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1 **Effect of carrier oil on the properties of sodium caseinate stabilized O/W**
2 **nanoemulsions containing *Trans*-cinnamaldehyde**

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19 **Abstract**

20 In this study, the impact of different contents of medium-chain triacylglycerols (MCT) and
21 long-chain triacylglycerols (LCT) as carrier oils mixed with *trans*-cinnamaldehyde (TCA) on the
22 properties of sodium caseinate-stabilized nanoemulsions was investigated. The obtained results
23 showed that the use of a carrier oil improves significantly the TCA emulsion stability, which can
24 be attributed to a reduction of TCA solubility in the aqueous phase followed by a decrease of
25 Ostwald ripening. This stability improvement was observed at different pHs (3, 7 and 11), salt
26 concentrations (0–300 mmol/L), and temperature (4, 25, 60, and 80 °C). However, the carrier oil
27 type (MCT or LCT) did not show any significant effect on the nanoemulsions properties whatever
28 the environmental conditions. Antibacterial testing revealed that the addition of carrier oil
29 increased the antibacterial activity of TCA against *Escherichia coli* and *Listeria innocua*. These
30 results are useful for expanding the application of TCA as food preservative in food industry.

31
32 **Keyword:** Nanoemulsion; Sodium caseinate; *trans*-Cinnamaldehyde; Carrier oil; Antibacterial
33 activity.

34 **1. Introduction**

35 It is well established that food preservation is a great challenge and a significant concern in
36 the food industries and the most serious concern of food spoilage remains that of foodborne
37 pathogens. To solve these problems, synthetic preservatives and chemical antimicrobial agents are
38 extensively applied in preventing microbial deterioration of food products and increasing their shelf
39 lives. However, given the growing public concern associated with the use of chemical preservatives,
40 there is an urgent need to be substituted by natural antimicrobial agents. Several natural
41 antimicrobial agents, such as essential oils (EOs), lysozyme, bacteriocins, chitosan and phenolic
42 compounds were applied for food preservation in different ways (Saeed et al., 2019).

43 *Trans*-Cinnamaldehyde (3-phenyl-2-propenal, TCA) is the major constituent of the
44 essential volatile oil extracted from cinnamon, a traditional flavoring agent. TCA is an aromatic
45 aldehyde regarded as a “Generally Regarded As Safe” (GRAS) molecule (Adams et al., 2004). In
46 addition to food applications as a flavoring agent in chewing gums, ice creams, candies, and
47 beverages, it has been used to provide a cinnamon flavor to medical products, cosmetics, and
48 perfumes (Shreaz et al., 2016). Over the years, numerous studies have indicated that TCA
49 possessed brilliant antibacterial activity with broad-spectrum against bacteria, yeasts, and molds,
50 such as *Salmonella spp.*, *Campylobacter jejuni*, *Cronobacter sakazakii*, *Listeria innocua* (Doyle
51 & Stephens, 2019a), *Laetiporus sulphureus*, *Clostridium difficile* (Mooyottu et al., 2014) and
52 *Saccharomyces cerevisiae* (Mochida et al., 1988). Thus, TCA may act as a good alternative to
53 synthetic chemical preservatives due to its potential antibacterial activity and safety. Nonetheless,
54 TCA, in its free form, has a low water-solubility and it is difficult to introduce this EO into aqueous-
55 based foods. Moreover, TCA is a volatile compound easily denatured at room temperature, which
56 limits its use in food processing (Jo et al., 2015).

57 Recently, encapsulation techniques, such as emulsion-based encapsulation process, are
58 generally used as delivery systems for EOs. Particularly, oil-in-water emulsion systems are
59 particularly suitable for encapsulating these lipophilic bioactive components and the average size
60 can play an important role in the efficiency of these systems. Indeed, nanoemulsions may have
61 better physical and chemical stability compared to conventional emulsions for encapsulating
62 bioactive components because of their nanometric size (20-500 nm) and high surface area
63 (Polychniatou & Tzia, 2018). Thus, nanoemulsion-based systems can provide many advantages,
64 such as protecting hydrophobic bioactive agents from chemical degradation, limiting their volatility,
65 increasing their biological activity, and minimizing the impact on the organoleptic characteristics
66 of food products (Donsi et al., 2011). In addition, nanoemulsions are more kinetically stable than
67 conventional emulsions but they are more susceptible to Ostwald ripening due to their small droplet
68 size (McClements & Rao, 2011). As a result, they tend to undergo physical changes during storage,
69 which may lead to undesirable alterations in appearance, texture, and shelf life of food products.
70 Chen et al. (2018) showed that nanoemulsions containing pure cinnamaldehyde encapsulated by
71 whey proteins is not physically stable due to a significant change in their size. This phenomenon
72 might be attributed to the high water solubility of cinnamaldehyde, which causes droplet growth
73 through Ostwald ripening (Riquelme et al., 2019). Accordingly, it is necessary to properly design
74 nanoemulsion-based delivery systems to ensure that they have good physical stability in different
75 conditions. Walker et al. (2017) indicated that carrier oil can be incorporated into the oily phase
76 of nanoemulsions to improve its physical and oxidative stability. In another study reported by
77 Donsi et al. (2012), the authors added sunflower oil to the lipid phase to overcome Ostwald ripening
78 of carvacrol, d-limonene and *trans*-cinnamaldehyde nanoemulsions prepared by high pressure
79 homogenization. In the same context, McClements (2012) developed beverage emulsions by

80 addition of poorly water soluble oils to inhibit Ostwald Ripening. [Walker et al. confirmed \(2017\)](#)
81 that the key to enhance the stability of nanoemulsions depends on the physical properties of the
82 carrier oil, such as viscosity, density, and water-solubility. For instance, it has been shown that : (i)
83 combining poor water-solubility carrier oils with hydrophobic bioactive phase, has been shown to
84 enhance physical stability of nanoemulsions by inhibiting Ostwald ripening (Julian McClements et
85 al., 2012); (ii) combining low-viscosity carrier oils with hydrophobic bioactive phase can be used
86 to reduce the size of nanoemulsion droplets during high-pressure homogenization ([Qian &](#)
87 [McClements, 2011](#)); and (iii) combining carrier oils containing natural antioxidant or antibacterial
88 agents with hydrophobic bioactive phase may endow different bioactive properties ([Li et al., 2015](#);
89 [Meroni & Raikos, 2018](#)).

90 In order to study, the effect of the chain length of oil carrier, two different triacylglycerols
91 were used in this work to improve the properties of TCA oil-in-water nanoemulsions: a medium
92 chain triacylglycerol (MCT) and a long chain triacylglycerol (LCT). The used MCT is typically rich
93 in saturated medium chain fatty acids (C_{8:0} and C_{10:0}) and the LCT is derived from the linoleic acid
94 and oleic acid (C_{18:0}). Both carrier oils show low water solubility compared with TCA, and they
95 can be used to prepare nanoemulsions to inhibit Ostwald ripening. In addition, the consequences
96 of these structural differences on the stability of emulsions, and consequently on their functional
97 properties, deserve to be evaluated. Sodium caseinate was selected as emulsifier in this study
98 because it is one of the most widely used proteins and has considerable emulsification, gelling and
99 nutritional properties (Choi et al., 2009). The effects of parameters such as pH, ionic strength, and
100 temperature on the stability as well as on the antibacterial activity of the nanoemulsions were also
101 compared. The information generated by this study could be valuable for improving the functional
102 performance and physical stability of food emulsion systems containing essential oil.

103 **2. Materials and methods**

104 **2.1. Materials**

105 Sodium caseinate powder was purchased from Fisher Scientific (United Kingdom). Protein
106 content in sodium caseinate determined by the Kjeldahl method was 93.20% (nitrogen conversion
107 factor $N = 6.38$). A medium chain triacylglycerol oil (MCT) was purchased from Gattefossé (Saint-
108 Priest, France). A long chain triacylglycerol oil (sunflower oil, LCT) was purchased from a local
109 Market. Trans-cinnamaldehyde (CA, 99%) was purchased from Sigma-Aldrich (St Quentin
110 Fallavier, France). The summary of some physicochemical properties of three different oils are
111 shown in Table 1. Analytical grade sodium chloride, imidazole, acetic acid, sodium hydroxide, and
112 hydrochloric acid, were purchased from Sigma-Aldrich Chimie (St Quentin Fallavier, France). All
113 the solutions were prepared in deionized water.

114 **2.2. Preparation of nanoemulsions**

115 A pre-emulsion was formulated by dispersing the lipid phase (10 g/100 g) in the continuous
116 phase, which consisted of (1 g/100 g) sodium caseinate and (89 g/100 g) buffer solution (5 mmol/L
117 acetic acid-imidazole, 50 mg/100 g sodium benzoate, pH 7). Firstly, the resulting suspensions were
118 stirred at room temperature until the protein was completely dissolved. The oily phase (10 g/100
119 g) and the aqueous phase (90 g/100 g) were mixed using high-speed homogenization for 5 min
120 under 13 500 rpm to form coarse emulsions. Finally, nanoemulsions were obtained by passing
121 coarse emulsions through a microfluidizer (Microfluidics LM20, Microfluidics Corp., Newton,
122 MA, USA) 5 times at 5×10^7 Pa. The pH of the nanoemulsion was then immediately adjusted to
123 7.0. Each emulsion was produced in duplicate. All samples were stored in 100 mL sealed glass
124 bottles at room temperature in the dark for physical stability evaluation.

125 **2.3. Effect of carrier oil concentration**

126 The overall oil content of the nanoemulsions (TCA and carrier oil) was kept constant at 10
127 g/100 g. TCA was mixed with MCT or LCT in different ratios (100/0, 90/10, 70/30, 50/50, 30/70
128 and 0/100, m/m) under stirring (15 min, 750 rpm). In order to study colloidal stability under storage,
129 the prepared nanoemulsions were stored for 28 days and characterized in terms of hydrodynamic
130 size and zeta potential as a function of time ranging from 1 day to one month.

131 **2.4. Droplet hydrodynamic size and size distribution**

132 Droplet size and size distribution were determined by dynamic light scattering using a
133 Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK) at fixed angle of 90°. To perform
134 measurement, nanoemulsions were diluted to an oil droplet concentration around 0.1 wt.% using
135 imidazole acetate buffer to eliminate multiple scattering effects. After 90 s of equilibrium, each
136 sample was analyzed three times. Then the average droplet size and polydispersity index (Pdl) were
137 obtained.

138 **2.5. Zeta potential (ζ)**

139 Nanoemulsions were diluted to a concentration around 0.05wt% with imidazole acetate
140 buffer to avoid multiple scattering effects. Diluted samples were transferred to the cell of
141 disposable cuvettes for electrophoretic mobility measurement (Malvern Instruments,
142 Worcestershire, UK). All measurements were performed three times and the ζ -potential values
143 were reported as the average of three individual injections (\pm SD (standard deviation)).

144 **2.6. Nanoemulsion stability to environmental stress**

145 According to preliminary results, nanoemulsions containing mixed oily phase in the ratio
146 TCA/MCT or LCT of 100/0 and 70/30 were investigated in the remainder of the study. The

147 resultant nanoemulsions were referred to as T100, T70M30 and T70L30 according to their oil
148 compositions. To evaluate the effect of environmental stress on the properties of nanoemulsions,
149 different pHs, salt concentrations, and temperatures were used to mimic scene which might be
150 experienced during manufacture, cooking or utilization. For each dispersion, droplet size
151 distribution and ζ -potential of samples were measured.

152 *pH stability*: Nanoemulsion samples were adjusted to pH values from 9.0 to 3.0 by adding 1.0
153 mol/L HCl or 1.0 mol/L NaOH and then stirred for 20 min to make sure the value of pH stable.

154 *Salt stability*: Nanoemulsion samples (pH 7.0) were mixed with different ratios of NaCl solution
155 and distilled water while continuously stirring. The final NaCl concentration in the studied
156 nanoemulsions were 0, 50, 100, and 300 mmol/L. Oil content and emulsifier contents after dilution
157 were 5 g/100 g and 0.5 g/100 g, respectively.

158 *Temperature stability*: Selected nanoemulsion samples (pH 7.0) were heated in a water bath at
159 different temperatures 60 °C and 80 °C for 1 h, and then cooled down to room temperature prior to
160 analysis. Nanoemulsions stored in refrigerator (4 °C) and at room temperature (25 °C) were used
161 as controls.

162 **2.7. Viscosity**

163 Viscosity of nanoemulsions was measured by Viscometers (LP-22, Brookfield, Canada) at
164 150 s^{-1} at ambient temperature. Sample volume (0.5 mL) was kept constant and each measurement
165 was repeated three times.

166 **2.8. Interfacial tension**

167 The oil-water interfacial tension was measured using an optical tensiometer equipped with
168 an oscillating drop accessory (Tracker Teclis/IT Concept, France) at room temperature. In brief,

169 the aqueous phase was placed in a glass cuvette, and the oil phase was injected into the aqueous
170 phase using a syringe. The shape of the oil drop formed was recorded using a high-speed digital
171 camera and the interfacial tension (mN/m) was calculated using the Tracker Software (Tracker
172 Teclis/IT Concept, France). According to the density of the oil phase, the rising drop method or
173 pendant drop method were used. Five different oil compositions (T100, T70M30, T70L30, M100
174 and L100) were studied and the initial volume of the oil drop was ~15 μ L. The aqueous phase
175 contained 0.005 wt% caseinate in imidazole-acetate buffer (pH 7.0). The test was carried out at
176 room temperature for 7200 s and each experiment was run in triplicate.

177 **2.9. Antibacterial activity of nanoemulsions**

178 The “zone of inhibition” assay on solid media was used for the determination of the
179 antimicrobial effects of nanoemulsions against *Listeria innocua* (ATCC 33090) and *Escherichia*
180 *coli* (ATCC 10536). Strains were stored at -20 °C in Tryptone Soy Broth (TSB) (Biokar diagnostics,
181 Beauvais, France) with glycerol (15 mL/100 mL). One milliliter of the stock culture was transferred
182 to 9 mL of TSB and incubated for 8 h at 37 °C. Then one milliliter of this pre-culture was transferred
183 in 9 mL of TSB and incubated for 16 h at 37 °C. After that, pre-cultured bacteria were diluted in
184 TSB to a final concentration of 10⁶ CFU/mL. One mL of bacterial suspension was incorporated at
185 5 mL/100 mL in melted Tryptone Soy Agar (TSA) and cooled in Petri dishes. A sterilized glass
186 pipette (6 mm in diameter, Fisher Scientific) was used to make a well in the TSA Petri dishes
187 inoculated. To maintain the same TCA concentration, 50 μ L of T70M30 and T70L30 and 35 μ L
188 of T100 were placed in different wells. M100, L100, pure MCT and pure LCT were used as controls.
189 The Petri dishes were then incubated for 2 h at 4 °C for making nanoemulsion diffusion and then
190 incubated at 37 °C. Inhibition zone diameter was measured after 24 h and each experiment was
191 repeated three times.

192 **2.10. Statistical analysis**

193 All assays were performed at least in triplicate. The data were presented as means \pm
194 standard deviation of different samples. Statistical analyses were performed using one-way analysis
195 of variance and multiple comparisons of means were done by Fishers Least Significant Difference
196 (LSD) test. Statistical significance was defined as $p < 0.05$.

197 3. Results and discussion

198 3.1. Effect of carrier oil content on the properties of TCA nanoemulsions

199 The mean droplet size and polydispersity index of nanoemulsions containing different
200 ratios of TCA/carrier oil (100/0, 90/10, 70/30, 50/50, 30/70 and 0/100) stored during 4 weeks were
201 measured and the obtained results are reported in [Fig. 1](#). Initially, all nanoemulsions had relatively
202 small average hydrodynamic particle diameters ranging between 105.7 ~ 211.3 nm, and the lowest
203 droplet size was obtained at the ratio TCA/LCT of 90/10. Except for nanoemulsions containing
204 pure TCA (100/0), the other nanoemulsions have lower PDI value (<0.15) after one day storage.
205 Low PDI value indicated that the droplet are narrowly size distributed. According to Stokes law,
206 the smaller the droplet size was, the lower the sedimentation or creaming rate was
207 ([Paraskevopoulou et al., 2005](#)). For nanoemulsions containing pure TCA, a transparent layer was
208 detected in the top of the sample indicating the movement of the oil droplets to the bottom of the
209 test tube during storage (data not shown). Results showed that, when the content of carrier oil was
210 above 30% in the oil phase, caseinate-coated nanoemulsions containing the mixed oil phase can
211 remain stable for a long time (> 28 days), which suggests that they were stable to Oswald ripening,
212 coalescence, and flocculation. In fact, it is known that low size of droplets is more prone to Ostwald
213 ripening during storage, *i.e.*, diffusion of oil molecules from the smaller to the larger droplets
214 ([McClements et al., 2012, p.;](#) [Wooster et al., 2008](#)). For this reason, it is possible for small droplets
215 to be produced during homogenization process and then grow rapidly during storage. In our case,
216 increased droplet size and relatively higher PDI value were present in the nanoemulsions containing
217 oil phases with 100% and 90% TCA after two weeks. This is in agreement with results obtained
218 for nanoemulsions containing pure thyme oil which was attributed to Ostwald ripening ([Chang et](#)
219 [al., 2012](#)). In addition, it is worth noting that, by comparing the PDI values of nanoemulsions

220 containing mixed oil phase of the 70/30, LCT as a carrier oil showed lower value of PDI than that
221 of MCT, which may be attribute to the difference in properties between the two carriers. Similar
222 results were reported by [McClements \(2012\)](#) who concluded that the properties of the droplets
223 produced during homogenization depends on the viscosity and interfacial tension of the lipid phase.

224 Compared to nanoemulsions containing TCA, the improved stability observed after
225 addition of a carrier oil could be attributed to a decrease in the solubility of the oily phase causing
226 a drop in Ostwald ripening. In fact, TCA is slightly soluble in water ([Seraj & Seyedarabi, 2019](#)),
227 while MCT and LCT have a relatively low water-solubility. Thus, the carrier oil can act as a
228 ripening inhibitor through an entropy of mixing effect during homogenization ([McClements et al.,
229 2012](#)). Density of oil is another factor that may affect the formation and stability of nanoemulsions.
230 In fact, TCA has a higher density ($1\ 050\ \text{kg.m}^{-3}$) than water ($997\ \text{kg.m}^{-3}$) at $25\ ^\circ\text{C}$, while MCT
231 ($945\ \text{kg.m}^{-3}$) and LCT ($920\ \text{kg.m}^{-3}$) have a relatively lower density than water. The density of the
232 mixed oil phase could be consequently close to that of the water phase which can reduce droplet
233 creaming and sedimentation during long time storage. In addition, strong electrostatic repulsions
234 between caseinate coated oil droplets is another important factor of nanoemulsion stability. On the
235 other hand, the mean particle diameter of nanoemulsion increased with increasing carrier oil
236 content in the organic phase, which could be probably attributed to the decreased diffusion of TCA
237 from the oil droplets, resulting in an increase of particle diameter after homogenization.

238 To further explore the effect of the properties of carrier oil on the stability of nanoemulsions,
239 the viscosity of nanoemulsions containing mixed oil phases in the ratio 100/0 and 70/30 were
240 investigated ([Fig. 2a](#)). According to the results reported in this figure and measured at $22\ ^\circ\text{C}$, pure
241 LCT showed highest viscosity ($30.68\ \text{mPa.s}$), while pure TCA showed the lowest one ($5.08\ \text{mPa.s}$)
242 and the mixtures TCA/MCT and TCA/LCT showed intermediate viscosity values ($7.56\ \text{mPa.s}$ and

243 9.87 mPa.s, respectively). Therefore, the addition of carrier oil significantly increased the viscosity
244 of the oily phase. As stated by Qian et al., (2011), the increased viscosity could improve stability
245 of nanoemulsions, but their droplet size will be slight increased, which was consistent with our
246 previous results (Fig. 1). Fig. 2b showed the interfacial tension reduction kinetics between different
247 oily phases and sodium caseinate aqueous solution. Whatever the oily phase composition, the
248 interfacial tension decreased over time, which can be attributed to the adsorption of caseinate
249 molecules to the oil-water interface. Pure LCT and pure MCT showed that the equilibrium
250 interfacial tensions were relatively high (around 10.9 mN/m and 7.3 mN/m, respectively), while
251 pure TCA showed an equilibrium interfacial tension around 2.7 mN/m. This result can be attributed
252 to the difference in polarity between TCA, LCT and MCT. Interestingly, the equilibrium interfacial
253 tensions of the two mixed oil systems showed almost the same equilibrium interfacial tensions than
254 that of pure TCA, suggesting that the interfacial tension between oil and water was mainly
255 dependent on the presence of TCA. Similar results were found by Chen et al. (2018) as well as
256 Felix et al. (2019). Indeed, Chen et al. (2018) found that, in the presence of TCA, the surface of the
257 oil droplets stabilized by whey protein became crinkly, while the shape of the oil droplets without
258 TCA did not change. This result indicates that TCA has the ability to improve the adsorption of
259 caseinate to the oil/water interface, resulting in smaller size oil droplets after homogenization (Fig.
260 1).

261 As shown in Fig. 3, the ζ -potential of the nanoemulsions containing the two different carrier
262 oils were measured within 4 weeks. At the first day, all nanoemulsions had a relatively high
263 negative surface potential (-50 to -30 mV), which can be attributed to the fact that the aqueous
264 phase pH (7.0) during the preparing process was above the isoelectric point of the caseinate
265 molecules (Wang, Dumas, & Gharsallaoui, 2019). As described by Lieberman and Rieger (2008),

266 when the absolute value of ζ -potential is greater than 25 mV, the electrostatic repulsions are high
267 enough to maintain good colloidal stability of the prepared emulsion. Thus, it could be deduced
268 that the presence of caseinate could provide high repulsive forces between the droplets to have
269 good physical stability whatever the oil composition. However, the surface potential of oil droplets
270 changed significantly after one week, especially for nanoemulsions containing pure TCA or a low
271 amount of carrier oil (T90M10 and T90L10), which could indicate a change in the interface
272 composition and/or structure.

273 Except for the system containing pure TCA (TCA100), the ζ -potential of the nanoemulsions
274 became more negative with increasing carrier oil content, which means that the electrostatic
275 repulsions between oil droplets are more intense when the carrier oil content was increased. Several
276 different physicochemical mechanisms might account for the observed changes in surface charge
277 of nanoemulsions. Firstly, the composition and structure of the adsorbed caseinate layer may
278 depend on the properties of the oil phase (such as size, polarity and viscosity), which could
279 indirectly change their surface potential (li Zhai et al., 2013). Secondly, there is a hypothesis that
280 TCA may have chemically reacted with the adsorbed caseinate due to the presence of an aldehyde
281 group in TCA molecules generally used as a cross-linking agent (Marin et al., 2014), and then
282 changed their structure and electrical characteristics. Thirdly, there may be charged surface-active
283 impurities in TCA, MCT and LCT that may affect the electrical properties of the droplets. Finally,
284 as stated by Zhu (2017), cinnamaldehyde can rapidly be oxidized to cinnamic acid (Fig.1.
285 Supplementary data) during storage. Thus, the presence of cinnamic acid can affect the
286 environmental properties, like pH, and then affect the ζ -potential of caseinate coated droplets.

287 To explore this perspective, five nanoemulsions containing different oil mixtures (T100,
288 T70M30, T70L30, T0M100 and T0L100) were selected and the pH of each sample was measured

289 within 4 weeks (Fig. 4). The pH of nanoemulsions containing pure TCA or mixtures of TCA and
290 carrier oils decreased with storage time, while the pH of nanoemulsions containing pure carrier oils
291 (MCT and LCT) remained unchanged. This result can also explain the fact that the zeta potential
292 of nanoemulsions containing high contents of carrier oil was relatively stable (Fig.3). Interestingly,
293 compared to pure TCA nanoemulsions, the nanoemulsions containing mixed oil phase (T70M30
294 and T70L30) appeared to be able to slow down the pH to the same extent after three weeks. In
295 addition, LCT as carrier oil in nanoemulsions exhibited more ability ($p < 0.05$) to change the pH
296 than MCT as carrier oil. This result can be explained by comparing the changes in PDI values of
297 T70M30 and T70L30 as a function of time (Fig. 1c and Fig. 1d). In fact, a significant increase of
298 PDI value was found when MCT was used as carrier oil after one week, while the PDI of T70L30
299 nanoemulsions remained stable. These effects may therefore depend on the physical properties of
300 carrier oils. Similar tendency was also reported by Walker (2017) who found that the oxidation rate
301 of fish oil was dependent on the carrier oil type.

302 **3.2. Physical stability of nanoemulsions against environmental stress**

303 Caseinate stabilized nanoemulsions are especially sensitive to environmental conditions
304 which could affect their physical stability. The main three influencing parameters are pH, ionic
305 strength, and temperature whose effects have been studied in the following sections.

306 **pH stability:** Three different pHs (3.0, 7.0 and 11.0) were used to study the effect of pH on the
307 stability of nanoemulsions (Fig. 5). As shown in Fig. 5a, no significant difference was found in
308 particle size of the three samples prepared at different pH values. For droplet size distribution,
309 nanoemulsions containing mixed oil phases showed very low PDI values indicating that they were
310 highly concentrated, while nanoemulsions containing pure TCA showed very high PDI values in
311 each pH condition. In fact, as the pH increased from 3.0 to 11.0, the particle size of the three

312 nanoemulsions slightly increased. This may be attributed to the electrostatic repulsion forces
313 among amino and carboxyl groups of caseinate (Lovett et al., 2015). On the other hand, aqueous
314 pH is related to the solubility of caseinate which will affect the particle size distribution of
315 nanoemulsions after homogenization. As stated by Mezdour et al. (2008), the increase of the
316 caseinate solubility allows to form thicker adsorption oil-in-water layer. The ζ -potential of the
317 droplets in these emulsions was also evaluated (Fig. 5b). For all samples, the ζ -potential showed
318 the similar trend and changed from positive to negative as the pH changed from 3 to 9, which can
319 be attributed to the fact that the isoelectric point (I_p) of caseinate is around 4.5. The results are
320 consistent with our previous studies that have shown that caseinate-coated oil droplets are highly
321 unstable and aggregate in the vicinity of this pH (Wang et al., 2019). This result also showed that
322 ζ -potential of emulsions mainly depends on the caseinate whatever the composition of the oily
323 phase.

324 ***Ionic force:*** Nanoemulsions can be used as delivery systems in some food matrices containing
325 different salt concentrations, and therefore it is important to verify the effect of ionic strength on
326 droplet stability. Thus, the effect of different NaCl concentrations on the mean particle diameter
327 and ζ -potential of nanoemulsions was examined (Fig. 6). All nanoemulsions showed an increase
328 of the droplet size and PdI when NaCl concentration was increased for 0 to 300 mmol/L, especially
329 for nanoemulsions containing pure TCA ($p < 0.05$, significantly). For nanoemulsions containing
330 mixed oily phases, addition of low content of salt (< 200 mmol/L) to the nanoemulsions caused
331 little changes in the mean particle diameter after homogenization, but addition of higher salt content
332 promoted a certain degree of droplet aggregation ($p < 0.05$). This droplet aggregation observed at higher
333 salt concentrations is due to a reduction in the electrostatic repulsion between the protein-coated droplets
334 caused by electrostatic screening and ion binding effects

335
336 Similar phenomenon was found upon addition of NaCl to β -lactoglobulin-stabilized emulsions
337 (Qian et al., 2011). Moreover, our results showed that, in the presence of NaCl, the droplet size of
338 nanoemulsions containing pure TCA turned to bimodal distribution (two peaks in one curve, data
339 not shown), while the particle size distributions of nanoemulsions containing mixtures of TCA and
340 carrier oil remained monomodal, though their peak shapes and locations were changed slightly.
341 Thus, addition of carrier oil can prevent the emulsions to form bimodal distributions, which was
342 consistent with the results obtained by Zhao et al. (2015). This phenomenon can be attributed to
343 the fact that an increase in particle size leads to an increase in the rate of creaming of droplets in
344 nanoemulsions (Paraskevopoulou et al., 2005). Fig. 6b showed that the ζ -potential of
345 nanoemulsions was significantly affected by ionic strength. All samples showed a decrease trend
346 when the NaCl concentration was increased suggesting an accumulation of counter-ions around oil
347 droplets surfaces screening caseinate charged groups preventing repulsions between oil droplets
348 (Öztürk, 2017).

349 **Temperature stability:** In many commercial applications, it seems important that emulsions can
350 withstand high temperatures during their production, storage or use. For this reason, the effect of
351 heating treatments (60 °C and 80 °C) on the stability of nanoemulsions was examined, and 4 °C as
352 well as 25 °C were used as control (Fig. 7). With the exception of pure TCA-containing
353 nanoemulsions treated at 80 °C, all other nanoemulsions appeared to be relatively stable to heat
354 treatment and showed a unimodal particle size distribution (Fig. 7a). This could be attributed to the
355 good thermal stability of caseinate-coated droplets owing to their relatively high negative charge
356 at pH 7, which led to stronger electrostatic repulsions between them. Meanwhile, caseinate
357 stabilized emulsions generally have better stability during heating, because of the protein structure

358 which does not affected by the heat-induced conformation changes as for globular proteins
359 (Srinivasan et al., 2002). For nanoemulsions containing pure TCA, a slight increase in size was
360 observed at 60 °C, but when the heating temperature was increased to 80 °C, the size increased
361 significantly ($p < 0.05$). After heating, the ζ -potential of nanoemulsions were also measured.
362 According to Fig. 7b, there was no significant change in the ζ -potential for all nanoemulsions upon
363 thermal treatments, suggesting that heating did not strongly influence the overall composition or
364 structure of the caseinate coatings. The extreme condition may affect the physical stability of the
365 nanoemulsion, however, MCT oil and LCT oil as carrier oils into the oil phase showed better
366 tolerance than pure TCA.

367 **3.3. Antibacterial activity of nanoemulsions**

368 Nanoemulsions were studied for their antibacterial activity against a Gram-positive
369 (*Listeria innocua*) and a Gram-negative (*Escherichia coli*) bacteria. Based on the same TCA
370 content, three nanoemulsions (T100, T70M30 and T70L30) were added to each well of the
371 bacterial-containing medium, and the same formulation without TCA as a control. Inhibition zones
372 of nanoemulsions on medium containing bacteria after 24 h is shown in Fig. 8a. The three
373 nanoemulsions showed clear inhibition zones against the two bacteria, whereas the control without
374 TCA showed no inhibition zones. The size of the inhibition zones was measured by the software
375 Image J (National Institutes of Health, USA) and the obtained values are shown in Fig. 8b. The
376 antimicrobial activity of TCA is attributed to its aldehyde functional group and the antibacterial
377 mechanism of TCA is probably due to the increase in permeability of bacteria cell wall (Doyle &
378 Stephens, 2019b). As stated by Weiss et al. (2009), encapsulation of TCA at the nanoscale could
379 increase the physical stability of bioactive compounds and increases their bioactivity through
380 activation of cell absorption mechanism. In addition, there was no significant disparity between

381 antimicrobial activity of nanoemulsions containing MCT and LCT as carrier oils, but these two
382 nanoemulsions exhibited larger inhibition zones than nanoemulsions containing pure TCA
383 ($p < 0.05$). This phenomenon can be attributed to the fact that the addition of a carrier oil increases
384 emulsion stability during storage, which can further increase its biological activity. Another reason
385 may be considered to explain this result, is that nanoemulsions containing pure TCA may lose a
386 small amount of TCA, because TCA is volatile and subject to oxidation. Meanwhile, the size of
387 inhibition zones can be affected by several parameters, such as the amount of nanoemulsion, the
388 spread area of nanoemulsions, the mechanism of TCA release, the number of bacteria in the
389 medium, and the time of measuring inhibition zone. As far as possible, we have tried in this work
390 to keep all these parameters constant and to vary only the composition of the emulsion in order to
391 detect the effect of the carrier oil.

392 **4. Conclusions**

393 This study compared the ability of two different carrier oils to improve the properties of
394 emulsions containing trans-cinnamaldehyde and stabilized by sodium caseinate. The influence of
395 the oil type and content on the physical stability of nanoemulsions was investigated under different
396 pHs, salt concentrations and temperature as well as their antibacterial activity against two common
397 gram-positive and gram-negative bacteria. The analyses revealed that stable caseinate-coated
398 nanoemulsions are difficult to formulate using pure TCA. However, nanoemulsions containing
399 TCA mixed with carrier oil could keep stable for a relatively long period, especially when LCT is
400 used as carrier oil. This stability improvement can be attributed to a reduction of TCA diffusion in
401 the aqueous phase and an inhibition of Ostwald ripening. Antibacterial effect revealed that,
402 compared to nanoemulsions containing pure TCA, the activity of nanoemulsions containing
403 mixtures of TCA and carrier oils exhibited better antibacterial activity against *Escherichia coli* and
404 *Listeria innocua*. The development of these nanoemulsions is in line with the growing interest of
405 the food industry and consumers in using products based on natural substances and the
406 incorporation of carrier oils into nanoemulsion-based systems containing TCA is a promising
407 technique that can extend the shelf life of perishable foods.

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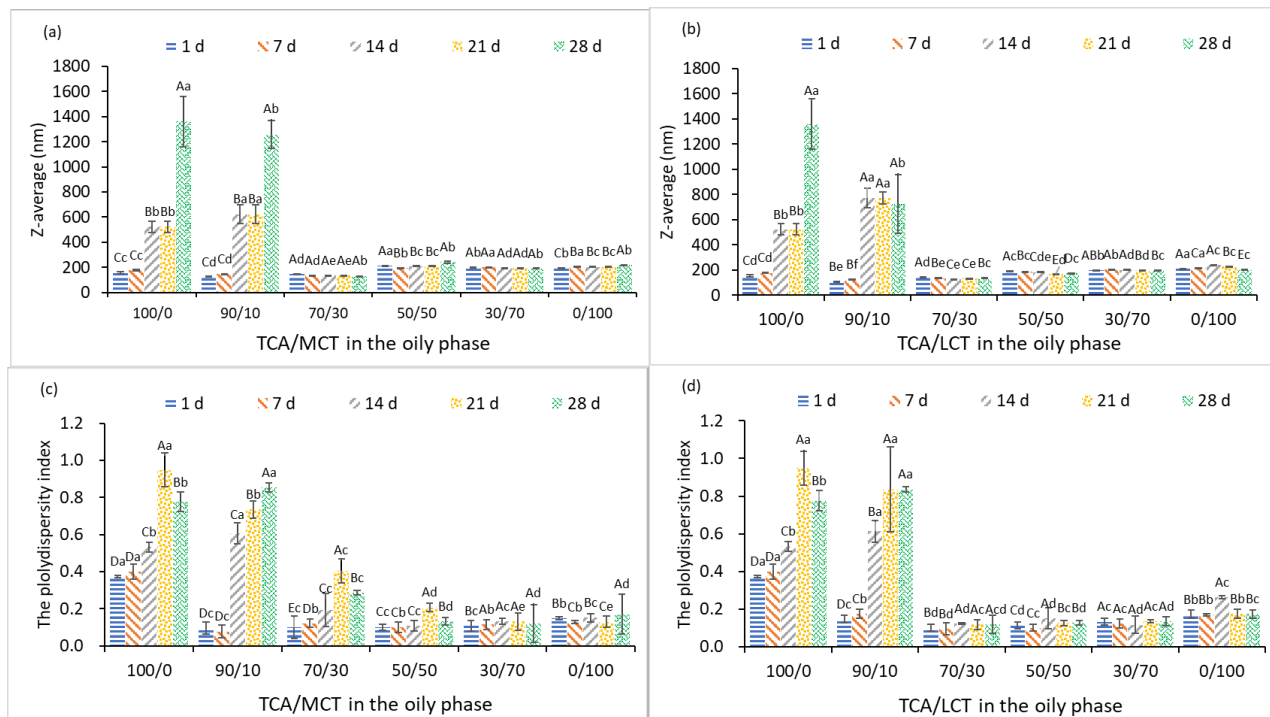
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512 **Table 1.** General physical properties of TCA, MCT and LCT.

Properties	(TCA) <i>trans</i>- Cinnamaldehyde	MCT	LCT (Sunflower oil)
Density (g/cm ³)	1.05	0.945	0.920
Viscosity (mPa.s)	5.08	22.88	30.68
Interfacial tension (mN/m)	2.70	7.30	10.90
Solubility in water (mg/100 mL)	1.42	Insoluble	Insoluble

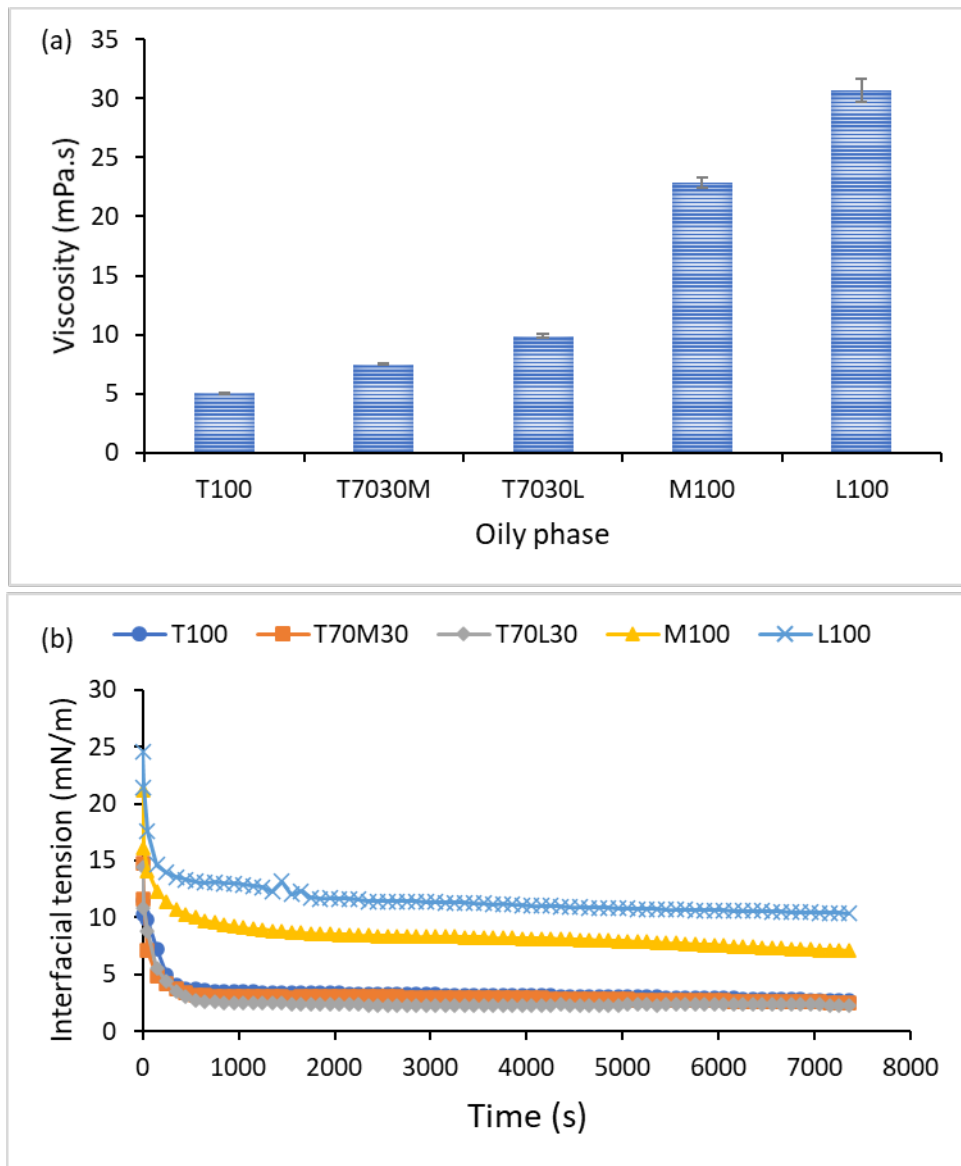
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515
 516 **Fig.1.** Effect of MCT or LCT content on particle size (a and b) and polydispersity index (c and d) of
 517 sodium caseinate stabilized TCA nanoemulsions as a function of storage time. Samples with different
 518 uppercase superscripts (A–E) differ significantly ($p < 0.05$) when different nanoemulsions are compared at
 519 same storage time; Samples with different lowercase superscripts (a–f) differ significantly ($p < 0.05$) when
 520 the same nanoemulsion is compared at different storage times.

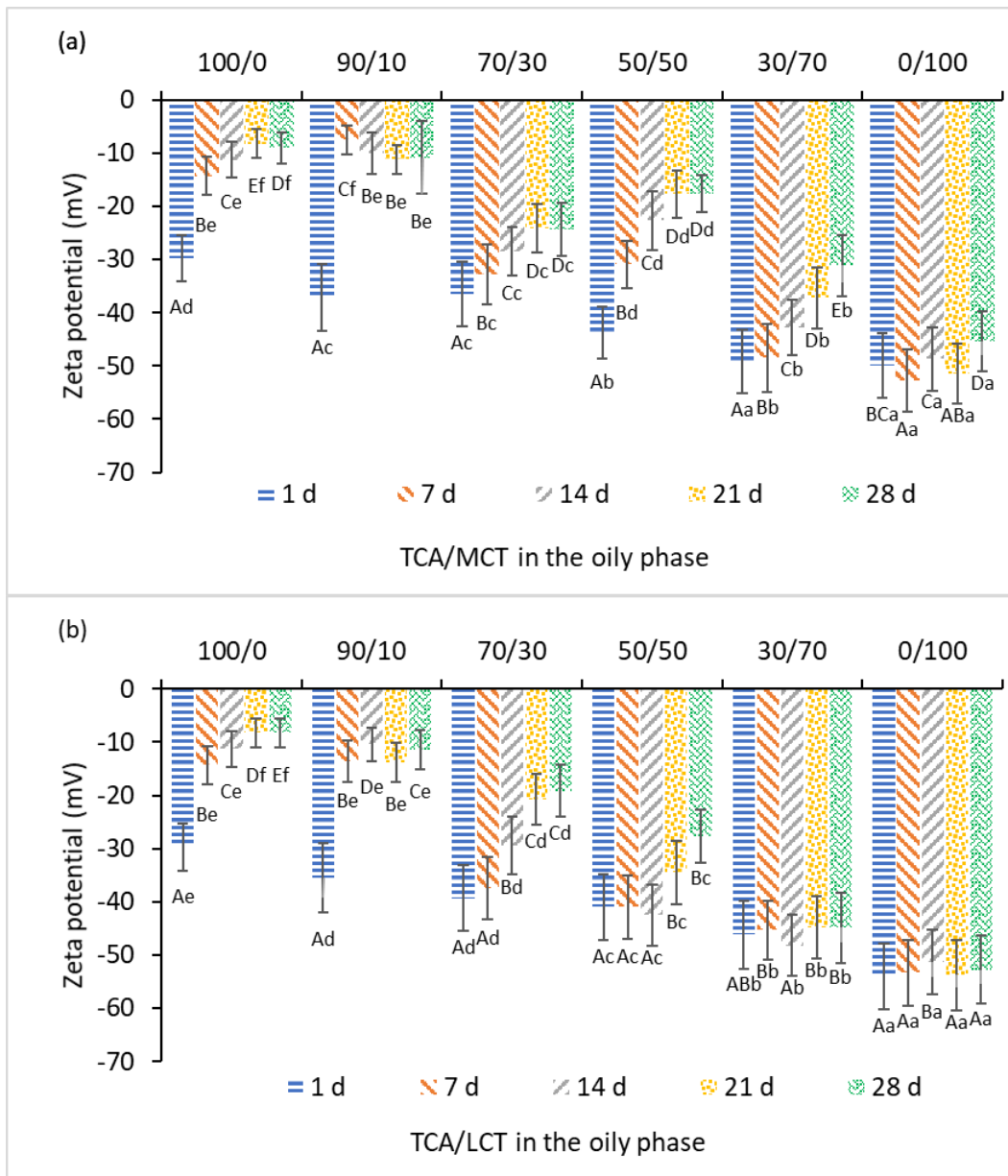
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522

523 **Fig. 2.** Viscosity of five different oil compositions (a) and interfacial tension over time of five different

524 oil compositions in the presence of 0.005wt% sodium caseinate in the aqueous phase (b).



525

526 **Fig.3.** Effect of MCT(a) or LCT (b) content on the ζ -potential of caseinate stabilized TCA nanoemulsions

527 as a function of storage time. Samples with different uppercase superscripts (A–E) differ significantly ($p <$

528 0.05) when different nanoemulsions are compared at same storage time; Samples with different lowercase

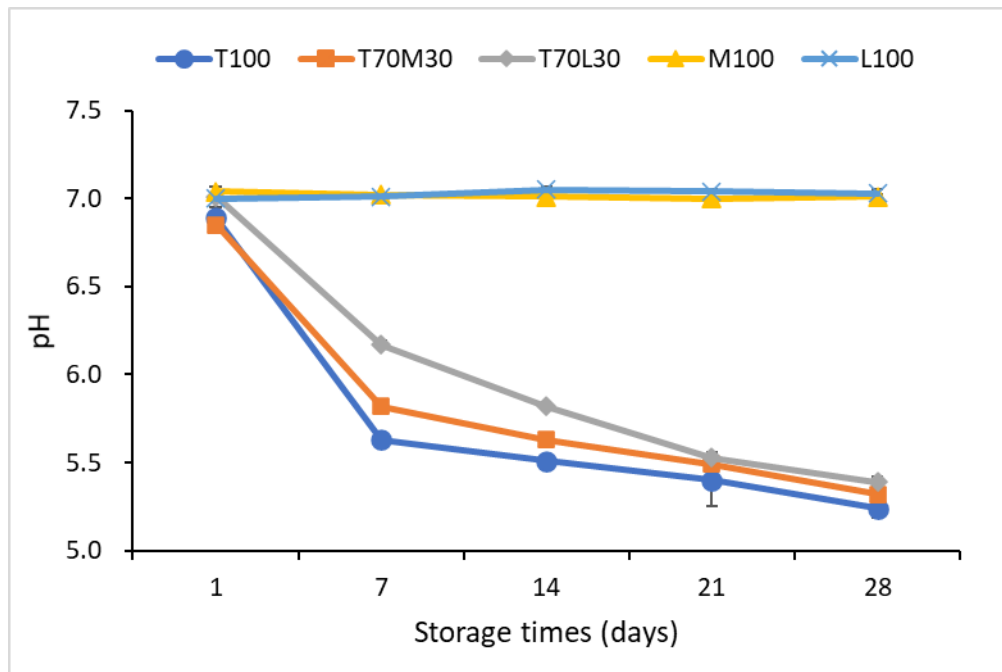
529 superscripts (a–f) differ significantly ($p <$ 0.05) when the same nanoemulsion is compared at different

530

storage times.

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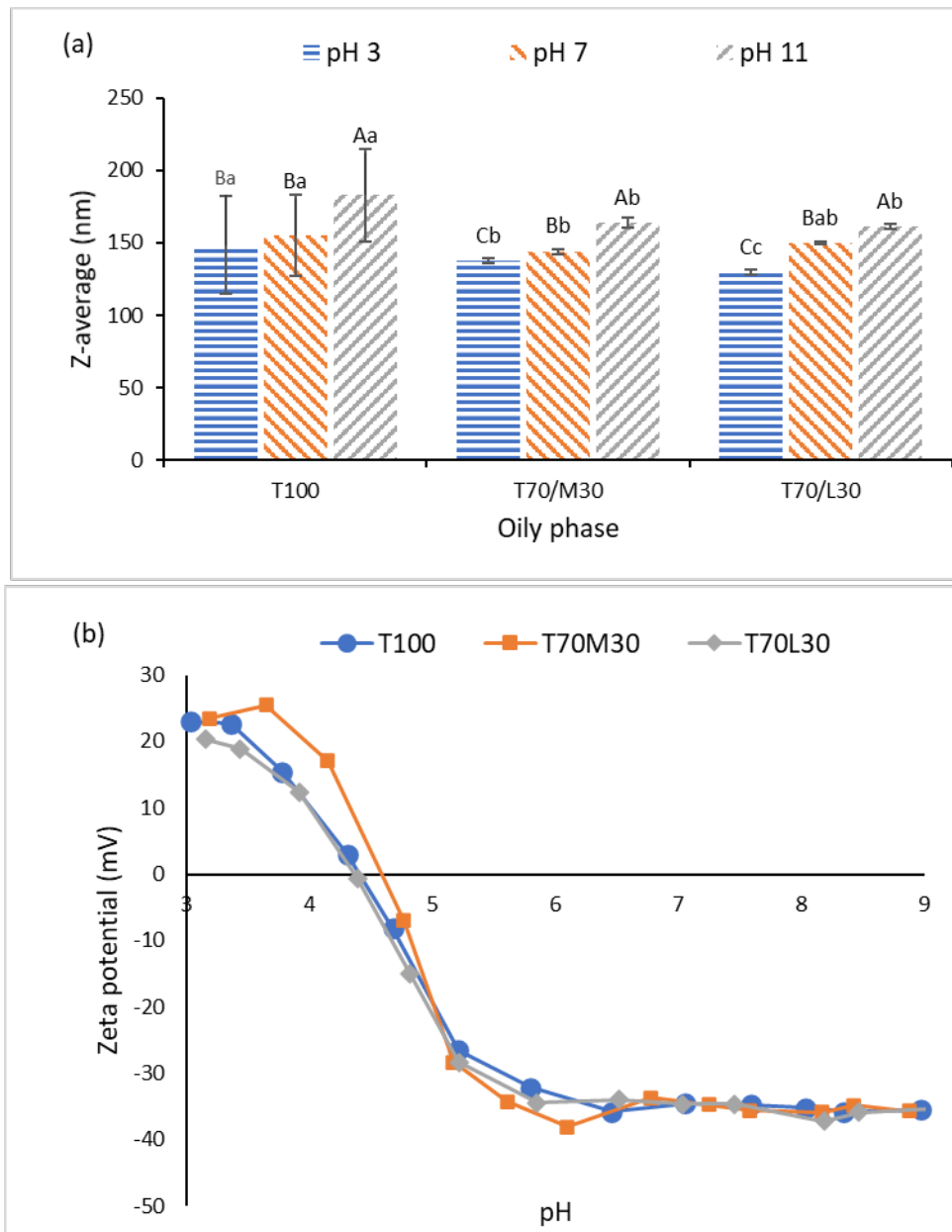
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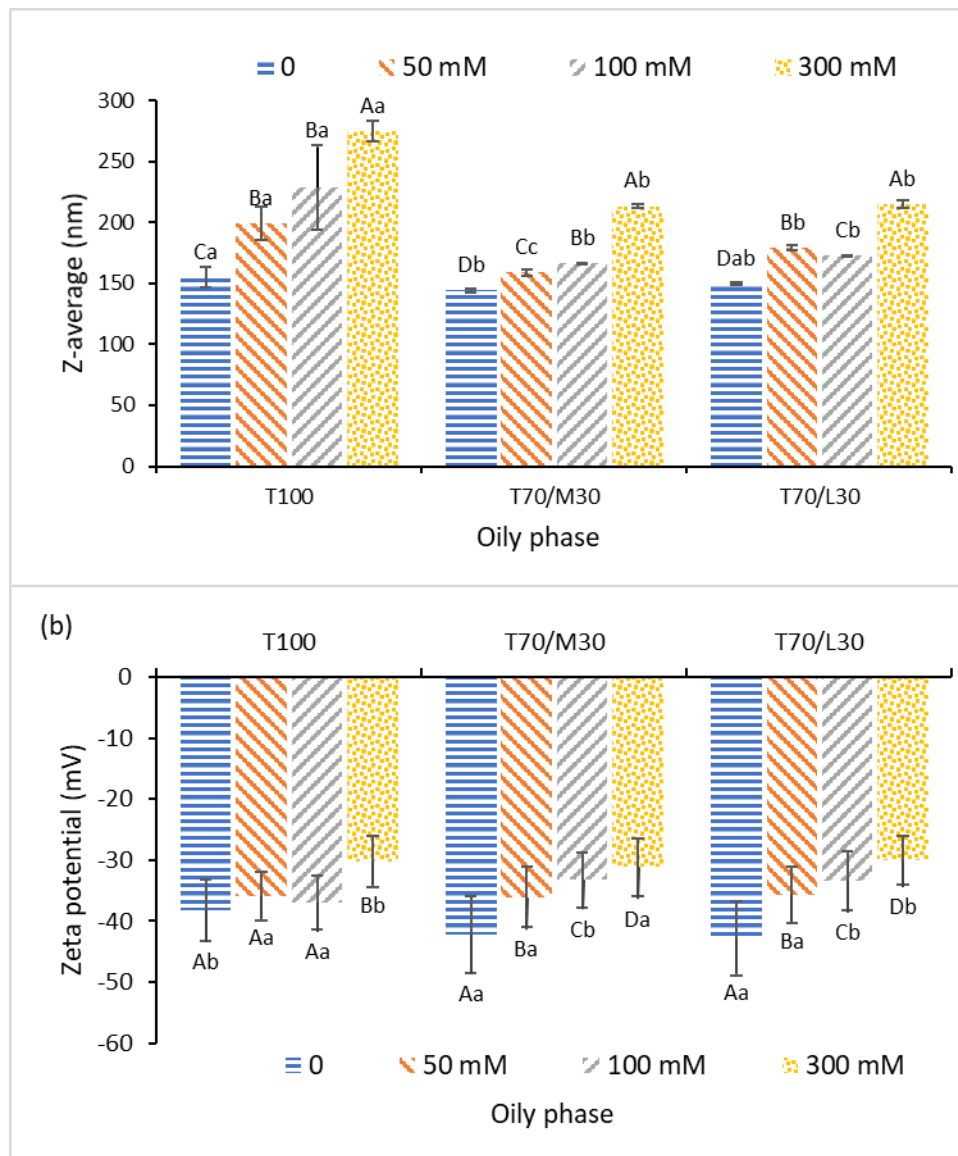
534 **Fig. 4.** pH values of caseinate stabilized nanoemulsions containing different oily phases during storage.

535



536

537 **Fig. 5.** Effect of pH on particle size (a) and ζ -potential (b) of caseinate-stabilized nanoemulsions
 538 containing different oily phases. The italic number above each bar refers to the PDI value. Samples with
 539 different uppercase superscripts (A–C) differ significantly ($p < 0.05$) when the same nanoemulsion is
 540 compared at different pHs; Samples with different lowercase superscripts (a–c) differ significantly ($p <$
 541 0.05) when the different nanoemulsions are compared at same pH



542

543 **Fig. 6.** The effect of salt concentration on particle size (a) and ζ -potential (b) of caseinate-stabilized

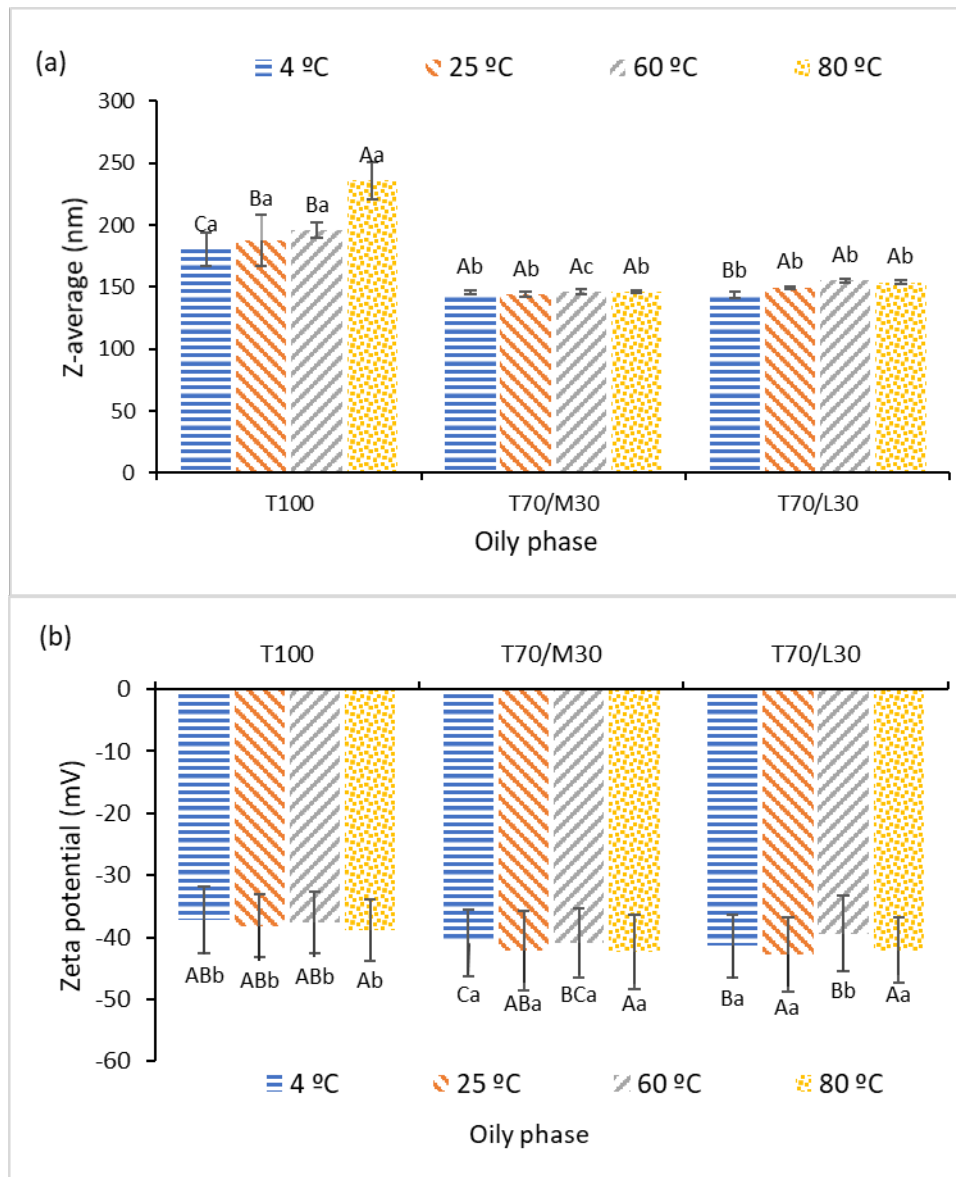
544 nanoemulsions containing different oily phases. The italic number above each bar refers to the PdI value.

545 Samples with different uppercase superscripts (A–D) differ significantly ($p < 0.05$) when the same

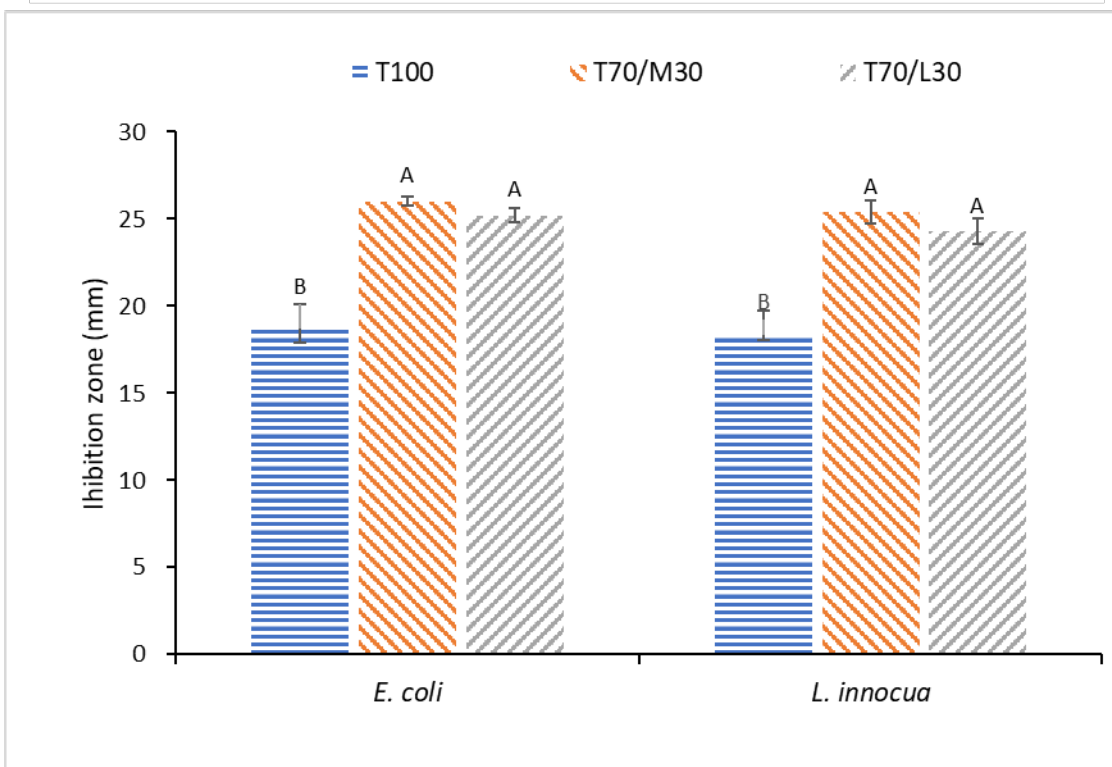
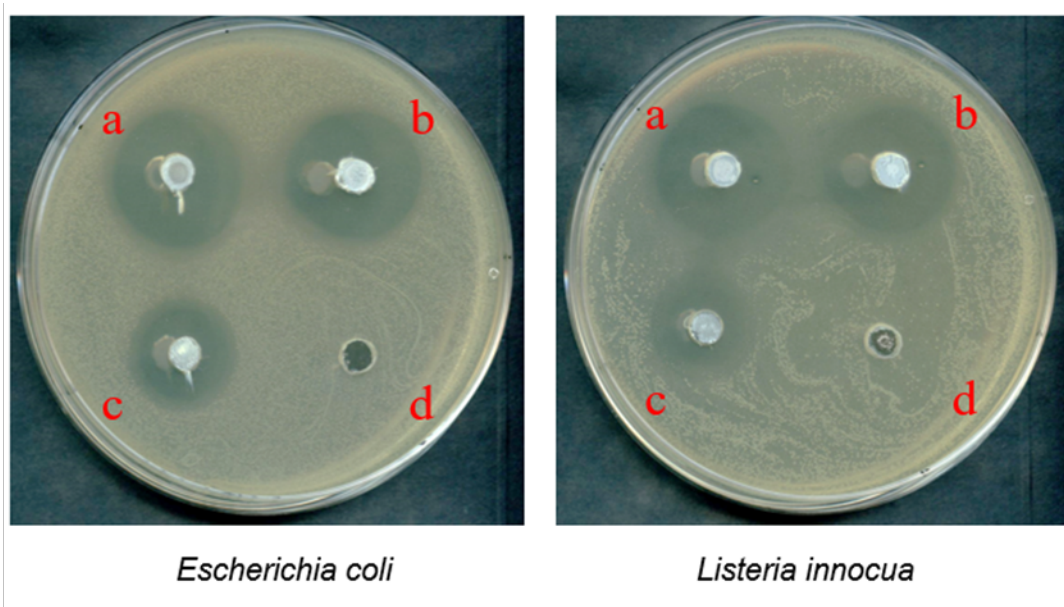
546 nanoemulsion is compared at different salt concentrations; Samples with different lowercase superscripts

547 (a–c) differ significantly ($p < 0.05$) when the different nanoemulsions are compared at same salt

548 concentration.



549
 550 **Fig. 7.** The effect of temperature on particle size (a) and ζ -potential (b) of caseinate-stabilized
 551 nanoemulsions containing different oily phases. The italic number above each bar refers to the PDI value.
 552 Samples with different uppercase superscripts (A–D) differ significantly ($p < 0.05$) when the same
 553 nanoemulsion is compared at different temperatures; Samples with different lowercase superscripts (a–c)
 554 differ significantly ($p < 0.05$) when the different nanoemulsions are compared at same temperature.



555
 556 **Fig. 8.** Top : Example of inhibition zones on TSA containing *Escherichia coli* and *Listeria innocua* with four
 557 nanoemulsions: T100 (a), T70M30 (b), T70L30 (c) and M100 (d). Bottom : Measured diameter of
 558 inhibition zone of three nanoemulsions against *Escherichia coli* and *Listeria innocua*: T100, T70M30 and
 559 T70L30. Samples with different uppercase superscripts (A–C) differ significantly ($p < 0.05$).

560