

The composition and oxidative stability of vegetarian omega-3 algal oil nanoemulsions
suitable for functional food enrichment

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

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Abstract

Background: Long chain omega-3 polyunsaturated fatty acid (LCn3PUFA) nanoemulsion enriched foods offer potential to address habitually low oily fish intakes. Nanoemulsions increase LCn3PUFA bioavailability, but may cause lipid oxidation. This study examined oxidative stability of LCn3PUFA algal oil-in-water nanoemulsions created by ultrasound using natural and synthetic emulsifiers during 5-weeks of storage at 4, 20 and 40°C. Fatty acid composition, droplet size ranges and volatile compounds were analysed.

Results: No significant differences were found for fatty acid composition at various temperatures and storage times.

Lecithin nanoemulsions had significantly larger droplet size ranges at baseline and during storage regardless of temperatures. While combined Tween 40 and lecithin nanoemulsions had low initial droplet size ranges, there were significant increases at 40°C after 5-weeks storage. Gas chromatograms identified hexanal and propanal as predominant volatile compounds, along with 2-ethylfuran; propan-3-ol; valeraldehyde. The Tween 40 only nanoemulsion sample showed formation of lower concentrations of volatiles compared to lecithin samples. Formation of hexanal and propanal remained stable at lower temperatures although higher concentrations were found in nanoemulsions than bulk oil. The lecithin only sample had formation of higher concentrations of volatiles at increased temperatures despite having significantly larger droplet size ranges than the other samples.

Conclusions: Propanal and hexanal were the most prevalent of five volatile compounds detected in bulk oil and lecithin and/or Tween 40 nanoemulsions. Oxidation compounds remained more stable at lower temperatures indicating suitability for enrichment of refrigerated foods. Further research to evaluate the oxidation stability of these systems within food matrices is warranted.

Keywords: Omega-3 fatty acids; Algal oil; Nanoemulsion; Oxidative stability; Lecithin; Tween 40.

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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.

EPA and DHA are characterised by long carbon chain lengths and a high degree of lipid unsaturation, which increases their susceptibility to oxidation when exposed to air, light and heat, all of which are inevitable during emulsion processing. The mechanism of lipid oxidation is proposed to include three stages initiation, propagation and termination ¹⁵. A high variety of volatile compounds of different polarity, stability and small molecular weight are formed which can be detected by dynamic headspace analysis ¹⁶. Once the initiation phase has begun the rate of oxidation increases exponentially and foods are quickly spoiled. Lipid oxidation in enriched/functional foods impacts the shelf-life, safety, nutritional value, functionality and flavour of the subsequent food products ¹⁷. The creation of LCn3PUFA nanoemulsions may further increase the oxidation susceptibility of these oils due to high droplet surface areas and the need for greater processing.

In previous work, novel oil-in-water nanoemulsion systems suitable for functional food enrichment were successfully created using ultrasound processing with commercially available high DHA vegetarian algal oil loads up 50% (w/w), which has not been previously achieved ¹⁸. The addition of a 50% (w/w) system is less likely to have a detrimental effect on food matrices than systems with lower oil loads as lower volumes of nanoemulsion can be added to achieve optimum enrichment levels ¹⁹. The systems were also demonstrated to significantly increase the bioavailability of total LCn3PUFA and DHA in a pilot randomised crossover trial ¹⁴, although additional larger, longer studies are needed to further ratify these findings. Preliminary evaluation to analyse total oxidation values (totox) indicated these systems remained within safe ranges when stored at 4°C for 37 days ¹⁸. However, there were significant detrimental changes in the sensory properties of yogurt when the nanoemulsion systems were used as a fortification vehicle, which may indicate the presence of volatile

oxidation compounds²⁰. A further 16-day shelf life sensory evaluation of 50% (w/w) algal oil and lecithin nanoemulsion enriched strawberry yogurt indicated that detrimental sensory changes were detected after 2 days of storage but significant improvements to several sensory attributes were found after 16 days of refrigerated storage, which warrants further investigation²¹.

Lecithin, a zwitterionic natural emulsifier has good emulsifying properties due to its molecular structure, which has hydrophilic and lipophilic groups and a hydrophilic-lipophilic balance (HLB) of 8 making it well suited to the successful creation of LC ω 3PUFA algal oil and marine based oil nanoemulsions²². Lecithin has been used successfully to create physically stable LCn3PUFA nanoemulsion systems in various previous studies^{16, 19, 23-25} and has been found to increase oxidative stability of emulsions whilst maintaining physical stability²⁶. The use of Tween 40 in the creation of LCn3PUFA nanoemulsions has also previously been demonstrated to have a protective effect on lipid oxidation²⁷⁻²⁹. However, a study to evaluate the oxidation stability of high load DHA algal oil lecithin and Tween 40 nanoemulsions using more comprehensive methods has yet to be undertaken. Therefore, the aim of the current study was to evaluate the oxidative stability of algal oil nanoemulsions created with ultrasound using lecithin and Tween 40 solely and in combination to maximise their physical and chemical stabilising properties, which coupled with the gas chromatography headspace method is a novel and useful study. The oxidative stability of algal oil was compared in bulk form and nanoemulsion under the same conditions with no other added components other than deionised water and the two types of emulsifier.

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. $\geq 30\%$ (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.

Both emulsion premixes (10g lecithin premix, 22g Tween 40 premix) were combined with algal oil (43g) and deionised water (25g) to give a 50% algal oil sample with a combination of lecithin and Tween 40 containing 3% w/w of each emulsifier.

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After premixing, the coarse emulsions were replaced in the water bath for a further 2 hours and hand stirred for 1 min at 30 min intervals. The temperature was controlled at 55°C. Samples underwent primary homogenisation using a L5 series Silverson rotor–stator mixer (Silverson Machines Ltd, England), on a medium setting (668.12 x g) for 2 min, then processed under an ultrasonic processor (BSP-1200 Ultrasonic processor, New York, USA) using Amplitude 100% with power 850w, operated at 19650Hz for 10 minutes to create nanoemulsions. A cold-water cooling jacket was used to ensure sample temperatures remained below 55°C.

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2.1.2 Fatty acid composition analysis by Gas Chromatography (GC)

The fatty acid composition analysis of algal oil and nanoemulsion samples was performed using fatty acid methyl ester (FAME) analysis whereby 0.5000 g algal oil /1.000 g of nanoemulsion sample and 10 ml Reagent A (2.5% w/v KOH solution in Methanol) were added into a MARSXpress vessel microwave digestion tube, then closed. The tube was placed into the Kevlar sleeves of a Mars 6 microwave (CEM Ltd., UK). The temperature was increased to 90°C in 5 min and held for 10 min. After cooling to room temperature, 15ml reagent B (2% 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

unit set 2400 rpm. For the emulsion samples, an absorption parameter value of 0.001 was selected and a refractive index ratio 1.488 for algal oil¹⁹. For the purposes of this study, Sauter 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 average value and the droplet size distribution and has been used in a number of previous studies^{19, 34}.

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

Gas chromatography (GC) was performed using 2 g nanoemulsion samples prepared with 1 ml 1% NaCl solution, added to HS vial and vortexed for 30 secs. The samples were heated in a Headspace (HS) sampler (TuborMatrix 40 PerkinElmer Inc, USA) at 100°C for 60 min and injected under the following conditions: vial pressure 30psi; pressurise time 0.2 min; needle temp 100 °C; injection time 4.8 sec; withdrawal time 6 sec.

To account for possible production of volatile compounds during heating, peak areas for heating times of 20, 40 and 60 min at 100°C were compared and 60 min chosen as this gave the most consistent peak composition results. The samples were analysed by Gas Chromatography Headspace Analysis (GCHS) Clarus 580, (PerkinElmer Inc, USA) equipped with a Flame Ionization Detector and 60 m 0.32 diameter column, 1.8 µm film thickness (Agilent Technologist, USA) under the conditions: Hydrogen flow rate 17 psi; injector temperature 230°C; detector temperature 230°C; oven temperature: from 40°C, ramp to 230°C at 20°C /min and hold at 230°C for 1 min, total time was 10.5 min. The volatile compounds were identified by reference to the retention time of standards. Analysis was 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 \pm 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.

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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

remained relatively stable during storage at lower temperatures, which is comparable to other work in the field ²⁸.

3.2 Emulsion Droplet Sizes

The droplet size ranges of the nanoemulsion samples were measured by laser light scattering particle sizer at baseline and intervals during storage at the different temperatures. The mean droplet sizes and droplet ranges of the nanoemulsions prepared with lecithin, Tween 40 and in combination are shown in Figures 1 and 2. At baseline the nanoemulsions prepared with Tween 40 (TN) and lecithin and Tween 40 combined (LTN) showed significantly smaller ($p < 0.05$) droplet sizes (242 ± 0.002 nm and 172 ± 0.002 nm respectively) than those prepared with lecithin alone (LN) (340 ± 0.001 nm). Statistical analysis using two-way ANOVA testing revealed the lecithin samples had significantly larger droplet ranges throughout the 5-week storage period at all temperatures ($p < 0.05$).

The combined LTN sample had the smallest droplet size ranges at baseline; however, there were significant increases in droplet sizes from baseline and 5-weeks at 20 and 40°C ($p < 0.05$). Larger droplet ranges for lecithin samples in this study may be explained by the molecular weight of lecithin, which is greater than that of Tween 40 ^{39, 40}. The sole use or use of high ratios of lecithin is more likely to produce larger droplets with thicker interfacial surface areas ⁴¹. The inclusion of Tween 40 may have reduced the droplet size ranges, because the lower molecular weight and higher hydrophilic-lipophilic balance (HLB) value of 15.6 ²². Tween 40 combined with lecithin which has a lower HLB of 8 ²² creates a balance giving a system with smaller droplet sizes.

3.3. Oxidation and volatiles produced in nanoemulsion preparation and storage

GCHS is used widely to measure volatile compounds produced in the terminal stages of lipid oxidation and is beneficial as no oil extraction methods are required⁴². The oxidised volatile compounds produced by hydroperoxides (ROOH) breakdown in the last stage of oxidation. Products with lower molecular weights than those of ROOH can therefore be used as a measure of lipid oxidation⁴³.

Hexanal and propanal were the predominant volatile compounds detected in this study (Figure 3) and these have also been previously identified as common indicators of secondary oxidation for LCn3PUFA nanoemulsions⁴⁴. Further gas chromatograms identified oxidised compounds 2-ethylfuran, propan-3-ol and valeraldehyde were produced by the algal oil and its nanoemulsions (see Figure 2 and Tables 3 to 6) all of which have been associated with rancid off flavours in oxidised LCn3PUFA oils and emulsions^{42, 45}. Significant differences were found for all volatiles except valeraldehyde in both lecithin nanoemulsion samples stored at 40°C after 2 weeks ($p < 0.05$). The Tween 40 samples showed formation of higher concentrations of hexanal in comparison to the lecithin only sample and this was further accelerated by increases in temperature.

The combined lecithin and Tween 40 sample showed formation of lower concentrations of volatiles at week 1 than the lecithin only sample; however, the Tween 40 only sample had the lowest concentrations of the five volatile compounds when analysing storage time and temperature. These differences may due to the nature of the emulsifiers, since lecithin consists of phospholipids that can also be susceptible to lipid oxidation^{13, 27}.

It may be that LA, which is abundant in lecithin ⁴⁶ positioned in the interfacial (outer) region of emulsion droplets and closer to the aqueous phase of the system was subjected to increased exposure to oxidation accelerants ⁴⁷, which could explain higher levels of volatile compounds in these samples. Following ultrasound processing, the DHA in these samples may have retained some stability as it was located within the interior of nanoemulsion oil droplets that were surrounded by lecithin molecules at the interfacial region ^{12, 37}.

All samples remained the most stable at 4°C with the least amount of significant differences in volatile compounds found at this temperature. Increases in sample storage temperature led to significant increases in the development of volatile compounds for all samples, so the degradation of fatty acids and production of oxidised compounds was temperature dependant particularly for the changes in the range of 20 °C to 40 °C.

Both lecithin samples demonstrated formation of higher concentrations of volatiles for storage times and temperature than the Tween only samples at 40°C, again indicating that Tween 40 may offer an overall protective effect whilst lecithin is less stable to oxidation particularly at increased temperatures. This is comparable to research by Uluata, McClements, & Decker ²⁷ and Arancibia et al ¹³ who found that lecithin nanoemulsions had higher propanal development at increased temperatures.

The fatty acid composition of samples in this study showed no significant differences for temperature and storage times although small non-significant changes were noted for LA and DHA in some samples at higher temperatures as discussed earlier. The droplet size range measurements showed the lecithin only samples had significantly larger droplet size ranges throughout the trial, which suggests that larger droplet size ranges do not offer a protective effect for the development of volatile compounds. Karthik & Anandharamakrishnan ²⁴ also found lecithin samples had larger droplet ranges and that Tween 40 could be used to create systems with smaller droplet ranges and offered a protective effect for oxidative stability. In the current study, samples created using the synthetic emulsifier Tween 40 had the smallest, most stable droplet ranges and developed fewer volatile compounds than the lecithin containing samples.

The present work evaluated volatile compounds found in the headspace of the bulk algal oil and its various nanoemulsions. Systems remained the most stable 4°C which coupled with our previous tottox work ²⁹ indicates the potential use of these systems as a refrigerated food enrichment vehicle. Further work is now needed to analyse the effect 50% (w/w) algal oil nanoemulsion enrichment may have on various appropriate food matrixes. In terms of application and further development for a larger bioavailability trial there may be concerns over the commercial acceptability of synthetic emulsifier Tween 40 in functional food products ¹², which would also need to be addressed.

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).

27. Uluata S, McClements DJ and Decker EA, Physical stability, autoxidation, and photosensitized oxidation of ω -3 oils in nanoemulsions prepared with natural and synthetic surfactants. *Journal of agricultural and food chemistry* **63**:9333-9340 (2015).
28. Karthik P and Anandharamakrishnan C, Fabrication of a nutrient delivery system of docosahexaenoic acid nanoemulsions via high energy techniques. *RSC Advances* **6**:3501-3513 (2016).
29. Lane KE, Zhou Q, Robinson S and Li W, Lipid oxidation within vegetarian long chain omega-3 polyunsaturated fatty acid oil nanoemulsions suitable for food fortification. *Proceedings of the Nutrition Society* **76**:E101 (2017).
30. Martek Biosciences Corporation, Application for the authorization of DHA and EPA-rich algae oil from *Schizochytrium sp.*, Ed by Gray RJH. Martek Biosciences corporation, Columbia, pp 1-51 (2010).
31. Kentish S, Wooster TJ, Ashokkumar M, Balachandran S, Mawson R and Simons L, The use of ultrasonics for nanoemulsion preparation. *Innovative Food Science & Emerging Technologies* **9**:170-175 (2008).
32. Sun Y, Xia Z, Zheng J, Qiu P, Zhang L, McClements DJ and Xiao H, Nanoemulsion-based delivery systems for nutraceuticals: Influence of carrier oil type on bioavailability of pterostilbene. *Journal of Functional Foods* **13**:61-70 (2015).
33. Horiba Scientific, A guidebook to particle size analysis, Ed by Horiba Instruments Inc, Irvine, USA, pp 1-28 (2010).
34. Yang Y, Leser ME, Sher AA and McClements DJ, Formation and stability of emulsions using a natural small molecule surfactant: Quillaja saponin (Q-Naturale®). *Food Hydrocolloids* **30**:589-596 (2013).
35. Medina I, Satue - Gracia MT and Frankel EN, Static Headspace Gas Chromatographic Analyses to Determine Oxidation of Fish Muscle Lipids During Thermal Processing. *Journal Of The American Oil Chemists' Society* **76**:231-236 (1999).
36. Iglesias J and Medina I, Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle. *Journal of Chromatography A* **1192**:9-16 (2008).
37. Taladrid D, Marín D, Alemán A, Álvarez-Acero I, Montero P and Gómez-Guillén MC, Effect of chemical composition and sonication procedure on properties of food-grade soy lecithin liposomes with added glycerol. *Food Research International* **100**:541-550 (2017).
38. Pingret D, Fabiano-Tixier A-S and Chemat F, Degradation during application of ultrasound in food processing: A review. *Food Control* **31**:593-606 (2013).
39. National Center for Biotechnology Information, *PubChem Database*. Polysorbate 40, 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, Shamoan 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