

1 **The oxidative stability of omega-3 oil-in-water nanoemulsion**
2 **systems suitable for functional food enrichment: A systematic**
3 **review of the literature**

4 **Authors**

5 Linda Bush, Leo Stevenson, Katie E. Lane.

6 Liverpool John Moores University, School of Sport Studies, Leisure and Nutrition, I.M.
7 Marsh Campus, Liverpool, L17 6BD, United Kingdom.

8 **Corresponding author**

9 Katie E. Lane, Liverpool John Moores University, School of Sport Studies, Leisure and
10 Nutrition, I.M. Marsh Campus, Liverpool, L17 6BD, United Kingdom,
11 k.e.lane@ljmu.ac.uk ORCID ID: 0000-0002-9092-2927

12 **Abstract**

13 There is growing demand for functional food products enriched with long chain omega-
14 3 fatty acids (LC ω 3PUFA). Nanoemulsions, systems with extremely small droplet
15 sizes have been shown to increase LC ω 3PUFA bioavailability. However,
16 nanoemulsion creation and processing methods may impact on the oxidative stability
17 of these systems. The present systematic review collates information from studies that
18 evaluated the oxidative stability of LC ω 3PUFA nanoemulsions suitable for use in
19 functional foods. The systematic search identified seventeen articles published during
20 the last 10 years. Researchers used a range surfactants and antioxidants to create
21 systems which were evaluated from 7 to 100 days of storage.

22 Nanoemulsions were created using synthetic and natural emulsifiers, with natural
23 sources offering equivalent or increased oxidative stability compared to synthetic
24 sources, which is useful as consumers are demanding natural, cleaner label food
25 products. Equivalent vegetarian sources of LC ω 3PUFA found in fish oils such as algal
26 oils are promising as they provide direct sources without the need for conversion in
27 the human metabolic pathway. Quillaja saponin is a promising natural emulsifier that
28 can produce nanoemulsion systems with equivalent/increased oxidative stability in
29 comparison to other emulsifiers. Further studies to evaluate the oxidative stability of
30 quillaja saponin nanoemulsions combined with algal sources of LC ω 3PUFA are
31 warranted.

32 **Keywords:** nanoemulsion, omega-3, functional foods, oil-in-water, oxidation,
33 oxidative stability

34 **Conflicts of interest:** None.

35 This research did not receive any specific grant from funding agencies in the public,
36 commercial, or not-for-profit sectors.

37 **Introduction**

38 There is increasing evidence in studies conducted over recent decades that numerous
39 health benefits are associated with the consumption of long chain omega-3 (ω -3)
40 polyunsaturated fatty acids (LC ω 3PUFA) throughout the human lifecycle (Bowen et
41 al. 2016, Calder 2014, Simopoulos 2011). An adequate LC ω 3PUFA status is a key
42 factor in the maintenance of health and may reduce the risk of chronic and
43 inflammatory diseases (Deckelbaum and Torrejon 2012, Yates et al. 2014). Despite
44 known health benefits, consumption of omega-3 fatty acids of which oily fish is the
45 most abundant source (Lenihan-Geels and Bishop 2016) remains lower than

46 recommended levels (table 1), with omega-3 intakes in Western regions being 5-fold
47 lower than Japanese intakes (Bates et al. 2016, Meyer 2011, Meyer 2016,
48 Papanikolaou et al. 2014). Supplementation may provide an alternative, however
49 supplement use is not widespread and a collaborative strategy of food fortification, in
50 addition to food sources (i.e., fish consumption) may need to be considered to achieve
51 recommended intakes in Western populations (Bates, et al. 2016, Papanikolaou, et al.
52 2014). To address this problem there has recently been an emphasis on the
53 incorporation of LC ω 3PUFA source oils into food products, which has led to increased
54 interest from consumers and the food industry (Decker et al. 2012, Jacobsen, Nielsen,
55 et al. 2013, Salvia-Trujillo et al. 2016).

56 **Omega 3 source oils**

57 Fish oils are currently the most prevalent source of the most beneficial LC ω 3PUFA
58 which are eicosapentaenoic acid (20:5 ω -3; EPA) and docosahexaenoic acid (22:6 ω -
59 3; DHA) (Lenihan-Geels and Bishop 2016). Fish oils contain a high concentration of
60 LC ω 3PUFA's and have a vast number of different fatty acids contained in their
61 triglycerides. The flesh of oily fish such as mackerel, salmon, sardines, anchovies and
62 pilchards is rich in EPA and DHA (Bailey 2009). The use of fish oils as a LC ω 3PUFA
63 source for supplementation and fortification is common place, however fish oil
64 supplementation may be disliked due to commonly reported adverse effects including
65 gastrointestinal upset, fishy aftertaste and gastric repetition (Fetterman and
66 Zdanowicz 2009). Krill oil provides a rich source of EPA and DHA, however as with
67 other marine based sources krill population numbers can fluctuate, therefore
68 sustainability cannot be guaranteed (Lane and Derbyshire 2015, Surette 2013,
69 Trivelpiece et al. 2011). Fish and krill based sources of LC ω 3PUFA are by their nature

70 unsuitable for vegetarians and non-fish eaters who abstain from eating marine and
71 fish sources for ethical reasons.

72 Further vegetarian sources are available in the form of flaxseed, echium seed, perilla
73 seed, blackcurrant seed and algal oils. (Asif 2011, Linnamaa et al. 2010, Mir 2008).

74 Flaxseed oil is currently the most significant vegetarian source of alpha- linolenic acid
75 (18:3 ω -3; ALA). Also known as linseed oil it can contain up to 57 per cent ALA when
76 cold pressed (Sharif et al. 2017). A considerable amount of research has examined
77 supplementation and food enrichment with ALA rich oils, however conversion of ALA
78 to its longer chain, more effective relatives EPA and DHA is limited in humans and
79 alternative direct sources of EPA and DHA are available (Lane, Derbyshire, et al.
80 2014).

81 Micro-algae oils are a fairly recent advance within the food and nutraceutical industry.
82 They are produced in tightly controlled, closed fermentation facilities or in the case of
83 phototropic algae produced in photobioreactors or open raceways and are entirely free
84 of animal products (Breivik 2007, Lenihan-Geels and Bishop 2016, Ryckeboesch et al.
85 2012). Capable of providing large amounts of EPA and DHA algae are also the primary
86 source of DHA in the food chain (Arterburn et al. 2006). Algae oils represent a
87 sustainable LC ω 3PUFA source suitable for vegetarians, vegans and non-fish eaters
88 (Lane, et al. 2014).

89 **Food fortification**

90 Functional foods provide an added health benefit over and above the food products
91 nutritional value (Bigliardi and Galati 2013, Khan et al. 2013). In recent years the food
92 industry has evolved, and there is an increased focus on innovative approaches in
93 processing and the introduction of novel foods that may help to optimise health and

94 wellbeing (Khan, et al. 2013). The use of LC ω 3PUFA source oils in functional foods
95 may offer considerable health benefits, however it also gives rise to a number of
96 challenges due to their low water solubility and poor chemical stability. The chemical
97 structure of LC ω 3PUFA also makes them particularly susceptible to oxidation
98 (Jacobsen 2010, Wang and Shahidi 2017). Oxidation occurs as the result of reactions
99 with PUFA, free radicals and oxygen (Walker et al. 2015b). Lipid oxidation is a complex
100 process which is influenced by many factors (Shahidi and Zhong 2010). Fatty acids
101 with a high degree of unsaturation can be less stable to oxidation when incorporated
102 into functional foods, which causes three main problems. Firstly, it gives rise to
103 objectionable 'off' flavours. It also reduces the nutritional value of foods containing
104 lipids (Wang and Shahidi 2017). Free radicals, which are formed during oxidation may
105 cause the formation of atherosclerosis following ingestion posing a potential health
106 risk to consumers (Jacobsen 2010).

107 **Emulsions and nanoemulsions**

108 In the case of LC ω 3PUFA, oil in water emulsion systems are commonly used in the
109 food industry as delivery vehicles, particularly in foods with an aqueous base. An
110 emulsion is a dispersion of two or more immiscible liquids consisting of a continuous
111 phase and a disperse phase (Coultate 2009). There is some debate within the
112 literature in relation to definitive nanoemulsion droplet size ranges. Solans and Solé
113 (2012) state that nanoemulsions are emulsion systems with extremely small droplet
114 sizes in the range of 20 to 500nm whereas McClements and Rao (2011) define
115 nanoemulsions also referred to as a mini emulsions as a conventional emulsion that
116 contains very small particles, with mean radii between about 10 to 100 nm. The
117 incorporation of LC ω 3PUFA oils into functional foods using nanoemulsions has the
118 potential to improve LC ω 3PUFA bioavailability (Lane, Li, et al. 2014). However this

119 may also create further concerns in relation to oxidative stability due to small lipid
120 droplet sizes and large droplet surface areas (Walker, et al. 2015b). Nanoemulsions
121 can be created using high mechanical energy, high surfactant levels or combinations
122 of both. Creation methods can normally be classed as high-energy and low-energy
123 methods (Walker, et al. 2015b).

124 Low energy methods can be described as condensation, low energy or phase
125 inversion methods. These processes make use of the phase transitions that take place
126 during homogenisation processes as a result of instantaneous curvature of the
127 surfactant (Solè et al. 2006). This change can be achieved using a number of
128 processes. Phase inversion temperature (PIT) involves changing the temperature
129 whilst maintaining the composition. Phase inversion composition (PIC), occurs when
130 the temperature is maintained and the environmental composition is amended. Phase
131 inversion can be triggered when amendments are made to the composition or
132 environment of an emulsion, examples of this include changes to the disperse phase
133 volume fraction, type of emulsifier, emulsifier concentration, solvent conditions,
134 temperature, or by mechanical agitation (Shahidi 2005). Nanoemulsions with droplet
135 sizes as small as 17nm have been achieved by Sole et al, (2006) using the PIC method
136 and 35nm using the PIT method by Ee, Duan, Liew & Nguyen (2008). However,
137 commercial applications for phase inversion are limited as only certain kinds of
138 emulsion are able to undergo inversion without being broken down into their
139 component phases (Shahidi 2005). These methods also require a large amount of
140 surfactants and are not applicable to large scale industrial productions (Jafari et al.
141 2006). Spontaneous emulsification involves the addition of one phase to another by
142 continuous stirring, and has also been used to create nanoemulsions with droplet sizes
143 <200nm (Walker et al. 2015a).

144 The high-energy approach is commonly used in the food sector. Devices with very
145 high energy input are utilised to give greater control of composition and size
146 distributions of the nanoemulsions produced (Karthik and Anandharamakrishnan
147 2016a). These methods use devices that are capable of generating intensely
148 disruptive forces that break up the oil and water phases leading to the formulation of
149 very small oil droplets (Acosta 2009) and include high speed homogenisation,
150 microfluidization and ultrasound. High speed homogenisation can be used to produce
151 very small droplets in an emulsion system by applying additional sheer force to break
152 down oil droplets using high speed, defined as rpm between 10000 and 24000
153 (Esquerdo et al. 2015, Karthik and Anandharamakrishnan 2016b). High pressure
154 homogenisation combines intense sheer, cavitation and turbulent flow to create
155 extremely small oil droplets (McClements 2015). A further high-power method,
156 microfluidization offers a flexible control over emulsion droplet sizes and can be used
157 to produce fine emulsions from a large variety of materials (Jafari, et al. 2006).
158 However, both methods can be disadvantaged by complex cleaning requirements,
159 high running costs and equipment wear rates making them prone to significant losses
160 in efficiency (Leong et al. 2009). Microfluidizers are applicable to large-scale
161 productions, although droplet sizes may be larger than some of the low energy
162 methods discussed earlier. Ultrasound refers to sound waves that are above and
163 beyond the frequency of human hearing (>18 kHz) (Ashokkumar et al. 2010,
164 Sanguansri and Augustin 2006). Ultrasound emulsification may be used instead of
165 high-pressure homogenisation and microfluidization to achieve similar results with
166 reductions in operating costs (Abbas et al. 2013).

167

168

169 Lipid oxidation

170 The reaction mechanism and factors that influence oxidation reactions are different for
171 emulsified fats and oils (lipids) than for bulk lipids (Hu and Jacobsen 2016). The
172 interfacial membrane of an emulsion system is of importance in lipid oxidation as it
173 represents the region where lipid and water soluble components are close enough to
174 interact, potentially giving higher concentrations of lipid peroxides and other volatiles
175 (Berton et al. 2011). Lipid oxidation in emulsions usually occurs at the oil in water
176 interface when free radicals interact with PUFA's within the lipid droplets or when water
177 soluble trace metal ions react with hydroperoxides located at the droplet interface
178 (Jacobsen, Horn, et al. 2013, Walker, et al. 2015b). Most LC3PUFA oils contain trace
179 levels of peroxides and foods suitable for enrichment can contain trace levels of
180 transition metals so metal-catalysed breakdown of peroxides is considered to be one
181 of the main quality issues for LC3PUFA enriched functional food products (Jacobsen,
182 Horn, et al. 2013, Jacobsen, Sørensen, et al. 2013). The creation of nanosized lipid
183 droplets in an aqueous continuous phase greatly increases the surface area of the
184 lipid phase and therefore the susceptibility to oxidation. In addition, when system
185 droplet ranges are smaller than the wavelength of light, the light waves are weakly
186 scattered giving the system transparent or turbid appearance. The increased
187 transmission of light waves through nanoemulsion systems may increase their
188 susceptibility to light induced oxidation (Uluata et al. 2015). The susceptibility of oil
189 droplets to lipid oxidation depends on whether the oxidation catalyst is electrostatically
190 attracted to the interfacial membrane (McClements and Decker 2000, McClements
191 and Rao 2011). If the oxidation catalysts are repulsed from the lipid water interface,
192 lipid oxidation in emulsions can be lowered (Yi et al. 2014). The choice of
193 homogenization equipment, emulsifier type and droplet size can also influence the

194 oxidative stability of the resultant systems. The use of high-power ultrasound methods
195 in the creation of nanoemulsion systems has been associated with increased oxidation
196 reactions in lipids. (Pingret et al. 2012, Pingret et al. 2013). The use of microfluidization
197 has been shown to result in decreased oxidation levels in comparison to high pressure
198 valve homogenization when whey protein is used as an emulsifier (Horn et al. 2012)

199 The main focus of this article is to compare and contrast the findings of studies
200 published during the last 10 years that have evaluated the oxidative stability of
201 LC ω 3PUFA nanoemulsions suitable for functional food enrichment. The aim of the
202 review is to evaluate some of the most recent key up to date papers in order fill a gap
203 in the literature in relation to this topic and to inform future decisions and research into
204 this promising area. This information should aid in the identification of safe, optimal
205 components including types of oils and emulsifiers, processing and storage conditions
206 to maintain the oxidative stability of LC ω 3PUFA nanoemulsions for use in in functional
207 foods.

208

209 **Methods**

210 The aim of this review was to fill a gap in the literature by evaluating studies that
211 focussed on the oxidative stability of LC ω 3PUFA nanoemulsions suitable for
212 integration into food vehicles. A systematic literature search was conducted in
213 accordance with the PRISMA checklist for systematic reviews and meta-analysis
214 (Table 2) (Moher et al. 2009, Moher et al. 2015). Search engines PubMed, Science
215 Direct, Google Scholar, and SCOPUS were used to identify English language, peer
216 reviewed articles published over a 10-year period between January 2007 and January
217 2017.

218 **Inclusion criteria**

219 Search terms including nanoemulsion(s), nanotechnology, emulsions and foods,
220 nutrients omega 3, ω 3, LC ω 3PUFA, DHA, EPA, ALA, fish/ vegetable oils (e.g. salmon,
221 tuna, carp, algae), or nut and seed oils (e.g. echium, walnut) identified a total of 1880
222 articles. These were then narrowed to 1420 articles with further inclusion of search
223 terms: food vehicles, food delivery and functional foods.

224 Further inclusion criteria were that studies:

225 1) Investigated products associated with the initiation, propagation and/or
226 termination stages of lipid oxidation including peroxide value (PV), anisidine
227 value (AV), total oxidation value (TOTOX or TV), iodine value (IV), thiobarbituric
228 acid reacting substances (TBAR's), gas chromatography headspace analysis
229 (GCHS), gas chromatography mass spectrometry headspace analysis
230 (GCMS,HA), high performance liquid chromatography (HPLC), fatty acid
231 analysis and sensory analysis.

- 232 2) Encompassed nanoemulsion system creation methods such including high
233 energy: ultrasound, ultrasonic, microfluidizer, high pressure valve and high
234 speed homogenization and low energy: phase inversion; temperature and
235 composition and spontaneous methods.
- 236 3) Examined the effect of different emulsifiers, hydrophilic lipophilic (HLB) balance
237 and processing conditions including pH and Zeta potential. Including amongst
238 others, emulsifiers lecithin, Tween products (all numbers), whey protein,
239 caseinate, glycerol dioleate, Span products (all numbers), sucrose
240 monolaurate, sodium steroyl, with HLB ranges from 1 to 20.
- 241 4) Examined the ability of antioxidants to retard or inhibit lipid oxidation in
242 LC3PUFA oil in water nanoemulsions

243

244 Exclusion criteria

245 Papers that were not written in English language or where the full article could not be
246 accessed were excluded. Studies that referred to non-food based nanoemulsions
247 (fuels and drug/pharmaceutical related); systems with droplet sizes outside the range
248 of 50-500nm, cosmetic applications and water in oil systems were removed. Papers
249 written prior to January 2007 were excluded alongside studies that did not evaluate
250 the oxidative stability of LC3PUFA oil in water nanoemulsion systems. Papers that did
251 not make specific reference to nanoemulsion systems/nanoliposome carriers were
252 also excluded from the review.

253

254

255

256 **Results and Discussion**

257 The literature search identified 17 key studies that have investigated the oxidative
258 stability of LC ω 3PUFA nanoemulsion systems suitable for use as food enrichment
259 vehicles. The 17 studies are summarised in Table 3.

260 **Fish oil**

261 As discussed previously, the chemical structure of LC ω 3PUFA makes them
262 particularly susceptible to oxidation. Source oils with greater fatty acid chain length
263 and higher numbers of double bonds generally demonstrate decreased oxidative
264 stability. Relative susceptibility for DHA (22:6) is increased by 30 times in comparison
265 to ALA (18:3) (Decker, et al. 2012). The majority of the studies examined in the review
266 used fish oil as an enrichment vehicle, which may be due to its current ease of
267 availability and high EPA and DHA content (Lenihan-Geels and Bishop 2016).

268 Rasti, Erfanian & Selamat (2017) evaluated the application, stability and suitability of
269 fish oil in water nanoliposomes in bread and milk products. Nanoliposomes had
270 significantly lower primary and secondary oxidation levels in comparison to
271 microencapsulated and bulk fish oil ($P < 0.05$) and were found to be suitable as
272 fortification vehicles in bread and milk. A further fish oil nanoliposome study was
273 conducted by Ghorbanzade, et al (2017) with nanoliposomes incorporated into
274 yoghurt. Peroxide value testing and sensory analysis established that liposomal
275 structures were successful for the encapsulation of DHA and EPA, which remained
276 stable during the 21-day storage period, nanoencapsulation was found to protect
277 LC ω 3PUFA from deterioration by oxidation. Esquerdo et al (2015) created chitosan
278 nanocapsules using a 15 per cent carp oil nanoemulsion. Peroxide values for the
279 nanocapsules remained stable during storage while bulk oil peroxide values increased

280 over a 45-day storage period. Salvia-Trujillo et al, (2016), Walker et al (2015a) and
281 Belhaj et al (2010) investigated the oxidative stability of 10 per cent oil in water
282 nanoemulsions using fish oil as the LCω3PUFA source. The authors investigated the
283 addition of antioxidants and effects of different emulsifiers on the oxidative stability of
284 the systems. The addition/presence of natural antioxidants such as lemon oil, marine
285 lecithin, astaxanthin and sodium alginate was found to increase the oxidative stability
286 of the resultant systems. Uluata et al (2015) investigated the oxidative stability of 1 per
287 cent fish oil in water nanoemulsions, creating systems with droplet ranges under
288 100nm. A range of primary and secondary oxidation tests were used to evaluate
289 synthetic and natural emulsifiers. Synthetic emulsifier Tween 80 was found to have
290 significantly higher radical scavenging capacity ($P < 0.05$) and quillaja saponin was
291 found to be an effective natural emulsifier due to its physical and oxidative stability.

292

293 Overall, the use of nanoemulsion technology appears to have increased or stabilised
294 oxidation reactions in studies using fish oil. However, the use of fish oil in food
295 fortification raises concerns in relation to their suitability, sustainability and issues with
296 contamination. The overall condition of global fisheries is in decline and scientific
297 concerns in relation to over fishing have frequently featured in the literature (Béné et
298 al. 2015). Seafood is particularly susceptible to contamination with organic lipophilic
299 pollutants and fish is a major source of exposure to heavy metals and organic
300 pollutants which may cause health concerns for consumers (Hong et al. 2015, Verbeke
301 et al. 2005).

302

303 **Krill oil**

304 Krill are small shrimp-like crustaceans that have particularly high content of EPA and
305 DHA attributed to their diet, which is based on microalgae. Krill oil has recently
306 emerged as a LCw3PUFA source oil and is similar to fish oil in terms of its EPA/DHA
307 content although 30-65 per cent of the fatty acids are in phospholipid form which may
308 increase bioavailability (Adarme-Vega et al. 2014, Lenihan-Geels and Bishop 2016).
309 Two studies in the review used high-power methods to create nanoemulsions with
310 droplet ranges <333nm. Wu et al, (2016) examined the physical and oxidative stability
311 of 1 per cent krill oil in water nanoemulsions and the influences of antioxidant polarity
312 with the addition of α -tocopherol and trolox antioxidants. The more polar trolox was
313 found to be a more effective antioxidant for these systems than α -tocopherol.

314 A further study by Zhu et al (2015) evaluated the chemical and physical stability of
315 lecithin stabilised nanostructured lipocarriers as a delivery system to encapsulate krill
316 oil. Nanostructured lipocarriers were found to offer significant protection against
317 photooxidation upon exposure to UV light ($P < 0.05$) in comparison to bulk krill oil.

318 Krill oil contains astaxanthin, which acts as a natural antioxidant enhancing the
319 potential associated health benefits and offering increased stability against oxidation
320 when processed for supplementation and addition to foods (Adarme-Vega, et al.
321 2014). Overall, the articles in this review found that the presence or addition of
322 antioxidants and encapsulation of krill oil increased oxidative stability and the process
323 of incorporation in to nanoemulsion systems did not have an adverse effect during
324 storage periods varying from 8 to 70 days. However as with fish oil, krill oil by its nature
325 may be unsuitable for consumption by vegetarians and vegans, furthermore concerns
326 have been raised in relation to sustainability due to global warming and exploitation
327 by over fishing in arctic areas (Trivelpiece, et al. 2011).

328 A further issue with krill oil is its unpleasant off-odour and flavour, which cannot usually
329 be removed by refining and deodorisation during processing. This makes it
330 unacceptable in terms of quality to consumers when used for food enrichment
331 purposes unless it is encapsulated and incorporated into novel nanocarriers to create
332 a sensory barrier (Henna Lu et al. 2011, Lu et al. 2013, Zhu, et al. 2015).

333 **Algal oil**

334 Algal oil is derived from algae, which forms the foundation of the seafood chain. Most
335 commercially produced algal oils are rich in DHA which is thought to be one of the
336 most beneficial LC ω 3PUFA sources (Baker et al. 2016). However, DHA is particularly
337 susceptible to oxidation due to its long carbon chain length and high number of double
338 bonds. Three of the articles in the review examined the oxidative stability of algal oil
339 nanoemulsions created using high-power methods. Karthik & Anandharamakrishnan
340 (2016a) investigated the physiochemical stability and in-vitro digestibility of DHA
341 nanoemulsions stabilised with Tween 40 (synthetic emulsifier), sodium caseinate and
342 soy lecithin (natural emulsifiers) created using microfluidization. Significant differences
343 were found in peroxide values of 10 per cent oil in water nanoemulsions stored over
344 20 days with soy lecithin stabilised systems significantly greater than Tween 40
345 systems ($P < 0.05$). There were no changes or differences in fatty acid profiles of the
346 different systems which suggests that soy lecithin may be susceptible to oxidation
347 reactions when processed using microfluidization. Tween 40 systems were found to
348 be most stable in terms of primary oxidation and in-vitro digestibility. Additional work
349 has also been completed to further evaluate algal oil nanoemulsions stabilised with
350 Tween 40 created using high speed/pressure homogenisation. There were no
351 significant differences in oxidative stability between systems created using high power
352 or pressure homogenization. A combination of high speed/pressure homogenization

353 was found to create better physical stability in 10 per cent systems stabilised with
354 Tween 40 (Karthik and Anandharamakrishnan 2016b). Research to evaluate spray
355 dried powders created from a 10 per cent algal oil nanoemulsion template was
356 conducted by Chen et al, (2016). Spray dried algal oil powders were found to have
357 excellent reconstructed behaviour during the 30 day trial. Enhanced oxidative stability
358 was found in systems formed with β -sitosterol & γ -oryzanol phytosterols ($P < 0.05$).
359 Spray dried powders also had lower levels of fishy off flavours which are associated
360 with oxidised oils. Algal oils offer a potentially viable source of LC ω 3PUFA, which is
361 sustainable and suitable for vegetarians and vegans. A review of 16 published clinical
362 trials found that consumption of algal oil may be beneficial in cardiovascular risk
363 factors and unlike fish oil, algal-DHA seldom caused gastrointestinal complaints such
364 as fishy taste and eructation (Ryan et al. 2009). The studies identified in the review
365 evaluated the oxidative stability of DHA oils, however more recently EPA/DHA algal
366 oils have become available and these have been found offer similar benefits to fish oil
367 for adults with hypertriglyceridemia (Maki et al. 2014). Research has yet to investigate
368 the suitability of EPA/DHA oils in functional foods. Further work is therefore warranted
369 to investigate integration of these oils into nanoemulsion systems with an additional
370 focus on oxidative stability, which may be improved in comparison to algal DHA alone
371 due to the shorter carbon chain and lower numbers of double bonds in EPA.

372 **Flaxseed oil**

373 Flaxseed oil is currently the most widely used source of vegetarian LC ω 3PUFA in
374 supplementation and food enrichment (Lane, et al. 2014, Lenihan-Geels and Bishop
375 2016). Flaxseed oil contains up to 57 per cent ALA, which may have increased
376 oxidative stability over its longer carbon chain counterparts EPA and DHA (Decker, et
377 al. 2012, Sharif, et al. 2017). Two studies identified in the review evaluated the

378 oxidative stability of 10 per cent flaxseed oil in water nanoemulsions created using
379 microfluidization. Primary products were identified using peroxide value testing in both
380 studies and the use of antioxidants eugenol and caseinate was found to significantly
381 reduce the formation of peroxides ($P < 0.05$) (Chen et al. 2017, Sharif, et al. 2017).
382 Analysis of secondary oxidation products was conducted using headspace analysis
383 and thiobarbituric acid reactive substances tests, the use of eugenol and caseinate
384 was also found to be significantly effective when compared to systems generated with
385 no addition of antioxidants. Flaxseed nanoemulsions were found to have significantly
386 increased total oxidation levels in comparison to bulk oil in both studies ($P < 0.05$),
387 which suggests that nanoemulsion processing does have an effect on the oxidative
388 stability of flaxseed oil.

389 **Walnut oil**

390 Walnut oil contains relatively low amounts of LC ω 3PUFA at around 10 per cent ALA
391 (Zhao et al. 2004), longer chain EPA and DHA are not present which may give
392 improved oxidative stability. Short term consumption of walnut oil has been found to
393 significantly decrease total and LDL cholesterol ($P < 0.05$), walnuts may also have
394 potential benefits on oxidative stress and inflammatory markers (Banel and Hu 2009).
395 Limited research has been conducted to examine the physiochemical properties of
396 walnut oil nanoemulsions with one study identified in the review. Emulsifying
397 conditions were investigated including processing time and concentration ratio using
398 8, 6 and 4 per cent walnut oil-in-water nanoemulsion systems created using
399 ultrasound. Loss of antioxidant activity testing over 35 days identified a quadratic effect
400 of ultrasound treatment leading to significant losses of antioxidant activity ($P < 0.05$)
401 (Homayoonfal et al. 2014).

402

403 **Antioxidants**

404 A number of studies in the review examined the use of added antioxidants to improve
405 the oxidative stability of LC3PUFA nanoemulsion systems. Wu et al, (2016)
406 determined how antioxidant polarity impacted the oxidative stability of 1% krill oil in
407 water nanoemulsion systems to reflect conditions in typical enrichment food vehicles.
408 Lipid oxidation was significantly accelerated by the addition of ferrous chloride and
409 trolox was found to be a better antioxidant than α -tocopherol. The antioxidant eugenol
410 was used in combination with Purity gum ultra surfactant by Sharif et al, (2017) who
411 noted significant improvements to physical and oxidative stability in these 10%
412 flaxseed oil in water nanoemulsion systems. Caseinate was used in combination with
413 the emulsifier quillaja saponin to create 10% oil in water flaxseed oil nanoemulsions
414 by Chen et al (2017). Peroxide and TBARS values increased at significantly slower
415 rates for the systems containing caseinate. The antioxidant properties of β -sitosterol
416 & γ -oryzanol were evaluated by Chen et al (2016) in the formation of 10 per cent algae
417 oil and quillaja saponin nanoemulsions. A significant protective effect was observed in
418 spray dried powders over 30 days of storage ($P < 0.05$). Overall results from the review
419 indicate that antioxidant addition is an effective strategy to stabilize LC3PUFA
420 nanoemulsions against oxidation during storage.

421 **The effect of emulsion stability and pH**

422 The zeta-potential of a conventional emulsion or nanoemulsion is the electrical
423 potential at the "shear plane," which is defined as the distance away from the droplet
424 surface below which the counter-ions remain strongly attached to the droplet when it
425 moves in an electrical field. Zeta potential is one of the fundamental parameters known

426 to affect the physical stability of emulsion systems (McClements and Rao 2011). Zeta-
427 potential and nanoemulsion oxidation stability was evaluated in 1% fish oil systems by
428 Uluata et al (2015) over a 5 day storage period. The effect of pH was also determined
429 over the range of 2 to 8. At pH 7 all lipid droplets were negatively charged. No
430 significant change in particle size was noted in pH range 2 to 8. Of the four natural and
431 synthetic emulsifiers used in the study quillaja saponin was found to create the most
432 physically and chemically stable systems. The physical and oxidative stability of fish
433 oil nanoemulsions was measured by Walker et al (2015a). The aqueous phase was
434 buffered at pH 3.0 to simulate the aqueous phase of a beverage system. Neither
435 particle size nor surfactant concentration had an impact on the oxidative stability of the
436 systems over 14 days of storage. Further work to evaluate the effect of physical
437 stability and pH ranges on the oxidative stability of nanoemulsions created with
438 different LC3PUFA oils, emulsifiers and antioxidants is warranted to simulate
439 conditions in food and beverage systems (Haahr and Jacobsen 2008).

440 **Nanoemulsion production methods**

441 The majority of studies identified used high power processing methods to create
442 nanoemulsion systems. High power processing has become more commonplace in
443 the creation of nanoemulsion systems in recent years, however interest in low-energy
444 methods for some applications is increasing due to their simple production methods,
445 lower costs and ability to create systems with smaller droplet size ranges than high-
446 energy methods (Walker, et al. 2015b). Both methods have benefits and
447 disadvantages. High-energy methods can be used to effectively create systems with
448 narrow droplet ranges, however the necessary equipment can be expensive. Low
449 energy methods are reasonably cheap in comparison, however high levels of
450 surfactants are required to generate stable systems (Walker, et al. 2015b). One study

451 in the review examined differences in the oxidative stability of fish oil in water
452 nanoemulsion systems created using lower-power spontaneous emulsification
453 compared to high-power microfluidization. Emulsions created using microfluidization
454 were found to have higher levels secondary oxidation products in comparison to
455 systems created using spontaneous emulsification with added iron over a 14-day
456 storage period. The authors concluded that fabrication methods may have an impact
457 on secondary oxidation products of nanoemulsions and that low energy methods can
458 be used to produce fish oil nanoemulsions without the use of expensive equipment
459 using high levels of synthetic surfactants (Walker, et al. 2015a). In addition to this study
460 previous research has identified that ultrasound processing may cause degradation in
461 edible oils with the increase of free radicals and oxidative products in sonicated oils
462 when compared to untreated oils. Microfluidization and ultrasound were the commonly
463 used processing methods identified in the review, with only the Walker et al, (2015a)
464 study examining the effect of processing treatment on oxidative stability of
465 nanoemulsions, further research in this area is therefore warranted.

466 **Type of emulsifier**

467 Emulsifiers are surface active substances that play a vital role in emulsion formation
468 and stability (Ozturk and McClements 2016). The type of emulsifier used to create
469 nanoemulsion systems can have a large impact on their oxidative stability with droplet
470 size ranges and attraction to prooxidants in the continuous phase being key factors.
471 Furthermore the oil/water ratio, emulsifier concentration and location of the emulsifier
472 within the aqueous phase interface are all important factors that can influence the
473 oxidation stability of resultant nanoemulsion systems (Jacobsen, Horn, et al. 2013,
474 Jacobsen, Sørensen, et al. 2013) Studies identified in the review by Nejadmansouri
475 et al, (2016) and Walker et al, (2015a) examined the influences of these factors and

476 found droplet ranges affected oxidation stability when a high molecular weight
477 emulsifier was utilized but there was no effect for a low weight molecular weight
478 surfactants although different levels of energy were used to create the systems.
479 Nejadmansouri et al, (2016) found droplet size ranges had a significant effect on
480 TBARS in 1% fish oil in water nanoemulsions ($P < 0.05$) created with ultrasound when
481 compared to conventional emulsions, both systems incorporated whey protein isolate.
482 Proteins usually adsorb at the interface with the lipophilic groups in the oil disperse
483 and the hydrophilic groups present in the aqueous continuous phase. Systems are
484 stabilized through electrostatic repulsion arising from charged groups on the protein
485 surface area (Genot et al. 2013, Nejadmansouri, et al. 2016). Conversely the study by
486 Walker et al (2015a) found neither particle size nor surfactant concentration had an
487 impact on the rate of oxidation in 10% fish oil nanoemulsions created using
488 spontaneous emulsification and low molecular weight synthetic surfactant Tween 80
489 to stabilize the nanoemulsion systems.

490

491 Consumer demand is dictating that the food industry should substitute synthetic
492 surfactants with more natural alternatives and there is considerable interest in food
493 products formulated with natural ingredients to provide cleaner labels (Ozturk and
494 McClements 2016, Román et al. 2017, Walker, et al. 2015b). Lecithin was the most
495 prevalent natural emulsifier identified in the review with five studies analyzing the
496 oxidative stability of systems created with lecithin from various sources. Uluata et al
497 (2015) compared systems created using sunflower lecithin to various natural and
498 synthetic emulsifiers and found that sunflower lecithin was less stable to oxidation
499 under light exposure which may impact its use in delivery systems in food and
500 pharmaceutical industries. The emulsifier quillaja saponin is a natural food-grade

501 surfactant isolated from the bark of the quillaja saponaria molina tree (Yang et al.
502 2013), it can be used produce systems with increased oxidative stability particularly
503 when additional antioxidants are utilized. Three of the studies in the review used
504 quillaja saponin and high power methods to create nanoemulsion systems using fish
505 oil, flaxseed and algae oil. Uluata et al (2015) analyzed 1 per cent fish oil ester systems
506 over a 5 day storage period and found quillaja saponin was an effective emulsifier due
507 to its physical and oxidative stability. F. Chen et al (2017) found the addition of sodium
508 caseinate gave a significant protective effect ($P < 0.05$) for quillaja saponin stabilised
509 flaxseed nanoemulsions in microgels stored over 14 days. X.- W. Chen et al (2016)
510 found the addition of β -sitosterol & γ -oryzanol in the formation of 10 per cent algae oil
511 and quillaja saponin nanoemulsions offered a significant protective effect over 30 days
512 of storage ($P < 0.05$). Further research to fully evaluate the use of quillaja saponin as
513 a natural surfactant in LC ω 3PUFA nanoemulsion enriched foods appears to be
514 warranted.

515 Other natural emulsifiers were identified in the review including systems created using
516 high power methods that were stabilised with whey protein isolate and modified
517 starches. Whey protein isolate was found to offer a protective effect for oxidation in 1
518 per cent fish oil nanoemulsion systems created with ultrasound by Nejadmansouri et
519 al (2016). Systems created with flaxseed oil and modified starch in the form of Purity
520 Gum Ultra by Sharif et al (2017) were found to be most stable to oxidation when
521 created in combination with eugenol, a phenolic compound derived from clove oil. This
522 was thought to be due to the formation of a compact thicker interfacial layer and the
523 free radical scavenging properties of eugenol.

524 Quillaja saponin and lecithin usually produce systems with a negative charge.
525 Negatively charged emulsion systems have increased susceptibility to lipid oxidation

526 when metals are present in the aqueous phase, this can be addressed by the use of
527 antioxidants as discussed earlier. Iron is thought to be the main prooxidant that
528 decomposes lipid hydroperoxides to products associated with the latter stages of
529 oxidation such as propanal (Walker, et al. 2015a). Iron was used as an accelerant in
530 the study by Wu et al (2016) that determined how typical conditions and antioxidant
531 use in food affects the stability of 1% krill oil nanoemulsions with a negative charge.
532 Krill oil contains natural phospholipids that can spontaneously form nanoemulsion
533 systems without the need for additional emulsifiers or surfactants. Iron was found to
534 be a strong prooxidant in the study and the antioxidant trolox produced systems that
535 were more stable to oxidation than α -tocopherol.

536 Synthetic emulsifiers are still extensively used to create nanoemulsions. The review
537 identified a that nonionic surfactants such as Span 80 and Tween 40 and 80 were
538 widely used to create systems with lower droplet ranges and high physical stability
539 than some of the available natural alternatives. Karthik et al (2016a) compared 10 per
540 cent algal oil nanoemulsions created using natural soy lecithin and Tween 40.
541 Refrigerated Tween 40 nanoemulsions exhibited lower lipid oxidation products and
542 there was a significant difference in peroxide values between the Tween 40 and
543 lecithin samples ($P < 0.05$). Uluata et al (2015) compared the oxidative stability of
544 nanoemulsions prepared with natural and synthetic surfactants over a 7 day storage
545 period. Systems were created using 1 per cent fish oil with natural emulsifiers lecithin
546 and quillaja saponin and synthetic emulsifiers Tween 80 and sodium dodecyl sulfate.
547 Lecithin stabilised emulsions showed increased oxidation with light exposure and
548 Tween 80 stabilised systems had significantly higher free radical scavenging capacity
549 ($P < 0.05$). Furthermore the nanoemulsions stabilised with quillaja saponin were found
550 to offer a suitable alternative to synthetic emulsifiers due to their physical and oxidative

551 stability. The authors concluded that quillaja saponin could be an outstanding natural
552 emulsifier for LC ω 3PUFA ethyl ester nanoemulsions.

553 **4.1 Recommendations**

554 Further studies should examine the potential of algal oils rich in EPA as well as DHA
555 for food enrichment in the form of nanoemulsions with a full evaluation of the oxidative
556 stability of the resultant systems in comparison to DHA algal oil products.

557 The effect of high/low processing methods has not been fully determined, further
558 research is necessary to compare the oxidative stability of LC ω 3PUFA systems
559 created with low-energy methods such as spontaneous emulsification to commonly
560 used high-power methods.

561 **Further work to evaluate the effect of physical stability and pH ranges on the oxidative**
562 **stability of nanoemulsions created with different LC3PUFA oils, emulsifiers and**
563 **antioxidants is warranted to simulate conditions in food and beverage systems.**

564 The review identified that quillaja saponin has the potential to provide an alternative to
565 synthetic emulsifiers using high power methods with a variety of source oils. Further
566 research is warranted to investigate the use of LC ω PUFA nanoemulsions systems
567 created with quillaja saponin over long term storage periods and when incorporated
568 into food matrixes.

569 Further research to determine primary and secondary oxidation products and the
570 effects of natural and synthetic emulsifiers for LC ω 3PUFA nanoemulsions created
571 using high and low processing methods is also warranted.

572 The review identified that use of nanoliposomes to encapsulate lecithin and fish oil
573 nanoemulsions provides a promising solution with significantly improvements to

574 primary and secondary oxidation stability. Further research should be conducted to
575 evaluate oxidation stability of systems created with a variety of source oils
576 incorporated into nanoliposomes.

577 **5.1 Conclusions**

578 There is considerable potential for LC ω 3PUFA functional foods that could act as
579 alternative sources to oily fish. Ready formulated vegetarian sources of EPA and DHA
580 such as algal oils are particularly promising as they provide direct sources of the more
581 effective longer chain ω 3 without the need for conversion in the metabolic pathway.
582 Using nanotechnology to incorporate these source oils into foods offers increased
583 bioavailability and, if processed under optimum conditions the oxidative stability of
584 these systems may remain similar or be improved in comparison to unprocessed/bulk
585 oils. Consumer demand dictates a clean label approach with considerable interest in
586 the use of natural ingredients. The emulsifier quillaja saponin appears to be a
587 particularly promising natural emulsifier that can produce systems with equivalent or
588 increased oxidative stability in comparison to other natural and synthetic emulsifiers,
589 particularly when additional antioxidants are used. Further studies to evaluate the
590 oxidative stability quillaja saponin in combination with algal sources of EPA and DHA
591 are warranted to enable the development of safe, clean label LC ω 3PUFA
592 nanoemulsion enriched functional food products.

593

594

595 **References**

- 596 Abbas S, Hayat K, Karangwa E, Bashari M, Zhang X. 2013. An Overview of
597 Ultrasound-Assisted Food-Grade Nanoemulsions. *Food Engineering Reviews*.
598 5(3):139-157.
- 599 Acosta E. 2009. Bioavailability of nanoparticles in nutrient and nutraceutical delivery.
600 *Current Opinion in Colloid and Interface Science*. 14(1):3-15.
- 601 Adarme-Vega TC, Thomas-Hall SR, Schenk PM. 2014. Towards sustainable sources
602 for omega-3 fatty acids production. *Current Opinion in Biotechnology*. 26:14-18.
- 603 Arterburn LM, Hall EB, Oken H. 2006. Distribution, interconversion, and dose
604 response of n-3 fatty acids in humans. *The American Journal of Clinical Nutrition*.
605 83(6):S1467-1476S.
- 606 Ashokkumar M, Bhaskaracharya R, Kentish S, Lee J, Palmer M, Zisu B. 2010. The
607 ultrasonic processing of dairy products – An overview. *Dairy of Science and*
608 *Technology*. 90(2-3):147-168.
- 609 Asif M. 2011. Health effects of omega-3,6,9 fatty acids: *Perilla frutescens* is a good
610 example of plant oils. *Oriental Pharmacy and Experimental Medicine*. 11:51-59.
- 611 Bailey N. 2009. Current choices in omega 3 supplementation. *Nutrition Bulletin*. 34:85-
612 91.
- 613 Baker EJ, Miles EA, Burdge GC, Yaqoob P, Calder PC. 2016. Metabolism and
614 functional effects of plant-derived omega-3 fatty acids in humans. *Progress in Lipid*
615 *Research*. 64:30-56.
- 616 Banel DK, Hu FB. 2009. Effects of walnut consumption on blood lipids and other
617 cardiovascular risk factors: a meta-analysis and systematic review. *The American*
618 *Journal of Clinical Nutrition*. 90(1):56-63.
- 619 Bates B, Cox L, Nicholson S, Page P, Prentice A, Steer T, Swan G. 2016. National
620 Diet and Nutrition Survey: Results from Years 5-6 (combined) of the rolling programme
621 (2012/2013 - 2013/14). London
- 622 Belhaj N, Arab-Tehrany E, Linder M. 2010. Oxidative kinetics of salmon oil in bulk and
623 in nanoemulsion stabilized by marine lecithin. *Process Biochemistry*. 45(2):187-195.
- 624 Béné C, Barange M, Subasinghe R, Pinstrup-Andersen P, Merino G, Hemre G-I,
625 Williams M. 2015. Feeding 9 billion by 2050 – Putting fish back on the menu. *Food*
626 *Security*. 7(2):261-274.
- 627 Berton C, Ropers M-HIn, Viau MI, Genot C. 2011. Contribution of the interfacial layer
628 to the protection of emulsified lipids against oxidation. *Journal of agricultural and food*
629 *chemistry*. 59(9):5052-5061.
- 630 Bigliardi B, Galati F. 2013. Innovation trends in the food industry: The case of
631 functional foods. *Trends in Food Science & Technology*. 31(2):118-129.

632 Bowen KJ, Harris WS, Kris-Etherton PM. 2016. Omega-3 Fatty Acids and
633 Cardiovascular Disease: Are There Benefits? Current Treatment Options in
634 Cardiovascular Medicine. 18(11):69.

635 Breivik H editor 2007. Long-Chain Omega-3 Speciality Oils. Bridgwater: The Oily
636 Press.

637 Calder PC. 2014. Very long chain omega-3 (n-3) fatty acids and human health.
638 European journal of lipid science and technology. 116(10):1280-1300.

639 Chen B, Rao J, Ding Y, McClements DJ, Decker EA. 2016. Lipid oxidation in base
640 algae oil and water-in-algae oil emulsion: Impact of natural antioxidants and
641 emulsifiers. Food Research International. 85:162-169.

642 Chen F, Liang L, Zhang Z, Deng Z, Decker EA, McClements DJ. 2017. Inhibition of
643 lipid oxidation in nanoemulsions and filled microgels fortified with omega-3 fatty acids
644 using casein as a natural antioxidant. Food Hydrocolloids. 63:240-248.

645 Chen X-W, Chen Y-J, Wang J-M, Guo J, Yin S-W, Yang X-Q. 2016. Phytosterol
646 structured algae oil nanoemulsions and powders: improving antioxidant and flavor
647 properties. Food & Function. 7(9):3694-3702.

648 Coultate TP. 2009. Food: the chemistry of its components. Royal Society of Chemistry.

649 Deckelbaum RJ, Torrejon C. 2012. The omega-3 fatty acid nutritional landscape:
650 health benefits and sources. The Journal of nutrition. 142(3):587S-591S.

651 Decker EA, Akoh CC, Wilkes RS. 2012. Incorporation of (n-3) fatty acids in foods:
652 challenges and opportunities. The Journal of nutrition. 142(3):610S-613S.

653 Ee SL, Duan X, Liew J, Nguyen QD. 2008. Droplet size and stability of nano-emulsions
654 produced by the temperature phase inversion method. Chemical Engineering Journal.
655 140(1):626-631.

656 Esquerdo VM, Dotto GL, Pinto LAA. 2015. Preparation of nanoemulsions containing
657 unsaturated fatty acid concentrate–chitosan capsules. Journal of Colloid and Interface
658 Science. 445:137-142.

659 Fetterman JW, Zdanowicz MM. 2009. Therapeutic potential of n-3 polyunsaturated
660 fatty acids in disease. American Journal of Health System Pharmacy. 66(13):1169-
661 1179.

662 Genot C, Kabri TH, Meynier A. 2013. 5 - Stabilization of omega-3 oils and enriched
663 foods using emulsifiers. In: Food Enrichment with Omega-3 Fatty Acids. Woodhead
664 Publishing. p. 150-193.

665 Ghorbanzade T, Jafari SM, Akhavan S, Hadavi R. 2017. Nano-encapsulation of fish
666 oil in nano-liposomes and its application in fortification of yogurt. Food Chemistry.
667 216:146-152.

668 Haahr A-M, Jacobsen C. 2008. Emulsifier type, metal chelation and pH affect oxidative
669 stability of n-3-enriched emulsions. European journal of lipid science and technology.
670 110(10):949-961.

- 671 Health Council of the Netherlands. 2015. Dutch dietary guidelines 2015. The Hague.
- 672 Henna Lu FS, Nielsen NS, Timm-Heinrich M, Jacobsen C. 2011. Oxidative Stability of
673 Marine Phospholipids in the Liposomal Form and Their Applications. *Lipids*. 46(1):3-
674 23.
- 675 Homayoonfal M, Khodaiyan F, Mousavi SM. 2014. Optimization of walnut oil
676 nanoemulsions prepared using ultrasonic emulsification: A response surface method.
677 *Journal of Dispersion Science and Technology*. 35(5):685-694.
- 678 Hong MY, Lumibao J, Mistry P, Saleh R, Hoh E. 2015. Fish Oil Contaminated with
679 Persistent Organic Pollutants Reduces Antioxidant Capacity and Induces Oxidative
680 Stress without Affecting Its Capacity to Lower Lipid Concentrations and Systemic
681 Inflammation in Rats. *The Journal of nutrition*. 145(5):939-944.
- 682 Horn AF, Nielsen NS, Jensen LS, Horsewell A, Jacobsen C. 2012. The choice of
683 homogenisation equipment affects lipid oxidation in emulsions. *Food chemistry*.
684 134(2):803-810.
- 685 Hu M, Jacobsen C. 2016. Oxidative stability and shelf life of foods containing oils and
686 fats. Elsevier.
- 687 International Society for the Study of Fatty Acids and Lipids. 2004. Report of the sub-
688 committee on recommendations for Intake of polyunsaturated fatty acids in healthy
689 adults. Brighton.
- 690 Jacobsen C. 2010. Enrichment of foods with omega-3 fatty acids: a multidisciplinary
691 challenge. *Annals of the New York Academy of Sciences*. 1190(1):141-150.
- 692 Jacobsen C, Horn AF, Nielsen NS. 2013. 12 - Enrichment of emulsified foods with
693 omega-3 fatty acids. In: *Food Enrichment with Omega-3 Fatty Acids*. Woodhead
694 Publishing. p. 336-352.
- 695 Jacobsen C, Nielsen NS, Horn AF, Sørensen A-DM. 2013. Food enrichment with
696 omega-3 fatty acids. Elsevier.
- 697 Jacobsen C, Sørensen ADM, Nielsen NS. 2013. 4 - Stabilization of omega-3 oils and
698 enriched foods using antioxidants. In: *Food Enrichment with Omega-3 Fatty Acids*.
699 Woodhead Publishing. p. 130-149.
- 700 Jafari SM, He Y, Bhandari B. 2006. Nano-emulsion production by sonication and
701 microfluidization - A comparison. *International Journal of Food Properties*. 9:475-485.
- 702 Karthik P, Anandharamakrishnan C. 2016a. Enhancing omega-3 fatty acids
703 nanoemulsion stability and in-vitro digestibility through emulsifiers. *Journal of Food
704 Engineering*. 187:92-105.
- 705 Karthik P, Anandharamakrishnan C. 2016b. Fabrication of a nutrient delivery system
706 of docosahexaenoic acid nanoemulsions via high energy techniques. *RSC Advances*.
707 6(5):3501-3513.

- 708 Khan RS, Grigor J, Winger R, Win A. 2013. Functional food product development –
709 Opportunities and challenges for food manufacturers. *Trends in Food Science &*
710 *Technology*. 30(1):27-37.
- 711 Lane K, Derbyshire E. 2015. Omega-3 Fatty Acids—A Review of Existing and
712 Innovative Delivery Methods. *Critical reviews in food science and nutrition*. (just-
713 accepted):00-00.
- 714 Lane KE, Derbyshire E, Li W, Brennan C. 2014. Bioavailability and Potential Uses of
715 Vegetarian Sources of Omega-3 Fatty Acids: A Review of the Literature. *Critical*
716 *reviews in food science and nutrition*. 54(5):572-579.
- 717 Lane KE, Li W, Smith C, Derbyshire E. 2014. The bioavailability of an omega-3-rich
718 algal oil is improved by nanoemulsion technology using yogurt as a food vehicle.
719 *International Journal of Food Science & Technology*. 49(5):1264-1271.
- 720 Lenihan-Geels G, Bishop KS. 2016. *Alternative Origins for Omega-3 Fatty Acids in the*
721 *Diet*. Cham: Springer International Publishing.
- 722 Leong TS, Wooster TJ, Kentish SE, Ashokkumar M. 2009. Minimising oil droplet size
723 using ultrasonic emulsification. *Ultrasonics sonochemistry*. 16(6):721-727.
- 724 Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin
725 B, Kris-Etherton P, Harris WS, Howard B, et al. 2006. Diet and lifestyle
726 recommendations revision 2006: a scientific statement from the American Heart
727 Association Nutrition Committee. *Circulation*. 114(1):82-96.
- 728 Linnamaa P, Savolainen J, Koulu L, Tuomasjukka S, Kallio H, Yang B, Vahlberg T,
729 Tahvonen R. 2010. Blackcurrant seed oil for prevention of atopic dermatitis in
730 newborns: a randomized, double-blind, placebo-controlled trial. *Clinical &*
731 *Experimental Allergy*. 40(8):1247-1255.
- 732 Lu FSH, Thomsen BR, Hyldig G, Green-Petersen DMB, Nielsen NS, Baron CP,
733 Jacobsen C. 2013. Oxidative Stability and Sensory Attributes of Fermented Milk
734 Product Fortified with Fish Oil and Marine Phospholipids. *Journal of the American Oil*
735 *Chemists' Society*. 90(11):1673-1683.
- 736 Maki KC, Yurko-Mauro K, Dicklin MR, Schild AL, Geohas JG. 2014. A new, microalgal
737 DHA- and EPA-containing oil lowers triacylglycerols in adults with mild-to-moderate
738 hypertriglyceridemia. *Prostaglandins, Leukotrienes and Essential Fatty Acids*
739 (PLEFA). 91(4):141-148.
- 740 McClements DJ, Decker E. 2000. Lipid oxidation in oil-in-water emulsions: Impact of
741 molecular environment on chemical reactions in heterogeneous food systems. *Journal*
742 *of food science*. 65(8):1270-1282.
- 743 McClements DJ, Rao J. 2011. Food-grade nanoemulsions: formulation, fabrication,
744 properties, performance, biological fate, and potential toxicity. *Critical reviews in food*
745 *science and nutrition*. 51(4):285-330.
- 746 McClements DJ. 2015. *Food emulsions: principles, practices, and techniques*. CRC
747 press.

- 748 Meyer B. 2011. Are we consuming enough long chain omega-3 polyunsaturated fatty
749 acids for optimal health? Prostaglandins, Leukotrienes and Essential Fatty Acids
750 (PLEFA). 85(5):275-280.
- 751 Meyer BJ. 2016. Australians are not Meeting the Recommended Intakes for Omega-
752 3 Long Chain Polyunsaturated Fatty Acids: Results of an Analysis from the 2011-2012
753 National Nutrition and Physical Activity Survey. *Nutrients*. 8(3):111.
- 754 Mir M. 2008. Echium oil: A valuable source of n-3 and n-6 fatty acids. *Sources D'*
755 *Omega-3*. 15(4):252-256.
- 756 Moher D, Liberati A, Tetzlaff J, Altman DG. 2009. PRISMA 2009 Checklist. York.
- 757 Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P,
758 Stewart LA. 2015. Preferred reporting items for systematic review and meta-analysis
759 protocols (PRISMA-P) 2015 statement. *Systematic Reviews*. 4(1):1.
- 760 National Health and Medical Research Council. 2013. Australian Dietary Guidelines.
761 In: Canberra.
- 762 Nejadmansouri M, Hosseini SMH, Niakosari M, Yousefi GH, Golmakani MT. 2016.
763 Physicochemical properties and storage stability of ultrasound-mediated WPI-
764 stabilized fish oil nanoemulsions. *Food Hydrocolloids*. 61:801-811.
- 765 Ozturk B, McClements DJ. 2016. Progress in natural emulsifiers for utilization in food
766 emulsions. *Current Opinion in Food Science*. 7:1-6.
- 767 Papanikolaou Y, Brooks J, Reider C, Fulgoni VL. 2014. U.S. adults are not meeting
768 recommended levels for fish and omega-3 fatty acid intake: results of an analysis using
769 observational data from NHANES 2003–2008. *Nutrition Journal*. 13(1):31.
- 770 Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, Cooney M-T,
771 Corrà U, Cosyns B, Deaton C, et al. 2016. 2016 European Guidelines on
772 cardiovascular disease prevention in clinical practice The Sixth Joint Task Force of the
773 European Society of Cardiology and Other Societies on Cardiovascular Disease
774 Prevention in Clinical Practice (constituted by representatives of 10 societies and by
775 invited experts) Developed with the special contribution of the European Association
776 for Cardiovascular Prevention & Rehabilitation (EACPR). *European Heart
777 Journal*. 37(29):2315-2381.
- 778 Pingret D, Durand Gg, Fabiano-Tixier A-S, Rockenbauer A, Ginies C, Chemat F. 2012.
779 Degradation of edible oil during food processing by ultrasound: electron paramagnetic
780 resonance, physicochemical, and sensory appreciation. *Journal of agricultural and
781 food chemistry*. 60(31):7761-7768.
- 782 Pingret D, Fabiano-Tixier A-S, Chemat F. 2013. Degradation during application of
783 ultrasound in food processing: A review. *Food Control*. 31(2):593-606.
- 784 Rasti B, Jinap S, Mozafari MR, Yazid AM. 2012. Comparative study of the oxidative
785 and physical stability of liposomal and nanoliposomal polyunsaturated fatty acids
786 prepared with conventional and Mozafari methods. *Food chemistry*. 135(4):2761-
787 2770.

- 788 Rasti B, Erfanian A, Selamat J. 2017. Novel nanoliposomal encapsulated omega-3
789 fatty acids and their applications in food. *Food chemistry*. 230:690-696.
- 790 Román S, Sánchez-Siles LM, Siegrist M. 2017. The importance of food naturalness
791 for consumers: Results of a systematic review. *Trends in Food Science & Technology*.
792 67:44-57.
- 793 Ryan AS, Keske MA, Hoffman JP, Nelson EB. 2009. Clinical Overview of Algal-
794 Docosaehaenoic Acid: Effects on Triglyceride Levels and Other Cardiovascular Risk
795 Factors. *American Journal of Therapeutics*. 16(2):183-192.
- 796 Ryckebosch E, Bruneel C, Muylaert K, Foubert I. 2012. Microalgae as an alternative
797 source of omega-3 long chain polyunsaturated fatty acids. *Lipid technology*. 24(6):128-
798 130.
- 799 Salvia-Trujillo L, Decker EA, McClements DJ. 2016. Influence of an anionic
800 polysaccharide on the physical and oxidative stability of omega-3 nanoemulsions:
801 Antioxidant effects of alginate. *Food Hydrocolloids*. 52:690-698.
- 802 Sanguansri P, Augustin MA. 2006. Nanoscale materials development – a food industry
803 perspective. *Trends in Food Science and Technology*. 17(10):547-556.
- 804 Shahidi F editor 2005. *Bailey's Industrial Oil and Fat Products, Volumes 1-6 (6th*
805 *Edition)*. London: Wiley.
- 806 Shahidi F, Zhong Y. 2010. Lipid oxidation and improving the oxidative stability.
807 *Chemical Society Reviews*. 39(11):4067-4079.
- 808 Sharif HR, Williams PA, Sharif MK, Khan MA, Majeed H, Safdar W, Shamoan M,
809 Shoaib M, Haider J, Zhong F. 2017. Influence of OSA-starch on the physico chemical
810 characteristics of flax seed oil-eugenol nanoemulsions. *Food Hydrocolloids*. 66:365-
811 377.
- 812 Simopoulos AP. 2011. Evolutionary Aspects of Diet: The Omega-6/Omega-3 Ratio
813 and the Brain. *Molecular Neurobiology*. 44(2):203-215.
- 814 Solans C, Solé I. 2012. Nano-emulsions: formation by low-energy methods. *Current*
815 *Opinion in Colloid & Interface Science*. 17(5):246-254.
- 816 Solè I, Maestro A, Gonzalez C, Solans C, Gutiérrez JM. 2006. Optimization of nano-
817 emulsion preparation by low-energy methods in an ionic surfactant system. *Langmuir*.
818 22(20):8326-8332.
- 819 Surette ME. 2013. Dietary omega-3 PUFA and health: Stearidonic acid-containing
820 seed oils as effective and sustainable alternatives to traditional marine oils. *Molecular*
821 *Nutrition & Food Research*. 57(5):748-759.
- 822 Trivelpiece WZ, Hinke JT, Miller AK, Reiss CS, Trivelpiece SG, Watters GM. 2011.
823 Variability in krill biomass links harvesting and climate warming to penguin population
824 changes in Antarctica. *Proceedings of the National Academy of Sciences*.
825 108(18):7625-7628.

826 Uluata S, McClements DJ, Decker EA. 2015. Physical stability, autoxidation, and
827 photosensitized oxidation of ω -3 oils in nanoemulsions prepared with natural and
828 synthetic surfactants. *Journal of agricultural and food chemistry*. 63(42):9333-9340.

829 Verbeke W, Sioen I, Pieniak Z, Van Camp J, De Henauw S. 2005. Consumer
830 perception versus scientific evidence about health benefits and safety risks from fish
831 consumption. *Public Health Nutrition*. 8(4):422-429.

832 Walker RM, Decker EA, McClements DJ. 2015a. Physical and oxidative stability of fish
833 oil nanoemulsions produced by spontaneous emulsification: Effect of surfactant
834 concentration and particle size. *Journal of Food Engineering*. 164:10-20.

835 Walker RM, Decker EA, McClements DJ. 2015b. Development of food-grade
836 nanoemulsions and emulsions for delivery of omega-3 fatty acids: opportunities and
837 obstacles in the food industry. *Food & Function*. 6(1):41-54.

838 Wang J, Shahidi F. 2017. Oxidative stability of marine oils as affected by added wheat
839 germ oil. *International Journal of Food Properties*. (just-accepted).

840 World Health Organisation. 2003. Joint WHO/FAO expert consultation on diet, nutrition
841 and the prevention of chronic diseases, WHO technical report series no 916, Geneva
842 WHO Geneva.

843 Wu Q, Uluata S, Cui L, Wang C, Li D, McClements J, Decker EA. 2016. Physical and
844 oxidation stability of self-emulsifying krill oil-in-water emulsions. *Food & Function*.
845 7(8):3590-3598.

846 Yang Y, Leser ME, Sher AA, McClements DJ. 2013. Formation and stability of
847 emulsions using a natural small molecule surfactant: Quillaja saponin (Q-Naturale®).
848 *Food Hydrocolloids*. 30(2):589-596.

849 Yates CM, Calder PC, Ed Rainger G. 2014. Pharmacology and therapeutics of omega-
850 3 polyunsaturated fatty acids in chronic inflammatory disease. *Pharmacology &*
851 *Therapeutics*. 141(3):272-282.

852 Yi J, Zhu Z, McClements DJ, Decker EA. 2014. Influence of Aqueous Phase
853 Emulsifiers on Lipid Oxidation in Water-in-Walnut Oil Emulsions. *Journal of agricultural*
854 *and food chemistry*. 62(9):2104-2111.

855 Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. 2004. Dietary
856 α -Linolenic Acid Reduces Inflammatory and Lipid Cardiovascular Risk Factors in
857 Hypercholesterolemic Men and Women. *The Journal of nutrition*. 134(11):2991-2997.

858 Zhu J, Zhuang P, Luan L, Sun Q, Cao F. 2015. Preparation and characterization of
859 novel nanocarriers containing krill oil for food application. *Journal of Functional Foods*.
860 19:902-912.

861

862

863

864 Table 1 – Recommendations for fish and LCω3PUFA intakes

Source	Quantity	Country/Organisation	Reference
Fish recommendations	1–2 Fish meals per week	FAO / WHO	(World Health Organisation 2003)
	2 Fish meals per week preferably oily or at least one oily	Netherlands, Australia, America, Europe	(Health Council of the Netherlands 2015) (National Health and Medical Research Council 2013) (Lichtenstein et al. 2006) (Piepoli et al. 2016)
LCω-3PUFA recommendations	200–500 mg/d EPA and DHA	FAO/WHO	(World Health Organisation 2003)
	450 mg/d EPA and DHA	The Netherlands	(Health Council of the Netherlands 2015)
	430–570 mg/d EPA and DHA	America	(Lichtenstein, et al. 2006)
	500 mg/d EPA and DHA	America, Australia, ISSFAL	(Lichtenstein, et al. 2006) (National Health and Medical Research Council 2013) (International Society for the Study of Fatty Acids and Lipids 2004)
	120 mg/d DHA min, 430 mg/d EPA, DPA and DHA women	Australia	(National Health and Medical Research Council 2013)
	610 mg/d EPA, DPA and DHA men	Australia	(National Health and Medical Research Council 2013)

865

866

867

868

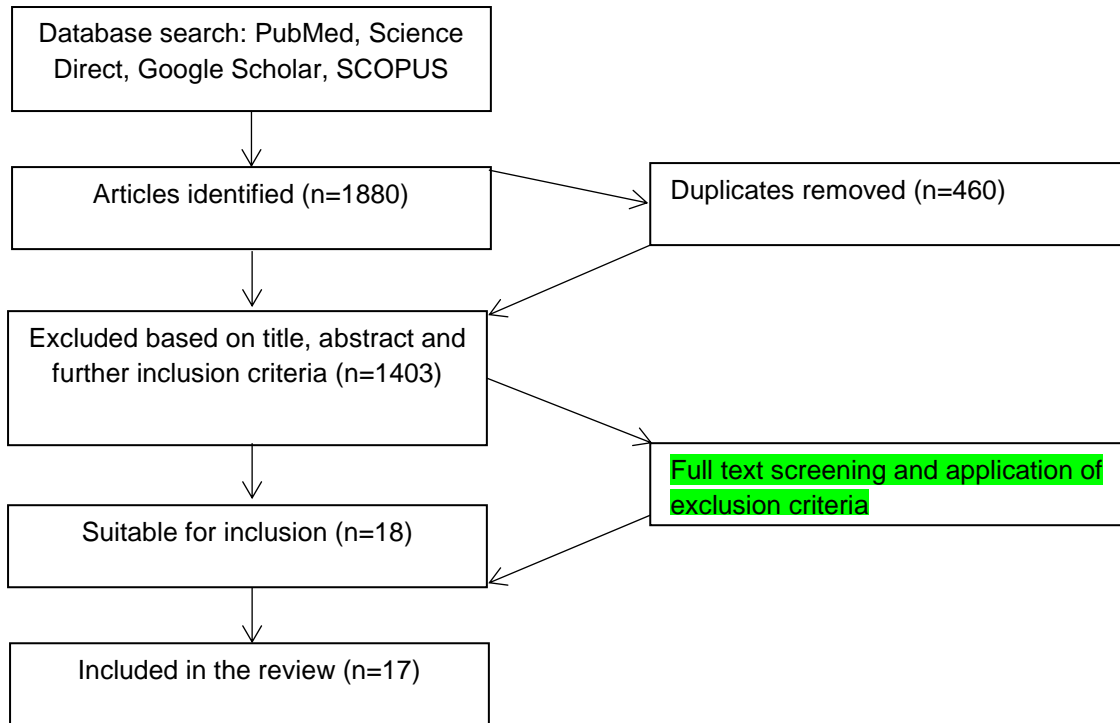
869

870

871

872 Table 2 – Summary of systematic review selection process

873



874

875

Table 3 – Results of the literature review

Article/Author/date	Study objectives	Emulsion type, % system droplet range and measure	Oil type and functional fatty acid	Emulsifier/surfactant and % of system	Antioxidant/other ingredients	Creation method	Oxidation test methods and storage periods	Main findings
Novel nanoliposomal encapsulated omega-3 fatty acids and their applications in food (Rasti, et al. 2017)	Evaluate the application, stability and suitability of ω 3 PUFAs incorporated nanoliposomes in food enrichment. Nanoliposomes compared to microencapsulated ω 3 PUFAs and bulk fish oil in milk and bread	Oil in water liposomes, fish oil and soy lecithin 0.4:2, mass ratio, with deionised water, 20-200nm, Zetasizer	Fish oil containin g EPA and DHA (3:2, 300mg/g) Microencapsulated fish oil, 10% EPA and DHA.	Soy lecithin, 1-4% nanoliposomes added to milk and bread.		Ultrasound	Peroxide values, anisidine values, 7 days bread, 3 days milk.	Peroxide and anisidine values for ω 3 enriched bread and milk samples increased significantly (P = 0.004) but not for the nanoliposomal enriched samples. Enriched bread would provide 170.6-174.8mg EPA and 113.3-117.6mg DHA/100g. Enriched milk 167.4-171.0mg EPA and 112.6-115.2mg DHA/100ml. Nanoliposomes can be used to fortify bread and milk.
Influence of OSA-starch on the physiochemical characteristics of flax seed oil-eugenol nanoemulsions. (Sharif, et al. 2017)	Examine the effect on oxidation of eugenol (EUG) and 2 modified starches as an emulsifiers for flaxseed oil nanoemulsions	Oil in water nanoemulsions. 99.73 to 558.2(nm). Mean droplet diameter (MDD) and polydispersity Index (PDI) using Zetasizer	Flaxseed oil, 57.0% ALA	Purity Gum Ultra (PG1), Purity Gum 2000 (PG2) starches, 10% flaxseed oil	Eugenol (EUG)	Microfluidizer	Peroxide value Headspace analysis of hexanal and propanal, 4 weeks	Higher % retention of ALA and EUG in PG1. Eugenol served an antioxidant role, PG1 showed improved physical and oxidative stability and provided better outer coverings to the encapsulated materials (P < 0.05). These findings would help in the development and incorporation of oxidatively stable ALA rich nanoemulsions in dairy and beverages.
Inhibition of lipid oxidation in nanoemulsions and filled microgels fortified with	Examine sodium caseinate as a natural antioxidant in nanoemulsion filled	Oil-in-water nanoemulsion Static light scattering (Mastersizer)	Flaxseed oil 71.4wt% of polyunsaturated fat	Quillaja saponin Emulsifier solution (1.12% (w/w) 10% (w/w)	Caseinate	Microfluidizer	Peroxide values TBARS Microstructure analysis, 14 days	Peroxide and TBARS values of nanoemulsions without caseinate increased significantly more than the other systems during storage (P < 0.05) Peroxide and TBARS values with caseinate increased moderately throughout storage, but at a much

omega-3 fatty acids using casein as a natural antioxidant. (Chen, et al. 2017)	hydrogel beads (microgels) fortified with omega-3 fatty acids.	Mean particle sizes - D ₃₂ or D ₄₃ after homogenization = <200nm D ₄₃ after homogenization = >200nm		flaxseed oil in water. , 5 mM phosphate 160 buffer, pH 7.0) 0.8 alginate beads injected into calcium chloride				slower rate than for the nanoemulsions without caseinate (P < 0.05). Encapsulating flaxseed oil droplets within an antioxidant protein-rich hydrogel bead is highly effective at protecting against oxidation.
Nano-encapsulation of fish oil in nano-liposomes and its application in fortification of yogurt. (Ghorbanzade , et al. 2017)	Incorporate nano-encapsulated fish oil by nano-liposomes into yogurt and evaluate the physicochemical and sensory effect on yogurt quality	Dynamic light scattering (Mastersizer) 300-500nm Encapsulation of fish oil by nano liposomes	Purified fish oil (fatty acid composition not specified)	Soy lecithin		Ultrasound	Fatty acid profile, Peroxide value, Sensory analysis, 21 days	Liposomal structures were successful for nanoencapsulation as DHA & EPA remained stable. Addition of nanoencapsulated fish oil to yogurt gave closer characteristics to control sample in terms of sensory parameters than yogurt with free (unencapsulated) fish oil.
Enhancing omega-3 fatty acids nanoemulsion stability and in-vitro digestibility through emulsifiers. (Karthik and Anandharama krishnan 2016a)	Evaluation of 3 different emulsifiers on the physiochemical stability and in-vitro digestibility of DHA nanoemulsions produced by microfluidization.	Oil in water nanoemulsion Laser light diffraction particle size analyser. Triplicate measurements T-40 NE & NaCa = smaller size (206 ±0.034nm) SL – larger (760 ± 0.131nm)	DHA algae oil (38.11% DHA)	10 w/w algae oil 2.8% w/w solution)	N/A	Microfluidizer	Particle size Peroxide value Fatty acid profile, 20 days	Refrigerated T-40 emulsion exhibited lower lipid oxidation than the other emulsions. There was a significant difference in PV between T40 and SL (P < 0.05). There were no changes in the functional group and fatty acid profile of DHA after nanoemulsification. The T-40 emulsion appears to be more advantageous in terms of oxidative stability and in-vitro digestibility
Physicochemical properties	Investigate effects of major	Oil-in-water nanoemulsion	Fish oil (FO)	Whey protein	N/A	Ultrasound	Peroxide value	A significant increase in TBARS for conventional emulsions compared to

and storage stability of ultrasound-mediated WPI-stabilised fish oil nanoemulsions. (Nejadmansouri, et al. 2016)	parameters of whey protein isolate, fish oil, weight ratio (WR) and pH) on characteristics of high intensity ultrasound mediated fish oil nanoemulsions. Main focus on physicochemical properties, oxidative stability and fatty acids profile changes of the nanoemulsions for 1m storage at different temperatures	1% (w/w) dispersed phase at different to-oil ratios (ranging from 0.5 to 1.5) and different pH values D ₄₃ & span Measured by static light scattering average particle size 84nm	EPA - 7% DHA - 18% of total fatty acids	isolate (WPI) into 5 mM buffer solution of pH7 containing 0.03% (w/w) sodium azide as an antimicrobial agent.		TBARS Fatty acid profile 28 days	ultrasound emulsions (P < 0.05). The increased antioxidant capacity of WPI in nanoemulsions was likely due to sonochemical reactions from ultrasound treatment. The oxidation rate of the nanoemulsion at 25°C was more than 4°C (P < 0.05) due to enhanced temperature.
Fabrication of a nutrient delivery system of docosahexaenoic acid nanoemulsions via high energy technique. (Karthik and Anandharama krishnan 2016b)	Investigate high pressure homogenization. (HPH) High speed homogenization (HSH) and combination of the HSH + HPH techniques to produce stable DHA nanoemulsions	Oil in water nanoemulsion Malvern zetasizer (z potential) Dynamic light scattering (particle size distribution) Refractive indices HSH & HPH combined mean particle range = 11.17 & 11.31	Algae oil 38.11% DHA	Tween-40 (2.8% w/w) Algae oil 10%, w/w	High pressure homogenizers/high speed homogenizers	Fatty acid profile TBARS, 100 days	There was no change in fatty acid profile or structural changes of DHA in any of the emulsions. Refrigerated HPH and HSH + HPH DHA exhibited lower lipid oxidation than the emulsion stored at other conditions (P < 0.05). Better stability achieved via HSH & HPH technique compared to HPH and could be used in future in the food industry to improve stability and bioavailability of omega 3 delivery

		HSH only = 87 (nm diameter)							
Influence of an anionic polysaccharide on the physical and oxidative stability of omega-3 nanoemulsions: Antioxidant effects of alginate. (Salvia-Trujillo, et al. 2016)	Assessment of the impact of an anionic polysaccharide on the physical properties and chemical stability of fish oil-in-water nanoemulsions	Oil in water Nanoemulsion Light scattering (Mastersizer) Particle size - D ₄₃ Initial droplet diameter 135nm	Fish oil (Ropufa ω-3 food oil) containin g 101 mg of EPA/g of oil, 148 mg of DHA/g oil, and a total of omega-3 PUFA of 312 mg/g of oil. Lemon oil	Tween 80 1% w/w Aqueous Oil phase 10% (fish and lemon oil 50:50 w/w) phase mM acetic-acetate buffer at pH 3.0	Sodium alginate (anionic) Chitosan (cationic) Methyl cellulose (non-ionic)	Microfluidizer	Hydroperoxides, TBARS 20 days	Chitosan and alginate were significantly more effective at inhibiting lipid oxidation (P < 0.05) Intermediate sodium alginate addition resulted in increases mean droplet sizes. Outcome - The use of alginate as a natural antioxidant in nanoemulsions can be effective; however, it also highlights the potential for this polysaccharide to promote physical instability	
Physical and oxidative stability of self-emulsifying krill oil-in-water emulsions. (Wu, et al. 2016)	To determine how conditions typical in foods impact the physical stability of krill oil-in-water emulsions and determine how antioxidants' polarity influences the oxidative stability of krill oil-in-water emulsions	1 wt% krill oil in water nanoemulsions Zetasizer (particle size distribution) determined after emulsion preparation and every day during each experiment. Particle size range – 150-165nm	Integral phospholipid emulsifiers within Krill oil (30% EPA and DHA 32% phospholipids)	1% krill oil 99 wt% mM acetic acid buffer pH7	α-tocopherol Trolox	Microfluidizer	Hydroperoxides, TBARS, 8 days	Lipid oxidation was accelerated by ferrous chloride (P < 0.05). All α-tocopherol concentrations decreased lipid hydroperoxides (P < 0.05). Addition of α-tocopherol after homogenisation inhibited hydroperoxide and TBAR formation (P < 0.05). Iron was a strong pro-oxidant and trolox was a better antioxidant than α-tocopherol	
Phytosterol structured algae oil nanoemulsion and powders: improving	Reduce or delay oxidation and off-flavours by phytosterols structured in	Algae oil (wt10%) in water nanoemulsion	DHA algae oil LCω3PUFA content 40%	Quillaja saponin 1.4 wt% Algae oil 10% wt surfactant dispersed	β-sitosterol γ-oryzanol campesterol	Ultrasound	Peroxide value GCHS Characterisation of spray dried	Spray dried algae oil powders from structured nanoemulsions exhibit excellent reconstructed behaviour up to 30 d of storage.	

antioxidant and flavour properties. (Chen, et al. 2016)	saponin-stabilised algae oil-in-water nanoemulsions and spray-dried powders made from the nanoemulsion templates	Dynamic light scattering (Zetasizer) Particle size range = 152 - 164nm	within an deionised water aqueous phase Phosphate buffer (pH 7.0)					powders (examining microstructure and reconstitution behaviour), 30 days	Formulation with β -sitosterol & γ -oryzanol resulted in enhanced oxidative stability ($P < 0.05$) Structured algae oil-loaded nanoemulsion and powder had lower levels of fishy off-flavour Phytosterols are an effective strategy to reduce off-flavours and maximize oxidative stability of both algae oil nanoemulsions and spray dried powders
Physical and oxidative stability of fish oil nanoemulsions produced by spontaneous emulsification: Effect of surfactant concentration and particle size. (Walker, et al. 2015b)	To examine the potential of spontaneous emulsification to fabricate fish oil nanoemulsions that are suitable for application in clear beverages.	Oil in water nanoemulsion (10 wt% total oil phase) Measured with either Zetasizer (dynamic) or Mastersizer (static light scattering) D_{32} (for large droplets) Z-average (small droplets)	Fish oil and lemon oil (FO) (Ropufa 30 ω -3 food oil) containin g 101 mg of EPA/g of oil, 148 mg of DHA/g oil, and 312 mg of total ω -3 PUFA/g of oil.	Tween 80 non-ionic (2.5–20 wt%) 5 wt % fish oil 5 wt% lemon oil Tween 80. Aqueous phase was 70–87.5 wt% double distilled water with 0.8 wt% citric acid and 0.08 wt% sodium benzoate at pH 3.0, Emulsions prepared using different surfactant - to-oil ratios (SOR)	Butylated hydroxytoluene. Sodium benzoate. Citric acid	Microfluidizer (MF), Spontaneous emulsification (SOR)	Peroxide value TBARS, 14 days	All emulsions reached a peak for hydroperoxides levels after 12 days. Low energy systems with added surfactant had slightly higher hydroperoxides values than other emulsions towards the end of the study. The MF emulsion reached the highest TBARS value within the 14 days. Neither particle size nor surfactant concentration had a major impact on the rate of lipid oxidation in the fish oil emulsions. Low-energy homogenization methods (spontaneous emulsification) can be used to produce fish oil emulsions that may be suitable to fortify transparent food or beverage systems.	

Physical Stability, Autoxidation, and Photosensitized Oxidation of ω -3 Oils in Nanoemulsions Prepared with Natural and Synthetic Surfactants. (Uluata, et al. 2015)	How synthetic and natural emulsifiers impacted the physical stability of nanoemulsions, autoxidation, and photosensitized lipid oxidation in oil-in-water emulsions.	Oil in water Nanoemulsion Particle electrophoresis instrument Z-potential Mean particle diameter of all samples was lower than 100nm	Fish oil ethyl ester containin g 55% omega-3 fatty acids	lecithin & quillaja saponin natural emulsifiers Tween 80 & sodium dodecyl sulfate (SDS) synthetic emulsifiers 1.5 wt % and 10 mM sodium 1% fish oil 99% aqueous phases. Phosphate buffer solution (pH 7).	Microfluidizer	Particle size Oxygen radical absorption assay (ORAC) Hydroperoxides, GCHS (propanal), 7 days	After 5 days storage hydroperoxide formation and propanal were in the order Tween 80 > SDS > lecithin > quillaja saponin and lecithin > Tween 80 > SDS > quillaja saponin respectively. Lecithin stabilised emulsions showed increased oxidation with light exposure. ORAC values showed Tween 80 had a significantly higher free radical scavenging capacity ($P \leq 0.05$) Quillaja saponin is an effective emulsifier for ω -3 ethyl ester nanoemulsions due to its physical and oxidative stability.	
Preparation & characterization of novel nanocarriers containing krill oil for food application. (Zhu, et al. 2015)	To Evaluate suitability and effectiveness of NLC (Nanostructured liposomes) as a delivery system to encapsulate krill oil and investigate chemical and physical stability of the prepared NLC.	Oil in water Nanoemulsion Zetasizer Nano ZS90 ZP value - 31.0mV, 332nm.	Antarctic krill oil (14.8% DHA, 22.5% EPA and 250 mg/kg astaxanthin). Total lipid phase (w/w) (X1)	Lecithin surfactant (w/w) (X2) in double distilled water to make aqueous solution Differing ratios of krill and palm oil used.	Ultrasound	Photostability and assay of bioactive constituents (DHA, EPA and astaxanthin), 70 days	NLC offers bioactives in krill oil giving significant protection against photooxidation upon exposure to UV light ($P < 0.05$). Good physical and chemical stabilities during long-term storage at different temperatures. Feasibilities of pasteurization and lyophilization were also demonstrated Novel nanocarriers containing krill oil could be used in functional drinks and milk powders	
Preparation of nanoemulsions containing unsaturated	To prepare nanoemulsions containing capsules of	Oil in water Zetasizer All nanoemulsion	Carp oil, PUFA content 35.6%	Tween 80. 1 % w/v chitosan powder, 5%	N/A	High speed homogenization at 10,000 rpm	Peroxide value (PV), 45 days	PV from carp oil and UFAC nanocapsules similar at baseline. PV for UFAC nanocapsules remained stable during storage while oil PV increased.

fatty acid concentrate–chitosan capsules (Esquerdo, et al. 2015)	unsaturated fatty acid concentrate (UFAC) using chitosan as wall material (UFAC–chitosan nanocapsules) and determine the stability	s presented capsules in the nanometric scale boundaries, smallest size 332nm	bleached oil and UFAC 50.1% respectively. 15 or 30% oil in ultra pure water	w/w Tween 80, Acetic acid solution (1% w/v) Then, the surfactant Tween 80 (5% w/w, in relation to chitosan) added.				PV values demonstrated that the microstructure was able to protect the UFAC against primary oxidation. The encapsulation efficiency was 74.1%, Chitosan has potential to be used as encapsulating agent for UFAC.
Optimization of walnut oil Nanoemulsions prepared using ultrasonic emulsification: A response surface method. (Homayoonfal, et al. 2014)	To investigate the emulsifying conditions including ultrasonic time (UT) & concentration ratio on the particle size, Span, and loss of antioxidant activity (LAA) of walnut oil-nanoemulsions	Oil in water nanoemulsion Lazer light scattering. D ₄₃ Average particle size 338 – 450nm	Walnut oil (Fatty acid composition not stated)	Tween 80/Span 80 0.7, 0.5 and 0.3 ratio (deionised water aqueous phase)Walnut oil disperse phase 8, 6 and 4% w/w. 0.01% w/w	Sodium azide (0.01% w=w)	Ultrasound	Response surface methodology (RSM) modelling. Loss of antioxidant activity (LAA), 35 days	The quadratic effect of UT was significant in LAA (P < 0.05). The enhancement of UT reduced the d ₄₃ and span, while this led to increased loss of antioxidant activity
Comparative study of the oxidative and physical stability of liposomal and nanoliposomal polyunsaturated fatty acids prepared with conventional and Mozafari methods (Rasti et al. 2012)	Evaluate and compare the physicochemical properties of PUFA liposomes and nanoliposomes created using the Mozafari method (liposomes prepared by direct hydration and without solving the PL	Oil in water liposomal suspensions, zetasizer, liposomes 362.5nm and nanoliposomes 316.5nm respectively	Fish oil DHA and EPA 2:3, 400mg/g	Lecithin. Fish oil and lecithin 2:0.4 mass ratio, 2% v/v oil in water	N/A	Ultrasound	Conjugated dienes and cyclic peroxides.	A significantly (P < 0.05) higher concentration of conjugated dienes and TBARS than was found in the initial values, was observed in liposomes prepared using the conventional method. In contrast, liposomes prepared with the Mozafari method did not show a significant increase (P < 0.05) in conjugated dienes and TBARS content

	and FAs in organic solvents)							
Oxidative kinetics of salmon oil in bulk and in nanoemulsion stabilised by marine lecithin. (Belhaj, et al. 2010)	To examine the preparation and characterisation of different formulations of nanoemulsions composed of salmon oil and marine lecithin with or without antioxidants	Oil in water nanoemulsion 5 different samples with different ratios of crude salmon oil, marine lecithin, alpha-tocopherol & water) Zetasizer Droplet range for most samples ranged between 200 – 207nm. One sample had a droplet size of 160nm due to high polar lipids	Salmon oil in 5 different formats, fatty acid composition of oils not presented.	Lecithin. 10% oil in deionised water marine lecithin quercetin α-tocopherol in different ranges)	α-tocopherol E307), astaxanthin, quercetin, lecithin from salmon heads:	High-pressure valve homogenizer	Polyene index, conjugated dienes (GC) fourier transform infrared spectroscopy (FT-IR), 40 days	Crude salmon oil was well-protected by its own natural antioxidant (tocopherol and astaxanthin). Salmon oil with marine lecithin was the most stable to oxidation. The use of marine phospholipids as emulsifiers in nanoemulsions preparation increases notably the stability of salmon oil against oxidation with a rise in LC-PUFA availability, especially in DHA.