Supporting Information

Fabrication, characterization, and application of chitosan-NaOH modified casein nanoparticles and its stabilized long-term stable high internal phase Pickering emulsions

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1. Materials and methods

1.1 Characterization of CS-CA NPs

The particle size, polydispersity index (PDI), and zeta potential of the prepared two kinds of NPs under different pH values were performed by dynamic light scattering using a Zetasizer Nano-ZS (Malvern Instruments).

The morphological characteristic of the two types of CS-CA NPs at pH 4.0 was characterized by transmission electron microscopy (TEM) analysis (JEOL H-7650, Hitachi, Japan). The interaction mechanism between CS and CA was characterized by Fourier transform infrared (FTIR) spectrum (IRPrestige-21, Shimadzu, Japan) over the range (500-4000 cm-1). Besides, the turbidity of CS-CA NPs was determined by the absorbance at a wavenumber of 600 nm from the UV-Vis spectrophotometer (UV-2550, Shimadzu, Japan).

The Germany DCAT21 (Data Physics Co.) surface tension meter with platinum pallet was used to detect the equilibrium tension at the corn oil-water interface. The CS-CA NPs1 (CS = 0.2 wt%, CA = 0.8 wt%), and CS-CA NPs2 (CS = 0.4 wt%, CA = 0.8 wt%) were tested according to Wilhelmy plate methods (25 ℃).

1.2 Lipid oxidation measurement of HIPPEs

The freshly prepared HIPPEs were placed in glass tubes and incubated in a thermostat at 60℃ for 14 d to accelerate the oxidation of the corn oil. An aliquot of HIPPEs was taken out at fixed time intervals to determine the primary oxidant products and the final concentration of the secondary oxidant products by previous reports with slight modification[1, 2]. For the primary oxidant products, HIPPEs (300 mg) was fully mixed with isooctane/2-propanol (1.5 mL, 3:1 v/v) and then centrifugated at 1000 g for 2 min. Subsequently, the supernatant (0.2 mL) was

transferred to 1-butanol/methanol (2.8 mL, 2:1 v/v) solutions with the addition of NH_4SCN (15 μL, 3.94M) and Fe^{2+} (15 μL, produced by mixing a 0.132 M $BaCl_2$ solution with 0.144 M $FeSO_4$) solution to render color. The absorbance of the obtained samples at 510 nm was measured after the 20 min dark reaction at ambient temperature using a UV-Vis spectrophotometer (UV-2550, Shimadzu, Tokyo, Japan). The lipid hydroperoxide (LH) concentration was examined according to the standard curve from cumene hydroperoxide. As for the secondary lipid oxidation products (malondialdehyde, MDA), 100 mg of the HIPPEs was blended with 0.9 mL of deionized water and 2.0 mL of TBA solution, consisting of TBA (0.375 g) and trichloroacetic acid (15 g) in 100 mL 0.25 M HCl solution. The mixtures were boiled in a water bath for 15 min, cooled to room temperature, and then centrifuged at 2000 g for 15 min. Finally, the absorbance at 532 nm of the supernatant was determined by the UV-Vis spectrophotometer described above and calculated by a standard curve from 1,1,3,3-tetraethoxypropane.

1.3 Chemical stability of BC

For the UV-light stability, the HIPPEs loaded with BC were spread onto a 60 × 15 mm (diameter × height) dish. Then, the samples were exposed in a UV chamber (WFH-204B, China) equipped with two UV lamps with a power of 12 W and wavelength of 365 nm at ambient temperature for 72 h. For the thermal stability, the HIPPEs loaded with BC were poured into the glass bottle and incubated in a thermostat at 80℃ for 8 h. As for the storage stability, the samples were stored at room temperature for 25 days. BC fully suspended in the corn oil phase was used as a control. At designed time intervals, the stability of BC was measured by the method reported previously[3]. Briefly, 1 mL dimethylsulfoxide (DMSO) and 0.5 g HIPPEs

samples were absolutely vortexed and centrifugated at 12000 rpm for 20 s. Then, 100 μL samples were taken out from the supernatant and mixed with 900 μL organic solvent (n-hexane: dichloromethane = 4:1, v/v) by vortexing completely. The absorbance of the extracts was measured at 450 nm by the UV-Vis spectrophotometer quantified with a standard curve by dissolving the BC in the organic solvent. All the collected data were illustrated by the function of Logistic supported by OriginPro 2021 (Learning Edition).

2. Results

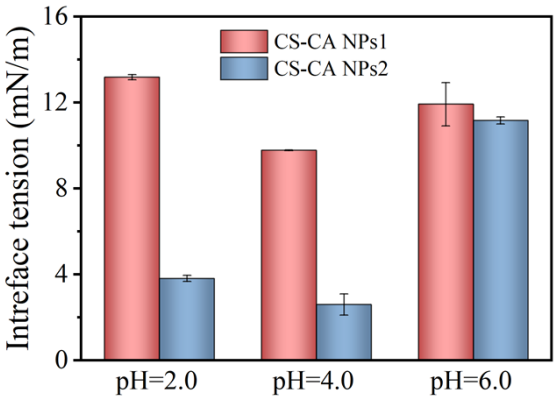

Fig. S1. Interface tension of CS-CA NPs.

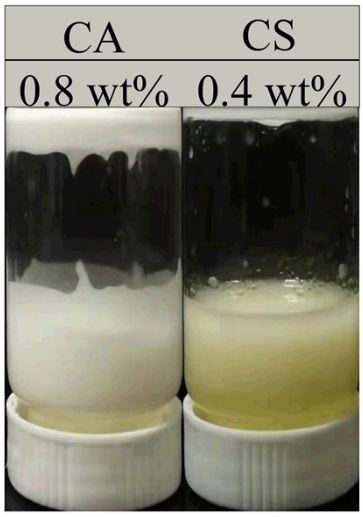

Fig. S2. Photographs of HIPPEs stabilized by solely CS or CA.

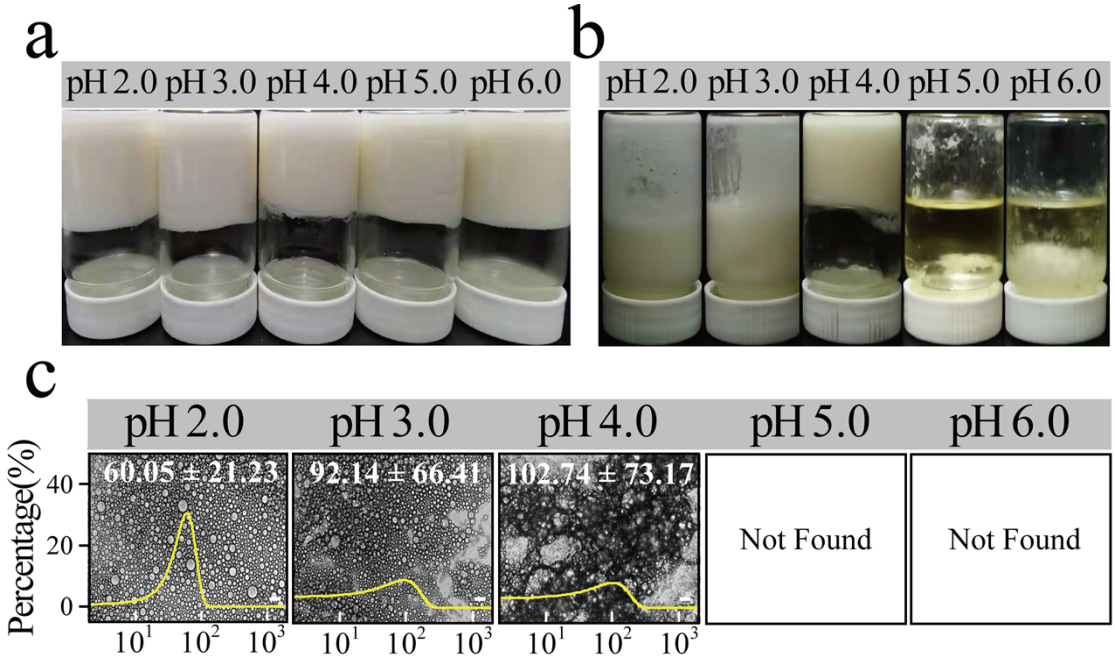

Fig. S3. Visual appearance of CS-CA NPs1 stabilized HIPPEs. Newly prepared (a). After 6 months of storage (b). Microscope images after 6 months of storage (c, scale bar: 200 μm, 10 ×).

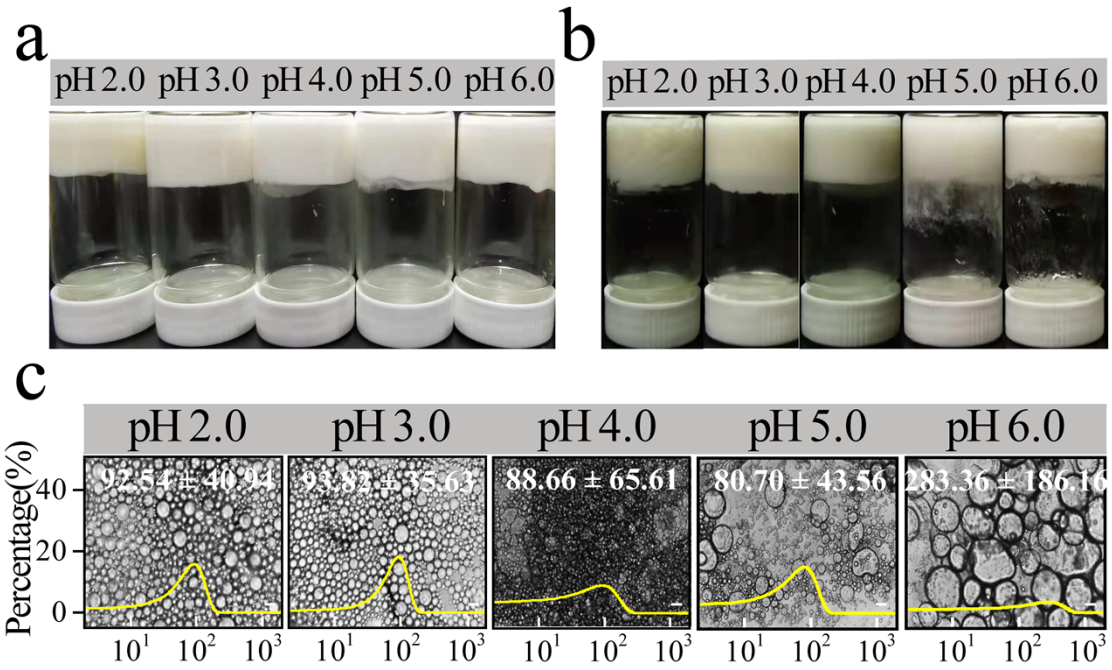

Fig. S4. Visual appearance of CS-CA NPs1 stabilized HIPPEs. Newly prepared (a). After 6 months of storage (b). Microscope images after 6 months of storage (c, scale bar: 200 μm, 10 ×).

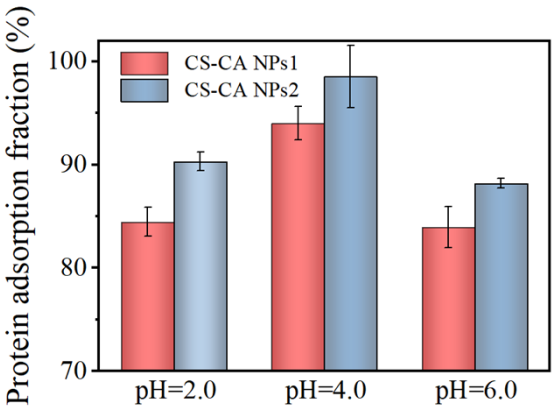

Fig. S5. Protein adsorption fraction of CS-CA NPs stabilized HIPPEs at pH 2.0, 4.0, and 6.0.

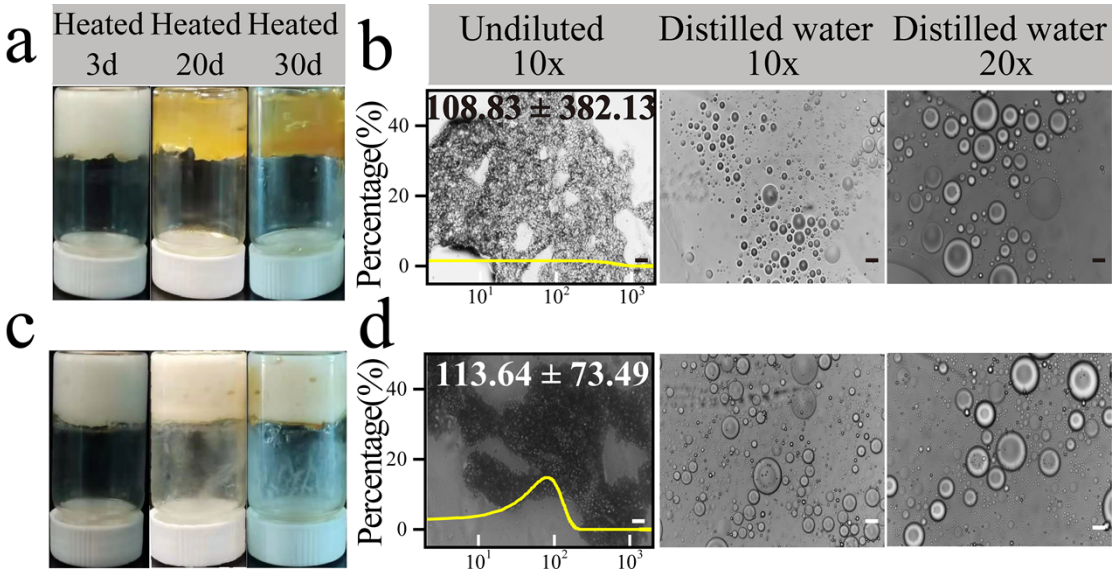

Fig. S6. Visual appearance of CS-CA NPs stabilized HIPPEs at pH 4.0 during the storage of 80℃ for 1 months (NPs1: a, NPs2: c). Microscope images of the HIPPEs after the storage of 80℃ for 1 months (NPs1: b, NPs2: d, scale bar: 200 μm, 10 ×).

Reference

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3. W. Li, Y. Nian, Y. Huang, X. Zeng, Q. Chen and B. Hu, High loading contents, distribution and stability of beta-carotene encapsulated in high internal phase emulsions, *Food Hydrocolloids*, 2019, **96**, 300-309.