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Incidental food nanoparticles from freshwater clam soup stabilize Pickering emulsions

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Daily foods contain abundant amounts of nanoparticles produced by the self-assembly of amphiphilic food components during processing. These nanoparticles are referred to as incidental food nanoparticles (iFNPs) because they are formed incidentally rather than through a specific nanoparticle fabrication method. Among them, freshwater clam soup nanoparticles (FCNPs) have potential as nanocarriers, but their capacity as stabilizers for pickering emulsions (PEs) has not yet been explored. This study investigated the feasibility of FCNPs as stabilizers for food-grade PEs. FCNPs were characterized and showed a contact angle of 54.64 ± 1.06°, indicating their suitability for stabilizing O/W PEs. FCNPs stabilized medium-chain triglycerides at different concentrations (1%-5%) and oil fractions (φ = 20%-80%) to form PEs or high internal phase PEs with excellent stability. These PEs were dominated by elastic gel-like and typical shear-thinning behavior. Increasing either the FCNPs concentration or the oil fraction was resulted in emulsions with smaller droplets and higher gel strength. These FCNPs-stabilized PEs were stable at 4 °C for 90 days and across broad ionic strength (0-600 mM) and pH (3-11) ranges, as well as under pasteurization treatments. These findings provide new insight into the development of food-grade PEs by utilizing iFNPs, widely present in daily foods, as a novel class of safe, effective, and 'clean-label' stabilizers.

Pickering emulsions (PEs) are stable emulsions that use solid particles to stabilise the interface between oil and water. These particles are irreversibly adsorbed onto the oil-water interface, providing steric hindrance and preventing droplet coalescence to create highly stable emulsions[1]. Due to this unique feature, PEs have emerged as a promising alternative to conventional surfactant-based emulsions in various industries, including cosmetics, food and pharmaceuticals[2].

The solid particles used to stabilise PEs can be either organic or inorganic, depending on their chemical composition[3,4]. Common inorganic particles, such as clay, silica, and magnetic Fe_3O_4 nanoparticles, have been extensively studied and widely applied due to their ease of surface modification and controllable size and structure[5–7]. However, concerns about their toxicity, low biodegradability and bioaccessibility have limited their use, particularly in the pharmaceutical, food and cosmetics industries[8].

Consequently, there has been growing interest in exploring and applying organic particles, particularly those based on natural biomacromolecules. Indeed, numerous studies have demonstrated the efficacy of natural biomacromolecule-based nanoparticles as stabilisers for PEs[9]. For example, a W/O/W double-layer Pickering emulsion was successfully prepared using one-step emulsification with zein nanoparticles and oppositely charged cellulose nanocrystals[10], and a stable high internal phase Pickering emulsion was prepared using a terpolymer containing zein, sodium caseinate, and propylene glycol alginate[11].

Daily foods are a rich source of micro/nanoparticles produced by the self-assembly of amphiphilic food components (e.g., proteins, polysaccharides, lipids) during routine processing (e.g., heating, cooling, emulsification)[12,13]. This formation mechanism stands in sharp contrast to conventional engineered nanoparticles (e.g., zein, casein nanoparticles) which typically require specific pH adjustments, anti-solvent precipitation, or the use of chemical reagents like ethanol[14,15]. These incidental food nanoparticles (iFNPs), formed as an inherent part of food preparation, widely occur in various foods with a long history of consumption[12]. For instance, loads of proteoglycan-lipid nanoparticles were produced during heating processing and have been isolated and characterized with size ranges from 40 nm to 149 nm from Freshwater Clams (*Corbicula fluminea* Muller) soup, a renowned folk remedy in China and other parts of East Asia[12,13]. Porcine bone soup, a traditional nourishing food, has been demonstrated to be rich in nanoscale colloidal particles with an average size of 230 nm[16].

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Numerous spherical nanoparticles of 150-200 nm in diameter were massively found in black tea infusion during tea brewing[17]. A large number of self-assembled micro/nanoparticles consisting of docosahexaenoic acid (DHA) and eicosatetraenoic acid (EPA) have been confirmed in big eye tuna (*Thunnus obesus*) head soup[18]. However, it is rarely explored whether these iFNPs can act as stabilizers for PEs.

Beyond their mere existence, iFNPs present a significant, untapped opportunity for food applications due to their unique safety profile. The safety of nanoparticles is a dual consideration: the safety of the constituent material and the safety of the nanostructure itself[19]. While many engineered nanoparticles are fabricated from food-grade biomacromolecules (e.g., zein, casein) and are thus materially safe, their novel nanostructures have not been subjected to long-term human consumption, and their toxicological profiles are still under active investigation[20,21]. In contrast, iFNPs, such as those from clam soup, have been safely consumed as part of the human diet for millennia. This long history of consumption provides robust, real-world evidence for the biosafety of their nanostructures. Therefore, exploring iFNPs as Pickering stabilizers is not just a search for a new functional ingredient, but a novel approach to developing 'clean-label' emulsions with an unparalleled, time-tested safety profile.

Previous studies have isolated proteoglycan-lipid iFNPs from freshwater clam soup (freshwater clam soup nanoparticles (FCNPs)), which could serve as a nanocarrier and encapsulated the majority of phytosterols (>80%) contained in the clam meat. Moreover, these iFNPs from freshwater clam soup could effectively inhibit the uptake of cholesterol in vitro[12]. In this study, we fabricated a Pickering emulsion by using proteoglycan-lipid iFNPs from freshwater clam soup as stabilizers. The obtained PEs including high internal phase PEs (HIPEs), were subsequently studied and characterized.

Results and discussion

Characteristics of the FCNPs

The mean particle size and ζ-potential of FCNPs separated by ultrafiltration were 68.23 ± 0.48 nm and −10.8 ± 0.25 mV, respectively (Fig. 1A, B; Table 1). Transmission electron microscopy (TEM) observations confirmed the FCNPs were spherical and well-defined (Fig. 1C). These FCNPs consist of a proteoglycan-lipid complex, as previously determined by ion exchange chromatography[12]. Furthermore, previous studies have demonstrated the outstanding pH, storage, ionic strength and temperature stability of FCNPs[12].

Particle wettability is a critical parameter for stabilizing PEs, as the solid particles must be partially wetted by both the oil and aqueous phases. The wettability of FCNPs was evaluated by the air-water contact angle, which was measured to be 54.64 ± 1.06° (Fig. 1D). This value, being below 90°, indicates that FCNPs are predominantly hydrophilic, making them suitable candidates for stabilizing oil-in-water (O/W) PEs. This observed hydrophilicity is consistent with the chemical composition of the FCNPs, which are predominantly composed of hydrophilic polysaccharides (71.18%, wt%) and proteins (10.35%, wt%), with only a minor fraction of hydrophobic lipids (2.00%, wt%) (Table 1).

To further confirm their interfacial activity, the dynamic interfacial tension (IFT) at the medium-chain triglycerides (MCT)-water interface was measured using the pendant-drop method. As shown in Fig. 1E, the IFT of the pure MCT-water interface (in the absence of FCNPs) was approximately 19.16 mN/m. Upon the addition of FCNPs to the aqueous phase, the IFT exhibited a clear concentration-dependent decrease, reaching a minimum value of 8.09 mN/m. Taken together, these results confirm that FCNPs are interfacially active and can spontaneously adsorb to the oil-water interface, providing the necessary stabilization for O/W Pickering emulsions.

Effect of oil fraction (φ) and particle concentration on PEs and HIPEs

The FCNPs-stabilized PEs could be diluted indefinitely in water but not in MCT, indicating the formation of an FCNPs-stabilized O/W PEs (Fig. 2A).

Confocal laser scanning microscopy (CLSM) was used to observe the interfacial micromorphology. As shown in the CLSM images (Fig. 2C-E), FCNPs (stained green with 5-DTAF; Fig. 2C) were clearly adsorbed at the interface of the oil droplets (stained red with Nile Red; Fig. 2D). The merged image (Fig. 2E) confirms the O/W structure, with FCNPs forming a distinct layer that acted as a physical barrier against creaming, flocculation, and coalescence.

The influence of the oil fraction (φ 20-80% v/v) on the formation of PEs was systematically investigated, holding the FCNPs concentration constant at 3.0 wt% (Fig. 2B). The volume-weighted mean diameter ($D_{4,3}$) of the FCNPs- stabilized PEs decreased gradually from 21.81 ± 0.24 μm to 4.21 ± 0.03 μm as the φ increased (Fig. 2F). This reduction in droplet size may be attributed to the increased efficiency of droplet break-up during emulsification at higher φ. Specifically, an increase in the φ typically leads to an increase in the overall viscosity of the emulsion, thereby facilitating more effective droplet breakup under the applied shear forces during mixing. This result is consistent with increased viscosity promoting improved droplet breakup[22]. FCNPs- stabilized PEs underwent a fast-creaming process and separated into cream and aqueous phases due to the density differences between water and oil[23]. The creaming index (CI) and $D_{4,3}$ of FCNPs-PEs did not change significantly during the storage period of 90 days (Fig. 2G), indicating that FCNPs-stabilized PEs had good creaming stability. Meanwhile, the value of the CI decreased gradually with the increase of φ, and no stratification (CI = 0%) was observed at φ = 80% (Fig. 2G). This may be attributed to the formation of a compact network and elastic gel-like structure as the φ increases, which prevents flocculation/agglomeration of emulsion droplets and thus improves the stability of the emulsion[24].

FCNPs were capable of stabilizing PEs with MCT across a wide range of φ (20-80%), including HIPEs at φ > 74%. The study evaluated the effect of FCNPs concentration (1-5%, w/v) on the formation and stability of emulsions at both φ = 60% and φ = 80%. As shown in Fig. 3, for PEs (φ = 60%), all tested FCNPs concentrations yielded stable emulsions. A clear concentration-dependent effect was observed, where the volume-weighted mean diameter ($D_{4,3}$) of the droplets systematically decreased from 39.45 ± 0.42 μm to 4.56 ± 0.01 μm as the FCNPs concentration increased from 1% to 5%. In contrast, for HIPEs (φ = 80%), stable emulsions were only formed at FCNPs concentrations of 2% and above. Within this effective range (2-5%), the $D_{4,3}$ value similarly decreased from 7.09 ± 0.13 μm to 3.12 ± 0.08 μm. This reduction in droplet size is attributed to the greater availability of particles to populate the oil-water interface, which lowers interfacial tension and facilitates the formation of smaller droplets during homogenization[25].

The long-term stability of the emulsions was also strongly influenced by particle concentration (Fig. 3E&F). The HIPEs formulated with 2-5% FCNPs exhibited exceptional stability over a 90-day storage period, with a CI near 0% and no significant changes in $D_{4,3}$ (Fig. 3D&F). Conversely, the 1% FCNP HIPE formulation was unstable and underwent rapid phase separation. The PEs also showed improved stability with higher FCNPs content; the CI value after 90 days decreased progressively as FCNPs concentration increased, reaching a minimum of approximately 8% at 5% FCNPs. These results collectively demonstrate the excellent capacity of FCNPs as a Pickering emulsifier for both conventional and high internal phase emulsions.

The rheological properties and apparent viscosity of FCNPs-stabilized PEs are related to their stability and microstructure. The storage modulus (G′), loss modulus (G″), and apparent viscosity of the FCNPs-stabilized PEs with various FCNPs concentrations and φ were further investigated. As shown in Fig. 3G-J, the G′ values of all samples were always higher than the corresponding G″ values over the frequency scanning range. Additionally, the apparent viscosity decreased with increasing shear rate in all investigated cases. This indicated that FCNPs-stabilized PEs predominantly exhibited elastic gel-like behavior and were typically shear-thinning[26]. Both moduli showed a weak frequency dependence, characteristic of an elastic gel-like structure resulting from densely packed particle layers[27,28]. The modulus and apparent viscosity increased with increasing FCNPs concentration (1%-5%) and oil fraction (φ = 60% and φ = 80%). Some oil droplet sizes may result in higher viscosity and gel

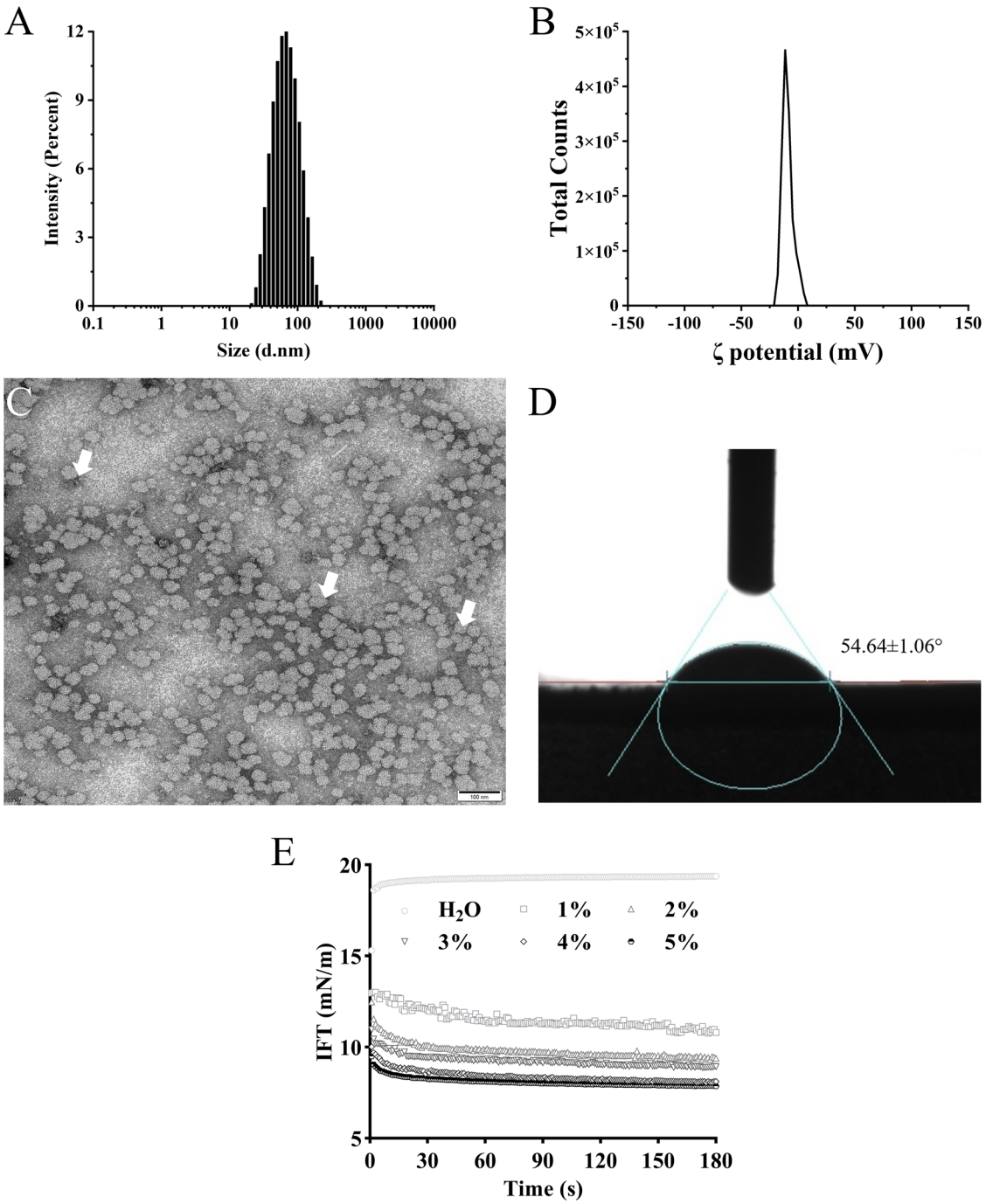

Fig. 1 | Structural and interfacial characterization of FCNPs. A Particle size distribution of FCNPs; **B** Zeta potential distribution of FCNPs; **C** TEM micrographs of FCNPs at magnification ×20,000; **D** The water-in-air contact angle of FCNPs; **E** Dynamic IFT of oil/water interfaces with or without FCNPs.

Table 1 | Properties of iFNPs Derived from C. *fluminea* Soup

	iFNPs
Hydrodynamic diameter (d.nm)	68.23 ± 0.48
ζ-potential (mV)	−10.8 ± 0.25
PDI	0.234 ± 0.003
Protein (wt %)	10.35 ± 0.30
Carbohydrates (wt %)	71.18 ± 0.18
Lipids (wt %)	2.00 ± 0.02

strength of the emulsion[1]. Additionally, as the oil fraction increased, the Pickering emulsion droplets formed a denser packing density structure, resulting in a higher modulus and viscosity[29].

Stability of PEs and HIPEs against ionic strength and pH

PEs in commercial products must withstand various salt concentrations and pH levels. The study, therefore, examined the impact of NaCl concentration (0-600 mM) and pH (3-11) on emulsion stability. As shown in Figs. 4 and 5, there were no noticeable differences in appearance or evidence of phase separation across all tested conditions. Changes in ionic strength and pH had minimal effect on the droplet size of FCNPs-stabilized PEs and FCNPs-stabilized HIPEs. The size distribution of these emulsions largely remained

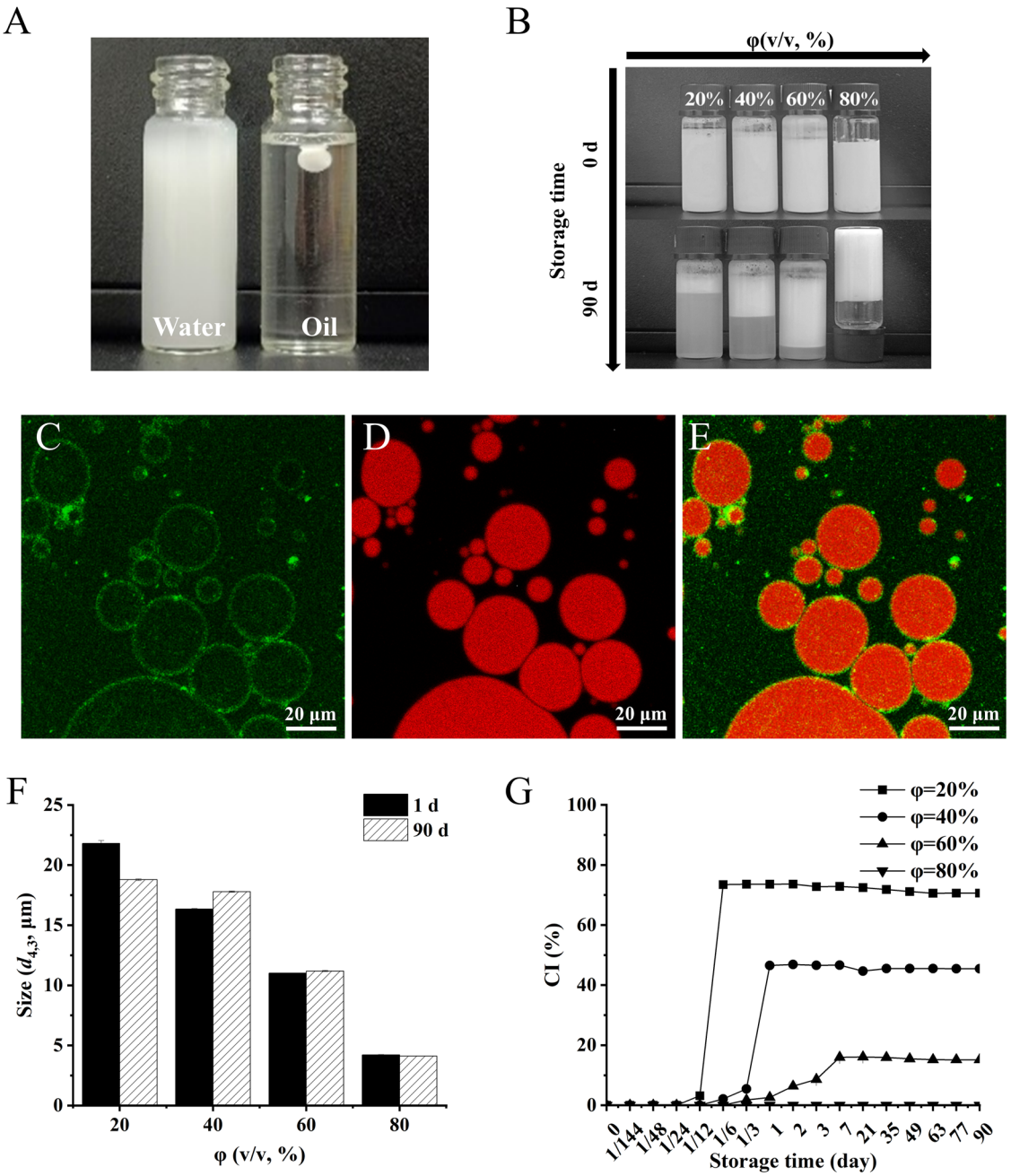

Fig. 2 | Storage stability and microstructural characterization of FCNP-stabilized Pickering emulsions. A The drop test of Pickering emulsions stabilized by FCNPs; Visual observation (**B**), average droplet size (**F**), and CI% (**G**) of Pickering emulsions stabilized by FCNPs with particle concentrations of 3.0 wt% at various oil fraction (20-80%), after a storage period of 90 days at 4 °C; CLSM images of FCNPs-stabilized PEs with the particle concentration of 3.0 wt% at the oil fraction of 60% (**C**-**E**). FCNPs dyed with 5-DTAF (**C**), Oil phase dyed with Nile red (**D**), overlapped image (**E**).

narrow and uniform. Moreover, after 7 days of storage, there were no significant changes in droplet size or size distribution. These results indicated that FCNPs-stabilized PEs and HIPEs possess excellent stability against ionic strength and pH variations.

Stability of PEs and HIPEs against thermal sterilization treatments

To prevent microbial contamination in commercial products, FCNPs-stabilized PEs and HIPEs may require thermal sterilization. The study systematically evaluated the impact of three heat treatments—pasteurisation (62 °C, 30 minutes), pasteurisation (75 °C, 15 minutes), and autoclaving (121 °C, 25 minutes)—on emulsion stability. Stability was assessed by visual appearance, droplet size and size distribution characteristics immediately after treatment (Day 0) and after 7 days of storage. As shown in Fig. 6, both FCNPs-stabilized PEs and FCNPs-stabilized HIPEs demonstrated excellent stability under both pasteurization conditions (62 °C for 30 minutes or 75 °C for 15 minutes). FCNPs-stabilized PEs also remained stable after autoclaving (121 °C for 25 minutes). For these stable samples, no significant changes in visual appearance, droplet size, or distribution were observed between Day 0 and Day 7 (Fig. 6A, C, E). In contrast, the FCNPs-stabilized HIPEs were unstable under autoclaving conditions. This harsh treatment induced severe droplet coalescence, evidenced by a dramatic increase in the oil droplet size from 3.82 ± 0.05 μm to 36.09 ± 1.40 μm (Fig. 6D) and a broadening of the size distribution (Fig. 6F). Although complete phase separation was not visible within the 7-day storage period, this significant microstructural disruption demonstrates that the HIPEs are unstable under

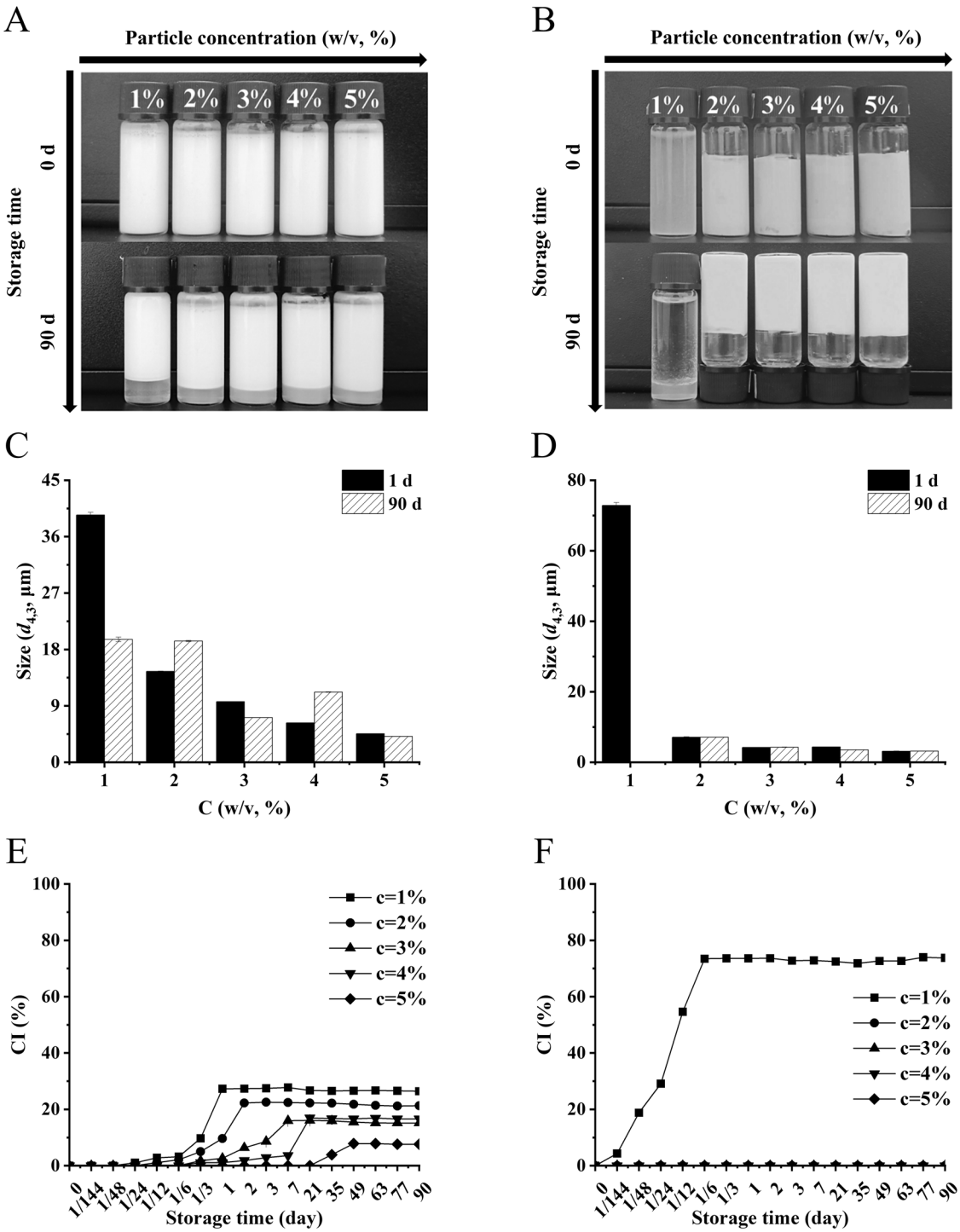

Fig. 3 | Impact of particle concentration on the storage stability and rheological properties of FCNP-stabilized emulsions. Visual observation (**A**, **B**), average droplet size (**C**, **D**), CI (**E**, **F**), apparent viscosity (**G**, **I**), and rheological properties (**H**, **J**) of the Pickering emulsions stabilized by FCNPs with the particle concentration of 1.0-5.0 wt% at the oil fraction of 60% or 80%, after a storage period of 90 days at 4 °C. Panels (**A**), (**C**), (**E**), (**G**), and (**I**) were the FCNPs-stabilized PEs at the oil fraction of 60%; (**B**), (**D**), (**F**), (**H**), and (**J**) were the FCNPs-stabilized HIPEs at the oil fraction of 80%. Data were expressed as the mean ± SD (n = 3).

these harsh thermal conditions and will likely lead to rapid creaming upon extended storage.

This study demonstrates that FCNPs can fabricate PEs that are highly stable against variations in pH, ionic strength and temperature. This indicates the broad potential of iFNPs, formed during food processing, to stabilize PEs and suggests a novel approach to developing new food-grade PEs. These findings validate FCNPs as a distinct class of stabilizers, differentiated by their unique origin and established safety profile. Their generation is both sustainable and simple: unlike engineered nanoparticles (NPs) requiring chemical synthesis, FCNPs form via spontaneous self-assembly during conventional thermal processing (i.e., boiling). This represents a simple, reproducible, and chemical-free 'bottom-up' fabrication method[12]. These processing-induced iFNPs (e.g., from soup) can even be directly utilized to formulate stable PEs and High Internal Phase Emulsions (HIPEs), linking culinary processes to functional food systems. Critically, their established safety profile

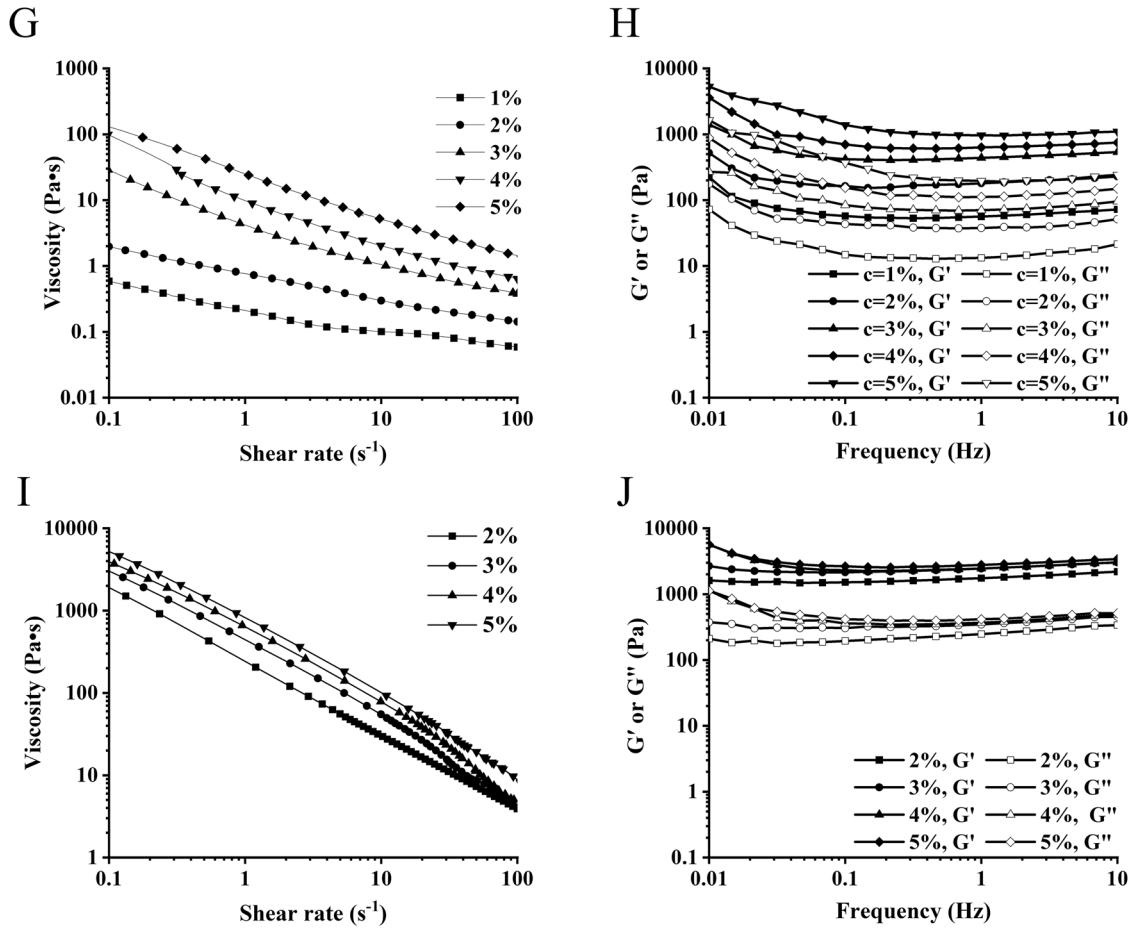

Fig. 3 | Continued

contrasts sharply with conventional Pickering stabilizers. Conventionally, the stabilization of PEs has relied on exogenous particles. For example, inorganic nanoparticles such as silica (SiO_2) are indigestible in vivo[30], posing potential biosafety risks and limits their application[31]. While nanoparticles synthesised from edible biomacromolecules (e.g., zein, soy protein isolate (SPI)) are chemically safe, the biosafety of their novel nanostructures requires thorough evaluation[20,21]. The distinct advantage of iFNPs, however, lies in their endogenous origin and long history of consumption. Composed of food constituents that are Generally Recognised as Safe (GRAS), their nanostructures have also been safely ingested as part of traditional foods for millennia. This long-term dietary exposure provides a critical, real-world validation of their biocompatibility that engineered nanoparticles lack. Therefore, utilising iFNPs as natural, safe Pickering stabilizers is a promising and innovative new line of research for food emulsions, addressing the growing consumer and regulatory demand for ingredients that are not only functional but also demonstrably safe and non-novel.

This study demonstrates that PEs can be successfully fabricated using FCNPs as a stabilizer. FCNPs could stabilize MCT with oil fractions ranging from 20% to 80%, forming PEs or HIPEs with excellent stability. After 90 days at 4 °C, all FCNP-stabilized PEs or HIPEs showed no change in appearance and minimal change in droplet size. The emulsions predominantly exhibited elastic, gel-like, and shear-thinning behavior. Increasing the particle concentration or oil fraction caused the droplet sizes to decrease and the PE gel strength to increase. These PEs stabilized by FCNPs possess excellent stability at a broad range of ionic strengths (0-600 mM) and pH levels (3-11), as well as under pasteurization treatments (62 °C for 30 minutes or 75 °C for 15 minutes). The PEs were also stable under autoclaving conditions (121 °C for 25 minutes), though HIPEs exhibited significant coalescence. This study successfully demonstrates the principle and feasibility of using iFNPs as effective and robust Pickering stabilizers. However, we acknowledge the limitations of this work. This study only investigated one type of iFNPs: FCNPs. Future research should explore other types of iFNPs from different food sources and their adsorption kinetics and in situ interfacial structures. Furthermore, a detailed comparison of the physicochemical properties and interfacial behaviors of these iFNPs and engineered nanoparticles is necessary. Validating the use of iFNPs from daily foods opens a new avenue for developing "clean-label" emulsions. This approach utilizes a novel class of stabilizers that are distinguished by their green culinary formation, "ready-to-use" functionality, and a proven, multi-millennial history of safe human consumption, which validates the safety of their nanostructure.

Methods

Materials

Freshwater clam (*Corbicula fluminea* Muller) was cultivated and collected by Hepu Aquaculture Farm (Hepu, Guangxi, China). MCT was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). 5-([4,6-Dichlorotriazin-2-yl]amino)fluorescein hydrochloride (5-DTAF) and Nile Red were purchased from Sigma-Aldrich Co., Ltd (St. Louis, MO, USA.). All other chemicals were of analytical grade and used without further purification. They were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA.) and Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

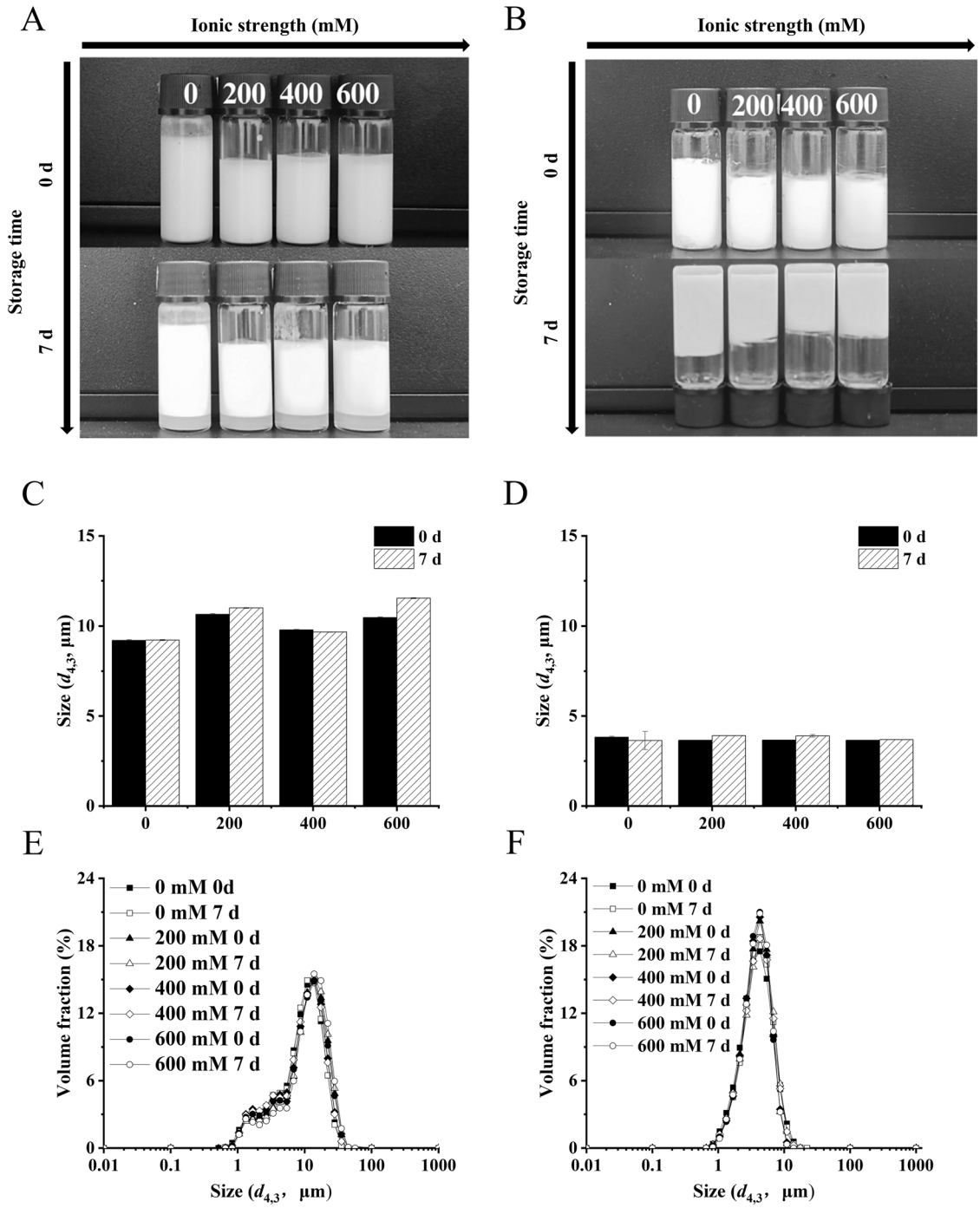

Fig. 4 | Influence of ionic strength on the physicochemical properties of FCNP-stabilized Pickering emulsions. Influence of ionic strength (0-600 mM) on the visual observation (**A**, **B**), average droplet size (**C**, **D**), and the particle size distribution (**E**, **F**) of the Pickering emulsions stabilized by FCNPs with the particle concentration of 3.0 wt% at the oil fraction of 60% or 80%. Panels (**A**), (**C**), and (**E**) were the FCNPs-stabilized PEs at the oil fraction of 60%; (**B**), (**D**), and (**F**) were the FCNPs-stabilized HIPEs at the oil fraction of 80%. Data were expressed as the mean ± SD ($n = 3$).

Preparation of freshwater clam soup nanoparticles

Freshwater clam soup nanoparticles were prepared as previously[12]. Briefly, freshwater clams were extracted with boiling deionized water (1:1, w/v) for 60 minutes. The soup was cooled to room temperature and centrifuged at 21,528 ×g for 10 minutes (Model CF16RX-II, Hitachi Koki Co., Ltd., Tokyo, Japan) to remove the solid residues. The supernatant was ultrafiltered using an Amicon™ Ultra Centrifugal Filter tube (*Mw* cut-off 100 kDa, Millipore Corporation, Billerica, MA, USA.) by centrifugation at 3500 ×g for 20 minutes. The retentate was collected, lyophilized, and designated as freshwater clam soup nanoparticles. The contents of protein, lipid and carbohydrate were determined using the Bicinchoninic acid (BCA) assay, GPO-PAP assay and anthrone-sulfuric acid assay, respectively.

Average hydrodynamic diameter and ζ-potential of FCNPs

The FCNPs were dispersed in aqueous solutions to achieve a concentration of 4 mg/mL. The average hydrodynamic diameter (D_h) and ζ-potential of the FCNPs were determined by DLS using a Nano-ZS instrument (NanoZS90, Malvern Instruments Ltd., Worcestershire, UK). All samples were equilibrated at 25 °C in 10 mm cuvettes with caps for 120 s and analyzed in triplicate[32].

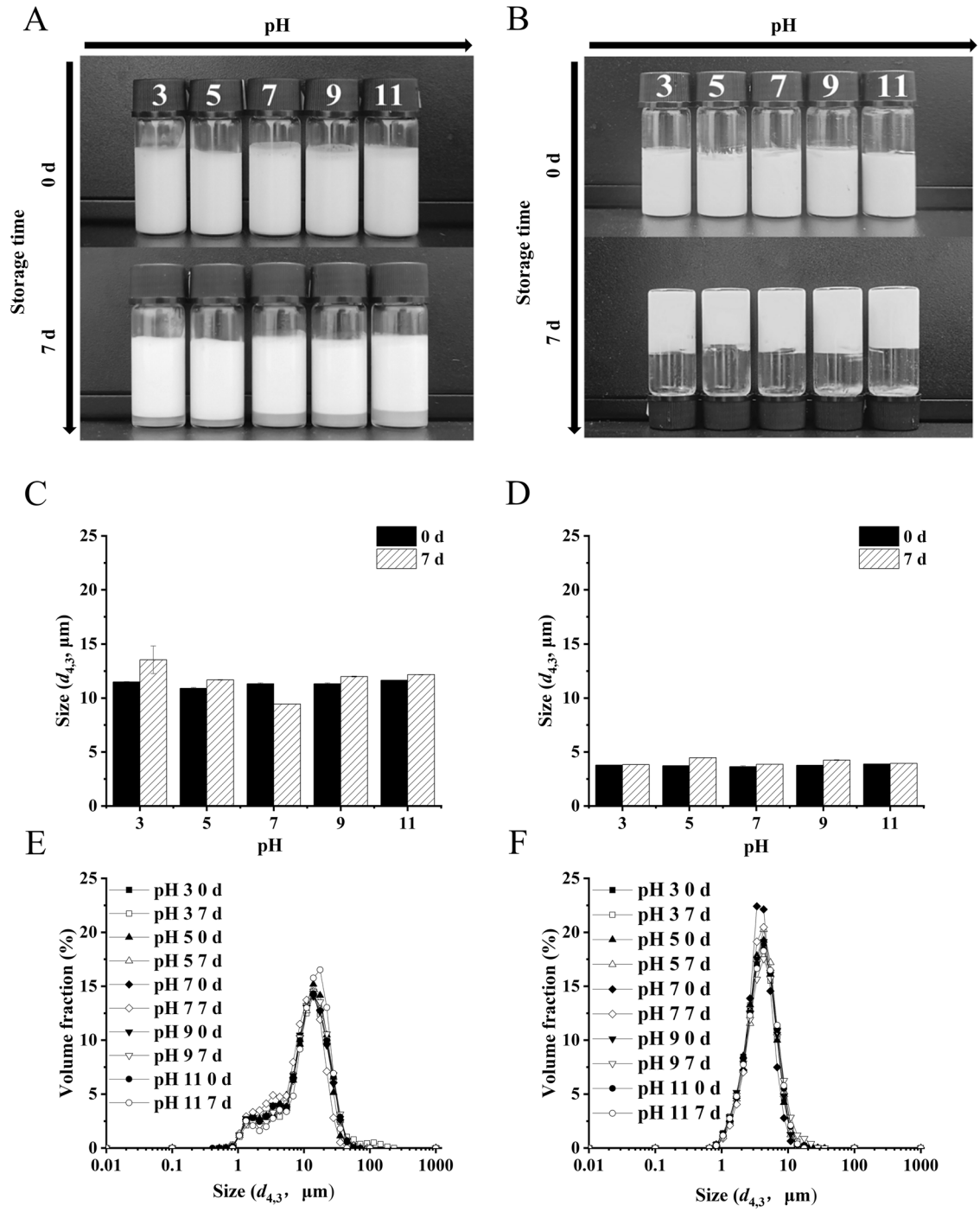

Fig. 5 | Effect of pH on the physicochemical properties of FCNP-stabilized Pickering emulsions. Influence of pHs (3-11) on the visual observation (**A**, **B**), average droplet size (**C**, **D**), and the particle size distribution (**E**, **F**) of the Pickering emulsions stabilized by FCNPs with the particle concentration of 3.0 wt% at the oil fraction of 60% or 80%. Panels (**A**), (**C**), and (**E**) were the FCNPs-stabilized PEs at the oil fraction of 60%; (**B**), (**D**), and (**F**) were the FCNPs-stabilized HIPEs at the oil fraction of 80%. Data were expressed as the mean ± SD ($n = 3$).

Morphology and contact angle (θ) of FCNPs

The microscopic morphology of FCNPs was observed using transmission electron microscope (TEM). Briefly, the samples were deposited on a 300-mesh copper grid, stained with 5% uranyl acetate, and then transferred to the TEM (JEOL JEM-1230, Tokyo, Japan). The image of FCNPs was obtained using the TEM at 80 kV acceleration voltage.

The contact angle (θ) of FCNPs was measured using a drop shape analyzer (Kruss-DSA25, KRÜSS Scientific-Kruss, Hamburg, Germany). FCNPs solutions (3.0 wt%) were poured onto cleaned glass substrates and dried at 40 °C for 24 hours. 2 μL of ultra-pure water was then dropped onto the film surface[33]. Contact angle values were obtained by analyzing the imaged droplets using the Laplace-Young equation[34]. At least six replicates were taken for each sample and the average value was used.

Dynamic IFT measurements

The dynamic IFT of the oil/water interface between the aqueous FCNPs dispersion (1.0-5.0 wt%) and the MCT oil phase was measured at room temperature using a pendant-drop shape analyzer (Kruss-DSA25, KRÜSS

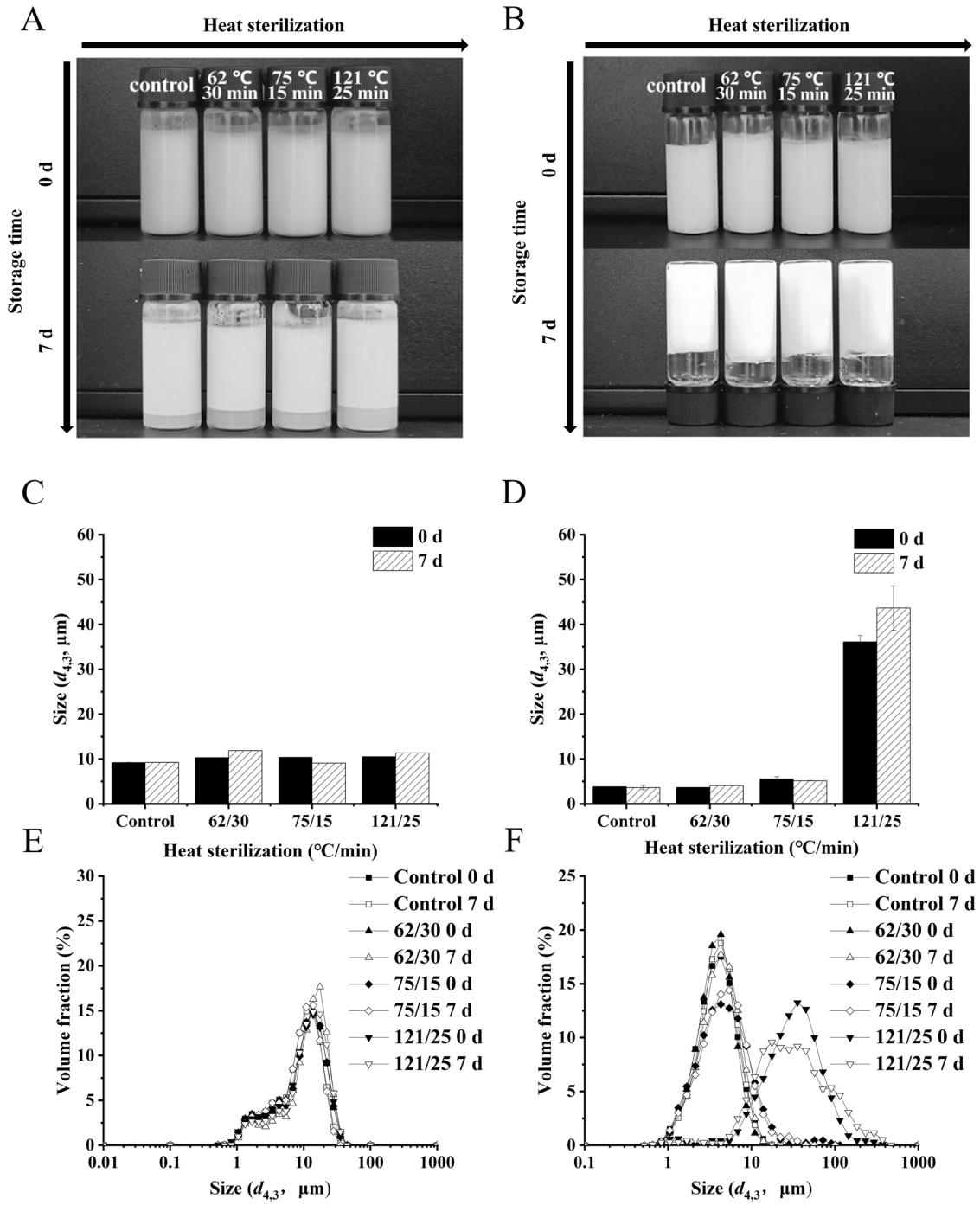

Fig. 6 | Impact of sterilization treatments on the physicochemical stability of FCNP-stabilized Pickering emulsions. Influence of sterilization treatments (62 °C for 30 minutes, 75 °C for 15 minutes, and 121 °C for 25 minutes) on the visual observation (**A**) (**B**), average droplet size (**C**) (**D**), and the particle size distribution (**E**) (**F**) of the Pickering emulsions stabilized by FCNPs with the particle concentration of 3.0 wt% at the oil fraction of 60% or 80%. Panels (**A**), (**C**), and (**E**) were the FCNPs-stabilized PEs at the oil fraction of 60%; (**B**), (**D**), and (**F**) were the FCNPs-stabilized HIPEs at the oil fraction of 80%. Data were expressed as the mean ± SD ($n = 3$).

Scientific-Kruss, Hamburg, Germany). An aqueous FCNPs drop was formed in the MCT oil phase, and the dynamic IFT was measured using continuously recorded droplet pictures collected at room temperature. The measurements were taken three times.

Emulsion preparation

A specific amount of FCNPs was dissolved in water to create an aqueous phase, while MCT was used as the oil phase. The two phases were mixed using a high-speed homogenizer (T18DS25, IKA, Staufen, Germany) equipped with a dispersing tool (S25N-18G) at 15,000 rpm for 3 minutes at room temperature to prepare PEs with oil fractions (φ) of 20, 40, 60, and 80% and FCNPs concentrations (c) of 1.0, 2.0, 3.0, 4.0, and 5.0 wt%. Sodium azide (0.02%, w/v) was added to prevent microbial growth. The resulting emulsions were stored at 4 °C for further analysis.

Particle size distribution of PEs

The particle size distribution of PEs was measured according to the methods described by Liu & Tang[35]. Briefly, the droplet size distribution of the

emulsions was measured using a LS-909 laser particle sizer (Zhuhai OMEC. Instruments Co., Ltd., Zhuhai, China). The samples were diluted in 500 mL of deionized water and stirred at 2,000 rpm. The volume-weighted mean diameter ($D_{4,3}$) was recorded[36]. All measurements were carried out in triplicate.

Measurement of the CI of PEs

The creaming stability of the emulsions was evaluated using the CI. Emulsion samples were carefully transferred into a 20-cm glass tube and the heights of the serum layer (H_s) and total height (H_t) of the samples were measured at 4 °C for 90 days. The creaming stability was quantified using Eq. (1)[37].

$$CI\% = (Hs/Ht) \times 100 \quad (1)$$

CLSM

The adsorption behavior of FCNPs at the PEs interfaces was analyzed using a Nikon A1 confocal microscope (Nikon, New York, NY, USA). FCNPs were covalently labeled with 5-DTAF in 100 mM Na_2CO_3 solution at pH 9.5 and left to react overnight at 4 °C. The reaction was stopped by adjusting the pH to 7.0, and free 5-DTAF was removed by dialysis. The labeled FCNPs were lyophilized and then used to prepare PEs with oil fractions (φ) of 60% and FCNPs concentrations (c) of 3.0 wt%. The oil phase was stained by adding Nile Red (0.1%). The dyed emulsions were placed on a glass microscope slide and covered with coverslips. The fluorescence images from the samples were collected at excitation (E_x) 492 nm/emission (E_m) 516 nm and E_x 559 nm/E_m 635 nm for 5-DTAF and Nile Red, respectively.

Rheological measurements

The rheological properties of FCNPs-stabilized PEs were evaluated using a rotational rheometer (Haake Mars 60, Thermo Fisher Scientific Inc., Germany) equipped with a parallel plate geometry (diameter 50 mm, gap 0.5 mm) according to a previous report[38]. Flow sweep measurements were carried out to obtain the apparent viscosity of PEs in the range of 0.1-100 s^{-1} shear rate. Dynamic frequency sweeps were performed at frequencies ranging from 0.01-10 Hz with a fixed strain of 0.2% to ensure all tests were conducted within the linear viscoelastic region. The frequency-dependent curves (within the frequency range of 0.1–100 rad/s) of the storage modulus (G′) and loss modulus (G″) of samples were measured. All tests were performed at 25 ± 0.1 °C and in triplicate.

Stability of FCNPs-stabilized PEs and HIPEs at different ionic strengths, pHs, and sterilization treatments

The effect of ionic strengths, pHs, and sterilization treatments on FCNPs-stabilized PEs and HIPEs was investigated. The FCNPs dispersions (3.0 wt%) mixed with MCT to obtain mixtures at oil fractions of 60% or 80%, followed by homogenization procedures as described above. Then, NaCl was added to FCNPs- stabilized PEs or HIPEs to achieve ionic strengths of 200 mM, 400 mM or 600 mM. In terms of pH effects, FCNPs- stabilized PEs or HIPEs were adjusted to pH values of 3, 5, 7, 9, or 11 by adding HCl or NaOH solutions. Meanwhile, a Pickering emulsion without NaCl was used as a control sample. In terms of sterilization treatment effects, Pickering emulsion stabilized by FCNPs (c = 3.0 wt%, φ = 60% or 80%) was sterilized in a water bath at 62 °C for 30 minutes, a water bath at 75 °C for 15 minutes, and autoclaving at 121 °C for 25 minutes, respectively[39]. All samples were stored at 4 °C for 7 days. Afterward, the appearance of the emulsions was observed and their particle size and particle size distribution were measured.

Statistical analysis

All experiments were performed in triplicate and data were analyzed with SPSS software (version 21.0, SPSS Inc., Chicago, IL, USA). Data were presented as mean ± SD ($n = 3$).

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on resonable request.

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Author contributions

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Competing interests

The authors declare no competing financial interest or non-financial interests. P.R. is Editor-in-Chief and L.K. is Associate Editor of npj Science of Food. P.R. and L.K. were not involved in the journal's review of, or decisions related to, this manuscript.

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