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## Modulation of Physicochemical Characteristics of Pickering Emulsions: Utilization of Nanocellulose- and Nanochitin-coated Lipid Droplet Blends

Hualu Zhou, Shanshan Lv, Jinning Liu, Yunbing Tan, Jorge Mundo, Long Bai, Orlando J. Rojas, and David Julian McClements

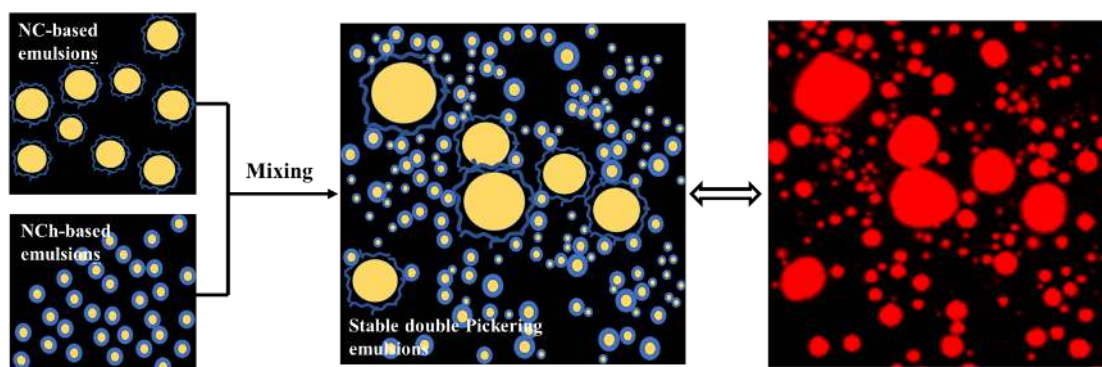
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## Graphical Abstract



1 **Modulation of Physicochemical Characteristics of Pickering Emulsions:**  
2 **Utilization of Nanocellulose- and Nanochitin-coated Lipid Droplet Blends**

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19

**20 ABSTRACT**

21 Mixed Pickering emulsions were prepared by blending anionic nanocellulose-stabilized lipid  
22 droplets with cationic nanochitin-stabilized lipid droplets. Changes in the surface potential,  
23 particle size, shear viscosity, and morphology of the mixed emulsions were characterized when  
24 the droplet mixing ratio was varied. Emulsion properties could be tailored by altering the pH and  
25 mixing ratio. Surface potential measurements suggested that the nanochitin-coated lipid droplets  
26 adsorbed to the surfaces of the nanocellulose-coated lipid droplets, thereby dominating the  
27 overall electrical characteristics of the mixed emulsions. As a result, the mixed emulsions had  
28 better stability to coalescence than the single emulsions containing only nanocellulose-coated  
29 lipid droplets. Our results suggest that the physicochemical properties, shelf-life, and functional  
30 performance of Pickering emulsions may be modulated by blending different kinds of particle-  
31 stabilized lipid droplets together.

32 **Keywords:** Pickering emulsions; heteroaggregation; nanochitin; nanocellulose; nanoparticles

33

34

## 35 INTRODUCTION

36 Oil-in-water emulsions consist of small oil droplets suspended within water <sup>1</sup>. From a  
37 thermodynamic perspective, emulsions are unstable systems due to the density contrast and  
38 interfacial tension between the oil and water phases.<sup>1</sup> Consequently, stabilizers such as  
39 emulsifiers and thickeners, have to be added to increase their kinetic stability and make them  
40 suitable for commercial applications. Most emulsifiers currently used in the food industry are  
41 surface-active molecules that adsorb to oil droplet surfaces and inhibit their aggregation by  
42 generating a repulsive force between them.<sup>2, 3</sup> Numerous kinds of natural and synthetic molecular  
43 emulsifiers are available for utilization in foods and beverages, including phospholipids,  
44 polypeptides, polysaccharides, and biosurfactants.<sup>4, 5</sup> More recently, researchers have focused on  
45 the potential of particle-based emulsifiers to form Pickering emulsions suitable for application in  
46 the food industry.<sup>6-8</sup> Colloidal particles with appropriate wetting characteristics adsorb strongly  
47 to oil droplet surfaces and form a protective coating that greatly increases their resistance to  
48 coalescence.<sup>9</sup> Various types of edible colloidal particles have already been shown to be suitable  
49 for this purpose.<sup>10, 11</sup>

50 In this study, we focused on two types of nature-derived solid nanoparticles capable of  
51 stabilizing Pickering emulsions: nanocellulose and nanochitin. Nanocellulose is produced from  
52 cellulose fibers, which are a natural, sustainable, and abundant biomaterial.<sup>12-14</sup> Numerous  
53 studies have reported that nanocellulose can be successfully used to form and stabilize Pickering  
54 emulsions.<sup>15, 16</sup> Indeed, nanocellulose has been shown to be highly effective at inhibiting the  
55 coalescence of oil droplets.<sup>17-19</sup> This phenomenon is due to the ability of nanocellulose fibers to  
56 strongly adsorb to the oil droplet surfaces and create a thick viscoelastic gel-like coating.<sup>16, 17</sup>

57 The functional performance of nanocellulose can also be extended by carrying out chemical<sup>20</sup> or  
58 enzymatic<sup>21</sup> modifications.

59 Chitin is a naturally abundant polysaccharide typically isolated commercially from  
60 crustacean shells, which is reported to be nontoxic, biocompatible, and biodegradable.<sup>12, 22-25</sup>  
61 Chemically, it consists of a linear polysaccharide chain comprised of repeating units of  $\beta$ -  
62 (1  $\rightarrow$  4)-N-acetyl-d-glucosamine. Many researchers have explored the potential applications of  
63 chitin as a functional biomaterial.<sup>24</sup> Recently, attention has been directed towards the  
64 development and utilization of chitin nanomaterials, such as chitin nanocrystals and  
65 nanofibers.<sup>24, 26, 27</sup> Chitin nanocrystals are usually produced industrially by carrying out an acid  
66 hydrolysis of shrimp shells, while chitin nanofibrils are prepared by applying mechanical forces  
67 to promote disintegration.<sup>28, 29</sup> Different processing treatments result in the generation of chitin  
68 nanomaterials with different physiochemical and functional properties<sup>25</sup>. For example,  
69 emulsions stabilized by chitin nanofibrils have been reported to have higher viscosities and better  
70 thermal stabilities than those stabilized by chitin nanocrystals.<sup>24</sup> Moreover, the thermal stability  
71 of nanochitin-coated oil droplets has been reported to be much better than nanocellulose-coated  
72 ones.<sup>26</sup> These studies indicate suggest that nanochitin has promising applications to form and  
73 stabilize Pickering emulsions.

74 In the present study, the potential of extending the functionality of particle stabilizers by  
75 creating mixed Pickering emulsion systems was investigated. In particular, we hypothesized that  
76 the physicochemical properties and stability of mixed emulsions could be controlled by altering  
77 the ratio of different types of droplets present. The mixed Pickering emulsions were formed by  
78 blending nanocellulose-coated oil droplets with nanochitin-coated oil droplets in different ratios.  
79 These two types of particle emulsifier were examined because they have opposite surface

80 charges: nanochitin is positive over most pH values found in food, whereas nanocellulose is  
81 negative. As a result, the different kinds of particle-coated oil droplets would be expected to be  
82 attracted to each other through electrostatic forces. There has been little previous work on mixed  
83 Pickering emulsions, and so, our study should provide some valuable information that may  
84 increase the utilization of nanoparticle-based emulsifiers in the food industry. Researchers have  
85 formed Pickering emulsions by mixing different kinds of inorganic colloidal particles together to  
86 form mixed interfaces, such as SiO<sub>2</sub> and Fe<sub>3</sub>O<sub>4</sub> nanoparticles<sup>30</sup> or silica and titania nanoparticles  
87<sup>31</sup>. To the authors knowledge, however, systems formed by mixing two different kinds of edible  
88 Pickering emulsions together have not been examined previously.

## 89 **MATERIALS AND METHODS**

### 90 **Materials**

91 Nanocellulose (NC) was prepared from birch tree fibers as described previously.<sup>32</sup> Fiber  
92 disintegration was achieved by passing them six-times through a high-pressure microfluidizer  
93 (M110P, Microfluidics Int. Co., Newton, MA). The average width of this nanocellulose was  
94 recently reported to be  $\sim 20 \pm 8$  nm.<sup>33</sup> The mean particle diameter measured by static light  
95 scattering was found to be  $18.6 \pm 1.2$   $\mu$ m. It should be noted that the mathematical model used  
96 to analyze the diffraction pattern in the light scattering instrument assumes that the particles are  
97 spherical, and so this value should be treated with caution.

98 Several steps were used to prepare the nanochitin (NCh), which have been described in a  
99 recent study<sup>26</sup>. Briefly, chitin was first isolated from fresh crab shells (*Callinectes sapidus*)  
100 purchased from a fish market in Finland (Helsinki Harbor, Finland). The isolated sample was  
101 then treated with concentrated alkaline solution (33 w/w% NaOH solution) for 3.5 hours at 90 °C



102 to partially deacetylate it, resulting in a degree of deacetylation (DD) of 27.3%. The deacetylated  
103 chitin (DE-chitin) was then washed, dried, and stored at 4 °C. The DE-chitin was then dissolved  
104 in acetic acid solution (pH = 3), and nanochitin was obtained by sonicating for 40 mins (FB505,  
105 Fisher Scientific, USA; 50% power, 3s on/2s off). The mean particle diameter of a nanochitin  
106 suspension measured by laser diffraction was  $0.220 \pm 0.002 \mu\text{m}$ , which should again be treated  
107 with caution for the reasons discussed for nanocellulose. Samples of both nanocellulose and  
108 nanochitin were kept at 4 °C before use.

109 Corn oil was purchased from a local store (Mazola, ACH Food Company, Memphis, TN).  
110 Nile red (a fat-soluble fluorescent dye) was purchased from Sigma-Aldrich Chemical Co. (St.  
111 Louis, Mo, USA). The other reagents used in the experiments were analytical grade and obtained  
112 from Sigma-Aldrich or Fisher Scientific (Hampton, NH, USA). Double distilled water was  
113 employed for all experiments.

#### 114 **Preparation of Pickering emulsions**

115 The water phases used to formulate the Pickering emulsions were prepared by dispersing  
116 nanoparticles (nanocellulose or nanochitin) into double distilled water to reach a level of 0.3  
117 wt%. Pure corn oil was used as the oil phase to formulate the Pickering emulsions. The water  
118 phase (90 wt%) and oil phase (10 wt%) were pre-mixed with a high-shear mixer (Bamix,  
119 BiospecProducts, Bartlesville, OK) for 2 mins at room temperature. Pickering emulsions were  
120 then produced by sonicating for 4 mins (FB505, Fisher Scientific, USA) inside an ice-water bath,  
121 using a power level of 50% strength and 3s/2s on/off cycles.

#### 122 **Preparation of mixed Pickering emulsions**

123 Nanochitin-stabilized emulsions were added to nanocellulose-stabilized emulsions in a glass  
124 beaker with continuous stirring. Mixed Pickering emulsions were prepared by blending varying

125 mass ratios of the two different emulsions: 1:0, 1:0.5, 1:1, 1:2, 0:1 NC:NCh. These values  
 126 correspond to mixed emulsions containing 0%, 33.3%, 50%, 66.7%, and 100% nanochitin. The  
 127 pure nanocellulose, pure nanochitin, and mixed emulsions were then kept under ambient  
 128 conditions for 24 hours prior to analysis.

### 129 **Storage stability of Pickering emulsions**

130 The Pickering emulsions were transferred into clean and sterile plastic test tubes, and then  
 131 stored under ambient conditions for 20 days.

132 *Particle size and charge:* The pH of the Pickering emulsions was determined with a digital  
 133 pH meter (Table 1). Then, the Pickering emulsions were diluted with pH-adjusted double  
 134 distilled water (same pH as measured sample) before measuring the size and charge of the  
 135 particles. A laser diffraction instrument was used to determine the mean particle diameter and  
 136 particle size distribution of the Pickering emulsions (Mastersizer 2000, Malvern Instruments,  
 137 Worcestershire, United Kingdom). The samples were stirred at 12,000 rpm during the  
 138 measurements to ensure they were homogeneous. The particle size is given as the volume-  
 139 weighted mean particle diameter ( $d_{43}$ ).

140 **Table 1.** pH values of Pickering emulsions prepared with various mixing ratios of nanocellulose  
 141 (NC)- and nanochitin (NCh)-coated lipid droplets.

	NC	NC/NCh = 1/0.5	NC/NCh = 1/1	NC/NCh = 1/2	NCh
<b>pH</b>	6.6	3.0	3.0	3.0	2.9

142

143 Particle electrophoresis was used to measure the surface charge ( $\zeta$ -potential) of the particles  
 144 within the Pickering emulsions (Nano-ZS, Malvern instruments, Worcestershire, United

145 Kingsom). Prior to analysis, the emulsions were diluted with pH-adjusted distilled water to be in  
146 the optimum intensity range to carry out particle charge measurements.

147 *Shear viscosity:* The rheological properties of the Pickering emulsions were characterized by  
148 dynamic shear rheometry (Kinexus, Malvern, Worcestershire, UK). The measurement cell used  
149 had a concentric cylinder geometry (25 mm internal and 27 mm external). The emulsions were  
150 poured or gently scooped into the measurement cell and then incubated at 25°C for 5 min before  
151 analysis to allow them to reach the measurement temperature. The shear stress-shear rate  
152 relationship was then recorded over the range from 0.1 to 100 s<sup>-1</sup> with 15 mins total measurement  
153 time per sample. The apparent shear viscosity was then calculated at each shear rate from these  
154 measurements.

155 *Microstructure:* The morphology of the Pickering emulsions was assessed using both electron  
156 and optical microscopy. For electron microscopy, the Pickering emulsions were diluted with  
157 distilled water 10-fold. Then, small aliquots (10 µL) of the diluted emulsions were placed on  
158 copper grids (400 mesh, carbon film, CF400-CU, Electron Microscope Sciences), and held for 2  
159 mins. Afterwards, filter paper was used to absorb any excess sample. 10 µL of phosphotungstic  
160 acid solution (PTA, 1 %) was then placed on the copper grids as a negative stain for 2 mins, and  
161 then any remaining dye was soaked up with filter paper. Afterward, the copper grids were washed  
162 3-times with distilled water then air-dried for 24 hours. Transmission electron microscopy (TEM)  
163 was then utilized to observe the morphology of the Pickering emulsions (JEOL JEM-2000FX).

164 The microstructures of the Pickering emulsions were also examined using optical and  
165 confocal laser scanning microscopy with a 40×objective lens (Nikon D-Eclipse C1 80i, Nikon,  
166 NY, United States). The Pickering emulsions were first stained with an oil-soluble dye (1

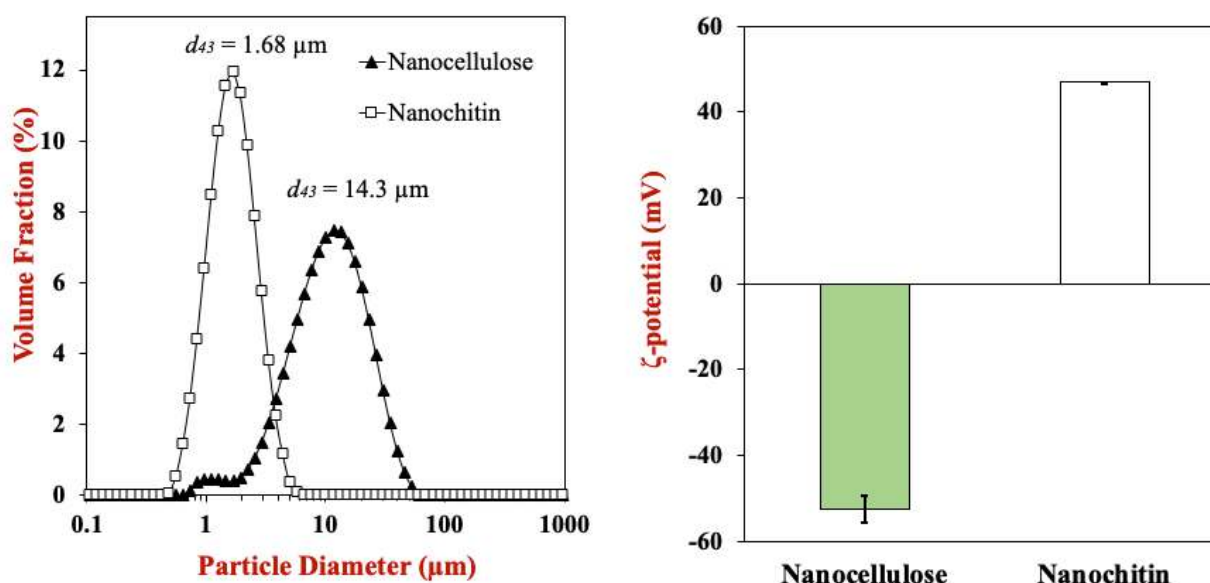
167 mg/mL Nile Red), then small aliquots (5  $\mu\text{L}$ ) of the stained emulsions were placed on the glass  
168 microscope slides and covered with a glass slip to avoid evaporation.

## 169 RESULTS AND DISCUSSION

### 170 Properties of Nanocellulose and Nanochitin emulsions

171 The physicochemical and structural properties of the initial nanocellulose- and nanochitin-  
172 emulsions were analyzed (Figs. 1 to 3).

### 173 Particle size and charge characteristics

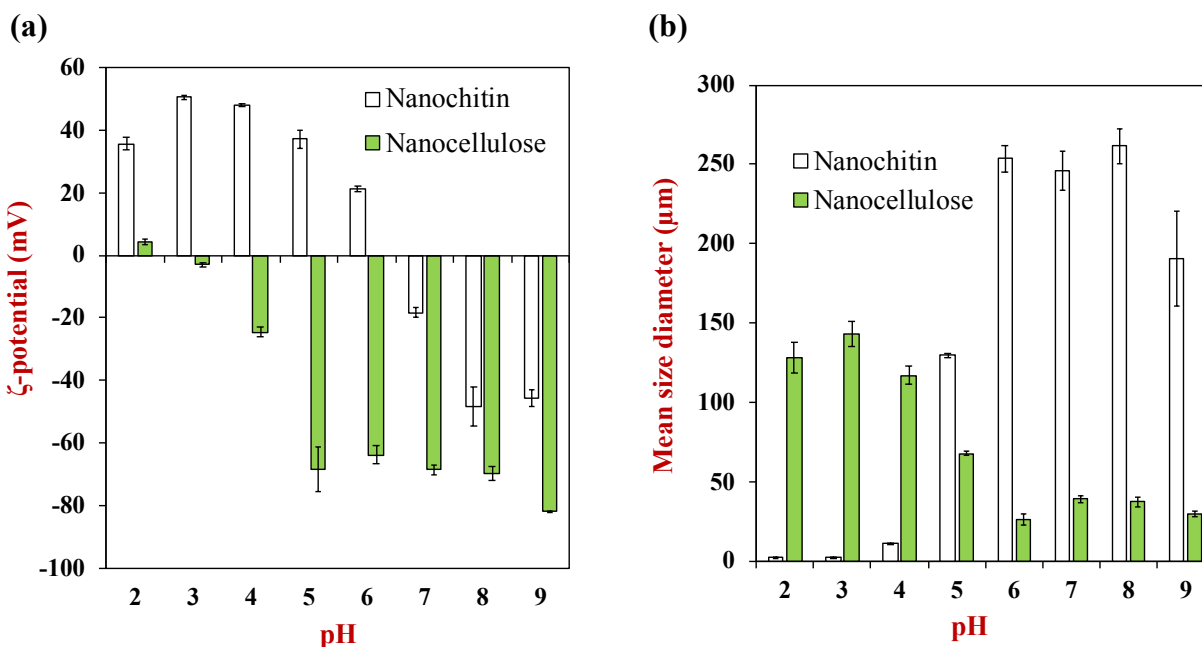


174  
175 **Figure 1:** Particle size distribution, mean particle diameter, and surface potential of oil-in-water  
176 Pickering emulsions stabilized by either nanocellulose or nanochitin.

177 Figure 1 shows the initial particle size characteristics of the Pickering emulsions determined  
178 by laser diffraction were considerably different. The nanocellulose-emulsions contained  
179 considerably larger particles ( $d_{43} = 14.3 \mu\text{m}$ ) than the nanochitin-emulsions ( $d_{43} = 1.68 \mu\text{m}$ ).

180 Additionally, the particle size distribution was much broader for the nanocellulose-emulsions (1  
181 to 100  $\mu\text{m}$ ) than the nanochitin-emulsions (0.5 to 5  $\mu\text{m}$ ).

182 The  $\zeta$ -potential measured for the nanocellulose-emulsions was  $-41.3 \pm 0.3$  mV (pH 6.6),  
183 while the value in the nanochitin emulsions was  $+46.6 \pm 1.2$  mV (at pH 2.9). As discussed later,  
184 the two emulsions had to be prepared at different pH values so that they were both physically  
185 stable, *i.e.*, extensive droplet aggregation was avoided. Moreover, the two emulsions contained  
186 oppositely charged particles of fairly similar charge magnitude at these pH values, which is an  
187 advantage when trying to promote droplet aggregation through electrostatic attractive forces.



188

189 **Figure 2.** The effects of pH on the  $\zeta$ -potential (a) and mean particle diameter (b) of  
190 nanocellulose- and nanochitin-emulsions.

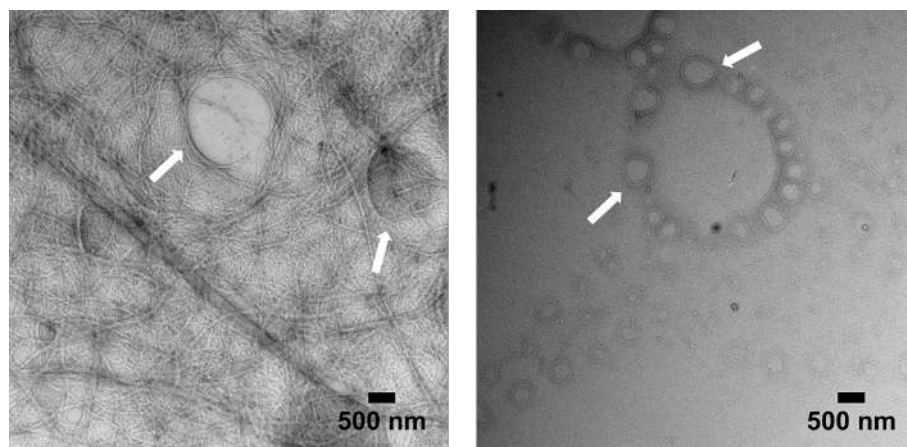
191 The pH-dependence of the physicochemical properties and structures of nanocellulose- and  
192 nanochitin-emulsions was then determined (Fig. 2). The particles in the nanocellulose-emulsions  
193 were highly negative from pH 8 to 5, but became less negative from pH 5 to 3, and eventually

194 became slightly positive at pH 2 (Fig. 2a). The anionic nature of the composite particles in these  
195 emulsions is a result of charged sulfate and carboxyl groups on nanocellulose<sup>34</sup>. Sulfate groups  
196 keep their strong negative character across the entire pH range used in our study because of their  
197 low pK<sub>a</sub> values (< pH 2), whereas the charge of carboxylic groups varies with pH because they  
198 have pK<sub>a</sub> values around pH 3.5<sup>34</sup>. Consequently, at relatively high pH values they are negatively  
199 charged (-COO<sup>-</sup>) but at lower values they lose their charge (-COOH). The surface potential of  
200 the nanochitin-emulsions was strongly positive from pH 2 to 6, but became highly negative at  
201 higher pH values (Fig. 2a). The strong positive charge under acidic conditions is a result of  
202 protonation of the amino groups (-NH<sub>3</sub><sup>+</sup>). The fact that there was also a charge at higher pH  
203 values suggests that there were also some ionizable anionic groups on the nanochitin, such as  
204 carboxyl groups (-COO<sup>-</sup>). Previous researchers have also reported that the charge on chitin  
205 nanoparticles goes from cationic to anionic as the pH is raised<sup>34</sup>. In that study, the presence of  
206 carboxyl groups was attributed to the chemical treatment of the nanochitin during the bleaching  
207 process. It should be noted that the nanochitin was strongly aggregated at the higher pH values,  
208 forming visibly large clumps, and therefore the particle electrophoresis measurements should be  
209 used with some caution.

210 The particle size of the two emulsions, which is influenced by any droplet aggregation that  
211 has occurred, also depended strongly on solution pH (Fig. 2b). For the nanocellulose-emulsions,  
212 the particle size was relatively large from pH 2 to 4, decreased at pH 5, then became relatively  
213 small from pH 6 to 10. This phenomenon arises from changes in the electrostatic forces  
214 operating amongst the droplets in the Pickering emulsions. At relatively low pH values (pH <  
215 5), the electrostatic repulsion amongst nanocellulose-coated oil droplets is relatively weak,  
216 leading to widespread droplet aggregation. Conversely, at higher pH values the electrostatic

217 repulsion amongst the anionic droplets is relatively strong, which inhibits aggregation. For the  
218 nanochitin-emulsions, the opposite effect was observed (Fig. 2b). The emulsions contained  
219 relatively small particles from pH 2 to 4, but much larger ones at higher pH values. In this case,  
220 there was a strong electrostatic repulsion between the positively charged nanochitin-coated oil  
221 droplets at low pH values, which would have prevented them from aggregating. At higher pH  
222 values, there may only have been a weak electrostatic repulsion amongst the droplets, which was  
223 not sufficiently high to inhibit aggregation. Alternatively, there may have been both positive ( $-$   
224  $\text{NH}_3^+$ ) and negative ( $-\text{COO}^-$ ) groups on the surfaces of the nanochitin-coated oil droplets at  
225 higher pH values, which promoted droplet aggregation due to electrostatic attraction. These  
226 results clearly show that solution pH profoundly influences the stability of both types of  
227 Pickering emulsion, which may limit their application in many foods and other commercial  
228 products.

### 229 **Microstructure Analysis**



230  
231 **Figure 3.** Transmission electron microscopy images of nanocellulose-emulsions (left) and  
232 nanochitin-emulsions (right), Some distinctive structures observed in the images are highlighted  
233 with white arrows.

234 The TEM images indicate the oil droplets within the nanocellulose-emulsions were coated  
235 by nanocellulose fibers, as well as being trapped within a gel network consisting of overlapping  
236 nanocellulose fibers in the surrounding aqueous phase (Fig. 3). The nanochitin-emulsions had a  
237 quite different microstructure. They appeared to contain relatively large oil droplets surrounded  
238 by approximately spherical nanochitin particles. Interestingly, the individual oil droplets within  
239 the nanocellulose-emulsions appeared to be quite small ( $< 5 \mu\text{m}$ ), suggesting that the large  
240 particle sizes determined using the laser diffraction method (Fig. 1) may have been due to the  
241 nanocellulose fibers, rather than the oil droplets.

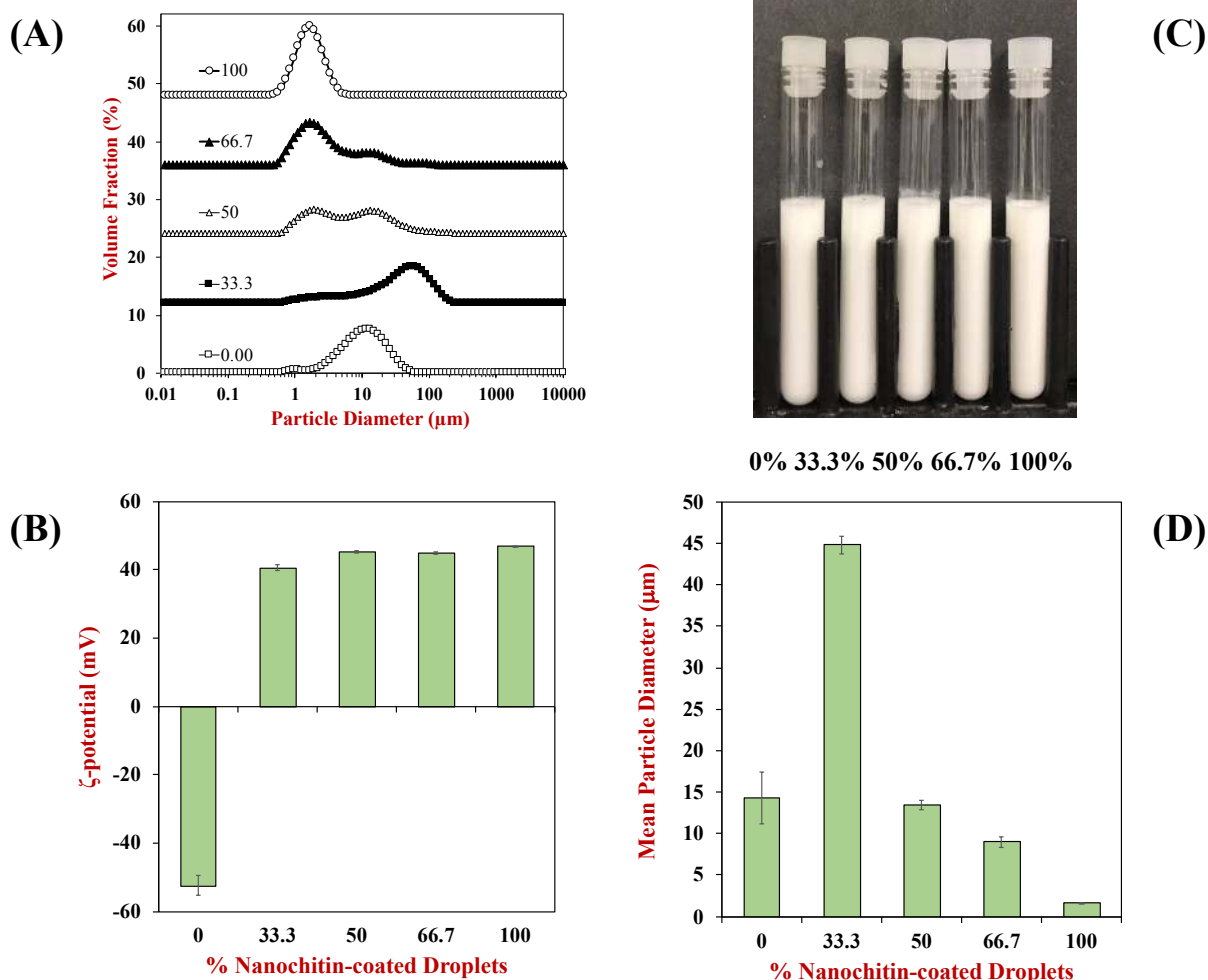
#### 242 **Structural and physicochemical characteristics of mixed Pickering emulsions**

243 The impact of mixing the anionic nanocellulose-emulsions with the cationic nanochitin-  
244 emulsions on their physicochemical and structural properties was then investigated.

245 *Particle size and surface charge:* The characteristics of the particles within the mixed  
246 nanocellulose- and nanochitin-emulsions was strongly influenced by the ratio of positive-to-  
247 negative droplets they contained (Figure 4). The mean particle diameter initially increased when  
248 the level of cationic droplets was raised from 0 to 33.3%, but then it decreased when their level  
249 was further raised from 33.3 to 100%. The initial increase in particle size might be attributed to  
250 hetero-aggregation, *i.e.*, the electrostatic attraction between positively and negatively charged  
251 droplets. Interestingly, however, the particles in the mixed emulsions were smaller than those in  
252 the two individual emulsions at higher levels of nanochitin-coated droplets. This result suggests  
253 that some other physicochemical phenomenon was occurring in these systems. For this reason,  
254 surface potential measurements were carried out to provide some insights into this effect.

255





256

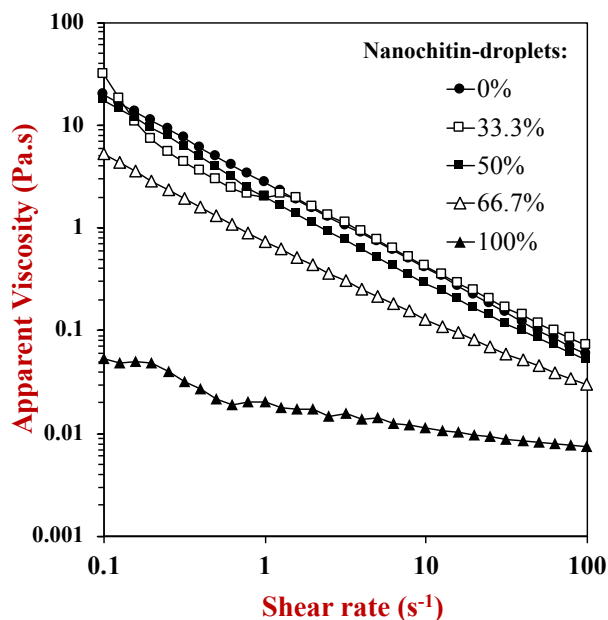
257 **Figure 4.** The particle size distributions, mean particle diameters,  $\zeta$ -potentials, and appearances  
 258 of mixtures of nanocellulose- and nanochitin-emulsions.

259 Surface potential analysis showed that the electrical charge on the particles was dominated  
 260 by that of the nanochitin-droplets in all of the mixed systems (Fig. 4). This suggests that the  
 261 smaller cationic nanochitin-coated oil droplets may have formed a layer around the larger  
 262 anionic nanocellulose-coated oil droplets. The  $\zeta$ -potential values determined by the  
 263 electrophoresis instrument used in the current study are mainly determined by the electrical  
 264 characteristics of the outer edges of particles. Consequently, an outer layer of cationic droplets  
 265 would make the overall charge of the composite particles in the system positive. In addition, the

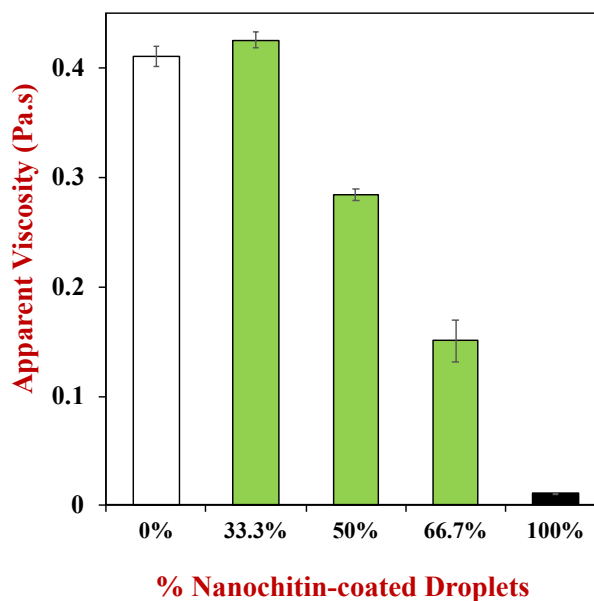
266 adsorption of the smaller cationic droplets around the larger anionic ones may have promoted  
267 some disruption of the aggregates in the nanocellulose-emulsions.

## 268 Viscosity

269 (A)



(B)



270

271 **Figure 5.** Impact of mixing ratio on the viscosity of mixed nanocellulose- and nanochitin-  
272 emulsions: (A) apparent viscosity *versus* shear rate; (B) apparent viscosity *versus* percentage of  
273 cationic nanochitin-coated droplets present (shear rate = 10 s<sup>-1</sup>).

274 The rheology of the mixed emulsions was characterized using shear rheometry (Fig. 5).  
275 Initially, the rheology of the individual nanocellulose- and nanochitin emulsions is compared.  
276 Both emulsions displayed shear thinning, *i.e.*, a decrease in apparent viscosity when the shear  
277 rate was raised. This effect, however, was much more pronounced in the nanocellulose-  
278 emulsions than in the nanochitin-emulsions. The magnitude of the apparent shear viscosity was  
279 much higher in the nanocellulose-emulsions than in the nanochitin-ones. This difference may be

280 because the nanocellulose-emulsions contained highly flocculated oil droplets, as well as a  
281 network of interlinked nanocellulose fibers in the surrounding continuous phase (Fig. 3).  
282 Flocculated droplets or nanofibers disturb the fluid flow in the emulsions, thereby leading to  
283 more energy dissipation due to friction, and a higher shear viscosity. Conversely, the nanochitin-  
284 emulsions appeared to contain individual oil droplets and did not contain a network of nanochitin  
285 particles in the aqueous phase (Fig. 3). The pronounced shear thinning behavior observed in the  
286 nanocellulose-emulsions may therefore have been due to deformation and disruption of the flocs,  
287 as well as alignment of the nanofibers with the flow field.

288 The mixed emulsions containing 33.3% nanochitin-coated droplets had a slightly higher  
289 apparent shear viscosity than the ones containing none of these droplets (Fig. 5). This effect may  
290 have been because low levels of cationic droplets promoted some additional aggregation of the  
291 anionic droplets due to the hetero-aggregation mechanism mentioned earlier. As the level of  
292 nanochitin-coated droplets was further increased, there was a decrease in the apparent shear  
293 viscosity. This may have been because the cationic oil droplets formed a layer around the  
294 anionic ones, thereby reducing their tendency to form large aggregates. **In particular, the  
295 nanofibers used in this study may have impacted the stability and rheology of the Pickering  
296 emulsions by altering the mechanical properties of the coatings around the oil droplets. Previous  
297 researchers have studied the impact of the adsorption of nanochitin and nanocellulose on the  
298 properties of oil-water interfaces.<sup>26, 35</sup> They showed that the adsorption of the nanofibers  
299 increased the interfacial elasticity, which may have increased the resistance of the nanofiber-  
300 coated oil droplets to coalescence or flocculation.** Overall, our results show that Pickering  
301 emulsions with different rheological characteristics can be prepared by blending positive and  
302 negative droplets together in different ratios.

### 303 **Storage stability of mixed Pickering emulsions**

304 The physical stability of emulsions determines their shelf life. For this reason, the  
305 physicochemical properties of the Pickering emulsions were characterized after 0 or 20 days  
306 storage at ambient temperature (Figs. 6 and 7). The relative increase in mean particle diameter  
307 after storage was used as an indicator of emulsion stability:

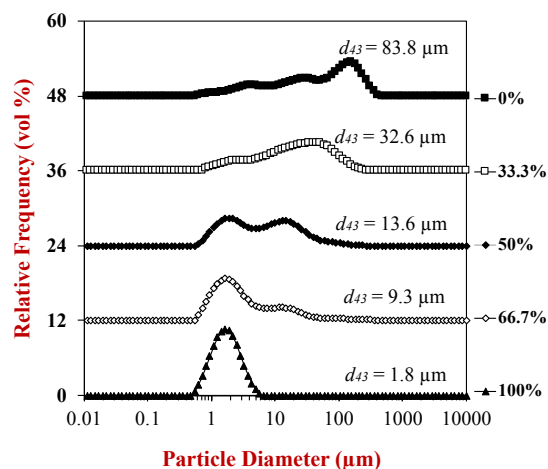
$$308 \quad \%Size\ Increase = 100 \times \frac{(d_{end} - d_{start})}{d_{start}}$$

309 Here,  $d_{end}$  and  $d_{start}$  are the mean particle diameters at the end and start of the storage period.

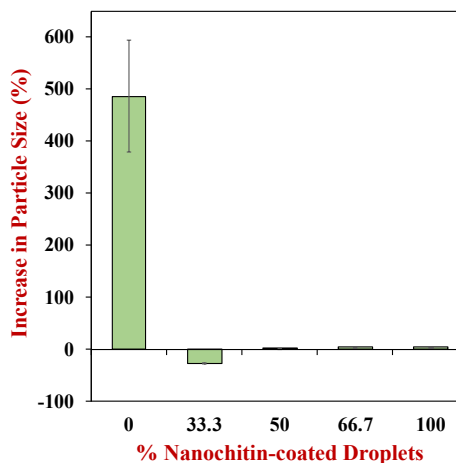
310 Initially, the stability of the individual Pickering emulsions was compared. The size of the  
311 particles in the nanocellulose-emulsions increased substantially after storage (Figs. 6A and 6B),  
312 which was mainly caused by coalescence of the oil droplets, as demonstrated by the increase in  
313 the size of the individual oil droplets seen in the microscopy images (Figs. 7 and 8). In contrast,  
314 only a small increase was observed in the size of the particles in the nanochitin-emulsions after  
315 storage, which was consistent with the microscopy images (few aggregated droplets). These  
316 results indicate that the nanochitin is much better at inhibiting droplet coalescence than the  
317 nanocellulose under the conditions used in this study. Interestingly, the apparent shear viscosity  
318 of the nanocellulose-emulsions was substantially lower after storage, but was significantly higher  
319 in the nanochitin-emulsions (albeit with a large variation between samples) (Fig. 6C). The  
320 observed viscosity decrease in the nanocellulose-emulsions may have been due to a reduction in  
321 the interfacial area of the oil droplets (resulting from the increase in droplet size), so there were  
322 less sites available for the nanocellulose fibers to bind to. Conversely, the viscosity increase in  
323 the nanochitin-emulsions may have been due to increased droplet aggregation during storage,  
324 possibly due to rearrangement of the nanoparticles in the system. For instance, a chitin

325 nanoparticle may have originally been attached to the surface of a single oil droplet but over time  
 326 it may have become attached to the surfaces of two or more different droplets, thereby promoting  
 327 bridging flocculation.

328 (A)

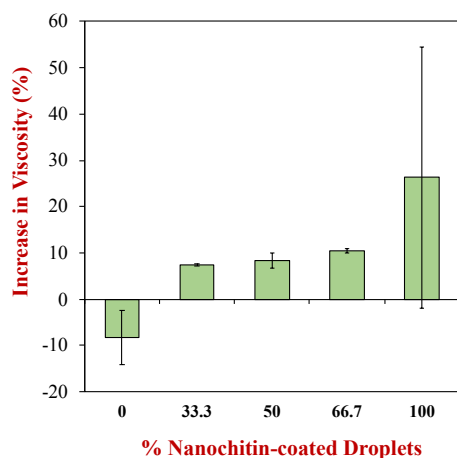


(B)



329

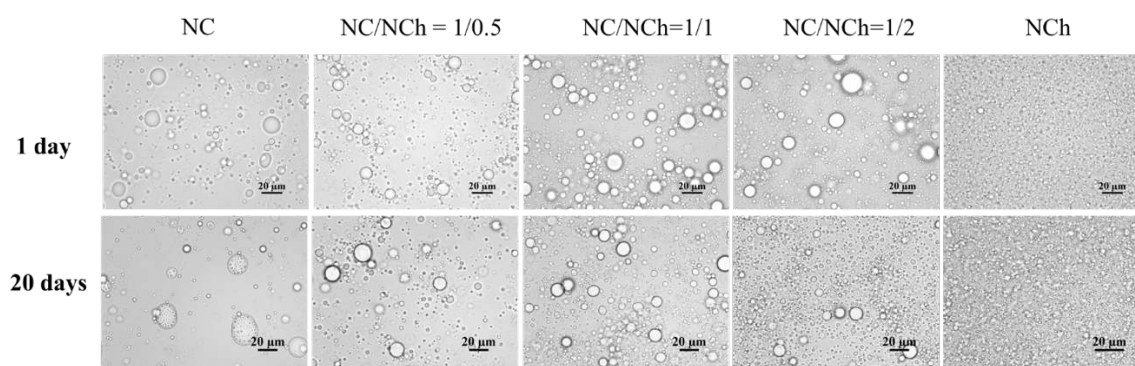
330 (C)



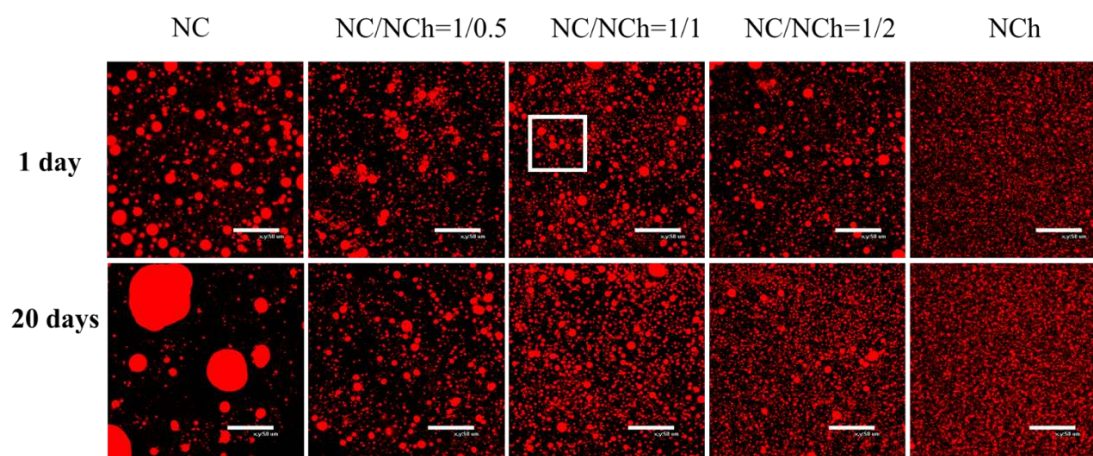
331

332 **Figure 6.** The particle size distribution (A), %increase in droplet size (B), and %increase in  
 333 viscosity (C) of mixed emulsions after 20 days storage. Some of the particle size distribution  
 334 data has been shifted up the y-axis to better compare the different samples.

335 In the mixed emulsions containing 33.3% nanochitosan-coated droplets, the mean particle  
336 size decreased slightly during storage, suggesting that there may have been some structural  
337 rearrangements in the system. For all the other mixed emulsions, the measured particle size  
338 remained fairly constant throughout storage, suggesting the presence of nanochitin-coated  
339 droplets improved the stability of the system. Moreover, the apparent viscosity of all the mixed  
340 Pickering emulsions only increased slightly after storage (Fig. 6C), suggesting that they were  
341 relatively stable.



342  
343 **Figure 7a.** Optical microscopy images of nanocellulose- and nanochitin-emulsions, as well as  
344 their mixtures. The scale bar represents 20 μm.

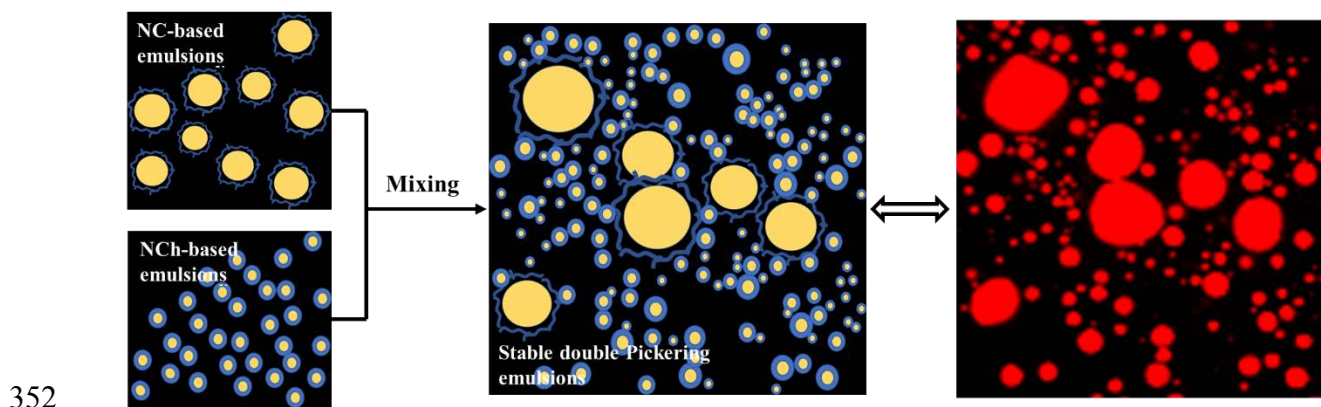


345

346 **Figure 7b.** Confocal fluorescence microscopy images of nanocellulose- and nanochitin-  
347 emulsions, as well as their mixtures. The oil phase was dyed red using Nile red. The scale bar  
348 represents 50  $\mu\text{m}$ .

### 349 Interaction mechanism

350 Finally, based on our experimental observations and knowledge of colloidal dispersions, we  
351 proposed a possible mechanism of interaction for the mixed Pickering emulsions (Fig. 8).



353 **Figure 8.** Schematic illustration of 1:1 mixed nanocellulose- and nanochitin-emulsions, where  
354 the confocal image is from the white box in Figure 7.

355 During homogenization, nanocellulose was less effective at generating fine oil droplets than  
356 nanochitin, which may have been because it consisted of long fibers, whereas the nanochitin  
357 consisted of small spheroids. The small nanochitin particles may therefore have moved to the oil  
358 droplet surfaces formed as a result of homogenization more rapidly. Moreover, the  
359 nanocellulose formed a network of interlinked fibers within the continuous phase of the  
360 emulsions, which may have contributed to their relatively high viscosity. This high viscosity  
361 may also have interfered with droplet disruption within the homogenizer, leading to the presence  
362 of relatively large oil droplets.

363 When low levels of nanochitin-coated droplets (33.3%) were mixed with high levels of  
364 nanocellulose-coated droplets (66.7%), some bridging flocculation occurred. In other words, the  
365 small cationic droplets formed electrostatic bridges between the anionic droplets. However, at  
366 higher levels of nanochitin-coated droplets ( $\geq 50\%$ ), the small cationic droplets formed a shell  
367 around the larger anionic droplets, which reduced the particle size and the viscosity, as well as  
368 increasing the coalescence stability.

369 Overall, our results show that Pickering emulsions properties can be controlled by varying  
370 the ratio of cationic and anionic droplets they contain. This knowledge may prove valuable for  
371 designing food products with novel rheological or stability characteristics. For instance, it may  
372 be possible to create reduced-calorie or slowly digestible pastes, spreads, dressings, or sauces  
373 with novel textures.

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