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1       **Effect of pectin on the properties of nanoemulsions stabilized by**  
2                           **sodium caseinate at neutral pH**

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21 **Abstract**

22 The effect of different concentrations of low methoxyl pectin (LMP) on lipid  
23 oxidation and physical stability of sodium caseinate (CAS) stabilized nanoemulsions  
24 under neutral pH was investigated. The addition of pectin at low concentration ( $\leq 0.10$   
25 wt%) had no significant effect on the average size of nanoemulsions, but a slight size  
26 increase and phase separation were observed at higher concentrations of pectin ( $\geq$   
27 0.25wt%). This result suggests that LMP can not adsorb at the oil/water interfacial  
28 CAS membrane at neutral pH. However, in the presence of LMP, the physical  
29 stability of nanoemulsions against high salt concentrations and freeze-thaw cycles was  
30 significantly enhanced. Moreover, nanoemulsions containing pectin have a better  
31 ability to inhibit lipid and protein oxidation than nanoemulsions without pectin after 3  
32 weeks, and the lowest lipid hydroperoxide content was observed for nanoemulsions  
33 containing 0.25 wt% pectin.

34

35 **Keywords:** Nanoemulsion ; Physical stability ; Lipid oxidation ; Sodium caseinate ;  
36 Pectin

## 37 **1. Introduction**

38 Over the last few years, substantial efforts have been concentrated on  
39 incorporating biologically active compounds into food formulations to endow food  
40 products with more functional properties. In addition, there is a great interest to  
41 produce delivery systems containing different types of natural lipophilic bioactive  
42 ingredients, such as carotenoids, phytosterols, vitamins, and essential oils ([Teng et al.,](#)  
43 [2020](#)). Nevertheless, there are many technical challenges to overcome such as low  
44 water solubility, chemical instability, and sensitivity to oxidative degradation of  
45 lipophilic compounds during production and storage. Fortunately, most of these  
46 problems can be successfully solved by encapsulation technologies. Among these  
47 technologies, nanoemulsion-based encapsulation systems have been widely used to  
48 achieve these goals, such as protecting bioactive compounds, delivering active  
49 molecules to target sites, increasing bioavailability, and extending their shelf  
50 life...(Aphibanthammakit & Kasemwong, 2021). Indeed, compared with the  
51 conventional emulsions, nanoemulsion-based systems exhibited better functionality,  
52 such as enhanced protection, increased stability and improved bioavailability of  
53 bioactive compounds, due to their nanometric size (20-500 nm) and high surface area  
54 ([Katsouli, Polychniatou, & Tzia, 2018](#)). Moreover, nanoemulsions are kinetically  
55 stable systems which are more stable to gravitational separation, coalescence, and  
56 flocculation than conventional emulsions, but they are more prone to Ostwald  
57 ripening due to their small droplet size ([McClements & Rao, 2011](#)) and generally  
58 narrowly size distributed.

59 The encapsulating wall material needs to be carefully selected, usually edible and  
60 inexpensive, especially in the food industry (Teng et al., 2020). At present, many  
61 encapsulation systems based on synthetic polymers have been established and  
62 explored. However, some food safety issues may arise because the preparation of  
63 these polymer-based synthetic microcapsules may involve the use of toxic organic  
64 solvents (Li et al., 2019). Thus, it is increasingly necessary in the food industry to  
65 replace synthetic emulsifiers with natural ones. Proteins and polysaccharides as  
66 natural biopolymers have been applied in food industries. Particularly, many types of  
67 food proteins, such as walnut protein (Cai et al., 2018), legume protein (Gumus,  
68 Decker, & McClements, 2017), whey protein (Berton-Carabin, Schröder, Rovalino-  
69 Cordova, Schroën, & Sagis, 2016; Khalloufi, Alexander, Douglas Goff, & Corredig,  
70 2008), soy protein (Hu, McClements, & Decker, 2003; Q. Li, Zheng, Ge, Zhao, & Sun,  
71 2020), casein proteins (Hu et al., 2003; Zhang, Cai, Ding, & Wang, 2021), etc, have  
72 been well studied as wall materials for encapsulation systems due to their commercial  
73 availability, film-forming, and excellent amphiphilic properties. Among them, casein  
74 proteins, which are generally sold industrially in the form of sodium caseinate (CAS),  
75 have been widely used as commercial emulsifiers in the food industry (Wang, Su, Jia,  
76 & Jin, 2013). CAS has rather flexible structure and composed of a soluble mixture of  
77 four different proteins,  $\alpha$ (s1)- and  $\alpha$ (s2)-caseins,  $\beta$ -casein, and  $\kappa$ -casein. As a result,  
78 they can be rapidly adsorbed by reducing the surface tension at the oil–water interface  
79 and form thick interfacial films during the emulsification process (Dagleish,  
80 Srinivasan, & Singh, 1995; Singh & Sarkar, 2011). CAS was also reported to be

81 effective in protecting emulsified oils from oxidation due to its unique iron chelating  
82 properties (Hu et al., 2003). Antioxidant proteins could slow down the oxidation of  
83 lipids in food, but during this process they are usually self-oxidized (Villiere, Viau,  
84 Bronnec, Moreau, & Genot, 2005). Meanwhile, the products of lipid oxidation  
85 generally could in turn promote the oxidation of proteins (Estévez, Kylli, Puolanne,  
86 Kivikari, & Heinonen, 2008). These changes typically result in modifications of the  
87 functional properties of proteins like their emulsifying ability (Berton-Carabin et al.,  
88 2016) and produce toxic reaction products (Obando, Papastergiadis, Li, & De  
89 Meulenaer, 2015). Therefore, it is necessary to simultaneously inhibit the oxidation of  
90 lipids and that of proteins in food emulsions.

91 Unlike proteins can be adsorbed at the oil-water interface, most polysaccharides  
92 such as pectin, xanthan gum, alginate, Arabic gum, etc. have little inherent surface  
93 activity because of their strong hydrophilicity (Surh, Decker, & McClements, 2006;  
94 Zang, Wang, Yu, & Cheng, 2019). Nevertheless, under the conditions that proteins  
95 and polysaccharides have opposite charges, they can be adsorbed on the surface of  
96 protein-encapsulated oil droplets through electrostatic interactions. Zhang et al. (2021)  
97 investigated the effect of pH on emulsions stabilized by chitosan and casein and their  
98 results indicated that emulsification pH range of casein could be effectively expanded  
99 as the non-covalent protein-polysaccharide complexes formed by electrostatic  
100 attractions between casein and chitosan. Zang et al. (2019) investigated the effect of  
101 different anionic polysaccharides on the properties of rice bran protein-stabilized  
102 emulsions and they found that the resistance of emulsions to environmental stresses

103 (salt, pH) was significantly improved. On the other hand, even if the polysaccharide  
104 has the same charge as the protein, the polysaccharide can act as an emulsion  
105 stabilizer by forming an extended network, thereby affecting the viscosity of the  
106 continuous phase (Cai et al., 2018). Khalloufi et al. (2008) demonstrated that flaxseed  
107 gum is a non-interacting polysaccharide at neutral pH when mixed with whey protein  
108 isolate stabilized emulsions and there was no visible phase separation at low addition  
109 levels. Zhang et al. (2020) developed almond proteins and *camellia* saponin based  
110 emulsions at pH 7. They found that the formation of lipid oxidation products was  
111 significantly lower in the almond protein stabilized emulsions containing *camellia*  
112 saponin, even though both the protein and the surfactant were negatively charged  
113 (Zhang et al., 2020). In this regard, many studies have begun to focus on the use of  
114 emulsifier blends to improve the emulsification effect and functional properties by  
115 combining the properties of many functional molecules. Particularly, based on  
116 electrostatic interactions between anionic polysaccharides and positively charged  
117 proteins below their isoelectric point (pI), the benefits of polysaccharides as an  
118 adsorbable layer in protein-stabilized emulsions have been widely reported (Cai et al.,  
119 2018; Qiu, Zhao, & McClements, 2015; Qiu et al., 2015). On the other hand, when the  
120 pH of the emulsions is higher than the pI of protein emulsifiers, anionic  
121 polysaccharides cannot be adsorbed on the emulsion as a protective layer due to the  
122 electrostatic repulsions. Several studies have suggested that unadsorbed  
123 polysaccharides can also improve the stability of protein-stabilized emulsion and  
124 enhance its nutritional value (Khalloufi et al., 2008; Zhang et al., 2020). Nevertheless,

125 there are still few reports on the influence of unadsorbed polysaccharides on the  
126 physicochemical properties of protein-stabilized emulsions.

127 The purpose of the present article was to study if anionic polysaccharides could be  
128 used to improve physical and chemical stability of proteins nanoemulsions at neutral  
129 pH. Our previous study has indicated that no significant interactions were found at pH  
130 7.0 between sodium caseinate and pectin by isothermal titration calorimetry ([Wang,  
131 Dumas, & Gharsallaoui, 2019](#)). Thus, sodium caseinate (CAS) and low methoxyl  
132 pectin (LMP) were selected as emulsifiers and stabilizers in this study to prepare  
133 nanoemulsions at pH 7.0. To achieve this goal, the effects of LMP concentration on  
134 particle size distribution, zeta potential ( $\zeta$ -potential), creaming behavior,  
135 microstructure, and environmental stresses (salt addition, heating, freeze-thaw) of  
136 CAS stabilized nanoemulsions were measured. Moreover, the encapsulated lipid and  
137 protein oxidation was also evaluated.



138 **2. Materials and methods**

139 **2.1. Materials**

140 Sodium caseinate (CAS) powder with 93.20% protein content (determined by the  
141 Kjeldahl method, N=6.38) was purchased from Fisher Scientific (United Kingdom).  
142 Low methoxyl pectin (LMP, esterification degree from 22% to 28% and acetylation  
143 degree from 20% to 23%) with 81.21% carbohydrate content was bought from Cargill  
144 (Baupre, France). Corn oil, trichloroacetic acid (TCA), thiobarbituric acid (TBA),  
145 1,1,3,3-tetraethoxypropane, cumene hydroperoxide, ammonium thiocyanate, ferrous  
146 chloride, imidazole, acetic acid, sodium azide, sodium hydroxide, and hydrochloric  
147 acid were purchased from Sigma-Aldrich Chimie (St Quentin Fallavier, France). All  
148 the solutions were prepared in deionized water.

149 **2.2. Preparation of oil-in-water (O/W) nanoemulsions**

150 Imidazole-acetate buffer solution was prepared by dispersing 5 mM imidazole and  
151 5 mM acetic acid in deionized water, and then adjusting the pH to 7.0 with 1 M NaOH.  
152 Stock solutions were prepared by dispersing separately 2.0 wt% CAS and different  
153 amounts of LMP into 5 mM imidazole-acetate buffer (pH 7.0) and stirring overnight  
154 at 4 °C to ensure complete hydration. The pH of the two stock solutions was adjusted  
155 to 7.0 before use.

156 Firstly, continuous phases were prepared by mixing 2.0 wt% CAS and 0.0, 0.1, 0.2,  
157 0.5, or 1.0 wt% LMP stock solutions in a ratio of 1:1 at pH 7. Then, oil-in-water  
158 coarse emulsions were formulated by mixing 10 wt% corn oil and 90 wt% emulsifier  
159 aqueous solution by high-speed homogenization (IKA, Staufen, Germany) under 13

160 500 rpm for 5 minutes. Finally, nanoemulsions were obtained by passing coarse  
161 emulsions through a microfluidizer (Microfluidics LM20, Microfluidics Corp.,  
162 Newton, MA, USA) 5 times at  $5 \times 10^7$  Pa. The pH of the nanoemulsions was then  
163 immediately adjusted to 7.0. Sodium azide (0.02% final concentration) was added into  
164 emulsions to prevent microbial growth (Chang, Gupta, Timilsena, & Adhikari, 2016).  
165 All nanoemulsions were stored in 100 mL sealed glass bottles at room temperature in  
166 the dark for three weeks. The hydrodynamic particle size of nanoemulsions as a  
167 function of time was recorded.

### 168 **2.3. Droplet hydrodynamic size and size distribution**

169 The hydrodynamic particle size (nm) and size distribution were determined by  
170 dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments,  
171 Worcestershire, UK) at a fixed angle of  $90^\circ$ . To eliminate multiple scattering effects,  
172 nanoemulsions were diluted to a droplet concentration around 0.1 wt.% by 5 mM  
173 imidazole acetate buffer (pH 7.0). After 90 s of equilibrium, each sample was  
174 analyzed at least three times. Then the average droplet size and polydispersity index  
175 (PDI) were obtained.

### 176 **2.4. Zeta potential ( $\zeta$ )**

177 To avoid multiple scattering effects, the nanoemulsions were diluted to a  
178 concentration of around 0.05 wt% in imidazole acetate buffer. Diluted samples were  
179 transferred to the zeta potential cell for electrophoretic mobility measurement using a  
180 Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK). All measurements  
181 were performed at least three times and the measured electrophoretic mobilities were

182 converted to Smoluchowski's  $\zeta$ -potential values via integrated software as the average  
183 of three individual measurements.

## 184 **2.5. Creaming stability measurement**

185 The creaming index can provide indirect information about the extent of droplet  
186 flocculation and aggregation in emulsion systems. Firstly, 10 mL of each  
187 nanoemulsion were transferred into a cylindrical test tube (inner diameter 1 cm,  
188 height 10 cm), tightly sealed with a rubber cap, and then stored at room temperature.  
189 If phase separation occurred, emulsions will be separated into an optically thin  
190 "creamed" layer at the top and a transparent (or turbid) 'serum' layer on the bottom  
191 during storage. The total height of the emulsions (HE) and the height of the serum  
192 layer (HS) were measured (Mwangi, Ho, Tey, & Chan, 2016). The creaming index (CI)  
193 was calculated as follows (Eq. (1)):

$$194 \quad CI(\%) = \frac{HS}{HE} * 100 \quad (1)$$

## 195 **2.6. Viscosity measurements**

196 The apparent viscosity of nanoemulsions was measured by a dynamic shear  
197 rheometer (Anton-Paar MCR 302, Graz, Austria) with a 40 mm parallel stainless-steel  
198 plate. The nanoemulsions were transferred to the platform, and the gap between the  
199 plate and the platform was adjusted to 1 mm. After equilibrating for 5 minutes at  
200 25 °C, the interfacial viscosity measurement was carried out for a shear rate ranging  
201 from 0.01 to 100 s<sup>-1</sup>. The flow data obtained was fitted with the Herschel-Bulkley  
202 model by Origin 8.0 software (Originlab, MicroCal, USA).

## 203 **2.7. Nanoemulsion stability to environmental stress**

204        *Heating stability:* 10 mL of each nanoemulsion were transferred into a cylindrical  
205 test tube and then placed into water bath under 100 °C. After heating for 15 min, 30  
206 min and 60 min, they were taken out and then cooled down to room temperature  
207 before measuring droplet size distribution.

208        *Freeze-thaw stability:* Nanoemulsion samples were transferred into 15 mL  
209 centrifuge tubes and incubated in a -20 °C freezer for 24 h. After incubation, the  
210 samples were thawed by incubating them in a water bath at 40 °C for 2 h. This  
211 freeze-thaw cycle was repeated from 0 to 3 times and the droplet size distribution of  
212 each nanoemulsion was recorded.

213        *Salt stability:* Nanoemulsions containing 0.1 wt% LMP was mixed with different  
214 ratios of NaCl solution under continuous stirring. The final NaCl concentrations of the  
215 studied nanoemulsions were 0, 50, 100, 200 and 300 mM. Lipid, CAS and LMP  
216 content after dilution were 5.00 g/100 g, 0.50 g/100 g and 0.05 g/ 100 g, respectively.  
217 The droplet size and zeta potential of these samples were measured. Meanwhile, the  
218 microstructure of the nanoemulsions was observed using conventional optical  
219 microscopy (DM2000, Wetzlar, Germany). Magnetic stirring is required before  
220 analysis to ensure that the nanoemulsions were homogeneous.

## 221 **2.8. Lipid and protein oxidation measurements**

222        According to the method of [Zhu et al. \(2018\)](#), the nanoemulsions were incubated  
223 at 45 °C in the dark for 21 days to accelerate the oxidation. The degree of the lipid  
224 oxidation in these nanoemulsions was taken out periodically to evaluate the formation  
225 of primary (lipid hydroperoxides) and secondary (2-thiobarbituric acid reactive

226 substances, TBARS) oxidation products. In addition, fluorescence spectroscopy was  
227 used to evaluate the impact of oxidation on the tertiary structure of CAS by means of  
228 tryptophan loss (Beveridge, Toma, & Nakai, 1974).

#### 229 2.8.1. Primary reaction products (lipid hydroperoxides)

230 For the lipid hydroperoxide analysis, 0.3 mL of each nanoemulsion sample was  
231 mixed with 1.5 mL of an isooctane:2-propanol mixture (2:1, v/v) to extract the oil  
232 phase and then, vortexed for 1 min. Thereafter, the mixed solution was centrifuged at  
233 2 000 g for 5 min and the organic solvent phase was collected. Two hundreds  $\mu\text{L}$  of  
234 the organic phase were subsequently mixed with 2.8 mL of a methanol:1-butanol  
235 mixture (2:1, v/v), followed by the addition of 50  $\mu\text{L}$  of 3.94 M ammonium  
236 thiocyanate and 50  $\mu\text{L}$  of 0.132 M ferrous iron solution. After 20 min, the absorbance  
237 of the mixtures was measured at 510 nm using a UV-visible spectrophotometer  
238 (Mettler Toledo, Columbus Ohio, USA). The concentration of lipid hydroperoxides  
239 was calculated based on a standard curve prepared with cumene hydroperoxide.

#### 240 2.8.2. Secondary oxidation products (TBARS)

241 For the TBARS analysis, 1.0 mL of each nanoemulsion was mixed with 2.0 mL of  
242 TBA reagent (formulated by mixing 15 g of trichloroacetic acid, 0.375 g of  
243 thiobarbituric acid (TBA), 1.76 mL of 12 M hydrochloric acid, and 82.9 mL of  
244 distilled water) in glass test tubes with screw caps and then vortexed for 1 min. These  
245 mixtures were then transferred in a boiling water bath for 15 min, cooled to room  
246 temperature for 10 min, and then mixed with 3 mL chloroform under stirring for 1 min.  
247 After centrifugation at 3000 rpm for 15 min, the supernatant was collected. The

248 absorbance of the samples was measured at 532 nm using a UV-visible  
249 spectrophotometer. The concentration of TBARS formed during storage was  
250 calculated based on a standard curve prepared with 1,1,3,3-tetraethoxypropane.

### 251 2.8.3. Protein oxidation measurements

252 Ten  $\mu\text{L}$  of each nanoemulsion were diluted in 2 mL of 10 mM phosphate buffer  
253 (pH 7) and then placed in a 4 mL quartz spectrofluorometer cell for analysis using a  
254 fluorescence spectrophotometer (Agilent, Santa Clara, USA). The excitation  
255 wavelength was 290 nm based on preliminary experiments, which showed that the  
256 oxidation of the protein decreased the fluorescence intensity, but did not change the  
257 maximum emission wavelength. Emission spectra of tryptophan were recorded in a  
258 wavelength range from 300 to 400 nm at a scanning speed of 5 nm/s.

## 259 **2.9. Statistical analysis**

260 All experiments were carried out in triplicate. Statistical analyses were performed  
261 utilizing a software package (SPSS 17.0, SPSS Inc., Chicago, IL, USA). Data are  
262 reported as means  $\pm$  standard deviations, and the mean values of lipid oxidation were  
263 compared using Duncan's multiple-range tests to identify significant differences  
264 ( $p < 0.05$ ).

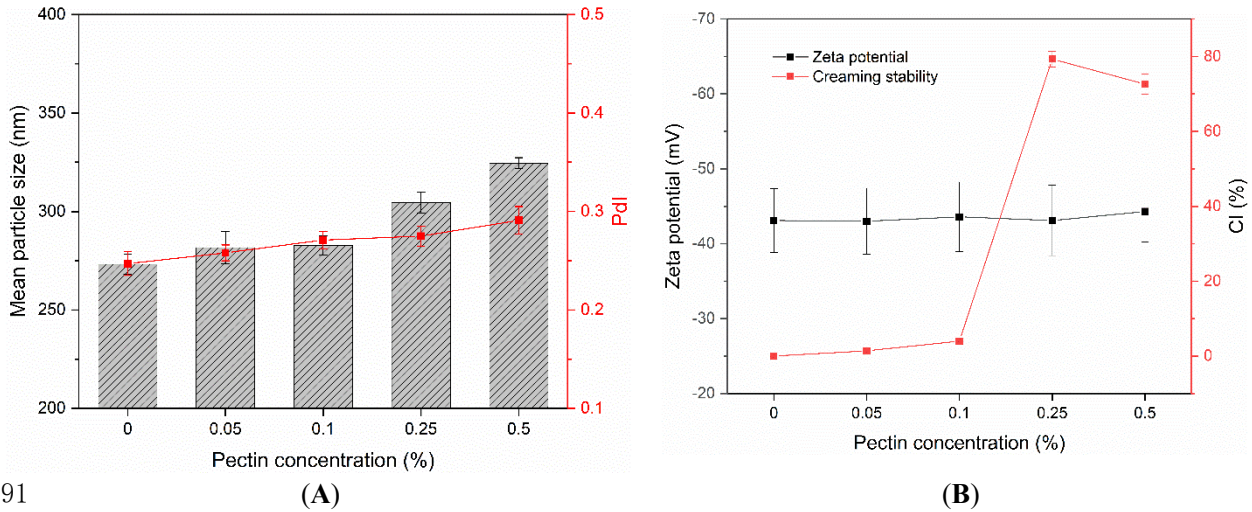
### 265 3. Results and discussion

#### 266 3.1. Effect of pectin concentration on the properties of nanoemulsions

267 To investigate the effect of adding LMP on the properties of CAS stabilized  
268 nanoemulsions at neutral pH, 2.0 wt% CAS solution was mixed with different  
269 concentrations of pectin to obtain nanoemulsions, and pH was then adjusted to 7.0.  
270 [Fig. 1A](#) depicts the particle size distribution and polydispersity index (PDI) of  
271 nanoemulsions. All nanoemulsions exhibited submicron size ranging from 273 nm to  
272 324 nm with monomodal size distribution and the lowest diameter was obtained for  
273 pectin free nanoemulsions. The addition of 0.05 and 0.10 wt% pectin has no obvious  
274 effect on the average diameter of the nanoemulsions. These results also confirm no  
275 adsorption of pectin on the surface of the oil droplets. In fact, the average  
276 hydrodynamic size of the formulated nanoemulsions measured by Dynamic Light  
277 Scattering was almost constant. Similar result tendency was observed in the case of  
278 wheat protein-coated emulsion after addition of pectin and xanthan gum ([Qiu et al.,](#)  
279 [2015](#)). However, as the pectin concentration increased from 0.1 to 0.5 wt%, the  
280 average size of nanoemulsions showed slight increase, which can be attributed to a  
281 slight flocculation that occurred in the original samples. In fact, the excess of free  
282 pectin in the medium could reduce polysaccharide adsorption which promote the  
283 flocculation of nanoemulsions *via* depletion process. Under neutral pH, increased size  
284 was also observed for nanoemulsions stabilized by soybean lipophilic protein as the  
285 concentration of hypromellose was increased ([Li et al., 2020](#)). Another possible  
286 explanation is that some anionic polysaccharides are able to adsorb on the surface of

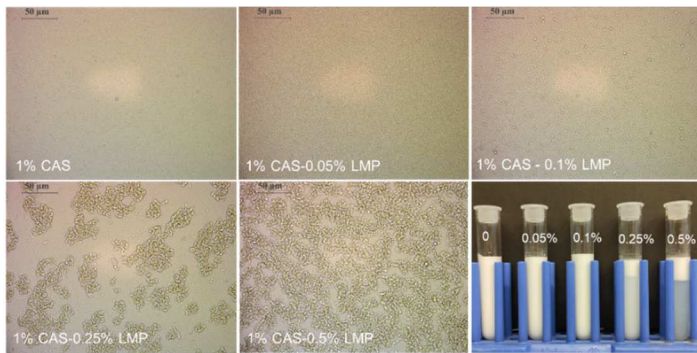
287 protein coated droplets even at neutral pH. Qiu et al. (2015) reported that xanthan  
 288 gum appeared to be capable of adsorbing to protein-coated droplet surfaces at neutral  
 289 pH, where both are strongly negatively charged.

290



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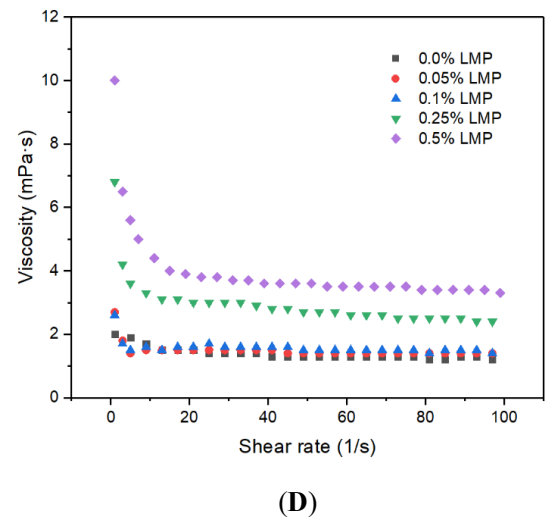
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294

295



296 **Fig. 1.** Effect of pectin concentration on the mean particle size (A), zeta potential,  
 297 creaming stability (B), visual appearance and microscopic structure (C) and viscosity  
 298 (D) of caseinate stabilized nanoemulsions at pH 7.

299 Surprisingly, an opposite result was observed in the case of ovalbumin stabilized  
 300 oil-in water emulsions after addition of xanthan gum (Xiao et al., 2021). These results  
 301 showed that at pH 7, the average particle size of prepared emulsions gradually  
 302 decreased as the concentration of xanthan gum increased in the medium. These



303 apparently contradictory results may be related to the used emulsifier, the type of  
304 polysaccharide, the formulation, and the emulsion preparation method. Meanwhile,  
305 with the gradual increase of the pectin concentration in the formulation, the  
306 polydispersity (PDI) of the nanoemulsions increased slightly, but remains lower than  
307 0.3, showing acceptable narrow size distribution.

308 Typically, the stability of emulsions can be reflected by the surface potential. The  
309 larger the absolute value of the  $\zeta$ -potential, the more stable the corresponding  
310 emulsion system. As shown in [Fig.1B](#), the  $\zeta$ -potential of CAS stabilized  
311 nanoemulsions for different pectin concentrations exhibited relatively high absolute  
312 value ( $\sim 42$  mV). As can be observed, there is no significant effect of LMP  
313 concentration on the  $\zeta$ -potential of the CAS-coated droplets. At pH values above the  
314 isoelectric point (pI) of the adsorbed protein, pectin molecules (pKa  $\sim 3.5$ ) and  
315 CAS-coated droplets are both negatively charged. Therefore, due to the relatively  
316 strong electrostatic repulsions between the negatively charged droplets and the  
317 negatively charged pectin molecules, the adsorption of pectin at pH 7 will be  
318 negligible or even there will have no adsorption at all. As a result, pectin is then  
319 mainly located in the aqueous phase of the emulsion rather on the droplets surface.  
320 [Zang et al. \(2019\)](#) investigated the effect of different anionic polysaccharides on the  
321 rice bran protein hydrolysate-stabilized emulsions. They found that, at pH 7, adding  
322 pectin and Arabic gum did not significantly change the  $\zeta$ -potential of rice brand  
323 protein-coated droplets, but the addition of xanthan gum and alginate showed slightly  
324 increases. This result was attributed to the high charge densities of alginate and

325 xanthan gum compared to pectin and Arabic gum, affecting the charge characteristics  
326 of protein-coated droplets. [Zhang et al. \(2020\)](#) developed oil in water emulsions using  
327 almond protein isolate / camellia saponin and they described that some of camellia  
328 saponin adsorbed to the almond protein-stabilized oil droplets despite the fact that  
329 both surfactant and protein were negatively charged. Moreover, steric repulsions  
330 could also participate in the maintenance of emulsion stability, and the magnitude of  
331 these repulsions was shown to be dependent on the properties of the polysaccharide  
332 ([Zang et al., 2019](#)). Creaming index is another indicator related to the long-term  
333 stability of the emulsion. In absence of pectin or in presence of a small amount of this  
334 polyanionic biopolymer ( $\leq 0.1$  wt%), CAS stabilized nanoemulsions were stable to  
335 creaming within 1 day ([Fig. 1B](#)). Indeed, even after 3 weeks of storage at room  
336 temperature, no obvious serum layer was detected in these nanoemulsions (data not  
337 shown), indicating that most of the droplets are small enough and the repulsions  
338 between them are sufficient to resist to creaming and did not aggregate during storage.  
339 However, when the concentration of pectin was increased to 0.25 and 0.50 wt%, the  
340 nanoemulsions were highly unstable to phase separation, which may be caused by  
341 depletion flocculation at neutral pH. The results of the microscopic structure ([Fig. 1C](#))  
342 could support this conclusion. Interestingly, based on particle size measurements ([Fig.](#)  
343 [1A](#)), the creaming of CAS-based emulsions arising from depletion flocculation is  
344 reversible, and the clusters of droplets could be disrupted by gentle stirring or dilution.  
345 This result was consistent with the study of [Zang et al. \(2019\)](#). The maximum  
346 creaming of protein-based emulsions at pH 7 was clearly observed at high

347 concentration of anionic polysaccharides, however, creaming had no significant effect  
348 on the hydrodynamic particle size. The viscosity (Fig.1 D) plays an important role in  
349 the taste and stability of food emulsions. The apparent viscosity decreased with  
350 increasing shear rate, which means that these nanoemulsions are pseudoplastic fluids  
351 (He, Peng, Wei, Li, & Tian, 2020). Meanwhile, there was no significant difference  
352 between the CAS-stabilized nanoemulsions containing no pectin or containing low  
353 pectin concentrations ( $\leq 0.1\text{wt}\%$ ), while at higher pectin concentration, a significant  
354 increase in viscosity was observed and the highest value was obtained for  
355 nanoemulsions containing  $0.5\text{wt}\%$  LMP. This clearly showed the formation of  
356 flocculated droplets at higher pectin concentrations due to depletion flocculation,  
357 which was also supported by the creaming results (Fig. 1C). In fact, pectin is a  
358 non-adsorbing linear polysaccharide, and its aqueous solution had an excellent  
359 viscoelasticity. Besides, Table 1 exhibited the Herschel-Bulkely parameters of these  
360 nanoemulsions and the obtained flow data is fitted well with this model ( $R^2 > 0.99$ ).  
361 The flow behavior index of all nanoemulsions was in a range of 0.810–0.957, which  
362 means that nanoemulsion containing no LMP or low concentrations of LMP exhibited  
363 a Newtonian behavior, whereas, at high concentrations of LMP, the nanoemulsions  
364 exhibited a shear-thinning behavior, clearly demonstrating the presence of flocculated  
365 oil droplets domains. Similar phenomena was found in nanoemulsions stabilized by  
366 whey proteins (Khalloufi et al., 2008). The yield stress ( $\tau_0$ ) is the instantaneous  
367 pressure required to initiate material flow, and the value obtained in these  
368 nanoemulsions is almost zero. The consistency coefficient (k) represents the viscosity

369 behavior of a fluid and the results demonstrate that the k value of nanoemulsions  
 370 increases gradually with increasing the pectin concentration.

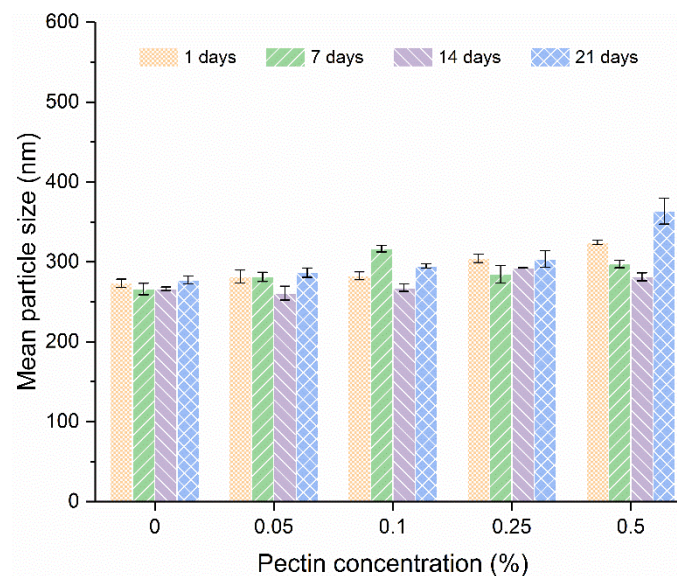
371 **Table 1.** The parameters of Herschel-Bulkely model for caseinate stabilized  
 372 nanoemulsions containing different pectin concentrations at pH 7.  
 373

<b>Pectin concentration</b> (%, w/w)	n	k (Pa.sn)	$\tau_0$ (Pa)	R <sup>2</sup>
0.00	0.937±0.008	0.0016±0.0001	0.0023±0.0004	0.999
0.05	0.957±0.009	0.0017±0.0001	0.0001±0.0050	0.999
0.10	0.876±0.011	0.0025±0.0001	0.0000±0.0007	0.998
0.25	0.810±0.009	0.0057±0.0003	0.0000±0.0011	0.998
0.50	0.841±0.004	0.0043±0.0001	0.0014±0.0006	0.999

374 Value are means ± standard deviation of triplicates.  
 375  
 376

### 377 **3.2. Effect of pectin concentration on the ageing stability and environmental** 378 **stress resistance of nanoemulsions**

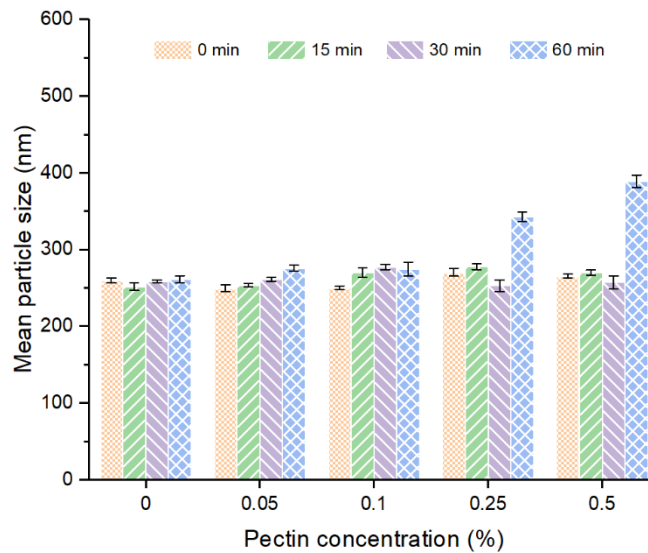
379 The mean droplet size of CAS-stabilized nanoemulsions containing different  
 380 pectin concentrations stored at room temperature for 3 weeks were measured and the  
 381 obtained results are reported in Fig. 2. From the particle size point of view, all  
 382 nanoemulsions showed excellent storage stability and there was no significant change  
 383 within three weeks. This could be attributed to CAS properties having relatively high  
 384 negative charge at pH 7, which led to stronger electrostatic repulsions between oil  
 385 droplets. Interestingly, a slight increased particle size was observed in the  
 386 nanoemulsions containing 0.5 wt% LMP after 21 days storage. This result suggests  
 387 that although flocculation caused by higher concentration of pectin in the  
 388 nanoemulsions is reversible as mentioned in the previous section, the long-term  
 389 storage effect may produce some irreversible growth of particle size.



**Fig. 2.** Effect of pectin concentration on the ageing stability of caseinate stabilized nanoemulsions at pH 7.

To evaluate the effect of environmental stress on the properties of nanoemulsions, different heating times, freeze-thaw cycles, and salt concentrations were used to simulate conditions which might be experienced during manufacture, cooking or utilization. For each dispersion, droplet size distribution of the nanoemulsions was measured to evaluate their stability. Fig. 3 presented the effect of heating time (at 100 °C) on the stability of nanoemulsions containing different concentrations of LMP. All nanoemulsions appeared to be very stable to heat treatment and showed stable unimodal particle size distribution within 1 hour of heating. This can be attributed to CAS as adsorbed layer which provides these nanoemulsions excellent thermal stability, because the structure and the conformation of caseinate is not significantly affected by thermal treatment (Srinivasan, Singh, & Munro, 2002). The  $\zeta$ -potential of nanoemulsions was also measured (data not shown) and was found to be not significantly affected by the thermal treatments, the surface charge was not

407 significantly changed upon thermal treatments, demonstrating, that such emulsions  
408 showed excellent stability against heating.



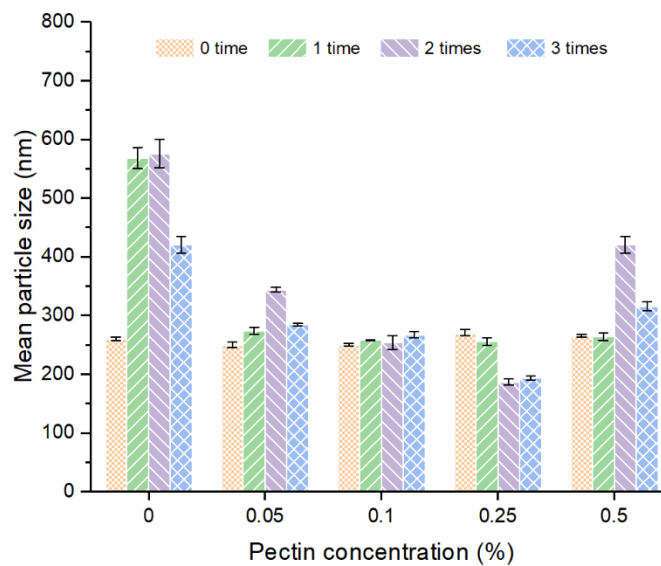
409

410 **Fig. 3.** Effect of heating time (100 °C) on caseinate stabilized nanoemulsions  
411 containing different concentrations of pectin at pH 7.

412 Food in emulsion forms may also undergo freezing and thawing treatments during  
413 the storage and consumption, so it is necessary to understand the stability of  
414 nanoemulsions during the freeze-thaw process. Fig. 4 presented the effect of  
415 freeze-thaw times on the average hydrodynamic size of nanoemulsions containing  
416 different concentrations of LMP. In the absence of LMP, there was a marked increase  
417 in the mean particle size after freeze-thaw cycles. Crystallization of either the lipid or  
418 water phase during freezing can explain this phenomenon. When a nanoemulsion is  
419 thawed, the droplets tend to aggregate, thereby resulting in an increase of  
420 hydrodynamic particle size (Liu, Sun, Xue, & Gao, 2016). However, in the presence  
421 of LMP, nanoemulsions were much more stable to droplet aggregation after three

422 freeze–thaw cycles, especially the nanoemulsions containing 0.10 wt% and 0.25 wt%  
423 LMP. This result could be attributed to the ability of pectin to increase the amount of  
424 non-frozen aqueous phase in the nanoemulsions. A similar result was found in the  
425 case of nanoemulsions stabilized by multilayered biopolymers ( $\beta$ -Lactoglobulin–  
426  $\kappa$ -carrageenan–gelatin) (Gu, Decker, & McClements, 2007). Meanwhile, some large  
427 individual oil droplets were observed in nanoemulsions after thawing treatment,  
428 which indicated that the lipid may leak from the droplet (because there were no large  
429 droplets in the untreated nanoemulsions). This phenomenon may explain the  
430 decreased size observed for nanoemulsions containing 0.25% pectin after 2  
431 freeze-thaw cycles. A decreased droplet size after freeze-thaw treatments was also  
432 observed in lecithin and modified starch-based nanoemulsions (Donsì, Wang, &  
433 Huang, 2011). In addition, some large irregularly shaped aggregates appeared in the  
434 nanoemulsions after dilution, especially for pectin free nanoemulsions, which may be  
435 caused by the massive aggregation of CAS during freezing and thawing. Thus, the  
436 presence of free pectin could prevent the aggregation of caseinate coated droplets to a  
437 certain extent during the freezing process and then participate on the dissociation of  
438 aggregates during the thawing step due to the repulsive forces between CAS and  
439 LMP.

440



442 **Fig. 4.** Effect of the number of freeze-thaw cycles on caseinate stabilized  
 443 nanoemulsions containing different concentrations of pectin at pH 7.

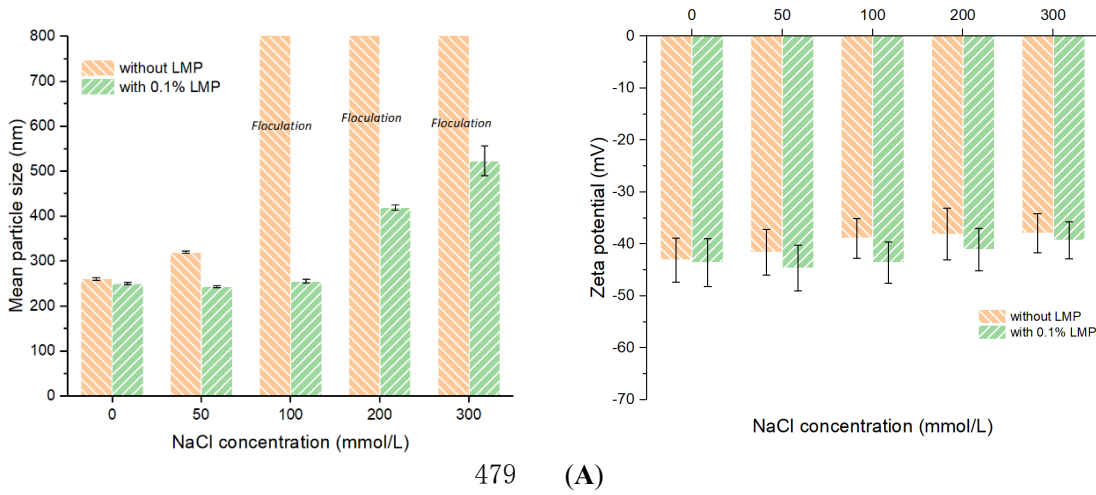
444

445 Adding edible salt to improve the taste of emulsion-based food products is a  
 446 common requirement in the diet, and so it is essential to understand the effect of salts  
 447 on the performance and stability of emulsions. Nanoemulsions containing 0.1 wt%  
 448 pectin were selected in this section because they are highly stable to creaming and  
 449 flocculation (Fig. 1C). The effect of different NaCl concentrations on the mean  
 450 particle diameter, zeta potential, visual appearance and microscopic image of  
 451 nanoemulsions containing or not 0.1 wt% pectin was examined (Fig. 5). The  
 452 nanoemulsions containing no pectin were colloiddally stable since no droplet  
 453 aggregation (Fig. 5A) and creaming (Fig. 5B) were observed in the absence of salt but  
 454 showed significantly increased mean particle size and phase separation in the  
 455 presence of ionic force above 100 mM NaCl. The microscopic images (Fig. 5C) of  
 456 nanoemulsions without LMP also showed a droplet aggregation phenomena. It was

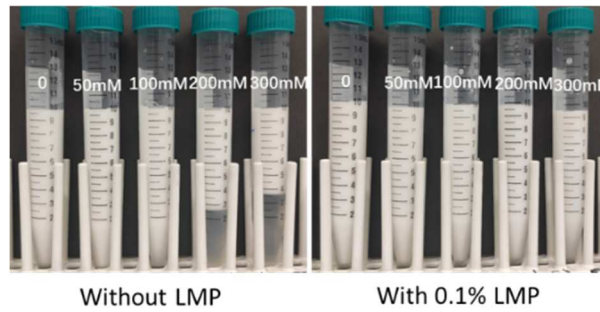


457 well established that at neutral pH, salt has the ability to promote the flocculation of  
458 droplets stabilized by protein based emulsifiers, which can be attributed to  
459 electrostatic screening and ion binding (McClements, 2004). The zeta potential  
460 measurements (Fig. 5A) showed that the addition of NaCl did not markedly change  
461 the magnitude of the droplet charge, but a slight decrease (from -43.1 to -37.9 mV)  
462 could be observed in the free pectin nanoemulsion. It is interesting to notice that, in  
463 the presence of pectin, the nanoemulsions exhibited better salty tolerance, but when  
464 the salinity was higher than 200 mM NaCl, the particle size of the nanoemulsions  
465 increased significantly. Through visual appearance and microscopic images, no phase  
466 separation (Fig. 5B) and obvious aggregation (Fig. 5C) occurred in the nanoemulsion  
467 containing 0.1 wt% pectin in the investigated range of salt concentrations, except for  
468 300 mM NaCl (some clusters were observed). This result indicates that even if both  
469 CAS and LMP are negatively charged at pH 7, LMP can still provide inhibition of  
470 aggregation. This may be attributed to the formation of gel-like network induced by  
471 free pectin in the aqueous continuous phase. Qiu et al. (2015) also reported that  
472 adding xanthan gum can improve the properties of wheat protein-stabilized emulsions  
473 against high ionic strengths at pH 7, but the addition of pectin had no obvious effect.  
474 They attributed this phenomenon to the fact that xanthan gum has a higher linear  
475 charge density than pectin.

476



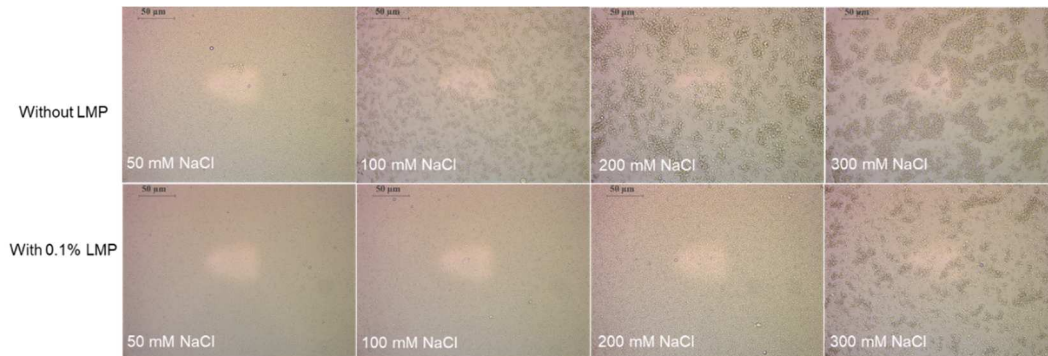
479 (A)



480 (B)

481 (C)

482 **Fig. 5.** Effect of ionic strength on the mean particle size and zeta potential (A), visual  
 483 appearance (B) and microscopic structure (C) of caseinate stabilized nanoemulsions  
 484 with 0.1 wt% or without pectin at pH 7.  
 485  
 486



487  
 488 **3.3. Effect of pectin addition on the oxidation of protein and emulsified lipid**

489 The influence of pectin addition on the lipid oxidation of nanoemulsions was

490 evaluated by monitoring the generation of primary (lipid hydroperoxides) and  
491 secondary (TBARS) reaction products for 3 weeks storage at 45 °C (Fig. 6). As  
492 expected, the lipid hydrogen peroxide and TBARS contents of all nanoemulsions  
493 gradually increased during storage, indicating that lipid oxidation was progressing.  
494 The results of primary reaction products (Fig. 6A) showed that, at neutral pH, the  
495 nanoemulsions containing pectin showed better ( $p<0.05$ ) antioxidant activity than  
496 nanoemulsions without pectin after 3 weeks, and the lowest ( $p<0.05$ ) lipid  
497 hydroperoxide content was measured in the case of nanoemulsions containing 0.25 wt%  
498 pectin. This may be related to several reasons. Firstly, it is reported that pectin have a  
499 certain antioxidant capacity and therefore the unadsorbed pectin molecules may react  
500 with free radical scavenging in the emulsion system (Celus et al., 2020; Kumar et al.,  
501 2021). Celus et al. (2018) reported that the antioxidant capacity of pectin was mainly  
502 determined by the degree of methyl esterification (DM) and the LMP exhibited higher  
503 antioxidant activity than the HMP (High Methoxyl Pectin). Indeed, the high numbers  
504 of active hydroxyl and carboxyl groups on galacturonic acid (GalA) residues of pectin  
505 are essential for its antioxidant activities (Cui et al., 2021). In addition, the antioxidant  
506 activity could be probably attributed to the non-pectic compounds, such as individual  
507 polyphenols and their conjugates in the extracted pectin (Ahn, Halake, & Lee,  
508 2017). Secondly, unadsorbed pectin may prevent water-soluble oxidants from  
509 reaching lipids by forming a weak gel-like network in the aqueous continuous phase  
510 (Zhang et al., 2020). The lipid oxidation was also reported to be affected by the  
511 viscosity of emulsions (Pernin, Bosc, Soto, Le Roux, & Maillard, 2019). In fact, the

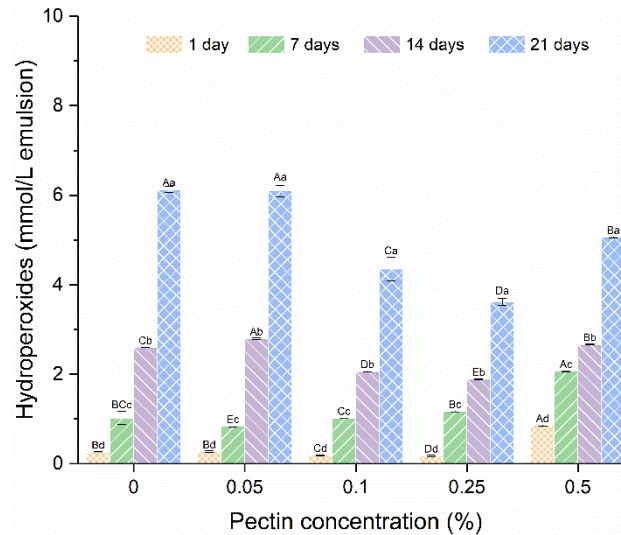
512 transfer of oxygen from the aqueous phase to lipid droplets could be retarded by  
513 higher apparent viscosity of nanoemulsions. The viscosity data (Fig. 1D) obtained in  
514 this study also support this hypothesis. However, the lowest lipid hydroperoxide  
515 content was obtained in nanoemulsions containing 0.25 wt% instead of 0.5 wt%. We  
516 hypothesized that flocculation might increase lipid oxidation since higher degree of  
517 droplet flocculation was observed for 0.5 wt% LMP. In fact, most droplets clump  
518 together and move upwards by gravity, and the aggregated droplets at the top of the  
519 emulsions are closer to the air oxygen, which could accelerate oxidation phenomena.  
520 In addition, previous studies showed that non-adsorbed proteins have the ability to  
521 bind cationic transition metal ions (pro-oxidants) and then inhibit their interactions  
522 with the encapsulated lipids (Gumus et al., 2017; Zhu et al., 2018). In this regards,  
523 unadsorbed CAS and unadsorbed LMP in the negatively charged nanoemulsions at  
524 pH 7 may also interact with the cationic transition metals leading therefore to possible  
525 inhibition of oxidation process.

526 The TBARS measurements (Fig. 6B) showed that nanoemulsions without pectin  
527 have a similar ( $p>0.05$ ) trend compared to the nanoemulsions containing pectin,  
528 except the one containing 0.5 wt%. This indicated that, at neutral pH, the addition of  
529 different concentrations of pectin demonstrated a limited impact on the TBARS  
530 formation in caseinate-stabilized nanoemulsions. In addition, after 3 weeks of storage,  
531 a significantly increase of TBARS value was observed in the nanoemulsions  
532 containing 0.5 wt% pectin. After three weeks of storage at 45 °C, the depletion  
533 flocculation induced by the high concentration of unadsorbed pectin may explain the

534 observed phenomenon.

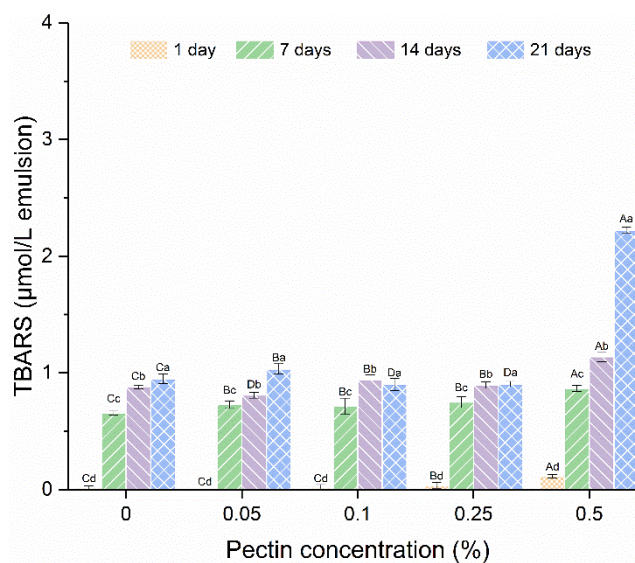
535

536



(A)

537



(B)

538

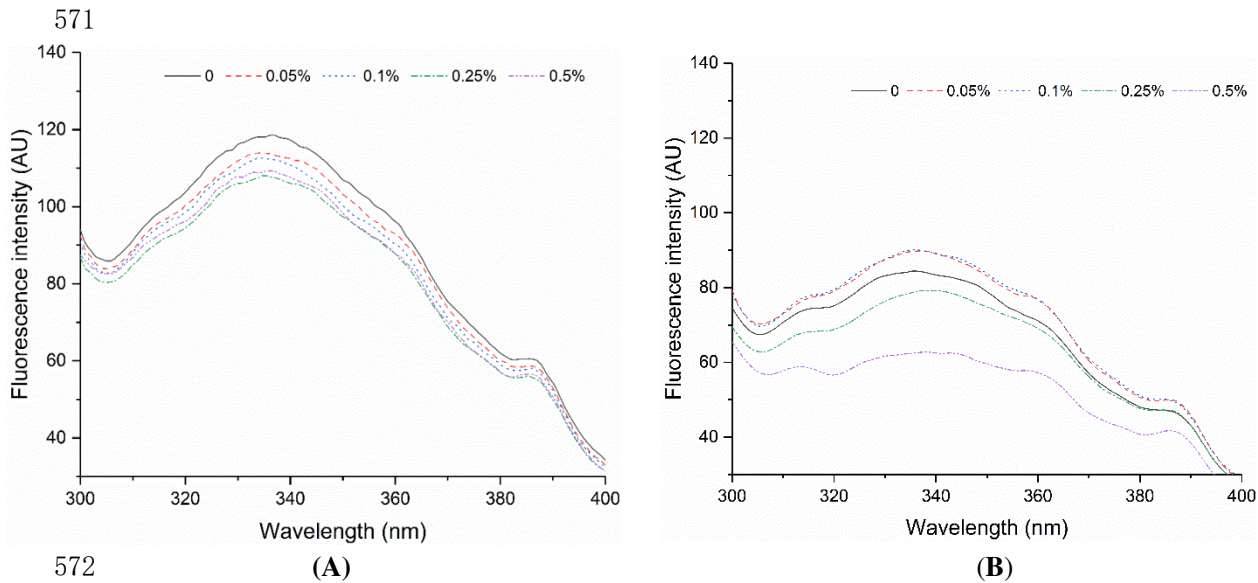
539

540 **Fig. 6.** Effect of pectin concentration on lipid oxidation of nanoemulsions during  
541 storage at 45 °C for 21 days. Lipid oxidation was monitored by measuring  
542 hydroperoxides (A) and TBARS (B). Samples with different uppercase superscripts  
543 (A–E) differ significantly ( $p < 0.05$ ) when different pectin concentrations are  
544 compared at the same oxidation time; Samples with different lowercase superscripts  
545 (a–d) differ significantly ( $p < 0.05$ ) when different oxidation times are compared at  
546 the same pectin concentration.

547

548 In food systems, proteins are crucial targets of lipid co-oxidation. It was reported  
549 that lipid oxidation products such as free radicals, hydroperoxides and carbonyl  
550 compounds can greatly destroy proteins (Elias, Kellerby, & Decker, 2008). In this  
551 regard, we monitored the loss of intrinsic tryptophan fluorescence of CAS in the  
552 nanoemulsion systems for 3 weeks storage at 45 °C. The fluorescence spectra of  
553 nanoemulsions after 0 day and 21 days of storage are shown in Fig. 7A and Fig. 7B,  
554 respectively. The maximum fluorescence emission was observed at a wavelength of  
555 around 336 nm for all nanoemulsions. In the beginning (0 day), nanoemulsions  
556 without pectin exhibited the highest fluorescence intensity and the intensity value  
557 decreased as the pectin concentration increased. Similar phenomenon was found in  
558 almond protein stabilized emulsions (Zhang et al., 2020). This may be attributed to  
559 free pectin in the continuous phase of nanoemulsions and these unadsorbed pectin  
560 molecules may affect the exposure of tryptophan residues. Consistent with other  
561 studies (Li et al., 2020; Zhu et al., 2018), during the storage process, the intrinsic  
562 fluorescence intensity of tryptophan in all nanoemulsions decreased after 21 days (Fig.  
563 7B), which can be attributed to the oxidative degradation of tryptophan leading to  
564 changes in the spherical structure of caseinate. The nanoemulsions containing 0.05 wt%  
565 and 0.10 wt% pectin showed better ability to inhibit protein oxidation than pectin free  
566 nanoemulsions after 3 weeks. However, in the case of nanoemulsions containing  
567 higher pectin concentration (0.5 wt%), the tryptophan fluorescence intensity  
568 decreased significantly. This observation further supports that under neutral  
569 conditions, an appropriate pectin concentration can contribute to the oxidation

570 stability of CAS-stabilized nanoemulsions.



573 **Fig. 7.** Effect of pectin concentration on the intrinsic tryptophan fluorescence of  
574 nanoemulsions during storage at 45 °C for 0 (A) and 21 (B) days.

575 In summary, even if both caseinate and pectin are both negatively charged at  
576 neutral pH, the addition of LMP can still improve the performance of CAS stabilized  
577 nanoemulsions, especially against high salt concentrations and freeze-thaw cycles,  
578 and appreciably enhance the antioxidant ability of nanoemulsions. The caseinate  
579 stabilized nanoemulsions containing 0.10 wt% and 0.25 wt% were the more stable  
580 formulations in this study.

#### 581 **4. Conclusion**

582 The effect of pectin on the physical and oxidation stability of caseinate-stabilized  
583 nanoemulsions under neutral pH was investigated as a function of several parameters  
584 and conditions. It emerges from this systematic study that the presence of pectin could  
585 improve the physical (especially against high salt concentrations and freeze-thaw  
586 cycles) and chemical stability of caseinate-stabilized nanoemulsions, depending on its

587 concentration. Nanoemulsions containing 0.10 wt% and 0.25 wt% are considered as  
588 the optimal formulation which exhibited the ability to inhibit lipid and protein  
589 oxidation more effectively than caseinate alone. There are at least two possible  
590 mechanisms for the antioxidant properties of pectin in nanoemulsions: i) the  
591 non-adsorbed pectin could have the ability to scavenge free radical and chelate metals;  
592 ii) the non-adsorbed pectin can reduce droplet mobility by forming a weak gel-like  
593 network that prevents water-soluble pro-oxidants reaching the lipids. In addition, a  
594 higher concentration of pectin (0.5 wt%) can promote the occurrence of depletion  
595 flocculation of nanoemulsions, resulting in phase separation, more secondary lipid  
596 oxidation products, and more tryptophan loss. This research could be interesting for  
597 the formulation of beverage emulsions with improved physical and chemical  
598 properties at neutral pH.



599 **References**

- 600 Ahn, S., Halake, K., & Lee, J. (2017). Antioxidant and ion-induced gelation functions  
601 of pectins enabled by polyphenol conjugation. *International Journal of Biological*  
602 *Macromolecules*, 101, 776–782.
- 603 Aphibanthammakit, C., & Kasemwong, K. (2021). Chapter 15—Nanoencapsulation  
604 in agricultural applications. In W. J. Lau, K. Faungnawakij, K. Piyachomkwan, & U.  
605 R. Ruktanonchai (Eds.), *Handbook of Nanotechnology Applications*: 359–382.  
606 Elsevier.
- 607 Berton-Carabin, C. C., Schröder, A., Rovalino-Cordova, A., Schroën, K., & Sagis, L.  
608 (2016). Protein and lipid oxidation affect the viscoelasticity of whey protein layers at  
609 the oil–water interface. *European Journal of Lipid Science and Technology*, 118(11),  
610 1630–1643.
- 611 Beveridge, T., Toma, S. J., & Nakai, S. (1974). Determination of SH-and SS-groups in  
612 some food proteins using Ellman’s reagent. *Journal of Food Science*, 39(1), 49–51.
- 613 Cai, Y., Deng, X., Liu, T., Zhao, M., Zhao, Q., et al. (2018). Effect of xanthan gum on  
614 walnut protein/xanthan gum mixtures, interfacial adsorption, and emulsion properties.  
615 *Food Hydrocolloids*, 79, 391–398.
- 616 Celus, M., Kyomugasho, C., Keunen, J., Van Loey, A. M., Grauwet, T., et al. (2020).  
617 Simultaneous use of low methylesterified citrus pectin and EDTA as antioxidants in  
618 linseed/sunflower oil-in-water emulsions. *Food Hydrocolloids*, 100, Article 105386.
- 619 Celus, M., Salvia-Trujillo, L., Kyomugasho, C., Maes, I., Van Loey, A. M., et al.  
620 (2018). Structurally modified pectin for targeted lipid antioxidant capacity in  
621 linseed/sunflower oil-in-water emulsions. *Food Chemistry*, 241, 86–96.
- 622 Chang, P. G., Gupta, R., Timilsena, Y. P., & Adhikari, B. (2016). Optimisation of the  
623 complex coacervation between canola protein isolate and chitosan. *Journal of Food*  
624 *Engineering*, 191, 58–66.
- 625 Cui, J., Zhao, C., Feng, L., Han, Y., Du, H., et al. (2021). Pectins from fruits:

626 Relationships between extraction methods, structural characteristics, and functional  
627 properties. *Trends in Food Science & Technology*, 110, 39–54.

628 Dalglish, D. G., Srinivasan, M., & Singh, H. (1995). Surface properties of  
629 oil-in-water emulsion droplets containing casein and Tween 60. *Journal of*  
630 *Agricultural and Food Chemistry*, 43(9), 2351–2355.

631 Donsì, F., Wang, Y., & Huang, Q. (2011). Freeze–thaw stability of lecithin and  
632 modified starch-based nanoemulsions. *Food Hydrocolloids*, 25(5), 1327–1336.

633 Elias, R. J., Kellerby, S. S., & Decker, E. A. (2008). Antioxidant activity of proteins  
634 and peptides. *Critical Reviews in Food Science and Nutrition*, 48(5), 430–441.

635 Estévez, M., Kylli, P., Puolanne, E., Kivikari, R., & Heinonen, M. (2008).  
636 Fluorescence spectroscopy as a novel approach for the assessment of myofibrillar  
637 protein oxidation in oil-in-water emulsions. *Meat Science*, 80(4), 1290–1296.

638 Gu, Y. S., Decker, Eric. A., & Julian McClements, D. (2007). Application of  
639 multi-component biopolymer layers to improve the freeze–thaw stability of  
640 oil-in-water emulsions:  $\beta$ -Lactoglobulin– $\iota$ -carrageenan–gelatin. *Journal of Food*  
641 *Engineering*, 80(4), 1246–1254.

642 Gumus, C. E., Decker, E. A., & McClements, D. J. (2017). Impact of legume protein  
643 type and location on lipid oxidation in fish oil-in-water emulsions: Lentil, pea, and  
644 faba bean proteins. *Food Research International*, 100, 175–185.

645 He, L., Peng, H., Wei, X., Li, B., & Tian, J. (2020). Preparation and Characterization  
646 of Emulsions Stabilized with Defatted Sesame Meal. *Food Science and Technology*  
647 *Research*, 26(5), 655–663.

648 Hu, M., McClements, D. J., & Decker, E. A. (2003). Lipid oxidation in corn  
649 oil-in-water emulsions stabilized by casein, whey protein isolate, and soy protein  
650 isolate. *Journal of Agricultural and Food Chemistry*, 51(6), 1696–1700.

651 Katsouli, M., Polychniatou, V., & Tzia, C. (2018). Optimization of water in olive oil  
652 nano-emulsions composition with bioactive compounds by response surface

653 methodology. *LWT*, 89, 740–748.

654 Khalloufi, S., Alexander, M., Douglas Goff, H., & Corredig, M. (2008).  
655 Physicochemical properties of whey protein isolate stabilized oil-in-water emulsions  
656 when mixed with flaxseed gum at neutral pH. *Food Research International*, 41(10),  
657 964–972.

658 Kumar, M., Potkule, J., Tomar, M., Punia, S., Singh, Surinder, et al. (2021). Jackfruit  
659 seed slimy sheath, a novel source of pectin: Studies on antioxidant activity, functional  
660 group, and structural morphology. *Carbohydrate Polymer Technologies and*  
661 *Applications*, 2, Article 100054.

662 Li, Q., Zheng, J., Ge, G., Zhao, M., & Sun, W. (2020). Impact of heating treatments  
663 on physical stability and lipid-protein co-oxidation in oil-in-water emulsion prepared  
664 with soy protein isolates. *Food Hydrocolloids*, 100, Article 105167.

665 Li, T., Teng, D., Mao, R., Hao, Y., Wang, X., et al. (2019). Recent progress in  
666 preparation and agricultural application of microcapsules. *Journal of Biomedical*  
667 *Materials Research Part A*, 107(10), 2371–2385.

668 Li, Y., Zhong, M., Xie, F., Sun, Y., Zhang, S., et al. (2020). The effect of pH on the  
669 stabilization and digestive characteristics of soybean lipophilic protein oil-in-water  
670 emulsions with hypromellose. *Food Chemistry*, 309, Article 125579.

671 Liu, S., Sun, C., Xue, Y., & Gao, Y. (2016). Impact of pH, freeze–thaw and thermal  
672 sterilization on physicochemical stability of walnut beverage emulsion. *Food*  
673 *Chemistry*, 196, 475–485.

674 McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid*  
675 *& Interface Science*, 9(5), 305–313.

676 McClements, D. J., & Rao, J. (2011). Food-grade nanoemulsions: Formulation,  
677 fabrication, properties, performance, biological fate, and potential toxicity. *Critical*  
678 *Reviews in Food Science and Nutrition*, 51(4), 285–330.

679 Mwangi, W. W., Ho, K.-W., Tey, B.-T., & Chan, E.-S. (2016). Effects of

680 environmental factors on the physical stability of pickering-emulsions stabilized by  
681 chitosan particles. *Food Hydrocolloids*, 60, 543–550.

682 Obando, M., Papastergiadis, A., Li, S., & De Meulenaer, B. (2015). Impact of lipid  
683 and protein co-oxidation on digestibility of dairy proteins in oil-in-water (O/W)  
684 emulsions. *Journal of Agricultural and Food Chemistry*, 63(44), 9820–9830.

685 Pernin, A., Bosc, V., Soto, P., Le Roux, E., & Maillard, M.-N. (2019). Lipid oxidation  
686 in oil-in-water emulsions rich in omega-3: Effect of aqueous phase viscosity,  
687 emulsifiers, and antioxidants. *European Journal of Lipid Science and Technology*,  
688 121(9), Article 1800462.

689 Qiu, C., Zhao, M., & McClements, D. J. (2015). Improving the stability of wheat  
690 protein-stabilized emulsions: Effect of pectin and xanthan gum addition. *Food*  
691 *Hydrocolloids*, 43, 377–387.

692 Singh, H., & Sarkar, A. (2011). Behaviour of protein-stabilised emulsions under  
693 various physiological conditions. *Advances in Colloid and Interface Science*, 165(1),  
694 47–57.

695 Srinivasan, M., Singh, H., & Munro, P. A. (2002). Formation and stability of sodium  
696 caseinate emulsions: Influence of retorting (121 C for 15 min) before or after  
697 emulsification. *Food Hydrocolloids*, 16(2), 153–160.

698 Surh, J., Decker, E. A., & McClements, D. J. (2006). Influence of pH and pectin type  
699 on properties and stability of sodium-caseinate stabilized oil-in-water emulsions.  
700 *Food Hydrocolloids*, 20(5), 607–618.

701 Teng, M.-J., Wei, Y.-S., Hu, T.-G., Zhang, Y., Feng, K., et al. (2020). Citric acid  
702 cross-linked zein microcapsule as an efficient intestine-specific oral delivery system  
703 for lipophilic bioactive compound. *Journal of Food Engineering*, 281, Article 109993.

704 Villiere, A., Viau, M., Bronnec, I., Moreau, N., & Genot, C. (2005). Oxidative  
705 stability of bovine serum albumin-and sodium caseinate-stabilized emulsions depends  
706 on metal availability. *Journal of Agricultural and Food Chemistry*, 53(5), 1514–1520.

707 Wang, J., Dumas, E., & Gharsallaoui, A. (2019). Low Methoxyl pectin/sodium  
708 caseinate complexing behavior studied by isothermal titration calorimetry. *Food*  
709 *Hydrocolloids*, 88, 163–169.

710 Wang, J., Su, Y., Jia, F., & Jin, H. (2013). Characterization of casein hydrolysates  
711 derived from enzymatic hydrolysis. *Chemistry Central Journal*, 7(1), 1–8.

712 Xiao, N., He, W., Zhao, Y., Yao, Y., Xu, M., et al. 2021. Effect of pH and xanthan gum  
713 on emulsifying property of ovalbumin stabilized oil-in water emulsions. *LWT*, 147,  
714 Article 111621.

715 Zang, X., Wang, J., Yu, G., & Cheng, J. (2019). Addition of anionic polysaccharides  
716 to improve the stability of rice bran protein hydrolysate-stabilized emulsions. *LWT*,  
717 111, 573–581.

718 Zhang, F., Cai, X., Ding, L., & Wang, S. (2021). Effect of pH, ionic strength, chitosan  
719 deacetylation on the stability and rheological properties of O/W emulsions formulated  
720 with chitosan/casein complexes. *Food Hydrocolloids*, 111, Article 106211.

721 Zhang, S., Tian, L., Yi, J., Zhu, Z., Decker, E. A., et al. (2020). Mixed plant-based  
722 emulsifiers inhibit the oxidation of proteins and lipids in walnut oil-in-water  
723 emulsions: Almond protein isolate-camellia saponin. *Food Hydrocolloids*, 109, Article  
724 106136.

725 Zhu, Z., Zhao, C., Yi, J., Liu, N., Cao, Y., et al. (2018). Impact of interfacial  
726 composition on lipid and protein co-oxidation in oil-in-water emulsions containing  
727 mixed emulsifiers. *Journal of Agricultural and Food Chemistry*, 66(17), 4458–4468.

728