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1 **The composition and oxidative stability of vegetarian omega-3 algal oil nanoemulsions**

2 **suitable for functional food enrichment**

3 **(Running title: Composition and oxidative stability of omega-3 algal oil nanoemulsions)**

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12

13 **Abstract**

14 **Background**: Long chain omega-3 polyunsaturated fatty acid (LCn3PUFA) nanoemulsion

15 enriched foods offer potential to address habitually low oily fish intakes. Nanoemulsions

16 increase LCn3PUFA bioavailability, but may cause lipid oxidation. This study examined

17 oxidative stability of LCn3PUFA algal oil-in-water nanoemulsions created by ultrasound using

18 natural and synthetic emulsifiers during 5-weeks of storage at 4, 20 and 40°C. Fatty acid

19 composition, droplet size ranges and volatile compounds were analysed.

20 **Results**: No significant differences were found for fatty acid composition at various

21 temperatures and storage times.

22   Lecithin nanoemulsions had significantly larger droplet size ranges at baseline and during

23   storage regardless of temperatures. While combined Tween 40 and lecithin nanoemulsions

24   had low initial droplet size ranges, there were significant increases at 40°C after 5-weeks

25   storage. Gas chromatograms identified hexanal and propanal as predominant volatile

26   compounds, along with 2-ethylfuran; propan-3-ol; valeraldehyde. The Tween 40 only

27   nanoemulsion sample showed formation of lower concentrations of volatiles compared to

28   lecithin samples. Formation of hexanal and propanal remained stable at lower temperatures

29   although higher concentrations were found in nanoemulsions than bulk oil. The lecithin only

30   sample had formation of higher concentrations of volatiles at increased temperatures despite

31   having significantly larger droplet size ranges than the other samples.

32   **Conclusions**: Propanal and hexanal were the most prevalent of five volatile compounds

33   detected in bulk oil and lecithin and/or Tween 40 nanoemulsions. Oxidation compounds

34   remained more stable at lower temperatures indicating suitability for enrichment of

35   refrigerated foods. Further research to evaluate the oxidation stability of these systems

36   within food matrices is warranted.

37

42

1   Introduction

Oily fish consumption continues to fall short of recommended levels in Western populations, causing potential implications for overall health and increased risk of other non-communicable diseases [1-3]. The use of long chain omega-3 polyunsaturated fatty acid (LCn3PUFA) rich oil-in-water nanoemulsion enriched foods offers a potential solution to this problem, particularly for population groups that consume little or no oily fish such as vegetarians and vegans.

New approaches, including food-based strategies have the potential to improve intakes and LCn3PUFA nanoemulsion enriched functional foods may offer an alternative solution for groups with low habitual oily fish consumption [4, 5]. Eicosapentaenoic acid (20:5 n3; EPA) and docosahexaenoic acid (22:6 n3; DHA) are thought to be the most beneficial forms of LCn3PUFA [6, 7]. The European Food Safety Agency [8] have approved health claims in relation to foods naturally rich or fortified with EPA and DHA. Microalgal oils have recently emerged to offer a sustainable alternative source of EPA and DHA that is also suitable for vegetarians and vegans [9-11].

Nanoemulsions, similar to conventional oil-in-water emulsion systems but with considerably smaller droplet sizes can be used to incorporate lipid based components into aqueous foods [12]. The use of nanoemulsions offers potential benefits including increases in water dispersibility of oils, good physical and chemical stability and improved bioavailability of various hydrophobic lipid components [12-14]. However, utilisation of LCn3PUFA rich oils in nanoemulsions may further promote lipid oxidation in these already vulnerable oils.

67 EPA and DHA are characterised by long carbon chain lengths and a high degree of lipid

68 unsaturation, which increases their susceptibility to oxidation when exposed to air, light and

69 heat, all of which are inevitable during emulsion processing. The mechanism of lipid oxidation

70 is proposed to include three stages initiation, propagation and termination [15]. A high variety

71 of volatile compounds of different polarity, stability and small molecular weight are formed

72 which can be detected by dynamic headspace analysis [16]. Once the initiation phase has begun

73 the rate of oxidation increases exponentially and foods are quickly spoiled. Lipid oxidation in

74 enriched/functional foods impacts the shelf-life, safety, nutritional value, functionality and

75 flavour of the subsequent food products [17]. The creation of LCn3PUFA nanoemulsions may

76 further increase the oxidation susceptibility of these oils due to high droplet surface areas

77 and the need for greater processing.

78

79 In previous work, novel oil-in-water nanoemulsion systems suitable for functional food

80 enrichment were successfully created using ultrasound processing with commercially

81 available high DHA vegetarian algal oil loads up 50% (w/w), which has not been previously

82 achieved [18]. The addition of a 50% (w/w) system is less likely to have a detrimental effect on

83 food matrices than systems with lower oil loads as lower volumes of nanoemulsion can be

84 added to achieve optimum enrichment levels [19]. The systems were also demonstrated to

85 significantly increase the bioavailability of total LCn3PUFA and DHA in a pilot randomised

86 crossover trial [14], although additional larger, longer studies are needed to further ratify these

87 findings. Preliminary evaluation to analyse total oxidation values (totox) indicated these

88 systems remained within safe ranges when stored at 4°C for 37 days [18]. However, there were

89 significant detrimental changes in the sensory properties of yogurt when the nanoemulsion

90 systems were used as a fortification vehicle, which may indicate the presence of volatile

4

91 oxidation compounds [20]. A further 16-day shelf life sensory evaluation of 50% (w/w) algal oil

92 and lecithin nanoemulsion enriched strawberry yogurt indicated that detrimental sensory

93 changes were detected after 2 days of storage but significant improvements to several

94 sensory attributes were found after 16 days of refrigerated storage, which warrants further

95 investigation [21].

96

97 Lecithin, a zwitterionic natural emulsifier has good emulsifying properties due to its molecular

98 structure, which has hydrophilic and lipophilic groups and a hydrophilic-lipophilic balance

99 (HLB) of 8 making it well suited to the successful creation of LCω3PUFA algal oil and marine

100 based oil nanoemulsions [22]. Lecithin has been used successfully to create physically stable

101 LCn3PUFA nanoemulsion systems in various previous studies [16, 19, 23-25] and has been found to

102 increase oxidative stability of emulsions whilst maintaining physical stability [26]. The use of

103 Tween 40 in the creation of LCn3PUFA nanoemulsions has also previously been demonstrated

104 to have a protective effect on lipid oxidation [27-29]. However, a study to evaluate the oxidation

105 stability of high load DHA algal oil lecithin and Tween 40 nanoemulsions using more

106 comprehensive methods has yet to be undertaken. Therefore, the aim of the current study

107 was to evaluate the oxidative stability of algal oil nanoemulsions created with ultrasound

108 using lecithin and Tween 40 solely and in combination to maximise their physical and chemical

109 stabilising properties, which coupled with the gas chromatography headspace method is a

110 novel and useful study. The oxidative stability of algal oil was compared in bulk form and

111 nanoemulsion under the same conditions with no other added components other than

112 deionised water and the two types of emulsifier.

113

114 **2   Materials and Methods**

2.1 Materials

Algal oil (Life DHATM S35-O300) was purchased from DSM Ltd., (Columbia, USA). The algal oil used in this study is a commercially available product that contains added antioxidants (tocopherols (0.025%) and ascorbyl palmitate (0.025%) [30]). L-α-Phosphatidylcholine (P3644-100G) of soybean and Type IV-S. ≥30% (enzymatic), Polyoxyethylenesorbitan monopalmitate (Tween 40, P1504) were purchased from Sigma-Aldrich, UK. Sodium chloride (99.5%) was purchased from ACROS, Spain. Hexane (HPLC Grade) was purchased from Fisher Scientific, (UK). Methanol (HPLC Grade), Sulphuric acid 95%. Sodium sulphate anhydrous were purchased from VER BDH PROLABO chemicals, EC (UK). Distilled and deionized water was added to all nanoemulsions.

2.1.1 Preparation of nanoemulsion samples

The 50% (w/w) oil-in-water nanoemulsions of LCn3PUFA algal oil were prepared by following the method developed by Lane et al, [19], in which 6% (w/w) of the selected emulsifiers i.e. lecithin, Tween 40, and equal ratio Tween 40 and lecithin (3% w/w of each) were used. A solution of lecithin premix containing algal oil (70g) combined with lecithin (30g) was placed in a water bath at 55°C for 2 hours to ensure the lecithin was completely dissolved. Tween 40 premixes containing deionised water (44g) mixed with Tween 40 (6g) were prepared and placed in a water bath at 55°C.

Appropriate measures of algal oil (36g) and water (44g) were combined with the lecithin premix (20g) to create the lecithin samples. Measures of algal oil (50g) were combined with 50g of Tween 40 premix to create the Tween 40 samples.

137 Both emulsion premixes (10g lecithin premix, 22g Tween 40 premix) were combined with algal

138 oil (43g) and deionised water (25g) to give a 50% algal oil sample with a combination of

139 lecithin and Tween 40 containing 3% w/w of each emulsifier.

140

141 After premixing, the coarse emulsions were replaced in the water bath for a further 2 hours

142 and hand stirred for 1 min at 30 min intervals. The temperature was controlled at 55°C.

143 Samples underwent primary homogenisation using a L5 series Silverson rotor–stator mixer

144 (Silverson Machines Ltd, England), on a medium setting (668.12 x g) for 2 min, then processed

145 under an ultrasonic processor (BSP-1200 Ultrasonic processor, New York, USA) using

146 Amplitude 100% with power 850w, operated at 19650Hz for 10 minutes to create

147 nanoemulsions. A cold-water cooling jacket was used to ensure sample temperatures

148 remained below 55°C.

149

150 **2.1.2   Fatty acid composition analysis by Gas Chromatography (GC)**

151 The fatty acid composition analysis of algal oil and nanoemulsion samples was performed

152 using fatty acid methyl ester (FAME) analysis whereby 0.5000 g algal oil /1.000 g of

153 nanoemulsion sample and 10 ml Reagent A (2.5% w/v KOH solution in Methanol) were added

154 into a MARSXpress vessel microwave digestion tube, then closed. The tube was placed into

155 the Kevlar sleeves of a Mars 6 microwave (CEM Ltd., UK). The temperature was increased to

156 90°C in 5 min and held for 10 min. After cooling to room temperature, 15ml reagent B (2%

157 sulphuric acid v/v in methanol) was added to the tube.

158 After closing, the tube was placed back into the Kevlar sleeves of the Mars 6 microwave and

159 the temperature was increased to 120 °C for 6 min. After cooling to room temperature, 10 ml

160 Hexane was added to the tube and inverted once.

161

162 The sufficient saturated salt solution was added to bring the hexane to the top layer. The

163 upper hexane layer containing fatty acid methyl esters was obtained for GC analysis using a

164 GC Clarus 480. 200μl of the upper hexane layer containing fatty acid methyl esters and 800μl

165 hexane was added into the GC vial with a small amount of added anhydrous sodium sulphate.

166 The samples were analysed by GC Clarus 480 system (PerkinElmer Inc, USA) equipped with an

167 auto sampler, Flame Ionization Detector, 30 m, 0.25 mm id 0.25 μm film thickness GC capillary

168 column (SGE Analytical Science Pty Ltd, Australia) and Total Chrom Navigator software system

169 (Version 6.3.2 PerkinElmer Inc, USA). The injector and detector temperature were 220°C and

170 250°C respectively, 1.5 μl of sample was injected in each time and hydrogen flow rate was set

171 at 8.4 psi. The temperature program for the column was increased from 60 to 170°C at a rate

172 of 20°C/min and to 200°C at a rate 1 °C/min, holding 1 min; the total run time was 36.5 min.

173 Fatty acids were identified by reference to the retention time of standards. Analysis was

174 performed in triplicate on individual vials for each time point.

175

176 **2.1.3 Measurement of emulsion droplet size**

177 Nanoemulsions are classed as systems with droplet sizes ranging from 50 to 500 nm [31, 32]. The

178 droplet size of emulsion samples prepared was determined by Mastersizer 3000 laser light-

179 scattering analyzer (Malvern Instruments Ltd, Malvern, UK) with a small sample dispersion

180   unit set 2400 rpm. For the emulsion samples, an absorption parameter value of 0.001 was

181   selected and a refractive index ratio 1.488 for algal oil [19]. For the purposes of this study, Sauter

182   mean ($d_{32} = \sum n_i d_i^3 / \sum n_i d_i^2$ [33]) has been reported as it reflects the surface diameter

183   average value and the droplet size distribution and has been used in a number of previous

184   studies [19, 34].

185

2.1.4   Lipid oxidation compound analysis: Gas Chromatography Headspace Analysis (GCHS)

187   Gas chromatography (GC) was performed using 2 g nanoemulsion samples prepared with 1

188   ml 1% NaCl solution, added to HS vial and vortexed for 30 secs. The samples were heated in

189   a Headspace (HS) sampler (TuborMatrix 40 PerkinElmer Inc, USA) at 100°C for 60 min and

190   injected under the following conditions: vial pressure 30psi; pressurise time 0.2 min; needle

191   temp 100 °C; injection time 4.8 sec; withdrawal time 6 sec.

192   To account for possible production of volatile compounds during heating, peak areas for

193   heating times of 20, 40 and 60 min at 100°C were compared and 60 min chosen as this gave

194   the most consistent peak composition results. The samples were analysed by Gas

195   Chromatography Headspace Analysis (GCHS) Clarus 580, (PerkinElmer Inc, USA) equipped

196   with a Flame Ionization Detector and 60 m 0.32 diameter column, 1.8 µm film thickness

197   (Agilent Technologist, USA) under the conditions: Hydrogen flow rate 17 psi; injector

198   temperature 230°C; detector temperature 230°C; oven temperature: from 40°C, ramp to

199   230°C at 20°C /min and hold at 230°C for 1 min, total time was 10.5 min. The volatile

200   compounds were identified by reference to the retention time of standards. Analysis was

201   performed in triplicate on individual vials for each time point.

202   Values for volatile compounds were determined by measuring the GC peak area to give

203   quantitative measurements compatible for statistical analysis as demonstrated in previous

204   studies [35, 36].

205   **2.1.5   The storage trial for algal oil and algal oil emulsion**

206   The algal oil and algal oil nanoemulsions were stored in the dark in single chamber incubators

207   set at 4 °C, 20 °C and 40 °C for 5-weeks. The droplet sizes and volatile compounds were

208   determined at baseline, week 1, week, 2 and week 5. Fatty acid composition was analysed for

209   the bulk oil at baseline and bulk oil and nanoemulsions during week 1 and week 5 of the

210   storage trial.

211

212   **2.1.6   Experimental design and data analysis**

213   All measurements were performed in duplicate or triplicate. Results are reported as the mean

214   ± the standard deviation where applicable. Statistical analysis was completed using IBM SPSS®

215   24.0, (SPSS Inc. Chicago, USA). Significant differences were identified ($p < 0.05$) by two-way

216   analysis of variance (ANOVA) with a Tukey post hoc test at confidence intervals of 95%.

217

218   **3.   Results and discussion**

219   **3.1   Fatty acid composition**

220   Changes in fatty acid profiles were monitored at baseline and during the storage trial. Fatty

221   acid composition levels for bulk oil were of a similar level to the manufacturer's specification

222   in Table 1. The linoleic acid (18:2 n6; LA) composition of samples containing lecithin was

223   increased in comparison to the bulk oil and Tween 40 only samples due to the fatty acid

224   composition of lecithin which is abundant in LA and contains small amounts of the other main

225   fatty acids that were measured [37].

226   Two-way ANOVA testing revealed no significant differences for overall fatty acid composition

227   when the results from the bulk DHA algal oil (baseline) were compared with the lecithin (LN),

228   lecithin and Tween 40 (LTN) and Tween 40 (TN) nanoemulsions during storage. No significant

229   differences in the percentage of DHA for sample, temperature and storage period, indicates

230   that there was no sign of DHA degradation in samples throughout the storage trial. However,

231   minor DHA degradation in samples may not have been detected within the storage timescales

232   using this method.

233

234   All of the nanoemulsion samples showed small non-significant reductions for percentage DHA

235   composition in comparison to the unprocessed oil with some more noticeable decreases at

236   the higher temperatures after 1 week of storage. In addition, small non-significant decreases

237   were found for LA in samples containing lecithin at higher temperatures. This indicates that

238   small amounts of oxidation products could have been created during ultrasound processing

239   and that accelerated temperatures may have further promoted the production of volatile

240   compounds although this was not significant [38]. Relatively small amounts of oxidation

241   products can have a detrimental effect on the flavour of LCn3PUFA enriched foods [17]. The

242   small though non-significant LA and DHA losses for the nanoemulsion samples could explain

243   previous detrimental changes to the sensory profiles of algal oil nanoemulsion enriched

244   yogurt [20]. The results of this study demonstrate that following the initial non-significant fatty

245   acid composition reductions for nanoemulsions created using ultrasound, DHA composition

246   remained relatively stable during storage at lower temperatures, which is comparable to

247   other work in the field [28].

248   **3.2   Emulsion Droplet Sizes**

249   The droplet size ranges of the nanoemulsion samples were measured by laser light scattering

250   particle sizer at baseline and intervals during storage at the different temperatures. The mean

251   droplet sizes and droplet ranges of the nanoemulsions prepared with lecithin, Tween 40 and

252   in combination are shown in Figures 1 and 2. At baseline the nanoemulsions prepared with

253   Tween 40 (TN) and lecithin and Tween 40 combined (LTN) showed significantly smaller ($p <$

254   0.05) droplet sizes (242 ± 0.002 nm and 172 ± 0.002 nm respectively) than those prepared

255   with lecithin alone (LN) (340 ± 0.001 nm). Statistical analysis using two-way ANOVA testing

256   revealed the lecithin samples had significantly larger droplet ranges throughout the 5-week

257   storage period at all temperatures ($p < 0.05$).

258

259   The combined LTN sample had the smallest droplet size ranges at baseline; however, there

260   were significant increases in droplet sizes from baseline and 5-weeks at 20 and 40°C ($p < 0.05$).

261   Larger droplet ranges for lecithin samples in this study may be explained by the molecular

262   weight of lecithin, which is greater than that of Tween 40 [39, 40]. The sole use or use of high

263   ratios of lecithin is more likely to produce larger droplets with thicker interfacial surface areas

264   [41]. The inclusion of Tween 40 may have reduced the droplet size ranges, because the lower

265   molecular weight and higher hydrophilic-lipophilic balance (HLB) value of 15.6 [22]. Tween 40

266   combined with lecithin which has a lower HLB of 8 [22] creates a balance giving a system with

267   smaller droplet sizes.

268

269   **3.3.   Oxidation and volatiles produced in nanoemulsion preparation and storage**

270   GCHS is used widely to measure volatile compounds produced in the terminal stages of lipid

271   oxidation and is beneficial as no oil extraction methods are required [42]. The oxidised volatile

272   compounds produced by hydroperoxides (ROOH) breakdown in the last stage of oxidation.

273   Products with lower molecular weights than those of ROOH can therefore be used as a

274   measure of lipid oxidation [43].

275   Hexanal and propanal were the predominant volatile compounds detected in this study

276   (Figure 3) and these have also been previously identified as common indicators of secondary

277   oxidation for LCn3PUFA nanoemulsions [44]. Further gas chromatograms identified oxidised

278   compounds 2-ethylfuran, propan-3-ol and valeraldehyde were produced by the algal oil and

279   its nanoemulsions (see Figure 2 and Tables 3 to 6) all of which have been associated with

280   rancid off flavours in oxidised LCn3PUFA oils and emulsions [42, 45]. Significant differences were

281   found for all volatiles except valeraldehyde in both lecithin nanoemulsion samples stored at

282   40°C after 2 weeks ($p < 0.05$). The Tween 40 samples showed formation of higher

283   concentrations of hexanal in comparison to the lecithin only sample and this was further

284   accelerated by increases in temperature.

285

286   The combined lecithin and Tween 40 sample showed formation of lower concentrations of

287   volatiles at week 1 than the lecithin only sample; however, the Tween 40 only sample had the

288   lowest concentrations of the five volatile compounds when analysing storage time and

289   temperature. These differences may due to the nature of the emulsifiers, since lecithin

290   consists of phospholipids that can also be susceptible to lipid oxidation [13, 27].

291 It may be that LA, which is abundant in lecithin [46] positioned in the interfacial (outer) region

292 of emulsion droplets and closer to the aqueous phase of the system was subjected to

293 increased exposure to oxidation accelerants [47], which could explain higher levels of volatile

294 compounds in these samples. Following ultrasound processing, the DHA in these samples may

295 have retained some stability as it was located within the interior of nanoemulsion oil droplets

296 that were surrounded by lecithin molecules at the interfacial region [12, 37].

297

298 All samples remained the most stable at 4°C with the least amount of significant differences

299 in volatile compounds found at this temperature. Increases in sample storage temperature

300 led to significant increases in the development of volatile compounds for all samples, so the

301 degradation of fatty acids and production of oxidised compounds was temperature

302 dependant particularly for the changes in the range of 20 °C to 40 °C.

303

304 Both lecithin samples demonstrated formation of higher concentrations of volatiles for

305 storage times and temperature than the Tween only samples at 40°C, again indicating that

306 Tween 40 may offer an overall protective effect whilst lecithin is less stable to oxidation

307 particularly at increased temperatures. This is comparable to research by Uluata, McClements,

308 & Decker [27] and Arancibia et al [13] who found that lecithin nanoemulsions had higher propanal

309 development at increased temperatures.

310

311 The fatty acid composition of samples in this study showed no significant differences for

312 temperature and storage times although small non-significant changes were noted for LA and

313 DHA in some samples at higher temperatures as discussed earlier. The droplet size range

314 measurements showed the lecithin only samples had significantly larger droplet size ranges

315 throughout the trial, which suggests that larger droplet size ranges do not offer a protective

316 effect for the development of volatile compounds. Karthik & Anandharamakrishnan [24] also

317 found lecithin samples had larger droplet ranges and that Tween 40 could be used to create

318 systems with smaller droplet ranges and offered a protective effect for oxidative stability. In

319 the current study, samples created using the synthetic emulsifier Tween 40 had the smallest,

320 most stable droplet ranges and developed fewer volatile compounds than the lecithin

321 containing samples.

322

323 The present work evaluated volatile compounds found in the headspace of the bulk algal oil

324 and its various nanoemulsions. Systems remained the most stable 4°C which coupled with our

325 previous totox work [29] indicates the potential use of these systems as a refrigerated food

326 enrichment vehicle. Further work is now needed to analyse the effect 50% (w/w) algal oil

327 nanoemulsion enrichment may have on various appropriate food matrixes. In terms of

328 application and further development for a larger bioavailability trial there may be concerns

329 over the commercial acceptability of synthetic emulsifier Tween 40 in functional food

330 products [12], which would also need to be addressed.

331 **4.   Conclusion**

332   This study was the first to use GCHS to analyse the oxidative stability of 50% (w/w) algal oil-

333   in-water nanoemulsions created using ultrasound. The algal oil nanoemulsion prepared with

334   lecithin was determined to have significantly larger droplet size ranges, which remained

335   significantly larger throughout the 5-week storage trial regardless of storage temperatures.

336   The nanoemulsion prepared with Tween 40 and lecithin combined had a low initial droplet

337   size range distribution; however, there were significant increases in droplet ranges at 40°C

338   after 5-weeks of storage. There were no significant differences in the DHA percentage

339   composition for all samples throughout the storage trial at all temperatures.

340

341   Five oxidised compounds were detected in bulk oil algal and its nanoemulsions prepared with

342   lecithin and/or Tween 40 over 5-weeks at the tested temperatures. Propanal was the main

343   component of oxidised compounds followed by hexanal. It was noted that propanal

344   production was temperature dependent and it was relatively stable at lower temperatures

345   (4°C and 20°C) compared to 40°C. However, the nanoemulsions prepared in this study were

346   less stable than bulk oil at low temperatures due to formation of higher concentrations of

347   volatiles overall and particularly propanal and hexanal.

348   In terms of application, this work indicates good potential for further development in

349   collaboration with the food industry to create innovative functional foods that offer

350   alternative vegetarian sources of LCn3PUFA with improved bioavailability to alleviate

351   habitually low oily fish consumption. The use of an algal oil source of LCn3PUFA offers a

352   solution for vegetarians, vegans and non-fish eaters. Further research to evaluate the

353   oxidation stability and potential improvements to LCn3PUFA bioavailability using algal oil-in-

354   water nanoemulsions within food matrixes on a larger scale is warranted.

356   **References**

357   1.   Roberts C, Steer T, Mablethorpe N, Cox L, Meadows S, Nicholson S, Page P and Swan
358   G, National Diet and Nutrition Survey Results from Years 7 and 8 (combined) of the Rolling
359   Programme (2014/2015 to 2015/2016), Ed. Public Health England, London (2018).

360   2.   Papanikolaou Y, Brooks J, Reider C and Fulgoni VL, U.S. adults are not meeting
361   recommended levels for fish and omega-3 fatty acid intake: results of an analysis using
362   observational data from NHANES 2003–2008. *Nutrition Journal* **13**:31 (2014).

363   3.   Kranz S, Jones N and Monsivais P, Intake Levels of Fish in the UK Paediatric Population.
364   *Nutrients* **9**:392 (2017).

365   4.   Lane KE and Derbyshire EJ, Omega-3 fatty acids – A review of existing and innovative
366   delivery methods. *Critical Reviews in Food Science and Nutrition*:1-8 (2017).

367   5.   Mori Cortés N, Califano AN and Lorenzo G, Physical and chemical stability under
368   environmental stress of microemulsions formulated with fish oil. *Food Research International*
369   **119**:283-290 (2019).

370   6.   Shahidi F, Omega-3 fatty acids and marine oils in cardiovascular and general health: A
371   critical overview of controversies and realities. *Journal of Functional Foods* **19**:797-800 (2015).

372   7.   Lenihan-Geels G and Bishop KS, *Alternative Origins for Omega-3 Fatty Acids in the Diet*.
373   Springer International Publishing, Cham (2016).

374   8.   European Food Safety Authority, Scientific Opinion: DHA/EPA related health claims.
375   *European Food Safety Authority Journal* **9**:1-30 (2011).

376   9.   Lane KE, Derbyshire EJ, Li W and Brennan CJ, Bioavailability and Potential Uses of
377   Vegetarian Sources of Omega-3 Fatty Acids: A Review of the Literature. *Critical Reviews in
378   Food Science and Nutrition* **54**:572-579 (2014).

379   10.   Sprague M, Betancor MB and Tocher DR, Microbial and genetically engineered oils as
380   replacements for fish oil in aquaculture feeds. *Biotechnology Letters* **39**:1599-1609 (2017).

381   11.   Lakra N, Mahmood S, Marwal A, Sudheep NM and Anwar K, Bioengineered Plants Can
382   Be an Alternative Source of Omega-3 Fatty Acids for Human Health, in *Plant and Human
383   Health, Volume 2: Phytochemistry and Molecular Aspects*, ed. by Ozturk M and Hakeem KR.
384   Springer International Publishing, Cham, pp 361-382 (2019).

385   12.   Walker RM, Decker EA and McClements DJ, Development of food-grade
386   nanoemulsions and emulsions for delivery of omega-3 fatty acids: opportunities and obstacles
387   in the food industry. *Food & function* **6**:41-54 (2015).

388   13.   Arancibia C, Riquelme N, Zúñiga R and Matiacevich S, Comparing the effectiveness of
389   natural and synthetic emulsifiers on oxidative and physical stability of avocado oil-based
390   nanoemulsions. *Innovative Food Science & Emerging Technologies* **44**:159-166 (2017).

391 14.   Lane KE, Li W, Smith CJ and Derbyshire EJ, The bioavailability of an omega‐3‐rich
392 algal oil is improved by nanoemulsion technology using yogurt as a food vehicle. *International*
393 *Journal of Food Science & Technology* **49**:1264-1271 (2014).

394 15.   McClements DJ and Decker E, Lipid oxidation in oil‐in‐water emulsions: Impact of
395 molecular environment on chemical reactions in heterogeneous food systems. *Journal of*
396 *Food Science* **65**:1270-1282 (2000).

397 16.   Rasti B, Erfanian A and Selamat J, Novel nanoliposomal encapsulated omega-3 fatty
398 acids and their applications in food. *Food Chemistry* **230**:690-696 (2017).

399 17.   Jambrak AR and Škevin D, Chapter 4 - Lipids, in *Nutraceutical and Functional Food*
400 *Components*, ed. by Galanakis CM. Academic Press, pp 103-128 (2017).

401 18.   Lane KE, Li W, Smith CJ and Derbyshire EJ, The oxidation stability of a vegetarian
402 omega-3 oil in water nanoemulsion with enhanced bioavailability. *The Proceedings of the*
403 *Nutrition Society* **75** (2016).

404 19.   Lane KE, Li W, Smith CJ and Derbyshire EJ, The development of vegetarian omega-3 oil
405 in water nanoemulsions suitable for integration into functional food products. *Journal of*
406 *Functional Foods* **23**:306-314 (2016).

407 20.   Lane KE, Derbyshire EJ, Smith CJ, Mahadevan K and Li W, Sensory evaluation of a
408 yogurt drink containing an omega-3 nanoemulsion with enhanced bioavailability. *Proceedings*
409 *of the Nutrition Society* **72**:E99 (2013).

410 21.   Lane KE, Li W, Smith CS and Derbyshire EJ, Product shelf life evaluation of an enriched
411 yogurt drink containing an omega-3 nanoemulsion with enhanced bioavailability. *The*
412 *Proceedings of the Nutrition Society* **74** (2015).

413 22.   Coultate TP, *Food: the chemistry of its components*. Royal Society of Chemistry,
414 London (2016).

415 23.   Ghorbanzade T, Jafari SM, Akhavan S and Hadavi R, Nano-encapsulation of fish oil in
416 nano-liposomes and its application in fortification of yogurt. *Food Chemistry* **216**:146-152
417 (2017).

418 24.   Karthik P and Anandharamakrishnan C, Enhancing omega-3 fatty acids nanoemulsion
419 stability and in-vitro digestibility through emulsifiers. *Journal of Food Engineering* **187**:92-105
420 (2016).

421 25.   Joyce P, Gustafsson H and Prestidge CA, Enhancing the lipase-mediated
422 bioaccessibility of omega-3 fatty acids by microencapsulation of fish oil droplets within porous
423 silica particles. *Journal of Functional Foods* **47**:491-502 (2018).

424 26.   Yesiltas B, García-Moreno PJ, Sørensen A-DM, Akoh CC and Jacobsen C, Physical and
425 oxidative stability of high fat fish oil-in-water emulsions stabilized with sodium caseinate and
426 phosphatidylcholine as emulsifiers. *Food Chemistry* **276**:110-118 (2019).

427 27.   Uluata S, McClements DJ and Decker EA, Physical stability, autoxidation, and
428 photosensitized oxidation of ω-3 oils in nanoemulsions prepared with natural and synthetic
429 surfactants. *Journal of agricultural and food chemistry* **63**:9333-9340 (2015).

430 28.   Karthik P and Anandharamakrishnan C, Fabrication of a nutrient delivery system of
431 docosahexaenoic acid nanoemulsions via high energy techniques. *RSC Advances* **6**:3501-3513
432 (2016).

433 29.   Lane KE, Zhou Q, Robinson S and Li W, Lipid oxidation within vegetarian long chain
434 omega-3 polyunsaturated fatty acid oil nanoemulsions suitable for food fortification.
435 *Proceedings of the Nutrition Society* **76**:E101 (2017).

436 30.   Martek Biosciences Corporation, Application for the authorization of DHA and EPA-
437 rich algae oil from *Schizochytrium sp.*, Ed by Gray RJH. Martek Biosciences corporation,
438 Columbia, pp 1-51 (2010).

439 31.   Kentish S, Wooster TJ, Ashokkumar M, Balachandran S, Mawson R and Simons L, The
440 use of ultrasonics for nanoemulsion preparation. *Innovative Food Science & Emerging
441 Technologies* **9**:170-175 (2008).

442 32.   Sun Y, Xia Z, Zheng J, Qiu P, Zhang L, McClements DJ and Xiao H, Nanoemulsion-based
443 delivery systems for nutraceuticals: Influence of carrier oil type on bioavailability of
444 pterostilbene. *Journal of Functional Foods* **13**:61-70 (2015).

445 33.   Horiba Scientific, A guidebook to partical size analysis, Ed by Horiba Instruments Inc,
446 Irvine, USA, pp 1-28 (2010).

447 34.   Yang Y, Leser ME, Sher AA and McClements DJ, Formation and stability of emulsions
448 using a natural small molecule surfactant: Quillaja saponin (Q-Naturale®). *Food Hydrocolloids*
449 **30**:589-596 (2013).

450 35.   Medina I, Satue‐Gracia MT and Frankel EN, Static Headspace Gas Chromatographic
451 Analyses to Determine Oxidation of Fish Muscle Lipids During Thermal Processing. *Journal Of
452 The American Oil Chemists' Society* **76**:231-236 (1999).

453 36.   Iglesias J and Medina I, Solid-phase microextraction method for the determination of
454 volatile compounds associated to oxidation of fish muscle. *Journal of Chromatography A*
455 **1192**:9-16 (2008).

456 37.   Taladrid D, Marín D, Alemán A, Álvarez-Acero I, Montero P and Gómez-Guillén MC,
457 Effect of chemical composition and sonication procedure on properties of food-grade soy
458 lecithin liposomes with added glycerol. *Food Research International* **100**:541-550 (2017).

459 38.   Pingret D, Fabiano-Tixier A-S and Chemat F, Degradation during application of
460 ultrasound in food processing: A review. *Food Control* **31**:593-606 (2013).

461 39.   National Center for Biotechnology Information, *PubChem Database. Polysorbate 40,
462 CID=24846075,*. https://pubchem.ncbi.nlm.nih.gov/compound/24846075 [03/06/2019.

463   40.   National Center for Biotechnology Information, *PubChem Database. Soybean lecithin,*
464   *CID=6438359, .* https://pubchem.ncbi.nlm.nih.gov/compound/Soybean-lecithin [03/06/2019.

465   41.   Yesiltas B, Torkkeli M, Almásy L, Dudás Z, Wacha AF, Dalgliesh R, García-Moreno PJ,
466   Sørensen A-DM, Jacobsen C and Knaapila M, Interfacial structure of 70% fish oil-in-water
467   emulsions stabilized with combinations of sodium caseinate and phosphatidylcholine. *Journal*
468   *of Colloid and Interface Science* **554**:183-190 (2019).

469   42.   Kolanowski W, Jaworska D and Weißbrodt J, Importance of instrumental and sensory
470   analysis in the assessment of oxidative deterioration of omega-3 long-chain polyunsaturated
471   fatty acid-rich foods. *Journal of the Science of Food and Agriculture* **87**:181-191 (2007).

472   43.   Choe E and Min DB, Mechanisms and factors for edible oil oxidation. *Comprehensive*
473   *reviews in food science and food safety* **5**:169-186 (2006).

474   44.   Sharif HR, Williams PA, Sharif MK, Khan MA, Majeed H, Safdar W, Shamoon M, Shoaib
475   M, Haider J and Zhong F, Influence of OSA-starch on the physico chemical characteristics of
476   flax seed oil-eugenol nanoemulsions. *Food Hydrocolloids* **66**:365-377 (2017).

477   45.   Shahidi F, Headspace Volatile Aldehydes as Indicators of Lipid Oxidation in Foods, in
478   *Headspace Analysis of Foods and Flavors: Theory and Practice*, ed. by Rouseff RL and
479   Cadwallader KR. Springer US, Boston, MA, pp 113-123 (2001).

480   46.   Ozturk B and McClements DJ, Progress in natural emulsifiers for utilization in food
481   emulsions. *Current Opinion in Food Science* **7**:1-6 (2016).

482   47.   Donsì F, Chapter 11 - Applications of Nanoemulsions in Foods, in *Nanoemulsions*, ed.
483   by Jafari SM and McClements DJ. Academic Press, pp 349-377 (2018).

484