The crystallinity of powdered samples were characterized using a model Q2000 calorimeter (TA Instrument, New Castle, DE, USA). Ten milligrams of powdered sample was sealed in hermetic aluminum pans and heated from 30 to 250 °C at a rate of 5 °C/min. Nitrogen was used as the transfer gas at a flow rate of 50 mL/min.

X-ray Diffraction (XRD). The XRD patterns of samples were characterized using an XRD-6000 powder X-ray diffractometer (Shimadzu Corp., Tokyo, Japan) with Cu Kα radiation at a wavelength (λ) of 1.5418 Å. Measurements were performed at a voltage of 30 kV and 10 mA. The 2θ angle was set from 5° to 55°, and the scanning rate was 2°/min.

Dynamic Light Scattering (DLS) and Zeta-Potential. In addition to the transparent dispersion with curcumin, two other dispersions were measured in DLS: native NaCas and processed NaCas hydrated in deionized water for 6 h. The dispersion pH was 6.8. Experiments were conducted with a Delsa Nano analyzer (Beckman Coulter, Atlanta, GA, USA) at a scattering angle of 165°. Samples were filtered through PTFE syringe filters with 0.45 μm pore size (Fisher Scientific, Pittsburgh, PA, USA). The time correlation functions were analyzed with a Laplace inversion program (CONTIN) to obtain the hydrodynamic diameter. Average hydrodynamic diameters, polydispersibility, and zeta-potential were reported on the basis of three replicates.

Fluorescence Spectroscopy. The fluorescence spectra were recorded using a RF-1501 spectrofluorometer (Shimadzu Corp.). The excitation wavelength was 419 nm, and the emission spectra from 450 to 700 nm were recorded. The slit width was set at 10 nm for both excitation and emission. The transparent curcumin dispersion was diluted to proper concentrations using deionized water to reach the instrument sensitivity range. For free curcumin, it was dissolved in ethanol before dilution in deionized water to the same final concentration as in the encapsulated curcumin. Dispersions of native and processed NaCas were prepared in deionized water and measured for fluorescence after being diluted to NaCas concentrations identical to the curcumin dispersion.

Fourier Transform Infrared (FTIR) Spectroscopy. FTIR spectra were compared for curcumin crystals, native NaCas, processed NaCas, and curcumin capsules prepared as above. The powdered sample was mixed and milled with KBr powder, and the mixture was pressed into a thin pellet. The FTIR spectra were measured by using a Nicolet Nexus 670 FT-IR spectrometer (Thermo Nicolet Corp., Madison, WI, USA) equipped with a germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector, and a KBr beam splitter. All spectra were taken via the ATR method with a resolution of 4 cm^{-1} using 64 scans.

Quantification of Total Antioxidant Activity (ABTS Assay). A literature assay protocol was adopted to quantify total antioxidant activity.[30] 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was dissolved in deionized water to a 7 mM concentration. Potassium persulfate was dissolved at an overall concentration of 2.45 mM in the ABTS solution and was allowed to stand in the dark at room temperature for 12−16 h before use. The ABTS radical solution was diluted with 10 mM phosphate buffer saline (PBS) to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. Curcumin crystal was first dissolved in 95% ethanol. Ethanol-dissolved and encapsulated curcumin were diluted 10 times with a 10 mM PBS buffer to achieve a 20−80% inhibition of the blank absorbance. Ten microliter samples or Trolox standards (at 0.5, 1.0, 1.5, 2.0, and 2.5 mM concentrations) were dissolved in 1 mL of ABTS radical solution, and the absorbance reading was taken at 30 °C exactly 1 min after initial mixing for up to 6 min. The 10 mM PBS was used as a blank in each run. Each sample was measured in triplicate, and the mean and standard deviation were calculated. The scavenging capability of test compounds was determined using eq 1 and converted to Trolox equivalent antioxidant capacity (TEAC) using the standard curve from Trolox solutions

$$\mathrm{ABTS}^{+}\text{scavenging}\ (\%) = (1 - A_s/A_c) \times 100 \quad (1)$$

where A_s and A_c are the absorbance of test and control (PBS) solutions, respectively.

In Vitro Cell Proliferation Assay. Human colon cancer cell line HCT-116 was purchased from American Type Culture Collection (Manassas, VA, USA) and was cultured McCoy's 5A medium (Mediatech, Manassas, VA, USA). The culture media contained 10% fetal bovine serum (Hyclone), 50 U/mL penicillin, and 50 μg/mL streptomycin. Cells were cultured at 37 °C under a humidified atmosphere of 5% CO_2. Anticancer activity of curcumin was tested by CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, WI, USA). Briefly, HCT-116 cells were seeded in 96-well microliter plates at a density of 1000 cells per well in a final volume of 100 μL of medium. After 24 h, the cells were treated with a medium containing dimethyl sulfoxide (DMSO)-dissolved or casein-encapsulated curcumin. Other cells were untreated (negative controls) or treated only with DMSO or NaCas at the concentrations as in the dispersions with encapsulated curcumin (positive controls). After 48 h, the cell culture medium was replaced with 20 μL of CellTiter 96 Aqueous One Solution and was incubated for 1 h at 37 °C. Absorbance at 490 nm was compared with a microplate reader (Bio-Tek Instruments, Winooski, VT, USA). Relative cell viability was expressed as the absorbance normalized by that of the untreated wells. The mean and standard deviation from four-well replicates were calculated. The cell viability was calculated according to eq 2. The normalized cell viability was obtained after normalizing the viability of a treatment by the viability of control cells treated with DMSO only.

$$\text{cell viability}\ (\%) = (A_{\text{treated}}/A_{\text{untreated}}) \times 100 \quad (2)$$

A_{treated} and $A_{\text{untreated}}$ are the absorbance of the treated and untreated cells, respectively.

Statistical Analysis. Statistical analyses were performed using the JMP program (JMP Statistical Software, SAS Institute, Cary, NC, USA). One-way analysis of variance was carried out. Differences between pairs of means were compared using a Tukey test. The significance level was set at 0.05.

RESULTS AND DISCUSSION

Encapsulation Properties. The spray-dried powder contained 16.7% (dry basis) curcumin. Compared to the theoretical loading before spray-drying (1.0085 g of curcumin and 4 g of NaCas, or 20.1% curcumin in total nonsolvent mass), the encapsulation efficiency (EE) was about 83.1%. The EE and loading level (LL) are higher than the 25.7−70.2% EE and 1.4−4.3% LL when encapsulated in Ploronic F68 block copolymers at different curcumin/polymer ratios.[15] The EE is also comparable to the EE of 85−90% for curcumin encapsulated in zein nanoparticles by electrohydrodynamic atomization[16] and the EE of 71.1−86.8% for curcumin encapsulated in zein nanoparticles by antisolvent precipitation.[31]

Impacts of Encapsulation on Crystallinity of Curcumin. To study the effects of encapsulation on the crystallinity of curcumin, XRD and DSC were performed. The XRD pattern of pristine curcumin showed several characteristic peaks resulting from its crystalline structure, whereas the typical amorphous XRD pattern was observed for NaCas (Figure 1A).

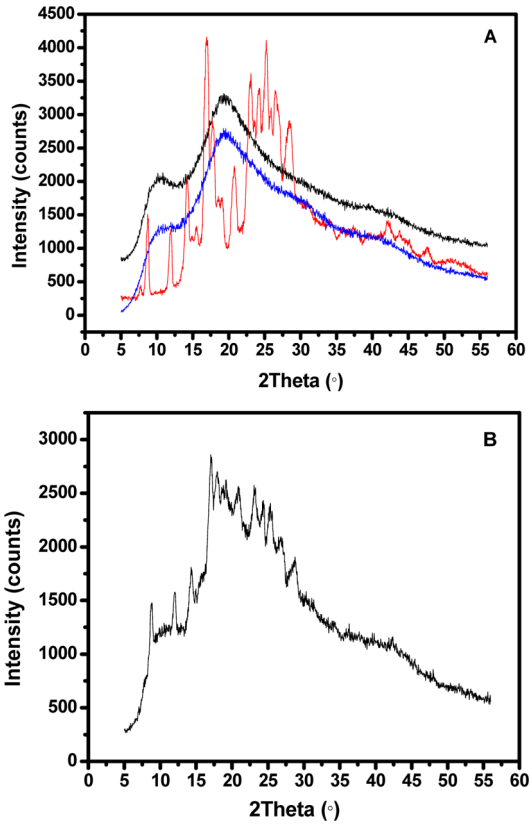

Figure 1. (A) XRD patterns of the processed sodium caseinate (NaCas, black), curcumin crystals (red), and curcumin encapsulated in NaCas (blue). (B) Simple mixture of curcumin crystals and NaCas at the same ratio as in the capsules in (A).

After encapsulation, the characteristic peaks of pristine curcumin disappeared (Figure 1A), whereas the simple physical mixture of pristine curcumin and NaCas powder at the same ratio maintained certain peaks of pristine curcumin (Figure 1B). The loss of crystalline structure of curcumin indicates successful encapsulation. Similar results have been reported after encapsulation of curcumin in zein nanoparticles, which

corresponded to turbid dispersions after hydration of freeze-dried powder in water.[31]

DSC results are shown in Figure 2. The smooth curve of NaCas indicates amorphous structure of caseins and thermal

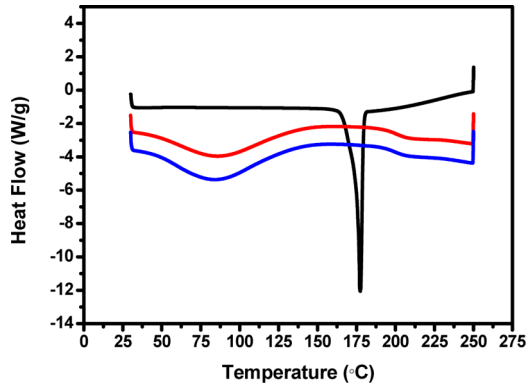

Figure 2. DSC thermograms of the processed sodium caseinate (NaCas, red), curcumin crystals (black), and curcumin encapsulated in NaCas (blue) during heating from 30 to 250 °C at a rate of 5 °C/min.

stability up to 250 °C. The sharp peak of pristine curcumin at 177.3 °C corresponds to the melting of curcumin crystals, which is consistent with the reported value of 176.4−177.5 °C.[32] The absence of the endothermic peak for curcumin capsules further confirms the loss of crystalline structure as observed in XRD.

Physical Properties of Transparent Dispersions. The transparent dispersion obtained by centrifuging the sample reconstituted with spray-dried capsules was determined to have 136.7 ± 1.3 μg/mL curcumin and 7.2 mg/mL casein. At the same curcumin concentration, free curcumin appeared as insoluble particulates (Figure 3) because of its low solubility of 11 ng/mL.[10] The encapsulation approach in this work thus dispersed curcumin more than 4 decades above the solubility while maintaining the transparent appearance.

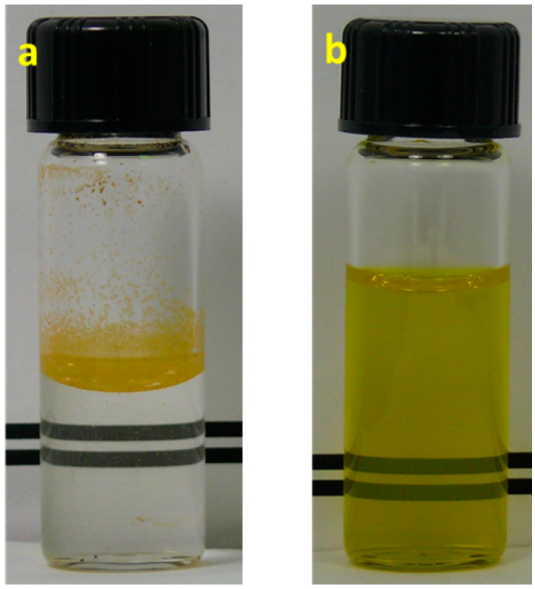

Figure 3. Free (a) and encapsulated (b) curcumin dispersed in deionized water at a concentration of 137 μg/mL.

It was previously reported that native casein micelles are capable of binding with curcumin in aqueous solution through hydrophobic interactions.[33] NaCas also contains four types of caseins, with α_{s1}-, α_{s2}-, and β-caseins being more hydrophobic than κ-casein. The curcumin concentration in the supernatant obtained by centrifuging the dispersion prepared by simply hydrating NaCas and curcumin in water was determined to be 30.2 ± 8.1 μg/mL, which was several-fold lower than the 136.7 μg/mL obtained by spray-drying warm aqueous ethanol solution, hydration of spray-dried powder, and centrifugation. Our results also were much higher than the 28 μg/mL curcumin dissolved in micelles of purified β-casein.[22]

The dispersion with curcumin encapsulated in NaCas had a zeta-potential of −31.1 ± 0.9 mV at pH 6.8. The hydrodynamic diameters of dispersions prepared from native NaCas, processed NaCas, and curcumin capsules are presented in Table 1. The dispersion of the processed NaCas had a

Table 1. Average Hydrodynamic Diameter of Native Sodium Caseinate (NaCas), Processed NaCas, and Curcumin Capsules after Hydration in Deionized Water

dispersion	mean diameter (nm)[a]	polydispersity[a]
native NaCas	216.0 ± 6.0a	0.222 ± 0.010a
processed NaCas	134.1 ± 3.5b	0.224 ± 0.005a
NaCas encapsulated with curcumin	168.7 ± 10.2c	0.280 ± 0.011b

[a]Numbers are the mean ± standard deviation. Different letters represent significant differences of mean.

significantly smaller hydrodynamic diameter than that of native NaCas, and the hydrodynamic diameter of the dispersion with curcumin nanocapsules was significantly larger than that of the processed NaCas but smaller than that of the native NaCas. Particles in dispersions of native NaCas and curcumin nanocapsules were further imaged using AFM, with the topography images presented in Figure 4 that also show the heights of selected particles. Native NaCas particles were mostly spherical and had a relatively narrow height distribution from 8 to 15 nm (Figure 4a). In comparison, curcumin-containing nanoparticles were less regular in shape and had a wider height distribution, ranging from 5 to 20 nm (Figure 4b), than those of native NaCas (Figure 4a).

There are considerable differences between NaCas and casein micelles. Compositionally, the two materials have different amounts of calcium phosphate.[34] Functionally, NaCas is more soluble than casein micelles.[35] Dispersions of NaCas have been reported for a much smaller hydrodynamic radius, from 10 to 100 nm,[36] than that of casein micelles. However, there are other studies reporting a hydrodynamic diameter larger than 200 nm in NaCas dispersions,[37,38] which agreed with transmission electron microscopy results in a study.[24] Sample preparation conditions including the source and concentration[39] of NaCas may have resulted in the discrepancies in the literature.

Nevertheless, structural changes of caseins in warm aqueous ethanol are expected to be driven by polarity that affects solvent quality and thus dissociation and reassociation of casein molecules.[40] The hypothesized mechanism of structural changes in the present study is then presented in Figure 5. When heated in aqueous ethanol, NaCas dissociates to smaller structures due to the improved solvent quality,[40] which was in

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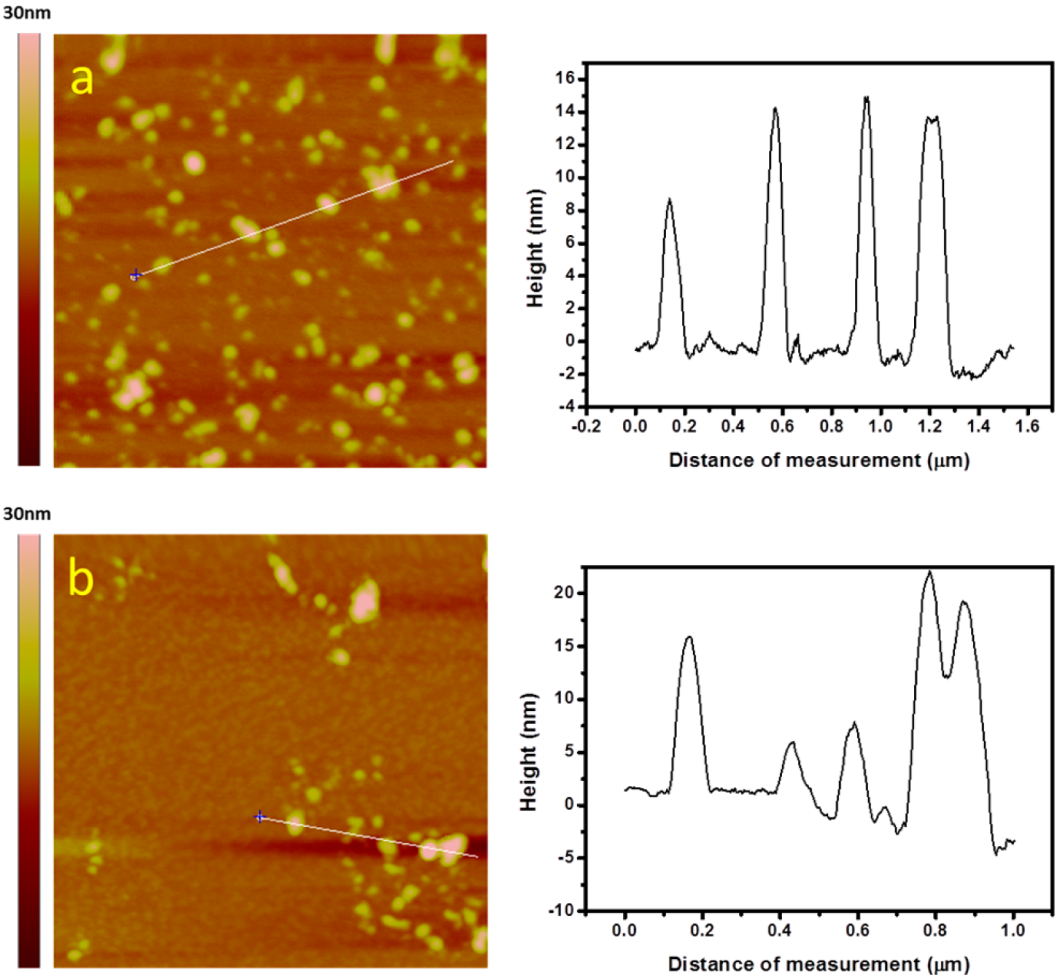

Figure 4. AFM topography images of the native sodium caseinate (a) and curcumin capsules (b). Plots indicate the height distribution along the measurement line.

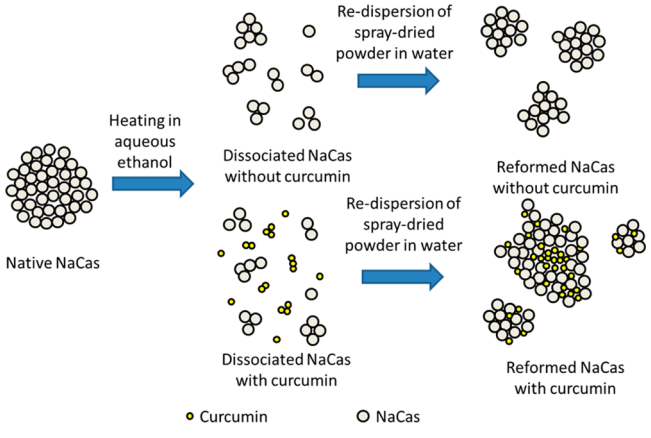

Figure 5. Schematic mechanism of the structural changes of sodium caseinate (NaCas) and encapsulation of curcumin.

agreement with our observation of the much reduced turbidity at 60 °C than at 21 °C (not shown). After spray-drying and rehydration, the dissociated caseins re-formed into nanoparticles smaller than those of native NaCas (Table 1), likely due to the irreversibility of disrupted physical forces (hydrophobic, hydrogen bonds, and calcium phosphate bridges). The dissociation of NaCas in warm aqueous ethanol increases the availability of hydrophobic sites of caseins for contact with curcumin during mixing. When spray-dried powder is hydrated, caseins reorganize into nanoparticles with curcumin, which increases the hydrodynamic diameter and broadens the particle size distribution of capsules when compared to the processed NaCas. For spray-dried particles with abundant curcumin, local precipitation of curcumin results in irregularly shaped nanoparticles with the surface being adsorbed by caseins (Figure 4b).

Interactions between Curcumin and Sodium Caseinate. The FTIR spectra of native NaCas, processed NaCas, pristine curcumin, and NaCas encapsulated with curcumin are shown in Figure 6 to study the interactions between curcumin and caseins. The characteristic absorbance peak around 3507 cm^{-1}, which corresponds to the −OH stretching vibration of curcumin, disappeared after encapsulation. There was a shift of the absorbance peak from 1529 to 1544 cm^{-1} after encapsulation of curcumin in NaCas, when compared with the native NaCas. This peak shift is due to structural changes of NaCas at the conditions used in encapsulation, as shown for the spectrum of the processed NaCas. The peak of native NaCas at 1658 cm^{-1} shifted to 1631 cm^{-1} after encapsulation of curcumin. Because this shift was not observed for processed NaCas, this change in FTIR spectrum after encapsulation is caused by the binding between curcumin and NaCas, possibly with the amide I group.

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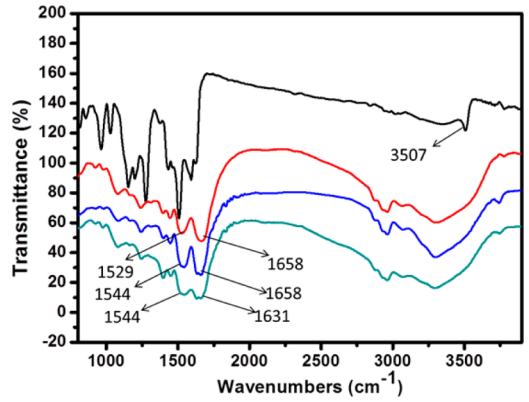

Figure 6. FT-IR spectra of the native sodium caseinate (NaCas, red), processed NaCas (blue), curcumin crytals (black), and encapsulated curcumin (green).

The binding between curcumin and NaCas was further confirmed by fluorescence spectroscopy (Figure 7). The

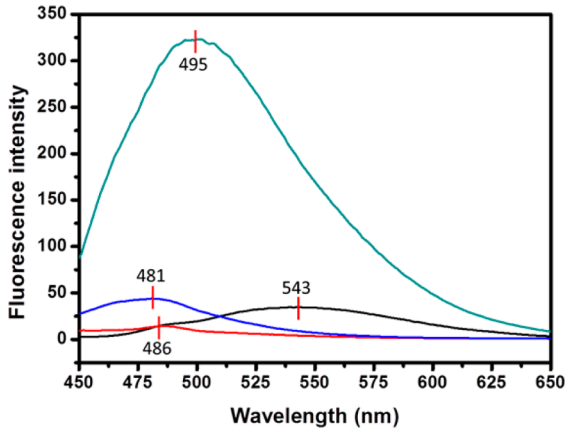

Figure 7. Fluorescence emission spectra of native sodium caseinate (NaCas, red), processed NaCas (blue), free curcumin (black), and encapsulated curcumin (green).

fluorescence spectrum of proteins can be affected by interactions with hydrophobic compounds and can be applied to study the microenvironment changes in aqueous systems.[41] It has been reported previously that curcumin binds with casein micelles through hydrophobic interactions,[33] which is also the case when curcumin binds with bovine serum albumin.[41] The blue shift of curcumin peak at 543 nm, to 495 nm, after encapsulation and the sharp increase in fluorescence intensity (Figure 7) agree with the results reported previously.[15,33] This indicates the environmental change of curcumin, from a more polar environment when dissolved in aqueous ethanol to the hydrophobic regions of caseins after encapsulation. This also suggests that curcumin is present at the core of nanocapsules in aqueous dispersions.

Bioactivity of Encapsulated Curcumin. The TEAC of curcumin before and after encapsulation is shown in Figure 8. The antioxidant properties have also been studied for caseins and casein-derived peptides,[42,43] which are ascribed to amino acid residues such as tryptophan and methionine cysteine that can be oxidized by free radicals.[44−46] Therefore, the TEAC of the processed NaCas was also determined, which was 300 μM (Figure 8). For curcumin, it potentially has a high antioxidant activity because its phenolic −OH groups play a major role in

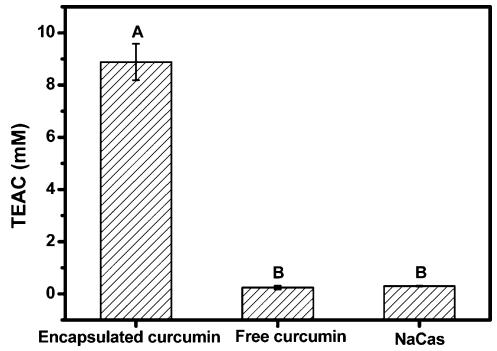

Figure 8. Trolox equivalent antioxidant capacity (TEAC) of the processed sodium casein (NaCas), free curcumin, and encapsulated curcumin at 30 °C. Different letters above the bars denote significant difference in the mean.

the oxidation reactions.[47] However, pristine curcumin has a low antioxidant activity (240 μM TEAC) when dissolved in 95% ethanol followed by dilution in water for testing, because the low solubility limits the amount of dissolved curcumin molecules to react with free radicals in the aqueous phase. The encapsulated curcumin had a much higher TEAC (8.87 mM) than those of pristine curcumin, processed NaCas, and their summation ($p < 0.05$). By dispersing in casein nanoparticles, the encapsulated curcumin is evenly distributed in the reaction medium, and the big surface area due to the nanometer-sized structures facilitates the kinetics of reaction with free radicals in the aqueous phase. The much improved antioxidant activity of curcumin after dispersion in colloidal particles such as β-casein and lecithins also has been reported.[22,48]

Figure 9 shows the normalized viability of human colon cancer HCT-116 cells after being treated with DMSO-dissolved

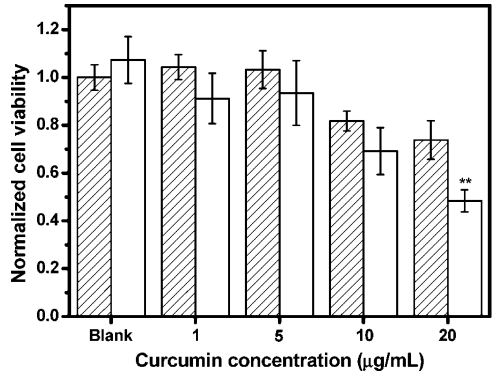

Figure 9. Normalized cell viability of DMSO-dissolved (hatched bars) and encapsulated (open bars) curcumin against HCT-116 cell line at different overall concentrations in media. The blanks were controls by using DMSO and sodium caseinate alone for free curcumin and encapsulated curcumin, respectively. The results were normalized by the viability of the control group treated with DMSO only. Error bars are standard deviations from four replicates. (**) Sample is significantly different from other treatments ($p < 0.05$).

or encapsulated curcumin at different concentrations. DMSO or NaCas alone did not show any significant inhibition of cell growth. However, the encapsulated curcumin exhibited a higher activity of cell growth arrest than curcumin dissolved in DMSO. The significant difference was noted when the curcumin concentration was >20 μg/mL ($p < 0.05$). The improved

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anticancer activity might have resulted from the much improved dispersibility after encapsulation in casein nanoparticles that provide a large surface area to contact with cancer cells. Similar improvements in anticancer activity have been reported for curcumin after binding with casein micelle[33] or complexing with hydrophobically modified starch.[18]

The approach in the present study does not require high-pressure homogenization and structural modification of food biopolymer, and the encapsulated curcumin is in the powdered form. These features are advantageous to encapsulate lipophilic bioactive compounds in industrial settings, particularly in the production of transparent beverages. It however should be noted that the eventual application of nanoencapsulated curcumin will require further testing of in vivo bioavailability and in vivo biological activities, as well as in vivo and in vitro toxicology studies to ensure safe application in foods and beverages. The allergenicity of caseins[49] is another limitation to some consumers.

In conclusion, dissociation of NaCas in warm aqueous ethanol exposed more hydrophobic regions of caseins for contacting curcumin. Upon hydration of spray-dried powder, the amount of curcumin in transparent dispersions improved 4-fold when compared to simply mixing NaCas and curcumin and was 4 decades higher than the water solubility of curcumin. Encapsulation reduced the crystallinity of curcumin. The bigger particle size of curcumin nanocapsules than of processed NaCas and the fluorescence spectroscopy together with the FTIR results suggested that curcumin was present in the capsule core due to hydrophobic interactions with caseins. The much improved dispersibility after encapsulation improved the reactivity of curcumin, resulting in enhanced biological activity as assessed by antioxidant and cell growth assays. The simple approach used in this work may be used to deliver a variety of lipophilic bioactive compounds to improve the health and wellness of consumers.

AUTHOR INFORMATION

Corresponding Author

*(Q.Z.) Postal address: Department of Food Science and Technology, The University of Tennessee, 2605 River Drive, Knoxville, TN 37996, USA. Phone: (865) 974-6196. Fax: (865) 974-7332. E-mail: qzhong@utk.edu.

Notes

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