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1 **Do oil-in-water (o/w) nano-emulsions have an effect on survival and growth of**  
2 **bacteria?**

3

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12  
13 **Abstract**

14 Nano-emulsions (typically droplet diameter <1µm) are common in foods, and have  
15 been extensively reported to present antimicrobial activity, however, the mechanism  
16 is not well defined, and some studies reported no effect. A review of the literature  
17 was conducted and revealed strongly contradictory reports regarding the  
18 antimicrobial effect of nano-emulsions even in reference to similar microbial species  
19 and formulations. Following up, this study aimed to investigate the effect of nano-  
20 emulsions on four bacterial species (*Staphylococcus epidermidis*, *Bacillus cereus*,  
21 *Lactobacillus acidophilus* and five *Escherichia coli* strains) possessing different  
22 surface charge and hydrophobicity. Model oil-in-water (O/W) emulsions with different  
23 size of oil droplets were prepared with sunflower oil stabilised by polysorbate 80  
24 (Tween80) emulsifier (hydrophilic), using high shear mixing followed by

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25 ultrasonication. The viability of bacteria was monitored by culture, membrane  
26 integrity was assessed with flow cytometric analysis with propidium iodide (PI)  
27 staining and fluorescence microscopy monitored the spatial distribution of cells within  
28 the O/W emulsions. The stability of the nano-O/W emulsions in the presence of  
29 bacteria was assessed by monitoring the droplet size [D (4, 3)] and creaming height.  
30 In contrast to other reports the survival and growth of bacteria was not affected by  
31 the size of the oil droplets, no damage to the bacterial membrane was evident with  
32 flow cytometry and emulsion stability was not affected by the presence of bacteria  
33 during 7 days of storage. Furthermore, the antimicrobial activity of caprylic acid (CA)  
34 was compared between O/W coarse and nano-emulsions while varying the  
35 concentration of the hydrophilic surfactant Tween80. The activity of CA was similar in  
36 nano-emulsion and coarse emulsion; however, it was higher than in bulk oil and was  
37 reduced with increasing Tween80 concentration, suggesting that its efficacy is  
38 dictated by formulation rather than oil droplet size. The results demonstrated no  
39 enhanced antimicrobial activity due to nano-sized oil droplets and that conclusions  
40 on nano-emulsions should be taken with caution.

41 **Keywords:** Nano-emulsion; Antimicrobial Activity; Flow Cytometry; Bacterial  
42 Membrane Integrity; Caprylic Acid; Emulsion Stability

Graphical Abstract

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## 53 1. Introduction

54 Nano-emulsions (typically with droplet diameter  $<1\mu\text{m}$ ) gained popularity in food  
55 production due to improving food properties and formulations, for example, use of  
56 less fat and emulsifiers, increased emulsion stability and improved optical  
57 appearance, enhancement of taste and sensory perception of ingredients or masking  
58 of certain ingredients (Chaudhry and Castle, 2011). Nano-emulsion manufacturing  
59 requires more energy than emulsions with larger droplet sizes (Gupta *et al.*, 2016)  
60 and they possess different physicochemical properties to coarse emulsions  
61 (McClements, 2010) due to their nano-sized droplets (Baglioni and Chelazzi, 2013)  
62 and increased interface. Nano-emulsions have shown antimicrobial activity against a  
63 variety of Gram-positive and Gram-negative bacteria including *Bacillus cereus*,  
64 *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Pseudomonas*  
65 *aeruginosa*, *Staphylococcus aureus*, *Bacillus megaterium*, *Bacillus subtilis* and  
66 *Bacillus circulans* (Hamouda *et al.*, 1999; Baker *et al.*, 2000; Teixeira *et al.*, 2007;  
67 Bharghava *et al.* 2015; Jo *et al.*, 2015; Majeed *et al.*, 2016; Lu *et al.*, 2017).  
68 Furthermore, nano-emulsions were found to selectively disrupt the membrane of  
69 prokaryotic cells but not eukaryotic cells (Baker *et al.*, 2000), which could expand  
70 their applications in managing safety and microbial growth in food through  
71 formulation. The antimicrobial effect of nano-emulsions has been attributed to their  
72 structure itself and the nano-sized droplets. When nano-emulsions are formed under  
73 high shearing forces (e.g. ultrasonication, high-pressure homogenisation or high-  
74 shear mixing) they acquire significant amount of energy as they are formed (Lee *et*  
75 *al.*, 2010). The nano-droplets are thermodynamically driven to fuse with lipid-  
76 containing micro-organisms and the energy that was stored during formation of the  
77 nano-emulsion will be released to destabilise the membrane's lipid bilayer leading to

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78 cell lysis and death (Hamouda et al., 1999; Hamouda and Baker, 2000; Myc *et al.*,  
79 2001; Hemmila *et al.*, 2010).

80 However, after summarising and reviewing the literature discussing the antimicrobial  
81 activity of nano-emulsions (Table 1), there is evidence of controversy and no  
82 consistency of effect on the same species of bacteria. For example, two studies  
83 found no correlation between droplet size and antimicrobial activity  
84 (Buranasuksombat *et al.*, 2011; Terjung *et al.*, 2012). Buranasuksombat et al. (2011)  
85 found that nano-emulsions (<300 nm droplet size) made from soybean oil and the  
86 non-ionic surfactant Tween80 had no antimicrobial effects on *E. coli*, *S. typhimurium*,  
87 *L. monocytogenes*, *B. cereus* and *P. aeruginosa* after exposure for 30 minutes,  
88 unless the oil phase itself contained antimicrobial properties. Terjung *et al.* (2012)  
89 found that the antimicrobial properties of nano-emulsion (80 nm droplet size) made  
90 from Miglyol 812N and Tween80 were less effective in inhibiting growth of *E. coli* and  
91 *Listeria innocua* compared to coarse emulsion (3 µm). Therefore, more work is  
92 required to confirm with confidence antimicrobial activity of nano-emulsions,  
93 exclusive to structure and droplet size. In other cases (Table 1) the antimicrobial  
94 activity was investigated in nano-emulsions containing antimicrobial components  
95 which were either added in the formulation or were natural components of the oil;  
96 surprisingly, in many studies the controls in place were not appropriate for supporting  
97 the conclusions, and antimicrobial activity was attributed to nano-emulsion structure  
98 instead of the formulation and the antimicrobial component.

99 The aim of this study was to comprehensively assess the effect of a model O/W  
100 nano-emulsion on bacteria, specifically, microbial survival in minimal growth medium  
101 at ambient temperature (M9 medium at 25°C), microbial growth in rich medium  
102 (30°C), and cell membrane integrity by flow cytometric analysis. As the O/W

103 emulsion structure can be affected by the interaction of bacterial cell properties with  
104 the emulsion interface (Ly *et al.*, 2006; Ly *et al.*, 2008), the study included different  
105 Gram-negative and Gram-positive bacterial species and strains of varying surface  
106 charge, hydrophobicity and ability to form the protein adhesin curli. Finally, in order  
107 to investigate the effect of O/W emulsion structure, i.e. size of the oil droplets, in  
108 combination with antimicrobial components in formulation, caprylic acid (CA) was  
109 added in the oil phase. CA is an eight-carbon short-chain fatty acid found naturally in  
110 milk with well documented antimicrobial activity in bulk against various species (Nair  
111 *et al.*, 2005; Annamali *et al.*, 2000; Andrews *et al.*, 2001), however, no study has yet  
112 assessed CA as part of an emulsion formulation. Since CA is minimally soluble in  
113 water and due to its fat solubility, it can be incorporated within the oil phase of O/W  
114 nano-emulsions, and highlights possible increases in antimicrobial activity due to  
115 increase in interface. Changes in the stability of O/W nano-emulsions in the  
116 presence of bacteria were monitored by measuring the droplet size and creaming  
117 height while fluorescence microscopy was employed to screen the localisation and  
118 distribution of bacteria within the emulsions.

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## 121 2. Materials and Methods

### 122 2.1. Materials

123 The water-soluble emulsifier polysorbate 80 (Tween80), hexane 95% and caprylic  
124 acid (CA)  $\geq 98\%$  were purchased from Sigma-Aldrich (United Kingdom). Sunflower oil  
125 (food grade) was purchased from a local retailer (United Kingdom). Nucleic acid  
126 stains 2-(4-amidinophenyl)-1H-indole-6-carboxamide (DAPI) and propidium iodide  
127 (PI) were purchased from Sigma-Aldrich (United Kingdom). Tryptic soy agar (Oxoid  
128 Ltd. CM0131), tryptic soy broth (Oxoid Ltd. CM0129), nutrient agar (Oxoid Ltd.  
129 CM0003), de Man, Rogosa and Sharpe (M.R.S) agar (OXOID CM0359) and broth  
130 (OXOID CM0361) were purchased from Fisher Scientific (United Kingdom).

### 131 2.2. Microbial cultures

132 *Escherichia coli* K-12 strains MG1655 (CGSC 6300), BW25113 (CGSC 7636),  
133 JM109 (NEB E4107), MC4100 (CGSC 6152) and its derivative PHL644  
134 (MC4100 *malA-kan ompR234*) (Vidal *et al.* 1998) were maintained on tryptic soy agar  
135 at 4°C. *Bacillus cereus* (NCTC 11143), and *Staphylococcus epidermidis* (NCIMB  
136 10387) were maintained on nutrient agar at 4°C. *Lactobacillus acidophilus* (ATCC  
137 4356) was maintained on M.R.S agar at 4°C. For obtaining cells in the exponential  
138 phase, cells were harvested by centrifugation (10,000 g, 10 minutes) and washed in  
139 PBS (phosphate buffered saline) solution twice. *E. coli*, *B. cereus*, and *S.*  
140 *epidermidis* cells were each transferred into 50 ml of tryptic soy broth, incubated at  
141 37°C for 24 hours shaking at 150 rpm and sub-cultured to 50 ml of tryptic soy broth  
142 for a further 2 hours (*E. coli*) or 4 hours (*B. cereus* and *S. epidermidis*). *L.*  
143 *acidophilus* cells were transferred into 50 ml of M.R.S broth, incubated at 37°C for 42  
144 hours and sub-cultured to 50 ml of M.R.S broth for a further 12 hours.



### 145 **2.3. Bacterial-adhesion-to-hydrocarbon (BATH) test**

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3 146 The hydrophobicity of bacterial cell surfaces was evaluated as by bacterial-adhesion-  
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6 147 to-hydrocarbon (BATH) according to the method proposed by Rosenberg *et al.*  
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8 148 (1980). The optical density ( $A_o$ ) of bacterial cells ( $\sim 10^9$  CFU/ml) harvested in the  
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10 149 exponential phase by centrifugation (10,000 g, 10 minutes) and washed twice in *PBS*  
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13 150 and re-suspended in M9 medium was measured at 600nm. Four millilitres of the  
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15 151 bacterial suspension were mixed with 1 ml hexane by vortexing for 2 minutes and  
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18 152 then left to stand for 15 min to allow separation of layers, at which time the optical  
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20 153 density at 600 nm ( $A_t$ ) was again measured by carefully removing a sample (1ml)  
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23 154 from the aqueous phase. The percentage of bacterial adhesion to hexane was  
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25 155 expressed by the difference of the absorbance of cell suspension before ( $A_o$ ) and  
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28 156 after ( $A_t$ ) mixing with the solvent:  $(1 - A_t/A_o) \times 100$ . The percentage of bound cells  
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30 157 was subsequently calculated by % adherence =  $(1 - A_t/A_o) \times 100$  where  $A_o$  is the  
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32 158 optical density measured at 600 nm of the bacterial suspension before mixing and  $A_t$   
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35 159 is the absorbance after mixing. The mean percentage of partitioning of an organism  
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37 160 into the hexane phase was calculated by using triplicate samples.

### 41 161 **2.4. $\zeta$ -potential (zeta potential) measurements**

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44 162 For measuring the  $\zeta$ -potential of bacteria, cells were harvested in exponential phase  
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46 163 by centrifugation, washed twice in *PBS*, re-suspended and diluted in M9 medium to a  
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49 164 density of  $10^7$  cells per ml. One millilitre of the samples was injected in a universal  
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52 165 folded capillary cell (Model DTS 1070, Malvern Instruments Ltd, UK) equipped with  
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54 166 platinum electrodes and a folded capillary, checking that all air bubbles were  
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57 167 removed. The electrophoretic mobility (EM) at 150V of the suspended bacteria was  
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59 168 then measured at 25°C using Malvern ZetaSizer Nano ZS (Malvern Instruments Ltd,  
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169 UK), which uses the scattering of incident laser light to detect the bacteria at relative  
170 low magnification. The instrument was calibrated using the  $\zeta$ -potential transfer  
171 standard (DTS1235) which has a  $\zeta$ -potential of  $-42\text{mV}\pm 4.2\text{mV}$ . The mobility of the  
172 bacteria under the applied voltage was converted to the  $\zeta$ -potential using the  
173 Smoluchowski equation and reported as the average and standard deviation of  
174 measurements made on two freshly prepared samples, with three readings made  
175 per sample. For measuring the  $\zeta$ -potential of single O/W emulsions, freshly made  
176 O/W emulsions were diluted 1:10 in M9 media and one millilitre of the diluted  
177 emulsions were then injected in a universal folded capillary cell and the  $\zeta$ -potential  
178 was measured as previous.

## 179 **2.5. Preparation of O/W emulsions**

180 Coarse O/W emulsions were prepared using a high shear mixer homogeniser  
181 (Silverson L5M) at 25°C. The continuous phase was prepared by dissolving  
182 Tween80 (8 wt%) in tryptic soy broth or M9 media at 60°C for 15 minutes. Nano-  
183 emulsions were prepared by homogenising sunflower oil in the continuous phase at  
184 5000 rpm for 60 seconds and the homogenised emulsions were sonicated with a  
185 probe sonicator (VCX 750 Sonics, USA) using a 22mm horn tip and operating at a  
186 frequency of 20 kHz and 750 watts for 4 minutes. Control coarse emulsions were  
187 prepared by homogenising sunflower oil in the continuous phase (ratio of 40:60 or  
188 20:80) at 3000 rpm for 60 seconds. For the CA study, 0.5% CA was dissolved in the  
189 oil phase prior to homogenisation. For microbial viability studies, bacterial cells ( $\sim 10^8$   
190 CFU/ml) were washed twice and re-suspended in ten millilitres O/W emulsions (M9  
191 media as continuous phase) or 6 ml M9 minimal growth medium (control) and  
192 incubated at 25°C for 2 and 7 days on a rotator (Stuart SB3, UK) at 2 rpm to ensure

193 homogenised mixing. For growth studies, bacterial cells ( $\sim 10^4$  CFU/ml) were washed  
194 twice in PBS and re-suspended in ten millilitres of O/W emulsions (tryptic soy broth  
195 as continuous phase) or 6 ml of tryptic soy broth (control) and inoculated with and  
196 incubated at 30°C over time on a rotator at 2 rpm. For the CA study, bacterial cells  
197 ( $\sim 10^8$  CFU/ml) were washed twice and re-suspended in ten millilitres O/W emulsions  
198 (M9 media as continuous phase) or 8 ml M9 minimal growth medium with 2 ml bulk  
199 oil with (0.5 or 1% CA) or without CA and incubated at 25°C for 1, 8 and 24 hours on  
200 a rotator at 2 rpm.

## 201 **2.6. Characterisation of emulsion stability during incubation**

### 202 **2.6.1. Measurement of oil globule size [D (4, 3)]**

203 The particle size distribution of the oil globules was measured immediately after  
204 preparation and as a function of storage time using a laser diffraction particle size  
205 analyser (Malvern Mastersizer 2000, Malvern Instrument Ltd, Worcestershire, UK),  
206 equipped with a He-Ne laser ( $\lambda = 633\text{nm}$ ). The dispersion unit stirring speed was  
207 kept at 2000 rpm and the measurement range was 0.02–2000 $\mu\text{m}$ . The optical  
208 parameters selected were: dispersed phase refractive index of  $n_D^{22}$  1.39; oil globule  
209 absorbance of 0.01; and a dispersant liquid (distilled water) refractive index  $n_D^{22}$   
210 1.33; obscuration between 10% and 20%. Sample was added dropwise to the  
211 system until the obscuration was within an acceptable range. Particle size  
212 calculations were based on the Mie Scattering theory and the volume mean diameter  
213 values (D [4, 3]), and the percentage of volume corresponding to each observed  
214 population were calculated using the Mastersizer 2000 software.

## 2.6.2. Observation of phase separation

The cream height fraction of the micro emulsion was measured immediately after preparation and as a function of storage time. Five millilitres of O/W emulsion were transferred to a graduated 10ml centrifuge tube and left standing upright for 1 hour. The apparition of a cream layer was observed and the cream height fraction was visually measured at 1-hour from the time creaming started. The expression used for calculation of the creaming percentage height is as follows:

$$H_{\text{Cream}} = \frac{H_{\text{Emulsion}} - H_{\text{Creamed phase}}}{H_{\text{Emulsion}}} \times 100\%$$

## 2.7. Determination of bacterial cell viability and growth

Serial dilutions in *PBS* and plating on tryptic soy agar using the Miles & Misra technique (Miles *et al.*, 1938) was conducted immediately after preparation and as a function of storage time to obtain bacterial cell counts as colony forming units per millilitre (CFU/ml).

## 2.8. Flow cytometric analysis of bacterial cells

Flow cytometric analysis was conducted immediately after preparation and as a function of storage time using a BD Accuri C6 flow cytometer (BD, Oxford, UK). From a 1 millilitre sample, the bacterial cells were harvested by centrifugation by centrifugation (10,000 g, 10 minutes) and washed twice and re-suspended in *PBS*. The bacterial cells were stained by adding PI (4µl/ml) and incubated in the dark for 30 minutes. Samples were excited using a 488nm solid state laser and particulate noise was eliminated using a Forward scatter height (FSC-H) threshold while 20,000 data points were collected at a maximum rate of 2500 events/s. Fluorescence was

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237 detected using 670 LP filters corresponding to PI fluorescence. The data was  
238 analysed using CFlow (BD).

## 239 **2.9. Fluorescent and optical imaging of bacteria in O/W emulsions**

240 The O/W emulsions with bacteria were observed using optical and fluorescent  
241 microscopy (Zeiss Axioplan) at ambient temperature. The sample was stained by  
242 adding DAPI (4µl/ml) and incubated in the dark for 30 minutes. The stained sample  
243 was placed on a microscope slide and gently covered with a cover slip. The images  
244 were acquired under objective lens 100x magnification (oil immersion) with a digital  
245 camera system AxioCam ICm1 using a 1.4 megapixel monochrome CCD camera via  
246 AxioVision Software (Zeiss). The samples were observed at room temperature using  
247 a fluorescent microscope (Zeiss AxioLab) equipped with a mercury arc lamp and the  
248 emission was observed at 461nm (DAPI). Micrographs were overlaid using analysis  
249 software (ImageJ).

## 250 **2.10. Statistical analysis**

251 Each experiment was conducted at least in duplicate (N=2) and some cases in  
252 triplicate (N=3). The generated results were collected in Excel (Microsoft Corp.) for  
253 calculating means, standard deviations and error bars. For Student's *t*-test to  
254 compare two means or one-way analysis of variance (ANOVA) and the Tukey's HSD  
255 *post hoc* test to compare several means were used for checking whether there is  
256 significant difference among samples using IBM SPSS Statistics software version  
257 21. Differences were considered significant at  $P < 0.05$ .

258 **3. Results and discussion**

259 **3.1. Effect of droplet size on the survival of bacteria**

260 The O/W emulsion formulations were characterised in terms of oil droplet size [(D (4,  
261 3)]. Two types of O/W emulsions with different D (4, 3) were achieved depending on  
262 the formulation: coarse emulsions (15-35µm) and nano-emulsions (170-650nm) (Fig.  
263 S1).

264 To understand the effect of droplet size on the survival of bacteria, the viability of  
265 bacteria in nano-emulsion was monitored and compared to coarse emulsion over  
266 time (Fig. 1). The M9 minimal growth medium used as continuous phase  
267 contains minimum nutrients that can sustain possible growth but lacks the presence  
268 of amino acids, therefore bacteria can grow in the exponential phase but slowly. As  
269 opposed to being in stationary phase (non-growing), bacteria in exponential phase of  
270 growth are more susceptible to stresses (Anderl *et al.*, 2003; Matsuo *et al.*, 2011)  
271 which allows for better detection any effects of nano-emulsion on bacterial survival.  
272 In this study, there was no significant difference observed in viability of different  
273 bacterial species in nano-emulsion compared to coarse emulsion and control M9  
274 minimal growth medium, after 2 and 7 days. *E. coli* presented no difference in growth  
275 between nano- and coarse emulsions, and the effect was not strain dependent as no  
276 variation in responses was observed between different strains (Fig. 1 A-D). For *S.*  
277 *epidermidis* the reduction in counts observed was comparable between nano- and  
278 coarse emulsions. In the case of *L. acidophilus* the M9 minimal growth medium could  
279 not support its survival after day 2 and therefore this species was discontinued from  
280 this part of the study. Also, microscopic observation showed that all bacteria grew as  
281 planktonic cells and no clustering or colony formation was observed (Fig. 2). Since

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282 colony formation in emulsion systems are associated with upregulation of stress  
283 genes (Prachaiyo and McLandsborough, 2003), it can be concluded that no such  
284 effects occurred in this study.

285 These results are in contrast to many studies reporting nano-emulsions possessing  
286 antimicrobial activity against bacteria. However, in some of these studies the  
287 mechanism behind the antimicrobial effects of nano-emulsions have not been clearly  
288 justified mainly due to lack in use of proper controls. For example, TEOP and BCTP  
289 are the most commonly reported nano-emulsion formulations to possess  
290 antimicrobial activity against several species of micro-organisms including bacteria  
291 such as *E. coli*, *S. aureus* and *L. monocytogenes* (Hamouda *et al.*, 1999; Teixeira *et*  
292 *al.*, 2007; Buranasuksombat *et al.*, 2011). BCTP is made of soybean oil containing  
293 the antimicrobial compound cetylpyridinium chloride (CPC) and stabilised with tri-*n*-  
294 butyl phosphate and Triton X-100 (emulsifier), while TEOP is made of ethyl oleate  
295 and stabilised with Tween80 and *n*-pentanol (co-emulsifier). In 2010, Ferriera *et al.*  
296 investigated the two nano-emulsion formulations and found that for the TEOP  
297 formulation, the antimicrobial effects were due to *n*-pentanol which sits at the O/W  
298 interface as no differences in reduction of bacterial counts were observed when the  
299 bacteria were treated with the TEOP formulation compared to a solution of *n*-  
300 pentanol with the same concentration. Moreover, for the BCTP formulation it was  
301 found that the antimicrobial effect was due to CPC (water soluble, cationic surface-  
302 active agent) and its efficacy was shown to be reduced when it was incorporated into  
303 the nano-emulsion compared to as a solution with the same concentration (Ferriera  
304 *et al.* 2010). The authors argued that controls were not included in studies reporting  
305 antimicrobial activity for the BCTP and TEOP formulations and that they could have  
306 evaluated the contributions of the different components of the emulsions for the

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307 observed antimicrobial activity. Hamouda and Baker (2000) investigated the  
308 antimicrobial activity of two nano-emulsion formulations: 8N8 and W60C against *E.*  
309 *coli*, *Salmonella typhimurium* and *Vibrio cholera*. 8N8 is a water-in-oil nano-emulsion  
310 made of soybean oil containing CPC and stabilised with tri-z-butyl phosphate and  
311 Triton X-100 while W60C is a liposome made of soybean oil stabilised with  
312 Tween60, glycerol monooleate and refined soya sterols. Furthermore, both nano-  
313 emulsion formulations showed antimicrobial effects against all the bacteria; however,  
314 no testing of antimicrobial effects of the individual components of the nano-emulsion  
315 formulation was carried out. In this case, it is not possible to attribute such effects to  
316 high surface tensions of nano-sized droplets. Thus, the process is probably not  
317 mechanical, but rather chemical. Chang *et al.* (2012) studied the antimicrobial effects  
318 of thyme oil nano-emulsion on *Zygosaccharomyces bailii*. They found that nano-  
319 emulsions made with corn and MCT oil did not exhibit any antimicrobial effects  
320 unless mixed with thyme oil indicating that the latter rather than the size of the  
321 droplets was the reason behind its antimicrobial activity. More recently, Ghost *et al.*  
322 (2014) found that sesame oil nano-emulsion possessed antimicrobial activity against  
323 *S. aureus* only when the antimicrobial compound eugenol was present in the oil  
324 phase and no such effects were occurring in the absence of eugenol. Therefore, it  
325 could be concluded that the antimicrobial activity of nano-emulsions reported in  
326 several cases in the literature can only be attributed to the antimicrobial agents that  
327 they carry and no such activity can result from high surface tensions and cell wall  
328 diffusion of nano-sized droplets.

### 3.2. Effect of droplet size on bacterial injury



330 In many cases, antimicrobial treatments can affect bacterial cells, and although they  
331 remain alive, result in stressed and injured subpopulations, which cannot be  
332 detected with analysis by culture. In this study, the membrane integrity of the  
333 bacteria was assessed using flow cytometry combined with PI staining (Table 2)  
334 which is non-permeant but can penetrate cells with a compromised membrane and  
335 binds to double stranded DNA by intercalating between base pairs (Zhang *et al.*,  
336 2001). According to the flow cytometry data (Table 2; Fig. S2), there was no  
337 significant increase in percentage of PI positive cells observed after incubation in  
338 nano-emulsion compared to coarse emulsion and controls in M9 medium. These  
339 results confirm that the membrane integrity of the bacteria was not affected by the  
340 nano-sized droplets and are in contrast to studies that reported extensive damage to  
341 the membrane of bacteria after exposure to nano-emulsions. Extensive  
342 disintegration of the cell membrane, disruption to cell wall and lysis of *S. mutans*  
343 after exposure to soybean oil nano-emulsion containing CPC was observed using  
344 SEM (Karthikeyan *et al.*, 2011). Ghosh *et al.* (2013) found that exposure to basil oil  
345 nano-emulsions against *E. coli* led to deformation in bacterial membrane  
346 phospholipids (confirmed by FT-IR analysis) and stained positive with ethidium  
347 bromide (EtBr) which only stains the DNA of cells with a membrane that lost its  
348 structural integrity. Exposure to eucalyptus oil nano-emulsion led to damage of cell  
349 membrane of *S. aureus* observed using SEM (Sugumar *et al.*, 2014). The exposure  
350 of *S. aureus*, *B. subtilis*, *E. coli* and *S. cerevisiae* to nano-emulsion made with D-  
351 limonene containing the antimicrobial nisin caused extensive membrane damage  
352 observed using SEM associated with release of cellular contents evident by leakage  
353 of the cytoplasmic content measured using UV absorbance (Zhang *et al.*, 2014). In  
354 another study, exposure to oregano oil nano-emulsion led to disruption of the

1 355 bacterial membrane in *L. monocytogenes*, *S. typhimurium* and *E. coli* 0157:H7  
2 356 observed using SEM (Bhargava *et al.*, 2015). However, in all these studies, the  
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4 357 antimicrobial activity of the nano-emulsion was compared to *PBS*, sterile water, or  
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7 358 broth as control rather than being compared to the individual components of the  
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10 359 nano-emulsion. In a study by Karthikeyan *et al.* (2012) reported that there were  
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12 360 higher antimicrobial effects against biofilm and planktonic forms of *S. mutans*, *L.*  
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14 361 *casei*, after a 1-minute exposure to soybean oil nano-emulsion containing CPC  
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16 362 compared to CPC solution only more damage to the cells membrane was evident by  
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18 363 increased fluorescence intensity of PI using fluorescence microscopy. However, no  
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20 364 effects of the nano-emulsion without the incorporation of CPC was compared. CPC  
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22 365 is water soluble and the lower antimicrobial activity with CPC solution would be  
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24 366 expected since the concentration of CPC in the continuous phase of the nano-  
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26 367 emulsion would be higher (due to the presence of the dispersed oil phase) thus  
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28 368 bacterial cells will be exposed to a higher concentration of CPC in nano-emulsion  
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30 369 compared to CPC solution. Although the study showed that nano-emulsion can  
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32 370 damage the membrane of bacteria (Karthikeyan *et al.*, 2012), it lacks use of full  
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34 371 controls and conclusions should be interpreted with caution.  
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### 42 372 **3.3. Effect of emulsion droplet size on cell growth**

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45 373 In order to investigate if nano-emulsions affect bacteria during growth and  
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47 374 proliferation, viability was compared between *O/W* nano-emulsion and coarse  
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49 375 emulsion made with tryptic soy broth as continuous phase (Fig. 3). Once again, there  
50  
51 376 was no significant difference in growth of bacteria between nano- and coarse  
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53 377 emulsion. Growth patterns were similar regardless of species and strains and  
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55 378 comparable to tryptic soy broth (control). Also, bacteria grew as planktonic cells and  
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57 379 no clustering or colony formation was observed as response to stress (Fig. 4).  
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380 Furthermore, the *E. coli* strain PHL644 which is a potent biofilm former that  
381 overexpress the protein adhesin curli (surface attachment structures) (Vidal *et al.*,  
382 1998; Perni *et al.*, 2013) maintained its planktonic form within the nano-emulsion. In  
383 support to our results, Naïtali *et al.* (2009) found that the growth kinetics of *L.*  
384 *monocytogenes* was not affected by incubation in nano-emulsions. In contrast, it  
385 was observed that as opposed to growing in planktonic form, *L. monocytogenes*  
386 were constrained to grow as colonies in O/W emulsions with higher oil phase  
387 concentrations (>80% vs 30 or 70%) and smaller droplet size (2 $\mu$ m vs 15 or 25 $\mu$ m)  
388 (Brocklehurst *et al.*, 1995). The authors argued that in such emulsions the oil  
389 droplets were sufficiently close-packed and viscous to prevent the mobility of the  
390 bacteria forcing growth in colonies, therefore it was a response to space and not a  
391 biological response of cells to interaction with nano-sized droplets. Also, the growth  
392 rates of bacteria were reduced due to restricted diffusion of nutrients and oxygen or  
393 accumulation of waste but this only occurred at lower pH (5 vs 7). However, in this  
394 study the conditions were not similar as the oil phase concentration was 40% and  
395 the pH value of the emulsions during inoculation were around ~7.3.

#### 396 **3.4. Activity of antimicrobial caprylic acid in O/W emulsion of different droplet** 397 **size**

398 In order to investigate the effect of oil droplet size in O/W emulsions in combination  
399 with antimicrobial components, caprylic acid (CA) was added in the oil phase. Since  
400 CA is minimally soluble in water and soluble in fat, its effect should be affected by  
401 the surface area of the oil phase. The antimicrobial activity of O/W emulsions  
402 containing CA was investigated in varying concentration of Tween80 (Fig. 5).

403 Both nano- and coarse emulsions became antimicrobial by adding CA, as evidenced  
404 by survival of <2log CFU/ml after 8 hours. The antimicrobial activity of 0.5% CA was  
405 enhanced in emulsions (CA), and resulted to comparable bacterial reductions with  
406 1% CA in bulk after 8 and 24 hours (Fig. 5). These results are in agreement with  
407 reports on higher activity of antimicrobial oils in emulsions compared to bulk form,  
408 including eucalyptus oil nano-emulsion against *B. cereus*, *S. aureus* and *E. coli*  
409 (Sugumar *et al.*, 2013) thyme oil nano-emulsion against *E. coli* O157:H7, *L.*  
410 *monocytogenes* and *S. enteritidis* (Wu *et al.*, 2014; Xue *et al.*, 2015), *Thymus*  
411 *daenensis* essential oil against *E. coli* (Moghimi *et al.*, 2016a), sage oil (*Salvia*  
412 *officinalis*) (Moghimi *et al.*, 2016b) anise oil against *L. monocytogenes* and *E. coli*  
413 O157:H7 (Topuz *et al.*, 2016), peppermint oil (PO) against *S. aureus* and *L.*  
414 *monocytogenes* (Liang *et al.*, 2012). The EO's possess antimicrobial properties and  
415 the increasing surface area of the oil interface in these studies, enhances the activity  
416 on bacterial membrane compared to bulk form, without however any EO-emulsion  
417 synergistic effect being observed, and therefore nano-size globules in emulsion do  
418 not directly contribute to the activity. The CA molecule is oriented so that the  
419 carboxyl group protrudes into the aqueous phase, while the hydrocarbon tail is in the  
420 oil phase (Andersson *et al.*, 2014). Therefore, it would be expected that the higher  
421 surface area in emulsion increases the amount of CA in contact with the bacterial  
422 membrane compared to bulk form. *S. epidermidis* was more susceptible than *E. coli*  
423 in bulk oil and 0.5% CA, and its viability significantly ( $P<0.05$ ) decreased (~4-log  
424 CFU/ml and <2-log CFU/ml at 8 and 24h respectively). The increased susceptibility  
425 of *S. epidermidis* compared to *E. coli* in bulk oil containing 0.5% CA could be due to  
426 the lack of the outer membrane in Gram-positive bacteria which provides extra  
427 protection to the peptidoglycan cell wall in Gram-negative bacteria. These results

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5 430 corroborate with studies reporting that Gram-positive bacteria are more sensitive to  
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8 431 the antimicrobial effects of CA (Nair *et al.*, 2005) and other fatty acids (Monk *et al.*,  
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10 432 1996) than Gram-negative bacteria.  
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16 433 The effect of Tween80 concentration on the antimicrobial activity of O/W emulsions  
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18 434 containing CA was investigated (Fig. 5). Interestingly, the CA antimicrobial activity  
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20 435 was evident for emulsions composed with 1% Tween80 but was not in samples with  
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22 436 8% Tween80. To ensure that these results were not due to differences in pH, the pH  
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24 437 was measured after 24 hours and all the samples had ~pH 6-6.5 (results not shown).  
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26 438 However, no differences between nano- and coarse emulsion were observed. The  
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28 439 responses were comparable for *E. coli* (MG1655) and *S. epidermidis*, showing,  
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30 440 overall, to be driven by formulation and not size of oil droplets. The concentration of  
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32 441 Tween80 can affect the efficacy of antimicrobials within nano-emulsions (Donsi *et*  
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34 442 *al.*, 2011; Terjung *et al.*, 2012). When the concentration of hydrophilic surfactants  
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36 443 increases in the continuous phase they form multilayer arrangement of interdigitated  
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38 444 surfactant chains that “wrap” the oil droplets (Tadros, 2013; El Kadri *et al.*, 2015).  
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40 445 Since Tween80 is a non-ionic surfactant that stabilises the emulsion by steric  
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42 446 repulsion, its increase in concentration could prevent contact of CA with the bacterial  
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44 447 membrane at the O/W interface.  
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48 448 Overall, the antimicrobial activity of CA was similar in nano- and coarse emulsions  
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50 449 (Fig. 5). These results are in agreement with previous work on lemon myrtle in  
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52 450 soybean oil nano-emulsion against *E. coli*, *L. monocytogenes*, *Salmonella*  
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54 451 *typhimurium*, *P. aeruginosa* and *B. cereus* (Buranasuksombat *et al.*, 2011) and  
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56 452 cinnamaldahehyde against *E. coli* (Bilbao-Sainz *et al.*, 2013), suggesting no  
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58 453 synergistic effect. Therefore, the antimicrobial effect of nano-emulsions could be  
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452 considered a derivative of antimicrobials and their delivery through nano-sized  
453 droplets. However, a recent study reported the antimicrobial effect of anise oil (AO)  
454 nano-emulsion on *E. coli* and *L. monocytogenes* to be higher than AO coarse  
455 emulsion (Topuz *et al.*, 2016) due to higher surface area. Donsi *et al.* (2011) found  
456 that O/W emulsions with smaller droplets have less antimicrobial effects compared to  
457 O/W emulsions with larger droplets due to mechanical stresses caused by the high-  
458 pressure homogenisation (HPH) process when forming nano-emulsions resulting in  
459 degradation of the antimicrobial agents such as phytophenols. In this study, it may  
460 be possible that the surface area provided by the coarse emulsion was enough to  
461 allow all the CA molecules to orient at the O/W interface comparably to nano-  
462 emulsion. Furthermore, the formation of nano-emulsion by ultrasonication generates  
463 heat which may affect the antimicrobial activity of CA. Pestana *et al.* (2015) showed  
464 that the amount of CA in milk samples was diminished after pasteurisation and ultra-  
465 high temperature (UHT) sterilisation.

### 3.5. Stability of nano-emulsions in the presence of bacteria

467 All O/W emulsions remained stable during the incubation period. The oil droplet size  
468 [(D (4, 3))] (Fig. 6; Table S1) and changes in creaming stability (data not shown) with  
469 or without bacteria in the continuous phase was monitored over time with no  
470 significant differences observed. Furthermore, there was no flocculation and  
471 aggregation of the oil droplets observed with any of the bacterial strains regardless  
472 of their surface characteristics. The most hydrophobic strains including *E. coli*  
473 (JM109), *S. epidermidis* and *B. cereus* (Table S2 and S3) resided within the  
474 continuous phase and did not aggregate around the oil droplets (Fig. 2 and 4).  
475 Similarly, the stability of O/W emulsions with CA was not affected by the presence of

1 476 bacteria (Fig. 6 and Fig. S1). Ly *et al.* (2006) found that the stability of O/W  
2 477 emulsions with bacteria was strain dependent and the negatively charged  
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4 478 *Lactococcus lactis* (LLD16) provoked creaming, flocculation and aggregation by  
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7 479 surrounding the positively charged oil globules whereas the positively charged *L.*  
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9 480 *lactis* (LLD18) caused no such effects. In another study, it was shown that as  
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11 481 opposed to the less negatively charged *E. coli* strain E21, the more negatively  
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13 482 charged *E. coli* JM109 promoted faster creaming rates, coalescence and flocculation  
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15 483 of O/W emulsions containing positively charged oil globules (Li *et al.*, 2001). In this  
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17 484 study, the oil globules in the nano-emulsion were stabilised by a non-ionic surfactant  
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19 485 (Tween80), hence, the absolute magnitude of the droplet charge is very low  
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21 486 (McClements, 2011; Tang *et al.*, 2012). Since the bacterial membranes were found  
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23 487 to be negatively charged (Table S2) and the oil droplets in all the O/W emulsions  
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25 488 were less negatively charged (Table S3), thus they repel each other and bacterial  
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27 489 cells will remain in the aqueous continuous phase. Therefore, the findings in this  
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29 490 work on the antimicrobial activity over time, could not have been affected by changes  
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31 491 in O/W emulsion stability.  
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#### 43 493 **4. Conclusion**

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47 494 The literature review identified controversy regarding the consistency and  
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49 495 mechanism of antimicrobial activity reported for nano-emulsions. In this study  
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51 496 reducing the size of oil droplets in O/W emulsions to the nano-scale had no direct  
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53 497 effect on the viability and growth of bacteria when no antimicrobial agents were  
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55 498 added and flow cytometry showed that the membrane integrity was intact.  
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57 499 Controversy seems to come from studies suggesting that nano-emulsions possess  
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1 antimicrobial properties due to high surface tensions and cell wall diffusion of the  
2 nano-sized droplets, however, many of these studies were found to lack appropriate  
3 controls to test the action of individual components of the nano-emulsion or the  
4 action of the nano-emulsion without active ingredients. Therefore, some of the  
5 findings that attribute direct antimicrobial activity to nano-emulsions should be taken  
6 with caution, and further work is needed before concluding. In contrast, there is  
7 strong evidence that O/W nano-emulsions present higher antimicrobial activity due to  
8 higher interface; however, the case study based on CA did not show increased  
9 antimicrobial activity in nano- compared to coarse emulsion. Therefore, it is indicated  
10 that these responses should not always be expected and the antimicrobial effect of  
11 nano-emulsions depends on the antimicrobial agent and is affected by the  
12 formulation. Nano-emulsions remain an extremely promising asset in food  
13 formulation applications and they are known to promote stability, improve sensory  
14 perception, and enhance food functionality. In contrast, their manufacturing requires  
15 more energy. Therefore, their antimicrobial capability must be fully realised for  
16 assessing the benefits of application.

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517 **References**

518

519 Abd-Elsalam, K. A., & Khokhlov, A. R. (2015). Eugenol oil nanoemulsion: antifungal  
520 activity against *Fusarium oxysporum* f. sp. *vasinfectum* and phytotoxicity on cotton  
521 seeds. *Applied Nanoscience*, 5, 255–265.

522

523 Andersson, M. P., Olsson, M. H. M., & Stipp, S. L. S. (2014). Predicting the pK<sub>a</sub> and  
524 Stability of Organic Acids and Bases at an Oil–Water Interface. *Langmuir*, 30, 6437–  
525 6445.

526

527 Andrel, J. N., Zahller., Roe, F., & Stewart, P. S. (2003). Role of Nutrient Limitation  
528 and Stationary-Phase Existence in *Klebsiella pneumoniae* Biofilm Resistance to  
529 Ampicillin and Ciprofloxacin. *Antimicrobial Agents and Chemotherapy*, 47, 1251–  
530 1256.

531

532 Andrews, J. M. (2001). Determination of minimum inhibitory concentrations.  
533 *Antimicrobial Agents and Chemotherapy*, 49 (6), 1049.

534

535 Annamalai, T., Nair, M. K. M., Marek, P., Vasudevan, P., Schreiber, D., Knight, R.,  
536 Hoagland, T., & Venkitanarayanan, K. (2000). In vitro inactivation of  
537 enterohemorrhagic *Escherichia coli* 0157: H7 in bovine rumen fluid by caprylic acid.  
538 *Journal of Food Protection*, 67, 884-888.

539

540 Baglioni, P., & Cherazzi, D. (2013). *Nanoscience for the conservation of works of art*.  
541 Royal Society of Chemistry, Dorchester, pp. 203-204

542

1  
2 543 Baker, J. R., Wright, D. C., Hayes, M. M., Hamouda, T., & Brisker, J. M. (2000).

3  
4  
5 544 Methods of inactivating bacteria including bacterial spores. U.S. Patent # 6015832.

6  
7 545

8  
9 546 Bhargava, K., Conti, D. S., da Rocha, S. R. P., & Zhang, Y. (2015). Application of an

10  
11  
12 547 oregano oil nanoemulsion to the control of foodborne bacteria on fresh lettuce. *Food*

13  
14  
15 548 *Microbiology*, 47, 69–73.

16  
17 549

18  
19 550 Bilbao-Sainz, C., Chiou, B., Du Sen, W. X., Gregorsky, K. S., & Orts, W. J. (2013).

20  
21  
22 551 Influence of disperse phase characteristics on stability, physical and

23  
24 552 antimicrobial properties of emulsions containing cinnamaldehyde. *Journal of*

25  
26  
27 553 *the American Oil Chemists' Society (AOCS)*, 90, 233–241.

28  
29 554

30  
31 555 Brocklehurst, T. F., Parker, M. J., Gunning, P. A., Coleman, H. P., & Robins, M. M.

32  
33  
34 556 (1995). Growth of food-borne pathogenic bacteria in oil-in-water emulsion: II-Effects

35  
36 557 of emulsion structure on growth parameters and form of growth. *Journal of Applied*

37  
38  
39 558 *Bacteriology*, 78, 609-615.

40  
41 559

42  
43 560 Buranasuksombat, U., Kwon, Y. J., Turner, M., & Bhandari, B. (2011). Influence of

44  
45  
46 561 emulsion droplet size on antimicrobial properties. *Food Science and Biotechnology*,

47  
48  
49 562 20, 793-800.

50  
51 563

52  
53 564 Chang, Y., McLandsborough, L., & McClements, D. J. (2012). Physical properties

54  
55  
56 565 and antimicrobial efficacy of thyme oil nanoemulsions: influence of ripening

57  
58 566 inhibitors. *Journal of Agriculture and Food Chemistry*, 60, 12056–12063.

59

60

61

62

63

64

65

567

1  
2 568 Chang, Y., McLandsborough, L., & McClements, D. J. (2015). Fabrication, stability  
3  
4 569 and efficacy of dual-component antimicrobial nanoemulsions: essential oil (thyme oil)  
5  
6  
7 570 and cationic surfactant (lauric arginate). *Food Chemistry*, 172, 298–304.

8  
9  
10 571

11 572 Chaudhry, Q., & Castle, L. (2011). Food applications of nanotechnologies: An  
12  
13  
14 573 overview of opportunities and challenges for developing countries. *Trends in Food*  
15  
16  
17 574 *Science & Technology*, 22, 595-603.

18  
19 575

20  
21 576 Donsì, F., Annunziata, M., Sessa, M., & Ferrari, G. (2011). Nanoencapsulation of  
22  
23  
24 577 essential oils to enhance their antimicrobial activity in foods. *LWT—Food Science*  
25  
26  
27 578 *and Technology*, 44, 1908–1914.

28  
29 579

30  
31 580 Donsì, F., Annunziata, M., Vincensi, M., & Ferrari, G. (2012). Design of  
32  
33  
34 581 nanoemulsion-based delivery systems of natural antimicrobials: effect of the  
35  
36  
37 582 emulsifier. *Journal of Biotechnology*, 159, 342–350.

38  
39 583

40  
41 584 El Kadri, H., Overton, T., Bakalis, S., & Gkatzionis, K. (2015). Understanding and  
42  
43  
44 585 controlling the release mechanism of *Escherichia coli* in double  $W_1/O/W_2$  emulsion  
45  
46 586 globules in the presence of NaCl in the  $W_2$  phase. *RSC Advances*, 5, 105098-  
47  
48  
49 587 105110.

50  
51 588

52  
53 589 Ferreira, J. P., Alves, D., Neves, O., Silva, J., Gibbs, P.A., & Teixeira, P. C. (2010).  
54  
55  
56 590 Effects of the components of two antimicrobial emulsions on food-borne pathogens.  
57  
58  
59 591 *Food Control*, 21, 227-230.

60  
61  
62  
63  
64  
65

592

1  
2 593 Ghosh, V. Mukherjee, A., & Chandrasekaran, N. (2012). Mustard oil microemulsion  
3  
4 594 formulation and evaluation of bactericidal activity. *International Journal of Pharmacy*  
5  
6  
7 595 *and Pharmaceutical Sciences*, 4, 497-500.

8  
9  
10 596

11 597 Ghosh, V. Mukherjee, A., & Chandrasekaran, N. (2013). Ultrasonic emulsification of  
12  
13 598 food-grade nanoemulsion formulation and evaluation of its bactericidal activity.  
14  
15  
16 599 *Ultrasonics Sonochemistry*, 20, 338–344.

17  
18  
19 600

20  
21 601 Ghosh, V., Mukherjee, A., & Chandrasekaran, N. (2014). Eugenol-loaded  
22  
23 602 antimicrobial nanoemulsion preserves fruit juice against, microbial spoilage. *Colloids*  
24  
25  
26 603 *and Surfaces B: Biointerfaces*, 114, 392–397.

27  
28  
29 604

30  
31 605 Gupta, A., Eral, H. B., Hatton, T. A., & Doyle, P. S. (2016). Nanoemulsions:  
32  
33 606 formation, properties and applications. *Soft Matter*, 12, 2826-2841.

34  
35  
36 607

37  
38 608 Hamouda, T., & Baker, J. R. (2000). Antimicrobial mechanism of action of surfactant  
39  
40 609 lipid preparations in enteric Gram-negative bacilli. *Journal of Applied Microbiology*,  
41  
42  
43 610 89, 397–403.

44  
45  
46 611

47  
48 612 Hamouda, T., Hayes, M. M., Cao, Z., Tonda, R., Johnson, K., Wright, D. C., Brisker,  
49  
50 613 J., & Baker J. R. Jr. (1999). A novel surfactant nanoemulsion with broad-spectrum  
51  
52 614 sporicidal activity against *Bacillus* spores. *The Journal of Infectious Diseases*, 180,  
53  
54  
55 615 1939–1949.

56  
57  
58 616

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65

617 Hemmila, M. R., Mattar, A., Taddonio, M. A., Arbabi, S., Hamouda, T., Ward, P. A.,  
618 Wang, S. C., & Baker, J. R. (2010). Topical nanoemulsion therapy reduces bacterial  
619 wound infection and inflammation after burn injury. *Surgery*, 148, 509-499.  
620  
621 Hwang, Y. Y., Ramalingam, K., Bienek, D. R., Lee, V., You, T., & Alvareza, R.  
622 (2013). Antimicrobial Activity of Nanoemulsion in Combination with Cetylpyridinium  
623 Chloride in Multidrug-Resistant *Acinetobacter baumannii*. *Antimicrobial Agents and*  
624 *Chemotherapy*, 57, 3568–3575.  
625  
626 Jo, Y. J., Chun, J. Y., Kwon, Y. J., Min, S. G., Hong, G. P., & Choi, M. J. (2015).  
627 Physical and antimicrobial properties of trans-cinnamaldehyde nanoemulsions in  
628 watermelon juice. *LWT: Food Science and Technology*, 60, 444–451.  
629  
630 Karthikeyan, R., Amaechi, B. T., Rawls, H. R., & Lee, V. A. (2011). Antimicrobial  
631 activity of nanoemulsion on cariogenic *Streptococcus mutans*. *Archives of Oral*  
632 *Biology*, 56, 437-445.  
633  
634 Karthikeyan, R., Amaechi, B. T., Rawls, H. R., & Lee, V. A. (2012). Antimicrobial  
635 activity of nanoemulsion on cariogenic planktonic and biofilm organisms. *Archives of*  
636 *Oral Biology*, 57, 15-22.  
637  
638 Kim, I. H., Lee, H., Kim, J. E., Song, K., Lee, Bin, Chung, Y. S., & Min, D. S. (2013).  
639 Plum coatings of lemongrass oil-incorporating carnauba wax-based nanoemulsion.  
640 *Journal of Food Science*, 78, E1551-9.

1  
2  
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60  
61  
62  
63  
64  
65

642 Lee, V. A., Karthikeyan, R., Rawls, H. R., & Amaechi, B. T. (2010). Anti-cariogenic  
643 effect of a cetylpyridinium chloride-containing nanoemulsion. *Journal of Dentistry*, 38,  
644 742-749.  
645  
646 Li, J., McClements, D. J., & McLandsborough, L. A. (2001). Interaction between  
647 emulsion droplets and *Escherichia coli* cells. *Journal of Food Science*, 66, 570-574.  
648  
649 Liang, R., Xu, S., Shoemaker, C. F., Li, Y., Zhong, F., & Huang, Q. (2012). Physical  
650 and antimicrobial properties of peppermint oil nanoemulsions. *Journal of Agriculture  
651 and Food Chemistry*, 60, 7548–7555.  
652  
653 Lu, W-C., Huang, D-W, Wang, C-C. R., Yeh, C-H., Tsai, J-C., Huang, Y-T., & Li, P-  
654 H. (2017). Preparation, characterization, and antimicrobial activity of nanoemulsions  
655 incorporating citral essential oil. *Journal of food and drug analysis*, 1-8,  
656 <http://dx.doi.org/10.1016/j.jfda.2016.12.018>  
657  
658 Ly, M. H., Naïtali-Bouchez, M., Meylheuc, T., Bellon-Fontaine, M-N., Le, T. M., Belin,  
659 J-M., & Waché, Y. (2006). Importance of bacterial surface properties to control the  
660 stability of emulsions. *International Journal of Food Microbiology*, 112, 26–34.  
661  
662 Ly, M. H., Aquedoc, M., Goudota, S., Lea, M. L., Cayotd, P., Teixeira, T. M., Leb, J-  
663 M., Belina, J-M., & Waché, Y. (2008). Interactions between bacterial surfaces and  
664 milk proteins, impact on food emulsions stability. *Food Hydrocolloids*, 22, 742–751.  
665

1 666 Majeed, H., Liu, F., Hategekimana, J., Sharif, H. R., Qi, J., Ali, B., Bian, Y. Y., Ma, J.,  
2 667 Yokoyama, W., & Zhong, F. (2016b). Bactericidal action mechanism of negatively  
3  
4 668 charged food grade clove oil nanoemulsions. *Food Chemistry*. 197, 75–83.  
5  
6  
7 669  
8  
9  
10 670 Matsuo, M., Oogai, Y., Kato, F., Sugai, M., & Komatsuzawa, H. (2011). Growth-  
11  
12 671 phase dependence of susceptibility to antimicrobial peptides in *Staphylococcus*  
13  
14 672 *aureus*. *Microbiology*, 157, 1786-97.  
15  
16  
17 673  
18  
19 674 McClements, D. J. (2010). Emulsion design to improve the delivery of functional  
20  
21 675 lipophilic components. *In Annual Review of Food Science and Technology*, 1, 241-  
22  
23 676 269.  
24  
25  
26 677  
27  
28  
29 678 McClements, D. J. (2011). Edible nanoemulsions: fabrication, properties, and  
30  
31 679 functional performance. *Soft Matter*, 7, 2297–2316.  
32  
33  
34 680  
35  
36 681 Miles, A., Misra, S., & Irwin, O. (1938). The estimation of the bactericidal power of  
37  
38 682 the blood. *The Journal of Hygiene*, 38, 732.  
39  
40  
41 683  
42  
43  
44 684 Monk, J. D., Beuchat, L. R., & Hathcox, A. K. (1996). Inhibitory effects of sucrose  
45  
46 685 monolaurate, alone and in combination with organic acids, on *Listeria*  
47  
48 686 *monocytogenes* and *Staphylococcus aureus*. *Journal of Applied Bacteriology*, 81, 7-  
49  
50 687 18.  
51  
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53 688  
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65

689 Moghimi, R., Ghaderi, L., Rafati, H., Aliahmadi, A., & McClements, D. J. (2016a).  
690 Superior antibacterial activity of nanoemulsion of *Thymus daenensis* essential oil  
691 against *E. coli*. *Food Chemistry*, 194, 410–415.

692

693 Moghimi, R., Aliahmadi, A., McClements, D. J., & Rafati, H. (2016b). Investigations  
694 of the effectiveness of nanoemulsions from sage oil as antibacterial agents on some  
695 food borne pathogens. *LWT - Food Science and Technology*, 71, 69-76.

696

697 Myc, A., Vanhecke, T., Landers, J. J., Hamouda, T., & Baker J. R. (2001). The  
698 fungicidal activity of novel nanoemulsion (X8W60PC) against clinically important  
699 yeast and filamentous fungi. *Mycopathologia*, 155, 195–201.

700

701 Nair, M. K., Joy. J., Vasudevan, P., Hinckley, L., Hoagland, T. A., &  
702 Venkitanarayanan, K. S. (2005). Antibacterial effect of caprylic acid and  
703 monocaprylin on major bacterial mastitis pathogens. *Journal of Dairy Science*, 88,  
704 3488-95.

705

706 Naïtali, M., Dubois-Brissonnet, F., Cuvelier, G., & Bellon-Fontaine, M-N. (2009).  
707 Effects of pH and oil-in-water emulsions on growth and physicochemical cell surface  
708 properties of *Listeria monocytogenes*: Impact on tolerance to the bactericidal activity  
709 of disinfectants. *International Journal of Food Microbiology*, 130, 101–107.

710

711 Pastana, J. M., Gennari, A., Monteiro, B. S., Lehn, D. N., & de Souza, C, F, V.  
712 (2015). Effects of pasteurization and ultra-high temperature processes on proximate



1 713 composition and fatty acid profile in bovine milk. *American Journal of Food*  
2 714 *Technology*, 10, 265-272.  
3  
4  
5 715  
6 716  
7 Perni, S., Hackett, L., Goss, R., J. M., Simmons, M. J., & Overton, T. W. (2013).  
8 717  
9 Optimisation of engineered *Escherichia coli* biofilms for enzymatic biosynthesis of L-  
10 718  
11 halotryptophans. *AMB Express*, 3, 66.  
12  
13  
14 719  
15  
16  
17 720 Prachaiyo, P., & McLandsborough, L. A. (2003). Oil-in-water emulsion as a model  
18  
19 721 system to study the growth of *Escherichia coli* O157:H7 in a heterogeneous food  
20  
21 722 system. *Journal of Food Science*, 63, 1018-1024.  
23  
24 723  
25  
26  
27 724 Rosenberg, M., Gutnick, D., & Rosenberg, E. (1980). Adherence of bacteria to  
28  
29 725 hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS*  
30  
31 726 *Microbiology Letters*, 9, 29–33.  
32  
33  
34 727  
35  
36  
37 728 Ruengvisesh, S., Loquercio, A., Castell-Perez, E., & Taylor, T.M. (2015). Inhibition of  
38  
39 729 bacterial pathogens in medium and on spinach leaf surfaces using plant-derived  
40  
41 730 antimicrobials loaded in surfactant micelles. *Journal of Food Science*, 80, M2522-  
42  
43 731 M2529.  
44  
45  
46 732  
47  
48  
49 733 Salvia-Trujillo, L., Rojas-Graü, A., Soliva-Fortuny, R., & Martín-Belloso, O. (2015).  
50  
51 734 Physicochemical characterization and antimicrobial activity of food grade emulsions  
52  
53 735 and nanoemulsions incorporating essential oils. *Food Hydrocolloids*, 43, 547-556.  
54  
55  
56 736  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 737 Sessa, M., Ferrari, G., & Donsì, F. (2015). Novel edible coating containing essential  
2 738 oilnanoemulsions to prolong the shelf life of vegetable products. *Chemical*  
3  
4  
5 739 *Engineering Transactions*, 43, 55–60.  
6  
7 740  
8  
9 741 Severino, R., Vu, K. D., Donsì, F., Salmieri, S., Ferrari, G., & Lacroix, M. (2014a).  
10  
11 742 Antibacterial and physical effects of modified chitosan based-coating containing  
12  
13 743 nanoemulsion of mandarin essential oil and three non-thermal treatments against  
14  
15 744 *Listeria innocua* in green beans. *International Journal of Food Microbiology*, 191, 82–  
16  
17 745 88.  
18  
19 746  
20  
21 747 Severino, R., Vu, K. D., Donsì, F., Salmieri, S., Ferrari, G., & Lacroix, M. (2014b).  
22  
23 748 Antimicrobial effects of different combined non-thermal treatments against *Listeria*  
24  
25 749 *monocytogenes* in broccoli florets. *Journal of Food Engineering*, 124, 1–10.  
26  
27 750  
28  
29 751 Severino, R., Ferrari, G., Vu, K. D., Donsì, F., Salmieri, S., & Lacroix, M. (2015).  
30  
31 752 Antimicrobial effects of modified chitosan based coating containing nanoemulsion of  
32  
33 753 essential oils, modified atmosphere packaging and gamma irradiation against  
34  
35 754 *Escherichia coli* O157:H7 and *Salmonella Typhimurium* on green beans. *Food*  
36  
37 755 *Control*, 50, 215–222.  
38  
39 756  
40  
41 757 Shah, B., Davidson, P. M., & Zhong, Q. (2013). Nanodispersed eugenol has  
42  
43 758 improved antimicrobial activity against *Escherichia coli* O157:H7 and *Listeria*  
44  
45 759 *monocytogenes* in bovine milk. *International Journal of Food Microbiology*, 161, 53–  
46  
47 760 59.  
48  
49 761  
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65

762 Sugumar, S., Nirmala, J., Ghosh, V., Anjali, H., Mukherjee., A., & Chandrasekaran,  
763 N. (2013). Bio-based nanoemulsion formulation, characterization and antibacterial  
764 activity against food-borne pathogens. *Journal of Basic Microbiology*, 53, 677–685.  
765  
766 Sugumar, S., Ghosh, V., Nirmala, M.J., Mukherjee, A., & Chandrasekaran, N.,  
767 (2014). Ultrasonic emulsification of eucalyptus oil nanoemulsion: antibacterial activity  
768 against *Staphylococcus aureus* and wound healing activity in Wistar rats. *Ultrasonics*  
769 *Sonochemistry*, 21, 1044–1049.  
770  
771 Tadros, T. F. (e.d.) 2013. *Emulsion formation and stability*, ed., Wiley-VCH  
772 Publishers, Weinheim, pp. 173–174.  
773  
774 Tang, S. Y., Manickama, S., Wei, T. K., & Nashiru, B. (2012). Formulation  
775 development and optimization of a novel Cremophore EL-based nanoemulsion using  
776 ultrasound cavitation. *Ultrasonics Sonochemistry*, 19, 330–345.  
777  
778 Teixeira, P. C., Leite, G. M., Domingues, R. J., Silva, J., Gibbs, P. A., & Ferreira, J.  
779 P. (2010). Antimicrobial effects of a microemulsion and a nanoemulsion on enteric  
780 and other pathogens and biofilms. *International Journal of Food Microbiology*, 118,  
781 15-19.  
782  
783 Terjung, N., Löffler, M., Gibis, M., Hinrichs, J., & Weiss, J. (2012). Influence of  
784 droplet size on the efficacy of oil-in-water emulsions loaded with phenolic  
785 antimicrobials. *Food and Function*, 3, 290–301.  
786

1 787 Topuz, O. K., Özvural, E. B., Zhao, Q., Huang, Q., Chikindas, M., & Gölükçü, M.  
2 788 (2013). Physical and antimicrobial properties of anise oil loaded nanoemulsions on  
3  
4 789 the survival of foodborne pathogens. *Food Chemistry*, 203, 117–123.  
5  
6  
7 790  
8  
9 791 Vidal, O., Longin, R., Prigent-Combaret, C., Dorel, C., Hooreman, M., & Lejeune, P.  
10  
11 792 (1998). Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on  
12  
13 793 inert surfaces: involvement of a new *ompR* allele that increases curli expression.  
14  
15 794 *Journal of Bacteriology*, 180, 2442-2449.  
16  
17  
18 795  
19  
20 796 Wu, J. E., Lin, J., & Zhong, Q. (2014). Physical and antimicrobial characteristics of  
21  
22 797 thyme oil emulsified with soluble soybean polysaccharide. *Food Hydrocolloids*, 39,  
23  
24 798 144–150.  
25  
26  
27 799  
28  
29 800 Xu, S., Ni, Z., Ma, L., & Zheng, X. (2017). Control of alternaria rot of cherry tomatoes  
30  
31 801 by food-grade *laurus nobilis* essential oil microemulsion. *Journal of Food Safety*, 37,  
32  
33 802 1-9.  
34  
35  
36 803  
37  
38 804 Xue, J., Michael Davidson, P., & Zhong, Q. (2015). Antimicrobial activity of thyme  
39  
40 805 oilco-nanoemulsified with sodium caseinate and lecithin. *International Journal of*  
41  
42 806 *Food Microbiology*, 210, 1–8.  
43  
44  
45 807  
46  
47 808 Zhang, S., & Crow, S. A. (2001). Toxic Effects of Ag(I) and Hg(II) on *Candida*  
48  
49 809 *albicans* and *C. maltosa*: A Flow Cytometric Evaluation, *Applied and Environmental*  
50  
51 810 *Microbiology*, 67, 4030–4035.  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 812 Zhang, Z., Vriesekoop, F., Yuan, O., & Liang, H. (2014). Effects of nisin on the  
2 antimicrobial activity of D-limonene and its nanoemulsion. *Food Chemistry*, 150,  
3 813 307–312.  
4  
5 814  
6  
7 815  
8  
9 816 Ziani, K., Chang, Y., McLandsborough, L., & McClements, D. J. (2011). Influence of  
10 surfactant charge on antimicrobial efficacy of surfactant-stabilised thyme oil  
11 817 nanoemulsions. *Journal of Agricultural and Food Chemistry*, 59, 6247–6255.  
12  
13  
14  
15 818  
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17 819  
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19  
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**Table 1.** The antimicrobial activity of nano-emulsions correlated with their mean droplet size, ingredients (oil phase, stabilisers, antimicrobials) and the micro-organism it is tested against. The impact of the nano-emulsion is classified as positive (+) when the nano-sized droplets improves the antimicrobial activity with respect to the control, as negative (-) when it decreases the antimicrobial activity, and as neutral (+/-) when no significant change is observed or a significant change was observed only due to incorporation of antimicrobials and not because of nano-size droplets. The method of emulsification is mentioned as: HPH – High Pressure Homogenisation, HSH – High Shear Homogenisation, US – Ultrasonication, MFZ – Microfluidizer, CPI – Catastrophic Phase Inversion.

Emulsion type	Oil phase	Stabilisers	Continuous phase	Antimicrobials	Mean droplet size (nm)	Method of emulsification	Impact of nano-emulsion	Micro-organism	Controls	Author
O/W nano-emulsion	Thyme oil and corn oil (from 0 to 100% (w/w) corn oil 5% (w/w)	Tween80 and lauric arginate (LAE) or sodium dodecyl sulfate (SDS)	Buffer solution (10mM acetate, pH 4)	None	163nm	HPH	+	<i>Zygosaccharomyces bailli</i> <i>Saccharomyces cerevisiae</i> <i>Brettanomyces bruxellensis</i> <i>Brettanomyces naardenensis</i>	Buffer solution (10mM acetate, pH 4)	Ziaini <i>et al.</i> (2011)
O/W nano-emulsion	Soybean oil (%N.A.)	Ethylenediaminete traacetic acid, glycerol, Tween20, and benzalkonium chloride	Saline solution 0.9% (w/w)	None	350nm	HSH	+	<i>P. aeruginosa</i>	Saline solution 0.9% (w/w)	Hemmilla <i>et al.</i> (2010)
O/W nano-emulsion	Eucalyptus oil 16.66% (v/v)	Tween80	Water	None	17.1nm	US	+	<i>B. cereus</i> <i>S. aureus</i> <i>E. coli</i>	Bulk eucalyptus oil or water containing Tween80 16.66% (v/v)	Sugumar <i>et al.</i> (2013)
O/W nano-emulsion	Eucalyptus oil 16.66% (v/v)	Triton X-100	Water	None	3.8nm	US	+	<i>S. aureus</i>	Untreated sample	Sugumar <i>et al.</i> (2014)
O/W micro-emulsion	<i>Laurus nobilis</i> essential oil 15% (w/v)	Tween20 and ethanol	Water	None	10nm	N.A.	+	<i>Alternaria alternata</i>	Water	Xu <i>et al.</i> (2017)
O/W nano-emulsion	Soybean oil 25% (v/v)	Triton X-100	Water	CPC 1% (v/v)	100-800nm	MFZ	+	<i>Acinetobacter baumannii</i>	Ethanol 30% (v/v) or untreated	Hwang <i>et al.</i> (2013)

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O/W nano-emulsion	Anise oil 75% (w/w)	Alcolec PC75 (soy lecithin)	Water	None	117.2–275.7nm	HPH	+	<i>L. monocytogenes</i> <i>E. coli</i> O157:H7	Anise bulk oil or coarse emulsion	Topuz <i>et al.</i> (2016)
O/W nano-emulsion	Miglyol 812N 10% (w/w)	Tween80	Water	Carvacrol and eugenol (5, 15, 30 and 50 (w/w %))	80nm	HPH	-	<i>E. coli</i> C 600 <i>Listeria Innocua</i>	Coarse emulsion	Terjung <i>et al.</i> (2012)
O/W nano-emulsion	Pure peppermint oil, medium chain triglyceride (MCT), and their mixture at ratios of 1:5, 1:1, and 5:1 (v/v)	Modified starch	Water	None	184-228nm	HPH	-	<i>L. monocytogenes</i> <i>S. aureus</i>	Bulk peppermint oil, MCT nano-emulsion or untreated sample	Liang <i>et al.</i> (2012)
O/W nano-emulsion	Thyme oil and corn oil or MCT medium chain triglyceride (MCT) (from 0 to 100% (w/w)) (10% (w/w))	Tween 80	Aqueous buffer solution (5mM citrate buffer, pH 3.5)	None	160-196nm	HPH	+	<i>Zygosaccharomyces bailii</i>	Nano-emulsion with corn or MCT but no thyme oil	Chang <i>et al.</i> (2012)
O/W nano-emulsion	Citral oil 10% (w/w) Surfactants:	Span 85, Brij 97 and ethylene glycol	Water	None	28nm	US	+	<i>S. aureus</i> <i>E. coli</i> <i>Pseudomonas aeruginosa</i> <i>Enterococcus faecalis</i> <i>S. typhimurium</i> <i>L. monocytogenes</i>	Sulphadiazine	Lu <i>et al.</i> (2017)
O/W nano-emulsion	D-Limonene 5% (w/w) or a mixture of terpenes 5% (w/w)	Soy lecithin Solec Ip, Tween 20 and glycerol monooleate and CLEARGUM CO 01	Water	D-limonene and a mixture of terpenes extracted from <i>Melaleuca alternifolia</i> (0.1-10% (w/w))	74.4-156.6nm	HPH	+	<i>E. coli</i> <i>L. delbrueckii</i> <i>S. cerevisiae</i>	Sunflower oil with D-limonene (50:50) 10% (w/w) or Palm oil with a mixture of terpenes (50:50) 10% (w/w)	Donsi <i>et al.</i> (2011)

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O/W nano-emulsion	Sunflower oil 8% (w/w)	Lecithin, pea proteins, sugar ester, and a combination of Tween20 and glycerol monooleate	Water	Carvacrol, d-limonene and cinnamaldehyde 2% (w/w)	170-240nm	HPH	+	<i>E. coli</i> <i>L. delbrueckii</i> <i>S. cerevisiae</i>	Water	Donsi <i>et al.</i> (2012)
O/W nano-emulsion	Soybean oil 25% v/v)	Triton X-100 10% (v/v)	Water	CPC 1% (w/v)	308nm Microfluidizer (M-110L, Microfluidics, Newton, MA) at 20,000 psi	MFZ	+	<i>S. mutans</i> (planktonic and biofilm)	Chlorhexidine digluconate 0.12% (v/v) or untreated sample	Karthikeyan <i>et al.</i> (2011)
O/W nano-emulsion	Soybean oil 25% (v/v)	Triton X-100 10% (v/v)	Water	CPC 1% (w/v)	308nm Microfluidizer (M-110L, Microfluidics, Newton, MA) at 20,000 psi for	MFZ	+	<i>S. mutans</i> (planktonic and biofilm) <i>L. casei</i> (planktonic and biofilm) <i>Actinomyces viscosus</i> <i>Candida albicans</i>	Chlorhexidine digluconate 0.12% (v/v) or untreated sample	Karthikeyan <i>et al.</i> (2012)
O/W nano-emulsion	<b>BCTP</b> Ethyl oleate 3% (v/v)  <b>TEOP</b> Soybean oil 16% (v/v)	<b>BCTP</b> <i>n</i> -pentanol and Tween80  <b>TEOP</b> Tri- <i>n</i> -butyl phosphate 2% (v/v), and triton X-100 2% (v/v)	Water	None	Not mentioned	HSH	+	<i>S. typhimurium</i> <i>E. coli</i> 0157:H7  <i>Pseudomonas aeruginosa</i>  <i>S. aureus</i>  <i>L. monocytogenes</i>	Untreated sample	Teixiera <i>et al.</i> (2007)
W/O nano-emulsion	<b>X8W60PC</b> Oil 64% (w/w)	Three non-ionic detergents and solvent	Water	None	400–800nm	HSH	+	<i>Candida parapsilosis</i>  <i>Fusarium oxysporum</i>  <i>Candida albicans</i>  <i>Candida tropicalis</i>	Untreated sample or bleach 6%	Myc <i>et al.</i> (2001)



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								<i>Microsporium gypseum</i> ,  <i>Trichophyton mentagrophytes</i> <i>Trichophyton rubrum</i> , and  <i>Aspergillus fumigatus</i>		
O/W nano-emulsion	Lemongrass oil 1% (v/v)	Tween80	Sodium alginate 1% (w/v)	None	4-35nm	MFZ	+	<i>E. coli</i>	Water	Salvia-Trujillo et al. (2015)
O/W nano-emulsion	<i>Thymus daenensis</i> oil 2% (w/w)	Tween80 and lecithin	Water	None	143nm	US	+	<i>E. coli</i>	Bulk <i>Thymus daenensis</i> oil or untreated sample	Moghimi et al. 2016a
O/W nano-emulsion	Sage oil ( <i>Salvia officinalis</i> ) 20% (w/w)	Tween80 and Span80	Water	None	222nm	US	+	<i>E. coli</i> <i>S. dysentery</i> <i>S. typhi</i>	Bulk sage oil or untreated sample	Moghimi et al. 2016b
O/W nano-emulsion	Soybean oil 25% (v/v)	Triton X-100 10% (v/v)	Water	CPC 1% (w/v)	168 nm	N.A.	+	<i>S. mutans</i>  <i>L. casei</i>	Chlorhexidine gluconate 0.12% (v/v) or untreated sample	Lee et al. (2010)
W/O nano-emulsion	<b>BCTP</b> Soybean oil 80% (w/w)  <b>BCTP 401</b> Soybean and peppermint oil 80% (w/w)	<b>BCTP</b> Tri- <i>n</i> -butyl phosphate and Triton X-100  <b>BCTP 401</b> Tri- <i>n</i> - butyl phosphate, Triton X-100, glycerol monosterate, refined soya sterols, Tween60	Water	CPC	400-800nm	N.A.	+	<i>B. cereus</i> spores  <i>B. circularis</i> spores  <i>B. megaterium</i> spores  <i>B. subtilis</i> spores	Different dilutions of BCTP and BCTP 401 (1:10, 1:100 and 1:1000)	Hamoud a et al. (1999)
W/O nano-emulsion	<b>8N8</b>	<b>8N8</b>	Water	<b>8N8</b>	400-800nm	N.A.	+	<i>E. coli</i>	Tris-EDTA	Hamoud

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	Soybean oil 64% (w/w)  <b>W60C</b> Soybean oil 20% (w/w)	Tri- <i>n</i> -butyl phosphate and Triton X-100  <b>W60C</b> Tween60, glycerol monooleate and refined soya sterols		None  <b>W60C</b> CPC 1% (w/w)				<i>Vibrio cholerae</i>  <i>S. typhimurium</i>	buffer solution	a & Baker (2000)
O/W nano-emulsion	Basil oil 6% (v/v)	Tween80	Water	None	29.3nm	US	+	<i>E. coli</i>	PBS	Ghosh et al. (2013)
O/W nano-emulsion	Mustard oil 6% (v/v)	Tween20	Water	None	18-430nm	Magnetic stirrer	+	<i>E. coli</i>	Untreated sample	Ghosh et al. (2012)
O/W nano-emulsion	Sesame oil 6% (v/v)	Tween20 or Tween80	Water	Euganol 1-6% (v/v)	20nm	US	+/-	<i>S. aureus</i>	PBS or sodium benzoate 0.3% (v/v)	Ghosh et al. (2014)
O/W nano-emulsion	<b>BCTP</b> Soybean oil 16% (v/v)  <b>BCTP-CPC</b> Soybean oil 16% (v/v)  <b>TEOP</b> Ethyl oleate 3% (v/v)	<b>BCTP</b> Tri- <i>n</i> -butyl phosphate, and Triton X-100  <b>BCTP-CPC</b> Tri- <i>n</i> -butyl phosphate, and Triton X-100  <b>TEOP</b> <i>n</i> -pentanol and Tween80	Water	<b>BCTP</b> Water  <b>BCTP-CPC</b> CPC 0.25% (w/v)  <b>TEOP</b> None	N.A.	US	+/-	<i>S. aureus</i>  <i>E. coli</i>  <i>L. monocytogenes</i>	Water, CPC solution 0.25% (w/v), tributyl phosphate solution, bulk soybean oil, Triton X-100, Tween80 or <i>n</i> -pentanol solution	Ferriera et al. (2010)
O/W nano-emulsion	<b>LMO</b> Lemon myrtle 5% (w/w)  <b>SBO</b> Soybean oil 16% (w/w)  <b>BCTP</b> Soybean oil 16% (w/w)	<b>LMO</b> Tween 80  <b>SBO</b> Tween80  <b>BCTP</b> Triton X-100, tributyl- <i>n</i> -phosphate	Water	None	97±2nm	MFZ	+/-	<i>E. coli</i>  <i>L. monocytogenes</i>  <i>S. Typhimurium</i>  <i>P. aeruginosa</i>  <i>B. cereus</i>	Coarse emulsion	Buranas uksomb at et al. (2011)

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O/W emulsion	Oregano oil 0.05 or 0.1% (w/w)	Tween80	Water	None	148nm	US	+	<i>L. monocytogenes</i> <i>S. Typhimurium</i> <i>E. coli</i> O157:H7	Water	Bhargha et al. (2015)
O/W emulsion	Clove or canola oil 10% (v/v) and a mixture at ratios of 1:9, 3:7 and 5:5 10% (v/v)	Tween80 and modified starch	Water	None	151.3-203.9nm	HPH	+/-	<i>L. monocytogenes</i> <i>S. aureus</i> <i>E. coli</i>	Nano-emulsion with canola oil with no clove oil	Majeed et al. (2016)
O/W micro-emulsion	Micelles of Tween20 0.6% (w/w)	Tween20	Water	Trans-cinnamaldehyde 0.2% (w/w)	127nm	HPH	+	<i>S. Typhimurium</i> <i>S. aureus</i> <i>E. coli</i> O157:H7	Water or Watermelon juice	Jo et al. (2015)
O/W nano-emulsion	Grindsted Acetem 90-50K 10-15% (w/w)	Tween60	Water	Cinnamaldehyde 3-10% (w/w)	79±2nm	HPH	-	<i>L. monocytogenes</i> <i>E. coli</i> O157:H7	Nano-emulsion without cinnamaldehyde	Bilbao-Sainz et al. (2013)
O/W nano-emulsion	D-limonene 4% (w/w)	Propylene glycol and Tween80	Water	Nisin 0%, 0.5, 1.5 or 3.0% (w/w)	16.34nm-18.92nm	CPI	+	<i>S. aureus</i> <i>B. subtilis</i> <i>E. coli</i> <i>S. cerevisiae</i>	Nutrient (bacteria), YPD broth (yeast) or of kanamycin sulphate (50 lg/ml) in broth	Zhang et al. (2014)
O/W nano-emulsion	Thyme 1% (w/v)	Sodium caseinate and lecithin	Water	None	82.5-125.5nm	HSH	+	<i>E. coli</i> O157:H7 <i>S. enterica</i> serovar Enteritidis <i>L. monocytogenes</i> Scott A	Bulk thyme oil or a mixture of water and milk	Xue et al. (2015)
O/W nano-emulsion	Hexane 10% (v/v)	Whey protein isolate	Water	Euganol 2% (v/v)	127-255 nm	HSH	-	<i>E. coli</i> O157:H7 <i>L. monocytogenes</i> Scott A	Untreated sample or eugenol (4.5 g/L) 2% reduced fat milk	Shah et al. (2013)
O/W nano-emulsion	Thyme oil 1% (w/w)	Propylene glycol and 1% sodium dodecyl sulfate	Water	None	279nm	HSH	+	<i>L. monocytogenes</i> Scott A <i>S. Enteritidis</i> <i>E. coli</i> O157:H7	Bulk thyme oil	Wu et al. (2014)

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O/W nano-emulsion	Euganol 5-12.5% (w/w)	Tween20	Water	None	50–110nm	US	+	<i>Fusarium oxysporum f. sp. vasinfectum</i>	Untreated sample	Abd-Elsalam et al. (2015)
O/W micro-emulsion	Micelles 1, 2, 3, 5, 7.5, and 10.0% (w/v)	Surfynol 485W or SDS or Tween20 or CG20 contains LAE 10% (w/v)	Water	Eugenol or carvacrol 0.01% to 8.0% (w/v)	Not mentioned	Magnetic stirrer	+	<i>E. coli</i> O157:H7 <i>S. enterica</i> serotype	Water	Ruengvi sesh et al. (2015)
O/W nano-emulsion	Sunflower oil 2-3% (w/w)	Tween20 and glycerol monooleate	Water	Carvacrol 2% (w/w), bergamot 3% (w/w), mandarin 3% (w/w) and lemon essential oils 3% (w/w)	133.4-176.4nm	HPH	+	<i>E. coli</i> O157:H7 <i>S. Typhimurium</i>	Untreated sample	Severino et al. (2015)
O/W nano-emulsion	Sunflower oil 2% (w/w)	Tween 20 and glycerol monooleate	Water	Mandarin essential oil 2% (w/w)	176.4 ± 14.5 nm	HPH	+	<i>L. innocua</i>	Untreated sample	Severino et al. (2014a)
O/W nano-emulsion	Sunflower oil 2-3% (w/w)	Tween 20 and glycerol monooleate	Water	Carvacrol 1% (w/w), bergamot 2% (w/w), mandarin 2% (w/w) and lemon essential oils 2% (w/w)	133.4-176.4nm	HPH	+	<i>L. monocytogenes</i> (5 strains)	Untreated sample	Severino et al. (2014b)
O/W nano-emulsion	Lemon, mandarin, oregano or clove essential oils 5% (w/w)	Glycerol monooleate or soy lecithin, whey protein isolate, pea proteins, Tween 20	Water	None	88-394nm	HPH	+	Endogenous flora of Rucola leaves	Untreated sample	Sessa et al. (2015)
O/W nano-emulsion	Lemongrass oil 0.5-4% (w/w)	Tween80 0.1, 0.5, 0.75 and 1% (w/w)	Water	None	56.5-87.6nm	HPH	+	<i>S. typhimurium</i> <i>E. coli</i> O157:H7	Untreated sample	Kim et al. (2013)
O/W nano-emulsion	Thyme and corn oil 10% (w/w)	Tween 80 or Tween and LAE	Water	None	<200nm	HPH	+	<i>Z. bailii</i>	Thyme or corn oil nano-emulsion with no LAE	Chang et al. (2015)

**Table 2.** Percentage of PI positive (dead) bacterial cells measured by flow cytometry at 0, 2 and 7-day incubation at 25°C. The O/W emulsions were prepared with 40% oil phase and stabilised with 8% Tween80 in the continuous phase (M9 minimal growth medium) in the presence or absence of bacteria. Results are taken from a minimum of 2 independent experiments.

	Sample	Day 0	Day 1	Day 7
<i>E. coli</i> (MG1655)	M9 medium	1±0 <sup>a</sup>	0.3±0 <sup>abc</sup>	0.15±0.07 <sup>c</sup>
	Coarse emulsion	1.15±0.35 <sup>ab</sup>	0.65±0.21 <sup>ac</sup>	0.2±0 <sup>c</sup>
	Nano-emulsion	0.75±0.21 <sup>abc</sup>	0.65±0.21 <sup>ac</sup>	0.25±0.07 <sup>c</sup>
<i>E. coli</i> (BW2115)	M9 medium	1.15±0.07 <sup>d</sup>	1.2±0 <sup>d</sup>	3.95±0.21 <sup>a</sup>
	Coarse emulsion	0.8±0.14 <sup>d</sup>	0.55±0.07 <sup>d</sup>	5.45±0.63 <sup>b</sup>
	Nano-emulsion	0.6±0 <sup>d</sup>	0.6±0.14 <sup>d</sup>	2.3±0.14 <sup>c</sup>
<i>E. coli</i> (JM109)	M9 medium	2.2±0 <sup>a</sup>	0.7±0.28 <sup>c</sup>	0.4±0 <sup>c</sup>
	Coarse emulsion	1.4±0.14 <sup>b</sup>	1±0.14 <sup>bc</sup>	0.55±0.07 <sup>c</sup>
	Nano-emulsion	1.3±0.14 <sup>b</sup>	0.45±0.07 <sup>c</sup>	0.4±0.14 <sup>c</sup>
<i>E. coli</i> (MC4100)	M9 medium	3.95±0.35 <sup>ab</sup>	5.2±1.27 <sup>ab</sup>	8.4±0.28 <sup>a</sup>
	Coarse emulsion	3.55±0.21 <sup>ab</sup>	3.2±0.85 <sup>ab</sup>	7.95±0.35 <sup>a</sup>
	Nano-emulsion	2.75±0.49 <sup>ab</sup>	2.6±0 <sup>b</sup>	6.55±3.6 <sup>ab</sup>
<i>E. coli</i> (PHL644)	M9 medium	0.3±0.14 <sup>acde</sup>	0.4±0.14 <sup>ade</sup>	0.3±0.14 <sup>acde</sup>
	Coarse emulsion	0.3±0.14 <sup>acde</sup>	0.15±0.07 <sup>e</sup>	0.3±0.14 <sup>acde</sup>
	Nano-emulsion	0.15±0.07 <sup>de</sup>	0.15±0.07 <sup>e</sup>	0.15±0.07 <sup>de</sup>
<i>B. cereus</i>	M9 medium	2.35±0.07 <sup>a</sup>	8.1±1.27 <sup>b</sup>	18.7±6.93 <sup>c</sup>
	Coarse emulsion	2.7±0 <sup>a</sup>	8.35±1.06 <sup>b</sup>	22.1±2 <sup>cd</sup>
	Nano-emulsion	2.95±0.35 <sup>a</sup>	6.9±0.42 <sup>b</sup>	30.75±0.63 <sup>d</sup>
<i>S. epidermidis</i>	M9 medium	0.1±0 <sup>a</sup>	0.1±0 <sup>a</sup>	0±0 <sup>c</sup>
	Coarse emulsion	0.05±0.07 <sup>b</sup>	0.1 ±0 <sup>a</sup>	0±0 <sup>c</sup>
	Nano-emulsion	0.1±0 <sup>a</sup>	0.1±0 <sup>a</sup>	0±0 <sup>c</sup>

The data was analysed with one-way ANOVA

<sup>a</sup> means ± standard deviation with different letters are significantly different

**Figure 1.** Changes in log CFU/ml of *E. coli* (MG1655) (A), *E. coli* (BW2115) (B), *E. coli* (JM109) (C), *E. coli* (MC4100) (D), *E. coli* (PHL644) (E), *B. cereus* (F) and *S. epidermidis* (G) within M9 minimal growth medium (control), nano-emulsion or coarse emulsion at day 0, 2, and 7 incubated at 25°C. The O/W emulsions were prepared with 40% oil phase and stabilised with 8% Tween80 in the continuous phase (M9 minimal growth medium). Bars represent mean  $\pm$  SEM taken from a minimum of 2 independent experiments. The data was analysed with one-way ANOVA.

**Figure 2.** Photomicrographs composed from the optical and fluorescence images of *E. coli* (MG1655) (A), *S. epidermidis* (B) and *B. cereus* (C) within coarse emulsion and nano-emulsion at the end of the incubation period (7 days). The O/W emulsions were prepared with 40% oil phase and stabilised with 8% Tween80 in the continuous phase. Scale bar: 10 $\mu$ m.

**Figure 3.** Changes in log CFU/ml of *L. acidophilus* (A), *E. coli* (MG1655) (B), *E. coli* (BW2115) (C), *E. coli* (JM109) (D), *E. coli* (MC4100) (E), *E. coli* (PHL644) (F), *B. cereus* (G) and *S. epidermidis* (H) within broth (control), nano-emulsion or coarse emulsion over 24 or 48 hours relative to hour 0 incubation at 30°C. The O/W emulsions were prepared with 40% oil phase and stabilised with 8% Tween80 in the continuous phase (tryptic soy broth). Bars represent mean  $\pm$  SEM taken from a minimum of 2 independent experiments. The data was analysed with one-way ANOVA.

**Figure 4.** Photomicrographs composed from the optical and fluorescence images of *E. coli* (MG1655) (A), *S. epidermidis* (B), *B. cereus* (C) and *L. acidophilus* (D) within coarse emulsion and nano-emulsion the end of the incubation period. The O/W emulsions were prepared with 40% oil phase and stabilised with 8% Tween80 in the continuous phase. Scale bar: 10 $\mu$ m.

**Figure 5.** Log CFU/ml of *E. coli* (MG1655) (A) and *S. epidermidis* (B) in bulk oil (control), nano-emulsion (NE), or coarse emulsion after 1, 8 and 24 hours incubated at 25°C. The O/W emulsions were prepared with 20% oil phase containing no or 0.5% CA and stabilised with 1 or 8% Tween80 in M9 minimal growth medium (continuous phase). The bulk oil was prepared from 20% oil phase containing 0, 0.5 and 1% CA and M9 minimal growth medium. Bars represent mean  $\pm$  SEM taken from a minimum of 3 independent experiments. Mean values with different letters are significantly different ( $P < 0.05$ ). The data was analysed with one-way ANOVA. Abbreviations: NE, nano-emulsion; CA, caprylic acid.

**Figure 6.** The mean diameter size ( $\mu$ m) of the oil droplets by light scattering [D (4, 3)] of *E. coli* (MG1655) (A) and *S. epidermidis* (B) nano-emulsion (NE) or coarse emulsion at 0 and 24 hours incubated at 25°C. The O/W emulsions were prepared with 20% oil phase containing 0.5% CA and stabilised with 1% or 8% Tween80 in M9 minimal growth medium (continuous phase) with or without bacteria. Abbreviations: NE, nano-emulsion; CA, caprylic acid.

Figure 1  
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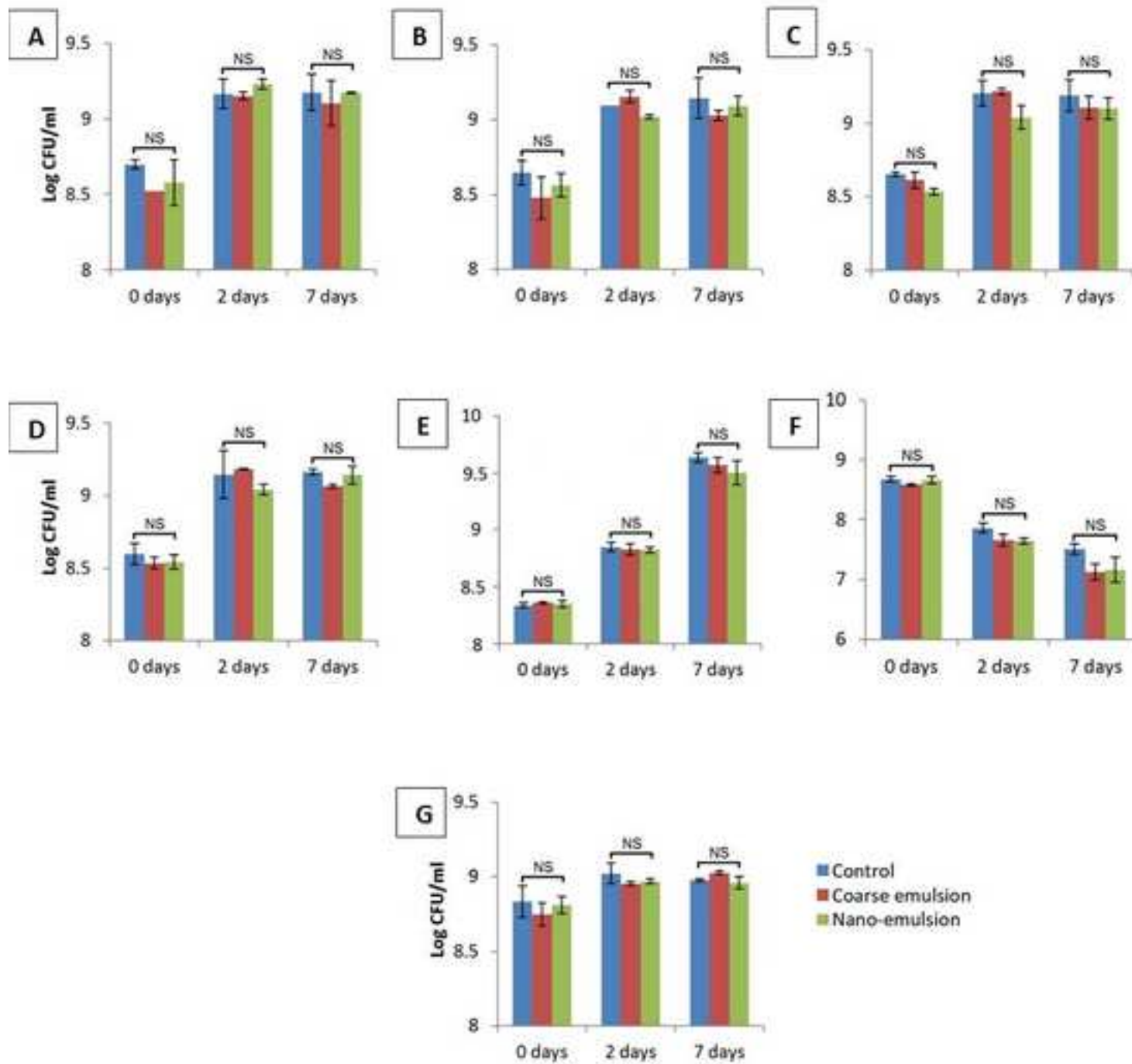


Figure 2  
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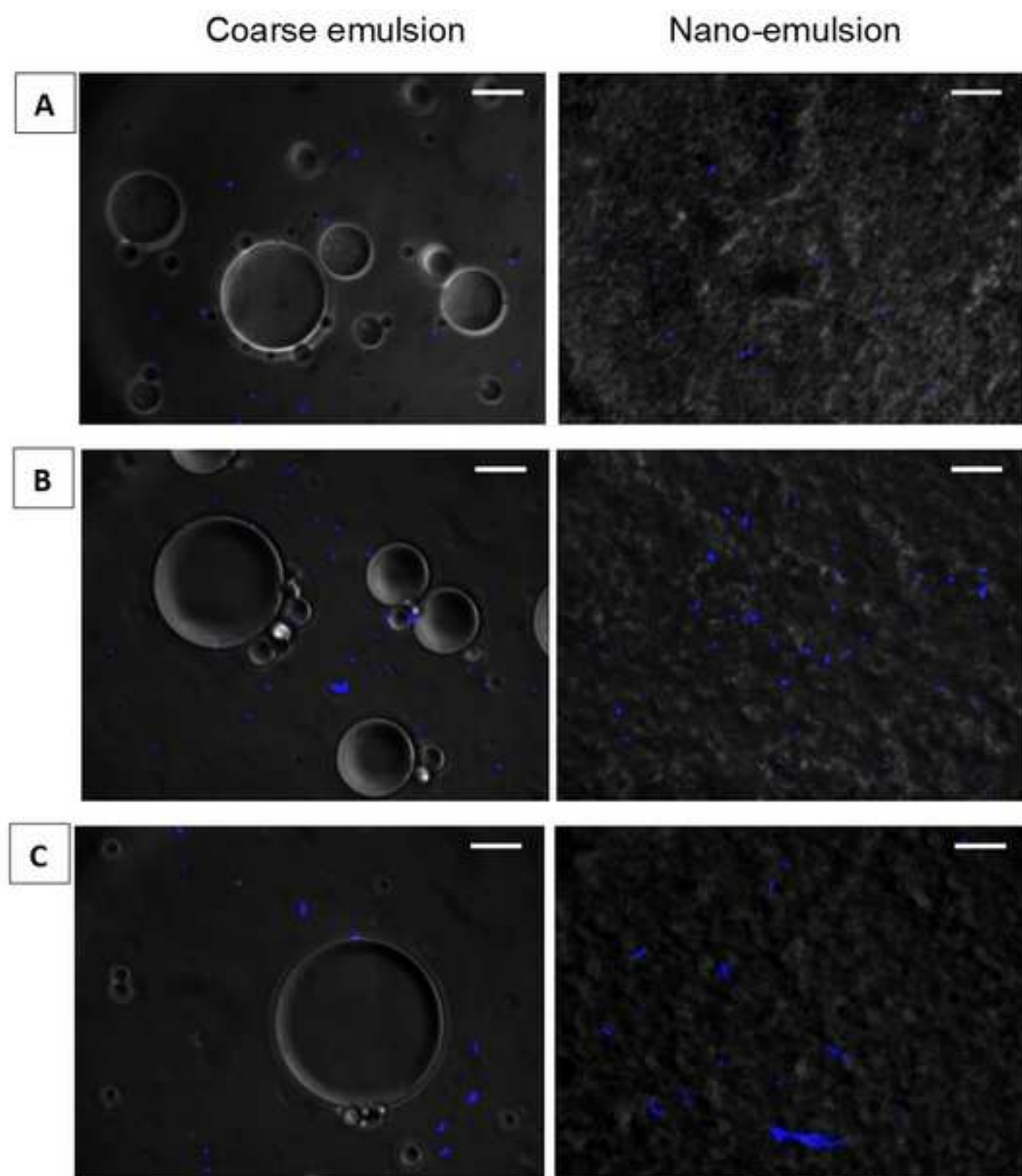




Figure 3  
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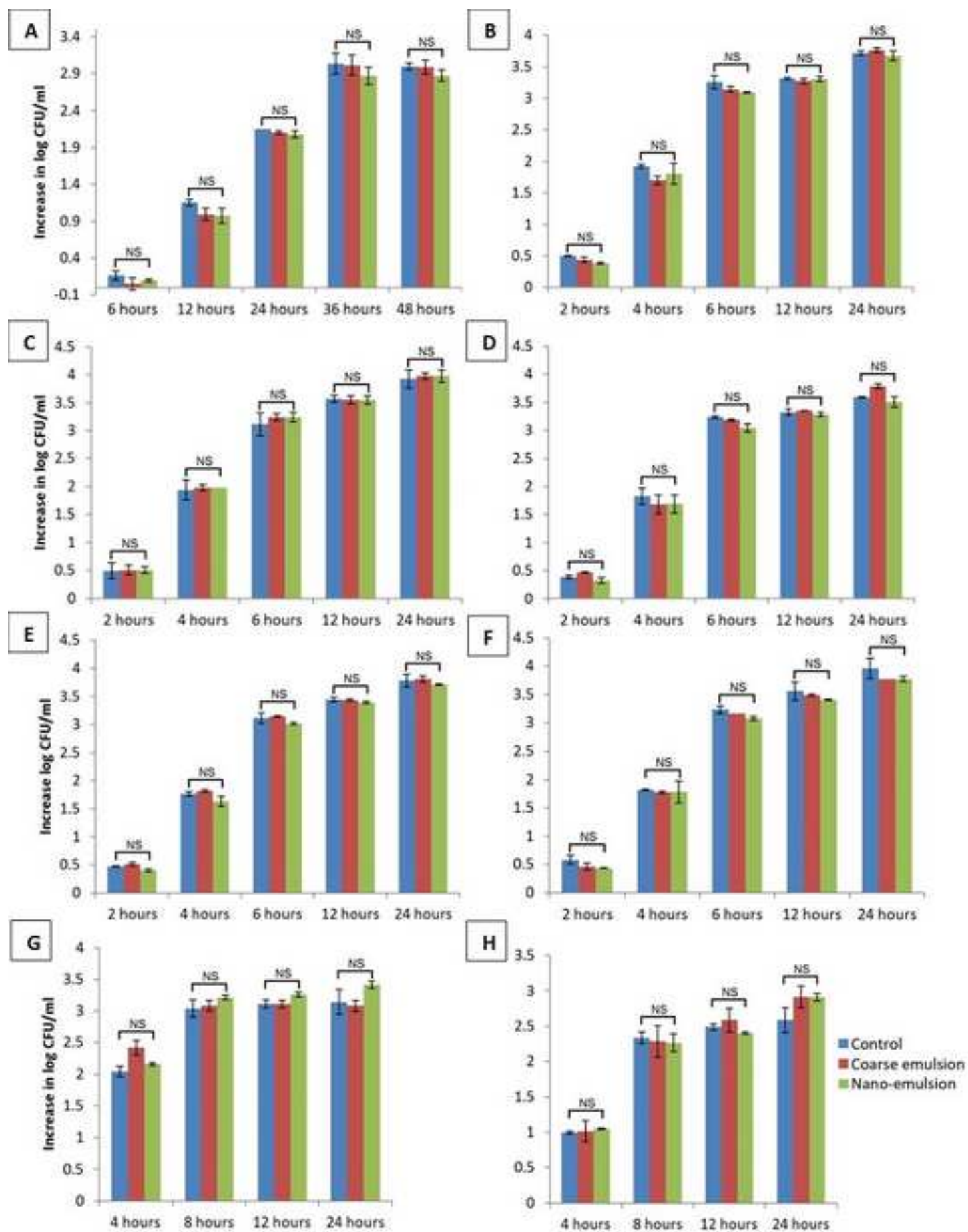
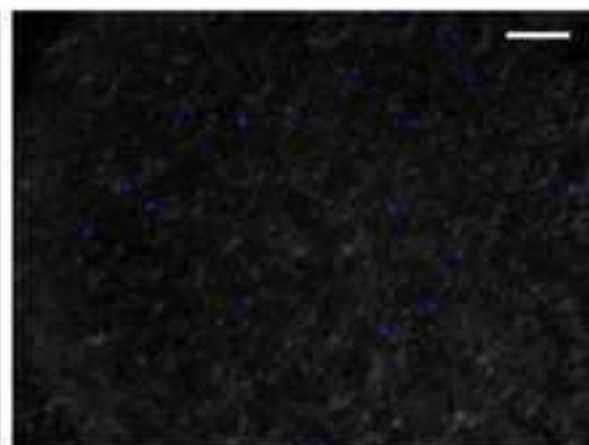
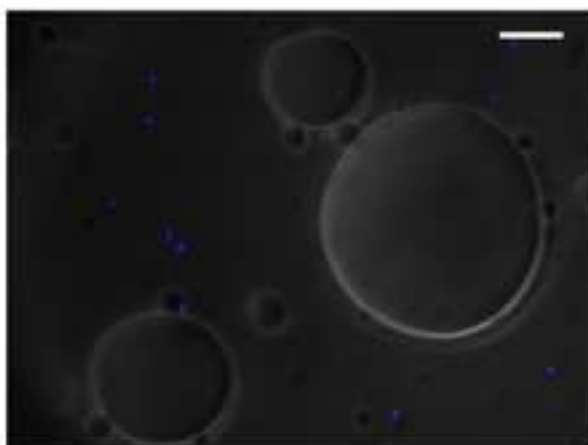


Figure 4

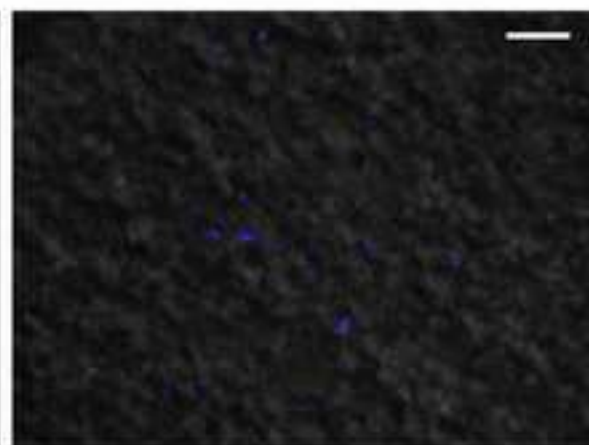
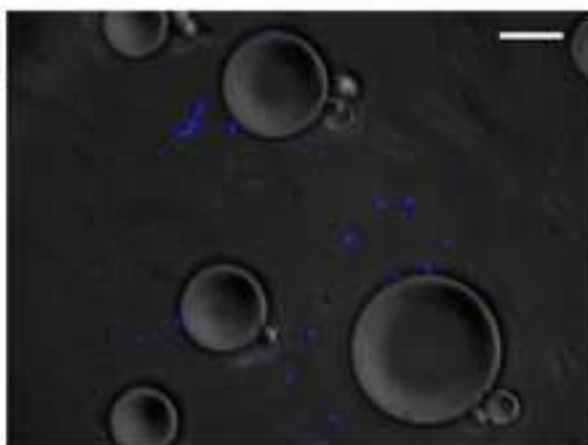
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Coarse emulsion

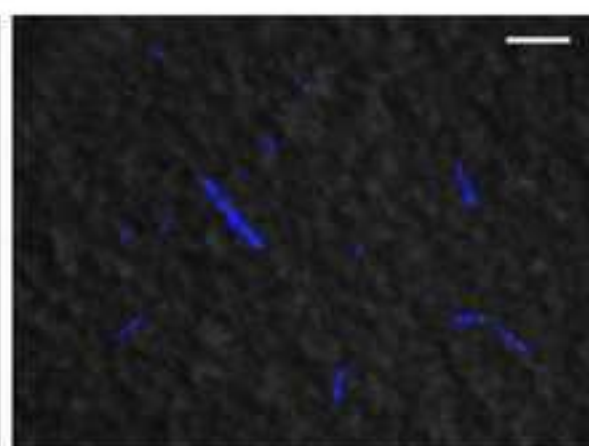
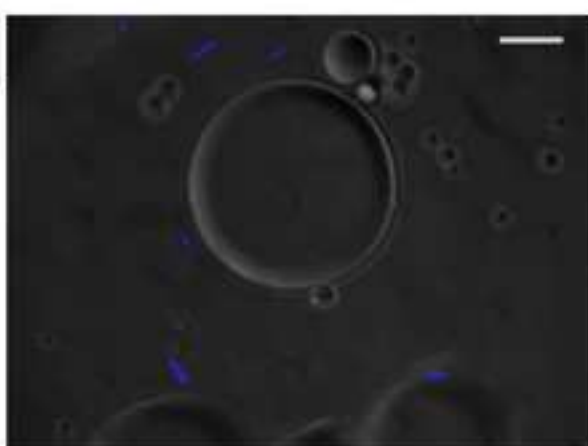
Nano-emulsion



**B**



**C**



**D**

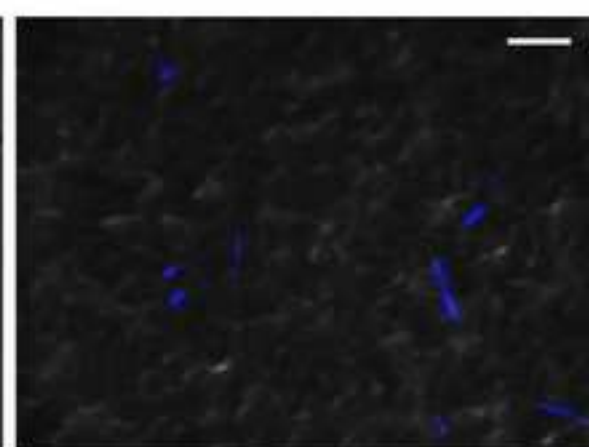
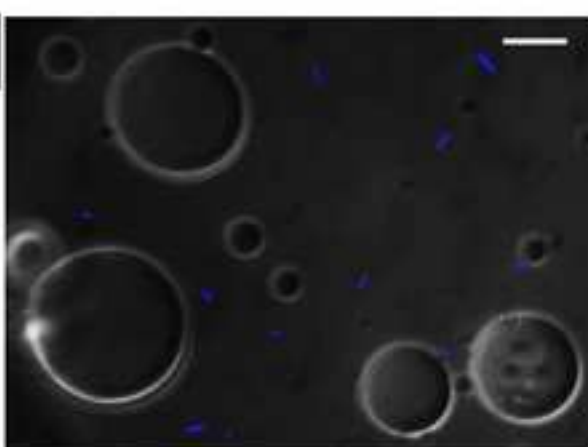


Figure 5  
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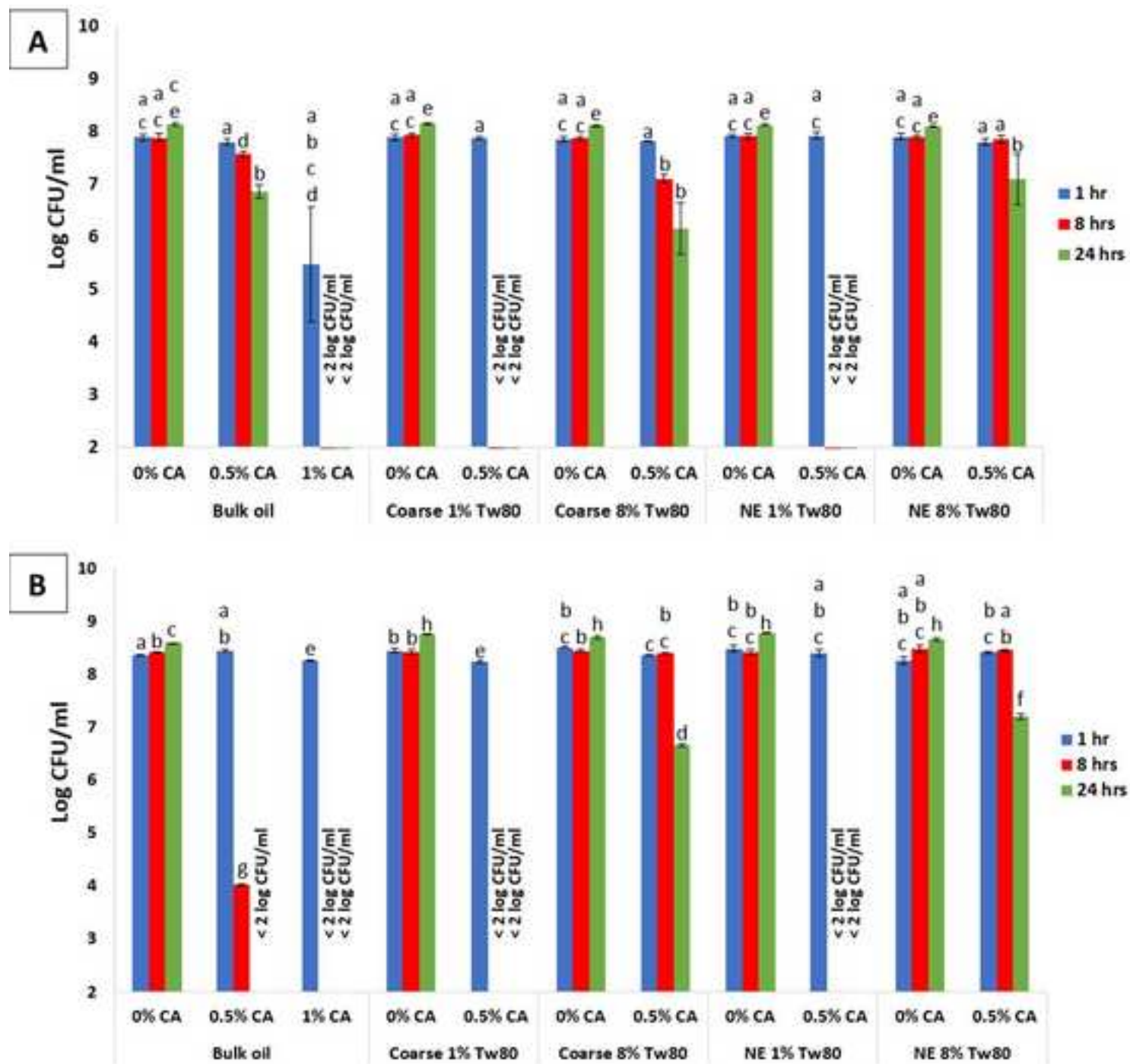
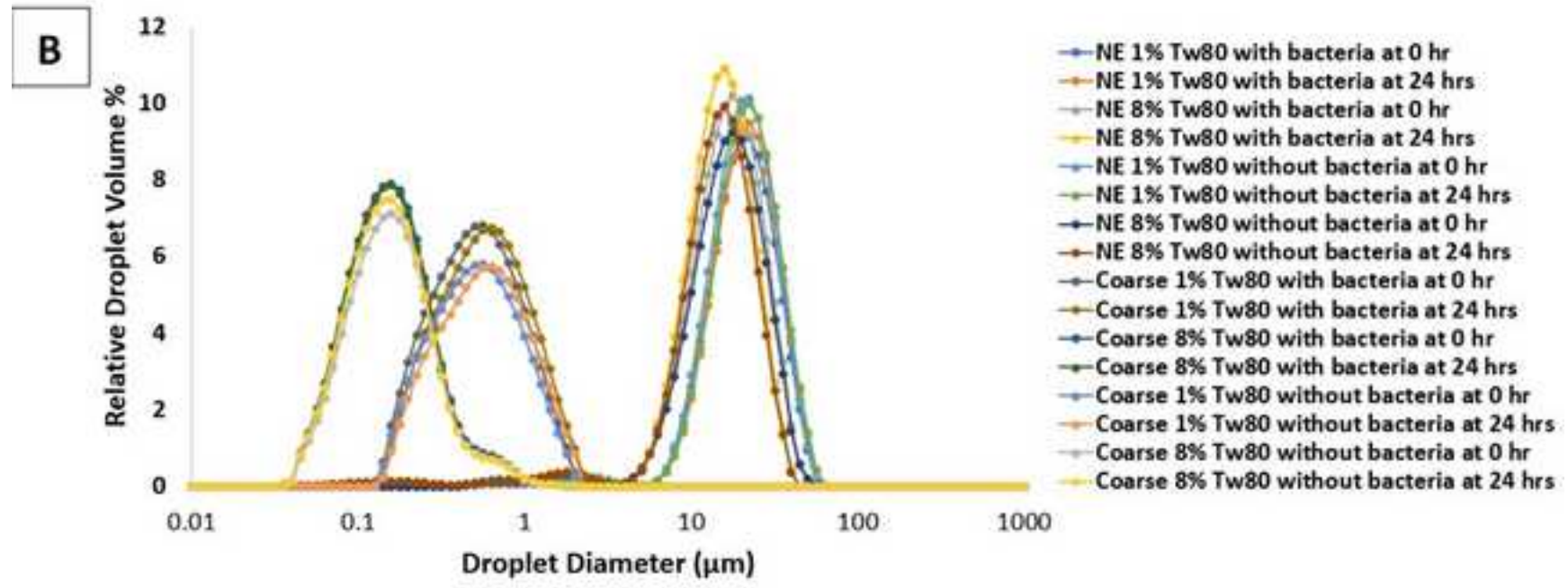
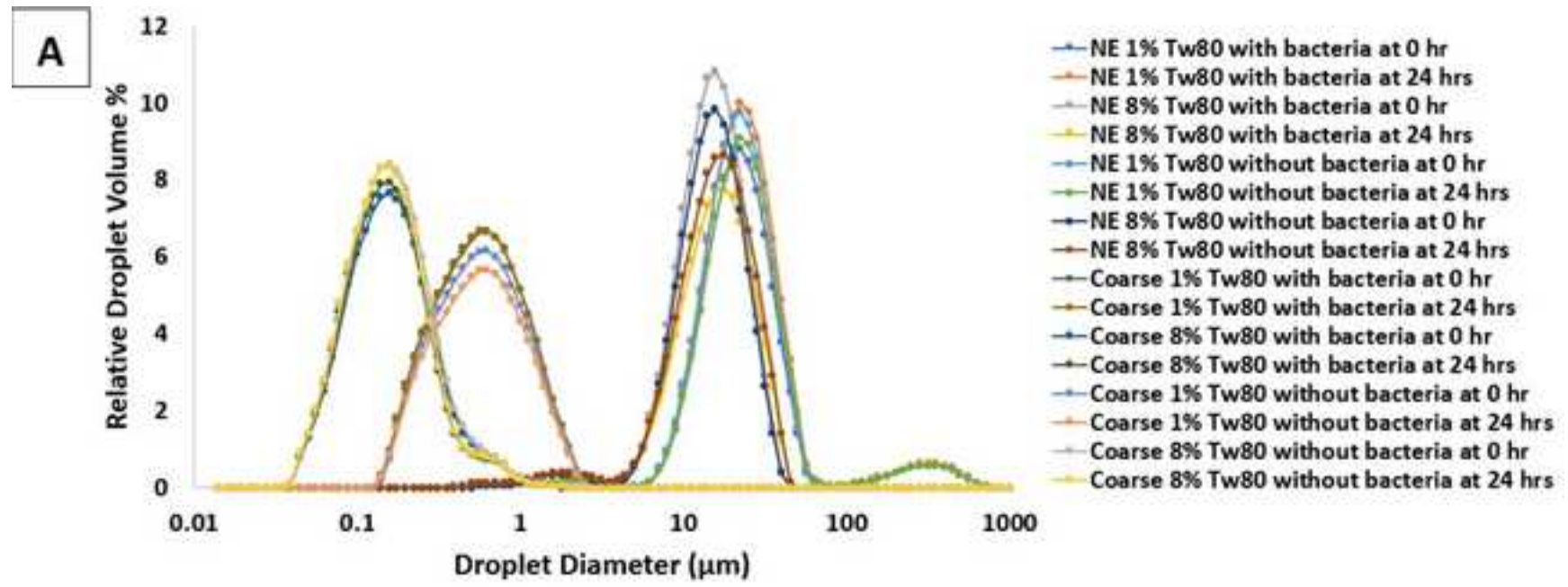


Figure 6  
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**Supplementary material**

[Click here to download Supplementary material for online publication only: Supplementary.DOCX](#)



