1.0. Introduction

Long chain omega-3 (*n*-3) polyunsaturated fatty acids (LCω3PUFA) in the human diet are mainly obtained from oily fish, fish oil or fish oil based supplements (Bourre, 2007). Recent evidence from Western countries indicates that certain population groups may not be consuming enough LCω3PUFA (Elmadfa & Freisling, 2009; Micha et al., 2015). Current UK recommendations state that two portions of fish should be consumed per week, one of which should be oily fish amounting to 140g per week of oily fish (Scientific Advisory Committee on Nutrition, 2004). However, average oily fish consumption in the UK is only around eight grams per day (Bates et al., 2014). Non fish sources of LCω3PUFA are particularly important for vegetarians, non-fish eaters and pregnant mothers (Lane, Derbyshire, Li, & Brennan, 2014a).

Eicosapentaenoic acid (20:5 *n*-3; EPA) and docosahexaenoic acid (22:6 *n*-3; DHA) comprise of the main LCω3PUFA in oily fish and have been linked to healthy aging throughout the life cycle (Swanson, Block, & Mousa, 2012). DHA plays a crucial role in normal human retinal and brain development and is considered by some as an essential fatty acid during early childhood development (Uauy, 2009). Further benefits have also been identified including cardiovascular health, decreased inflammation, improved cognitive function, health promotion and disease reduction (Aberg et al., 2009; Dawczynski, Martin, Wagner, & Jahreis, 2010; Mukaro et al., 2008; Murphy et al., 2007; Shahidi, 2015). Vegan diets are completely devoid of DHA and vegetarian diets contain smaller amounts of DHA than that of meat and particularly fish eaters (Ryan & Symington, 2015; Sanders, 2009).

The potential health implications of low LCω3PUFA intakes coupled with concerns about the sustainability of fish stocks call for innovative approaches to achieve a solution. The use of alternative sources of LCω3PUFA to fish oil is likely to be beneficial as based on current production methods, it is estimated that demand for fish oil will far exceed supply by 2025 (Jacobsen, Torstensen, & Undeland, 2013). Currently the most significant vegetarian dietary form of LCω3PUFA is alpha-linolenic acid (18:3 *n*-3; ALA), which can be found in flaxseeds, walnuts and other seed oils (Edel, Pierce, & Aliani, 2015; Lemahieu et al., 2015; Navas-Carretero et al., 2015). However, previous research has established that in humans, conversion of ALA into the more beneficial longer chain EPA and DHA found in oily fish is limited (Burdge & Calder, 2005; Burdge, Jones, & Wootton, 2002; Deckelbaum & Torrejon, 2012; Lane & Derbyshire, 2013b). Microalgal oils are produced in tightly controlled fermentation facilities and may offer a sustainable alternative source of LCω3PUFA in the forms of DHA and EPA that are also suitable for vegetarians and vegans (Arterburn et al., 2007; Ryan & Symington, 2015; Salem & Eggersdorfer, 2015; Sanders, 2009).

Supplements may provide a substitute, but the National Diet and Nutrition Survey (2014) found that supplements are only used by 11% of the general population (Bates et al., 2014). Supplements are widely available in capsule form, although in some cases their biological effects can be diminished or even lost due to incomplete absorption (Schuchardt & Hahn, 2013). Bioavailability is a measurement of the extent an active component reaches the systemic circulation and is available at the site of action (Huang, Yu, & Ru, 2010). Most sources of nutrients function differently when incorporated into food matrixes than in bulk forms, which may affect bioavailability, therefore food based approaches are recommended to optimise the bioavailability of fatty acids (Kris-Etherton & Hill, 2008).

A further solution may be offered by nanoemulsions, which have extremely small droplet sizes ranging from 50 to 500nm and can be used to encapsulate sensitive or volatile ingredients (Jafari, He, & Bhandari, 2006; Kentish et al., 2008; Sun et al., 2015).

When an emulsion consists of an entire droplet distribution below 80nm there may be advanced properties in comparison to conventional larger sized emulsions including transparency, increased colloidal stability and a large interfacial area in comparison to volume (Kentish et al., 2008). Materials at the nanometre scale equate to 10^{-9} m (Rao & McClements, 2011; Silva, Cerqueira, & Vicente, 2011).

The incorporation of nutrients into foods using nanotechnology has the potential to improve bioavailability due to small particle sizes and high surface to surface volume ratio (Acosta, 2009; Sun et al., 2015). Lipid emulsions behave differently in the digestive tract in accordance with droplet sizes (Armand et al., 1999). Small droplets of nutrients can easily be transported in the body through cell membranes giving increased blood plasma and erythrocyte concentrations (Huang et al., 2010). However, the use of nanoemulsions of omega-3 oils in food matrices may create challenges with consumer acceptability and oxidation stability, which must be considered (Augustin et al., 2015; Jacobsen, 2009; Tippetts & Martini, 2010; Walker, Decker, & McClements, 2015). The objective of this study was to develop stable vegetarian LCω3PUFA oil in water nanoemulsion systems suitable for incorporation into functional foods.

2.0. The creation of an oil-in-water nanoemulsion system

Materials and methods

2.1. Materials to create emulsion systems

Testing was conducted using vegetarian LCω3PUFA source oils rich in DHA or ALA (see Table 1).

DHA-S *schizochytrium sp* vegetarian algae oil containing 35% of fatty acids as DHA was kindly provided by DSM, London, UK. Flaxseed oil containing 52% of fatty acids as ALA was purchased online from Holland and Barrett, Manchester UK. The fatty acid content of flaxseed and algal oil was verified using lipid extraction and fatty acid analysis using the methods detailed by (Bell et al., 2002). Liquid soy lecithin was purchased from Now Foods, Bloomingdale, IL, USA. Tween 40 was purchased from Sigma-Aldrich Company Limited, Loughborough, UK.

2.2. Preparation methods

All emulsions were of the 'oil-in-water' (o/w) type and were prepared in accordance with methods that are patented by the authors (Lane, Derbyshire, Li, & Smith, 2012). The aqueous continuous phase was deionised water; the lipid dispersed phase was the oil. The emulsifier was either soy lecithin (LE), Tween 40 (TW) or a combination of soy lecithin and Tween 40 (TWLE).

A solution of 70% (w/w) LCω3PUFA oil in combination with 30% (w/w) lecithin was prepared two hours in advance and placed in a water bath at 55°C to dissolve. Tween 40 was introduced directly into deionised water, which had been brought to 55°C in a water bath.

Initially, coarse emulsions containing different compositions of oil, emulsifier and deionised water were prepared. Once prepared, samples were placed in a water bath at 55°C for two

hours and were hand stirred for 1 min at 30 min intervals. Samples underwent primary homogenisation using a Silverson rotor–stator mixer on a medium setting (4000rpm) for 2 min. Development trials took place with 15 and % (w/w) oil content. As stable systems were replicated, further trials were conducted using up to 70% (w/w) oil phase at various intervals.

Secondary homogenization was completed by ultrasound using a 24 kHz sonicator (Dr Hielscher series, Model UP 400S, Hielscher Ultrasound Technology, Teltow, Germany). This system consisted of a generator, converter and a sonotrode H22 titanium tip. The horn tip was immersed in the coarse emulsion for the designated time (max depth 45mm) then the ultrasonic processor was turned on at full power (Hielscher Ultrasound Technology, 2007).

After initial trials, all experiments were completed using a cold water cooling jacket to control temperature increases and each experiment was duplicated. The cooling jacket facilitated the treatment of a 250mg sample, which was agitated by hand throughout the process to ensure a more even distribution of ultrasound and to avoid hotspots in the sample. Samples were subjected to ultrasound for 30-second intervals then collected after each treatment and examined under a microscope at a 120 magnification using immersion oil and photographed.

Once stability and particle size had been established visually, further trials were completed using samples with 20, 25, 30, 40, 50 and 70% (w/w) oil with 2, 3, 4, 5 6, 7 and 8% (w/w) emulsifier. From visual analysis the 20, 50 and 70% (w/w) o/w emulsions consisting of 6% (w/w) lecithin (LE), 6% (w/w) Tween 40 (TW) or a combination of lecithin and Tween 40 in 50:50 (w/w) ratio (TWLE) were selected for particle size measurements.

2.3. Analysis of nanoemulsion systems

2.3.1. Measurement of temperature rises

Temperature increases were measured with a standard laboratory thermometer probe. Temperature increases can significantly reduce the oxidative stability of LCω3PUFA rich oils. Research by Alamed *et al.* (2006) indicated that LCω3PUFA emulsions can be heated to 90°C for up to 10 min without affecting oxidative stability. Temperature measurements were taken during the ultrasound process to maintain the oxidation stability of oils during product development. To monitor temperature increases during processing, 250ml of coarse emulsion was prepared for each sample using 50% (w/w) oil. Samples were then placed in a cold water cooling jacket and subjected to ultrasound treatment at maximum power output (100µm amplitude). Temperature measurements were taken at 30 sec intervals for up to 20 min using a thermometer probe, which was immersed directly into the sample.

2.3.2. Methods of measuring emulsion droplet sizes

Particle sizes were determined using a Malvern Mastersizer 2000 (courtesy of Glyndŵr University, Wrexham, UK). Droplet size (DS) distributions were measured for each interval in accordance with the methods used by Akhtar *et al.* (2006) and Akhtar and Dickinson (2003) using a Malvern Mastersizer MS2000 laser light-scattering analyser with a small sample dispersion unit set to 2000rpm. A drop of each emulsion sample amounting to approximately 10µl were pipetted into the dispersion unit. For the emulsion samples an absorption parameter value of 0.001 and the refractive index ratio of 1.488 for the algae oil and 1.4770 for the flaxseed oil were used (Breivik, 2007).

Samples were measured in duplicate to ensure accuracy with a 15-sec pause between measurements. For the purposes of this study the d_{32} Sauter mean ($d_{32} = \sum n_i d_i^3 / \sum n_i d_i^2$ (Horiba Scientific, 2010)) 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 (Abismaïl, Canselier, Wilhelm, Delmas, & Gourdon, 1999; Kentish et al., 2008; Yang, Leser, Sher, & McClements, 2013).

2.3.3. Statistical analysis

Statistical analyses were conducted on the Mastersizer droplet measurement results using SPSS version 19 to assess the effect of oil load, temperature, processing time and type of emulsifier on DS (d_{32} parameter). Prior to statistical analysis, all results were assessed for statistical compatibility using the Kolmogorov-Smirnov and Shapiro-Wilk tests to check for normality (Pallant, 2010). The effect of oil load for the three selected emulsifiers was assessed using the paired *t*-test function and non-parametric alternative Wilcoxon Signed Rank Test as described by Bell and Rowley (2011). The effect of emulsifier and temperature was measured using a one-way ANOVA test. The effect of the three different emulsifiers on processing time and droplet sizes was assessed using a two-factor repeated measures ANOVA with a Bonferroni adjustment to determine the main effect of the emulsifier and time interaction effects as described by Field (2013).

Where a main effect was observed a one-factor and one-factor repeated measures ANOVA test with post-hoc testing was conducted along with the non-parametric alternative Kruskal-Wallis test to identify where the differences were located. For the one-way ANOVA the d_{32} was added as dependant variable and the type of emulsifier was entered as the factor. The

Tukey and Scheffe post-hoc tests were selected to identify potential differences. For the Kruskal-Wallis test the d_{32} was entered as the test variable and the type of emulsifier was entered as the grouping variable as described by Pallant (2012).

3.0. Results and discussion

3.1. The creation of vegetarian omega-3 nanoemulsion systems

Table 2 shows the final oil to water emulsion ratios and experimental design processes used to create the vegetarian LCω3PUFA o/w nanoemulsions.

3.2. Temperature rises

The temperature rises of nanoemulsions created using ultrasound were measured to ensure that processing would not promote lipid oxidation. Recent research by Salvia-Trujillo *et al.* (2012) demonstrated that the processing of emulsions under ultrasound caused significant temperature increases, which were particularly prevalent at 100µm amplitude maximum ultrasound power, with increases of 27 to 47°C after 180 sec under ultrasound. The use of a cooling jacket in this study ensured that temperatures for samples processed at 100µm amplitude over 20 min rose by 20°C maximum and did not exceed 51°C (see Figure 2) which should not impact on oxidative stability (Alamed, McClements, & Decker, 2006).

3.3. Droplet sizes

Droplet measurement confirmed that the 7, 20 and 50% (w/w) oil samples could be classified as nanoemulsion systems in accordance previously defined measurements in the literature (figure 3) (Anton & Vandamme, 2011; Jafari et al., 2006; Kentish et al., 2008).

The 70% (w/w) oil load sample was not stable and particle sizes were in the µm range, so it could not be classed as nanoemulsion system. The particle size measurements for the 70% (w/w) flaxseed oil samples indicated that a nanoemulsion had not been successfully created. The oil load for this sample created instability resulting in a system that was prone to separation and appeared to have undergone phase inversion.

3.4. The impact of ultrasound processing energy on droplet measurements

To assess the impact of processing energy, samples were prepared using ultrasound at 30, 50, 70 and 100-µm amplitude. Initial visual analyses of samples under a standard laboratory microscope (see Figure 1) demonstrated that maximum ultrasound power caused maximum droplet disruption, creating small droplets with minimal processing times. All further samples were prepared at 100-µm amplitude to maximise droplet reduction.

Salvia-Trujillo *et al.* (2013) found that ultrasound amplitude and treatment time significantly reduced the droplet sizes of nanoemulsions, with 100µm amplitude facilitating the largest reductions in 1% (w/w) lemongrass oil–alginate nanoemulsions. Ultrasound treatment of the 50 % (w/w) samples at 100µm amplitude for up to 20 min created nanoscale droplet ranges, with optimum sizes achieved between 10 and 12 min for all emulsifiers (see Table 3). Overall ultrasound processing time had a significant effect on droplet sizes (P <0.001), although this

effect was only significant from 0 to 2 min of processing, changes were not statistically significant after 2 min of processing (see Table 4).

3.5. The effect of oil loading on droplet measurements

Statistical analyses assessed the effect of oil loading on droplet sizes and demonstrated that oil loads had a statistically significant effect on d_{32} measurements and that droplet sizes increased with higher oil loads ($P < 0.05$). This was the case for all emulsifiers except lecithin, which was approaching significance for parametric testing ($P = 0.051$) and statistically significant for non-parametric testing ($P = 0.038$) see Table 4.

Extensive droplet measurement research using high DHA algae oil nanoemulsions has yet to be published, indicating that this system is novel. However, a number of studies have made similar findings to this research using a variety of other lipid sources including flaxseed oil. Abismaïl *et al.* (1999) found that the d_{32} measurements of kerosene in water emulsions prepared with Tween 60 surfactant and generated using ultrasound, increased with oil load from approximately 250nm at 5% (w/w) to 900nm at 50% (w/w) oil load. Phase inversion was also noted when emulsions contained equal volumes of each phase.

Phase inversion did not occur in the present study at equal rates of algae or flaxseed oil and water, but was noted when samples reached a 70% (w/w) oil load. Phase inversion can occur when the droplet interface is only partially covered by surfactant particles and is therefore more likely to occur with increased oil loads (Lee, Niknafs, Hancocks, & Norton, 2012). A study by van Nieuwenhuyzen and Szuhaj (1998) further validates the findings from this study, o/w nanoemulsion droplet sizes were found to increase in lecithin or lecithin and o/w systems

from <100 to 300-500nm when the oil/lecithin concentration was increased from 0/1 (lecithin only) to 9/1 in ratio.

Previous research identifies the effect of increased oil loads on the droplet sizes of nanoemulsions and validates the findings from the current study. A study by Kentish *et al.* (2009) produced comparable results to this research, although the oil loads were lower. Flaxseed oil 15% (w/w) and 5.6% (w/w) Tween 40 emulsifier were used to produce nanoemulsions using ultrasound. The flaxseed study demonstrated that stable nanoemulsion systems with minimum droplet sizes of 120nm (d_{32}) were created with 15% (w/w) flaxseed oil loads. Further research using 10% (w/w) oil loads demonstrates that systems with a complete distribution below 100nm (d_{32}) can be created using ultrasound in combination with d-limonene oil and 1% surfactant (Li & Chiang, 2012). Oil loads of 1% lemongrass oil were combined with Tween 80 surfactant by Salvia-Trujillo *et al.* (2012) to successfully create translucent systems with extremely small minimum average droplet sizes of 4.31nm (d_{32}) with narrow size distributions. In addition to oil loads, the droplet sizes of nanoemulsions can be affected by the choice of emulsifier.

3.6. The effect of emulsifier on droplet measurements

The emulsifiers in this study were chosen in accordance with their hydrophilic-lipophilic balance (HLB) and favourable attributes identified in the literature (Coultate, 2009; Tadros, 2009; Tadros, Izquierdo, Esquena, & Solans, 2004). It was also hypothesised that the combination of the two emulsifiers in equal quantities would create a neutrally balanced HLB giving a system with very small droplet sizes that was less susceptible to lipid oxidation.

Temperature monitoring demonstrated that temperature increases were not significantly affected by the choice of emulsifier (see Table 4).

Emulsifiers act as surfactants and play an important role in deformation and break-up of droplets. Surfactants allow the existence of interfacial tension gradients, which are crucial for formation of stable oil droplets (Tadros et al., 2004).

During emulsification, interfacial tension is lowered causing a reduction in droplet sizes, which will further reduce with increased surfactant quantities until a plateaux value is reached. Emulsifiers that adsorb to the interface fastest will stabilise newly formed droplets more quickly than emulsifiers with slower adsorption rates (Lee et al., 2012). Small molecular weight surfactants are usually more efficient in emulsion stabilisation than biopolymers. They are adsorbed to the freshly formed surface of the droplet and stabilise the new interface in milliseconds preventing droplet coalescence (Jafari, He, & Bhandari, 2007). Visual analysis of samples under the microscope demonstrated that a 6% level of surfactant appeared to give optimum droplet reduction (Figure 1). Nanoemulsions have been created with emulsifier quantities around this level in comparable research by Kentish *et al.* (2009).

Statistical analysis indicated that there was a statistically significant interaction on droplet measurements between the type of emulsifier and the processing time ($P < 0.001$). It was also established that samples prepared using lecithin had larger droplet sizes than other emulsifiers for flaxseed and algae oils, although this was not statistically significant. Comparable research was conducted by Fomuso *et al.* (2002), who compared the droplet sizes of 10% fish o/w emulsions stabilised with Tween 20, whey protein, mono-diacylglycerols and lecithin. Most emulsions had an average droplet diameter of 0.30-0.37µm and the droplet

size was not found to be significantly influenced by the type of emulsifier; however, lecithin emulsions showed a population of particles with a larger diameter of 4.7 μm. During the emulsification process, lecithin forms water vesicles within the continuous phase, which must disperse before the emulsifier can adsorb to the surface of droplets.

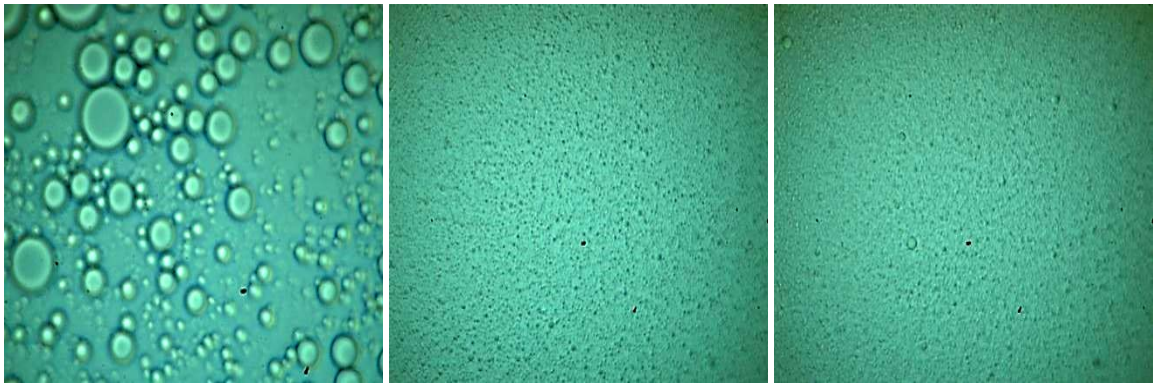
Interfacial tension is therefore reduced more slowly so larger droplets are formed by lecithin, which may explain why there were larger droplets for the lecithin samples in this study (Lee et al., 2012).

A review of current literature and patent applications demonstrates that the creation of o/w nanoemulsions using ultrasound and high DHA algae oil, which can be considered as an alternative to fish oil, has yet to be undertaken. Furthermore, papers and patent applications relating to the creation of a 50% nanoemulsion system using ultrasound have not been previously published, indicating that this system and the method of creating it is novel. This technique can be applied to create vegetarian LCω3PUFA nanoemulsions suitable for integration into enriched functional food products to provide a suitable alternative to fish oil with the potential to increase DHA bioavailability (Lane & Derbyshire, 2013a; Lane et al., 2014a; Lane, Li, Smith, & Derbyshire, 2014b, 2014c). The addition of a 50% (w/w) system is also 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.

4.0 Conclusion

Stable oil in water emulsion systems were successfully created using flaxseed and high DHA algae oil in combination with lecithin and Tween 40 emulsifiers. Particle size measurements

established that nanoemulsion systems had been created with up to 50% (w/w) oil loads. The ratio of oil to water was found to affect droplet sizes, which rose significantly with higher oil loads. It was not possible to create nanoemulsions with a 70% (w/w) oil load as phase inversion occurred at this level. Statistical analysis of the d_{32} means for the 50% flaxseed system showed that time under ultrasound significantly affected droplet sizes and that the optimum processing time to create the smallest droplets was between 10 and 12 minutes. Further research is now warranted to further develop appropriate food matrixes for fortification and to analyse the physical and oxidation stability of the 50% (w/w) o/w nanoemulsion systems.



Coarse emulsion 1 minute under ultrasound 2 minutes under ultrasound

Figure 1 - Visual microscopic slide pictures of 50% (w/w) TW emulsion at 120 magnification using immersion oil

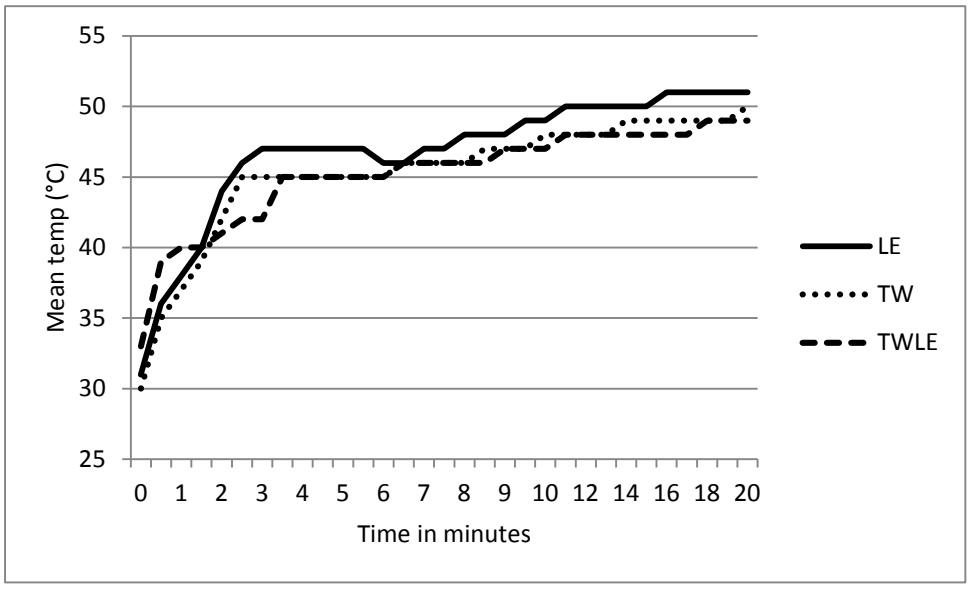
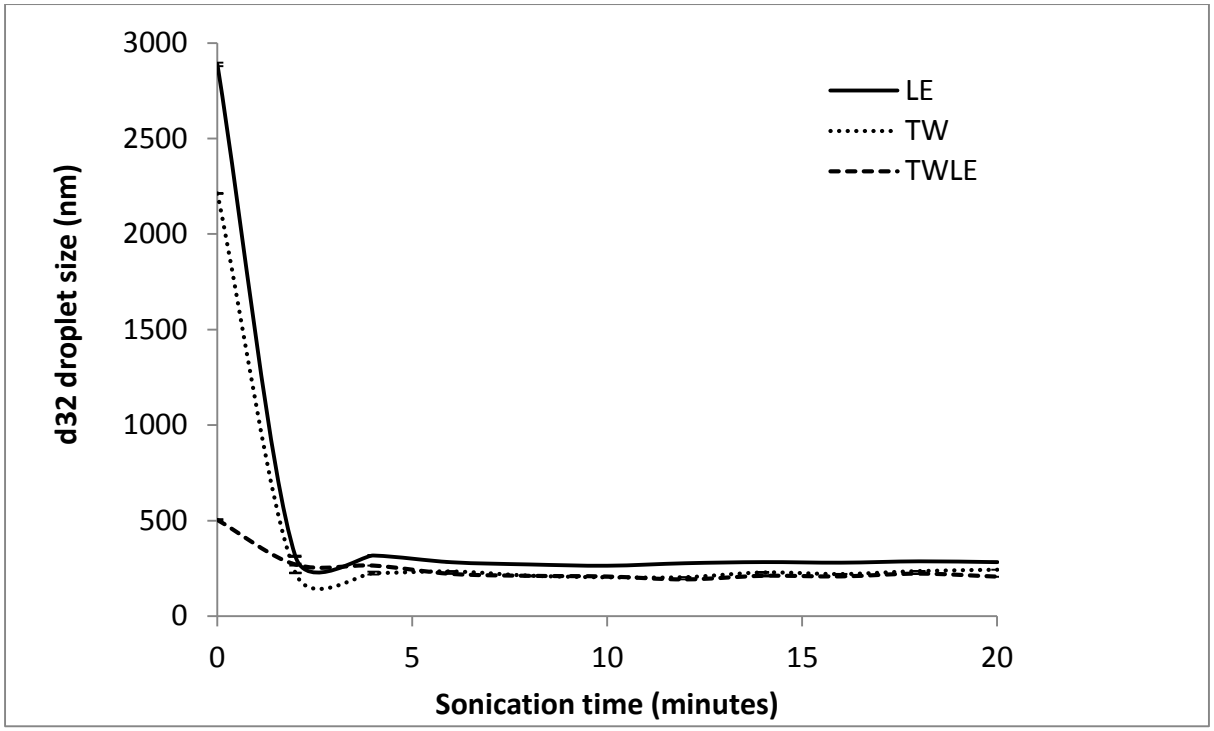


Figure 2 - Ultrasound temperature rises for 50% (w/w) flaxseed oil system processed at 100µm amplitude with a cold water cooling jacket



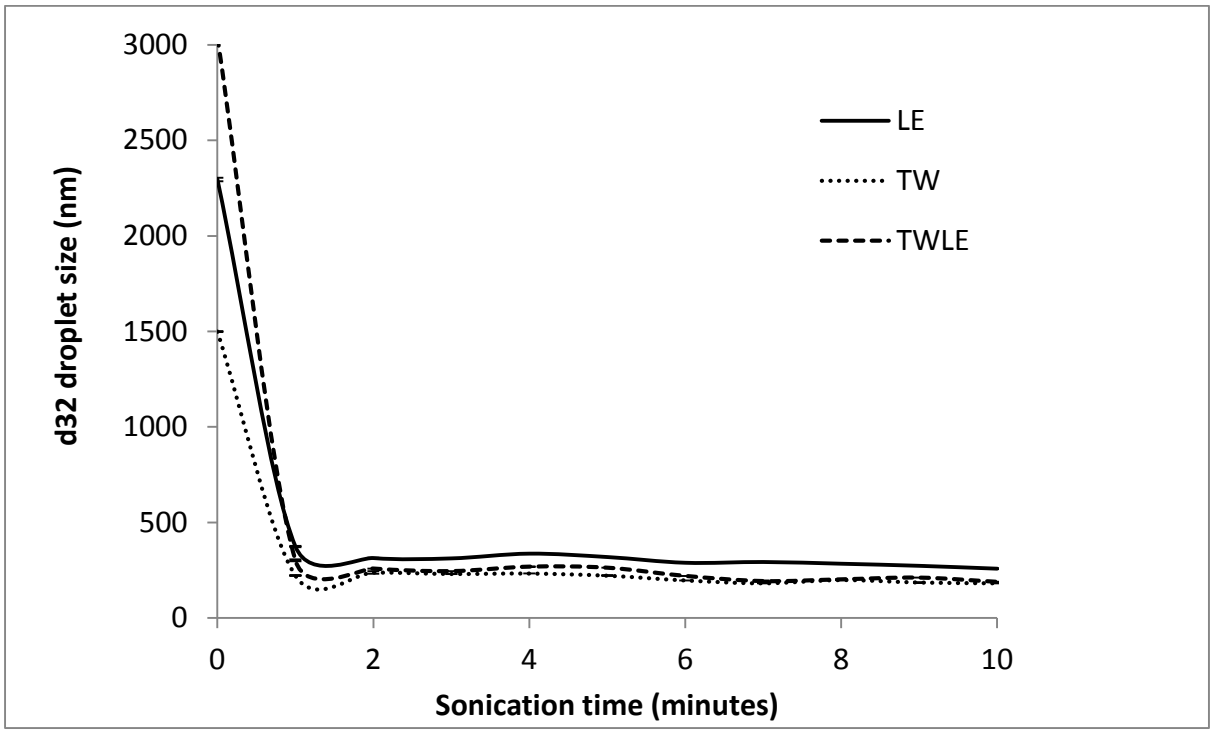


Figure 3 – The effect of sonication time for a 50% (w/w) 250ml flaxseed oil in water emulsion sample on the particle size (d_{32}). Error bars represent the standard error of measurements (top) and the effect of sonication time for a 50% (w/w) 250ml algae oil in water emulsion sample on the particle size (d_{32}). Error bars represent the standard error of measurements (bottom)

303 Table 1. - Fatty acid composition of flaxseed oil and DHA-S^{TM} algae oil

Fatty acid	**Flaxseed oil (g/100g)**	**DHA-S^{TM} algal oil (g/100g)**
16:0	6.00	0.00
18:0	3.00	0.00
18:1*n*-9	16.00	0.00
18:2*n*-6 (Linoleic acid)	17.00	1.27
18:3*n*-6		0.28
20:2*n*-6		0.00
20:3*n*-6		0.41
20:4*n*-6		1.06
22:4*n*-6		0.11
22:5*n*-6		15.63
18:3*n*-3 (ALA)	52.00	0.11
18:4*n*-3		0.36
20:3*n*-3		0.00
20:4*n*-3		0.82
20:5*n*-3 (EPA)		1.19
22:5*n*-3		0.47
22:6*n*-3 (DHA)		35.22
Total LCω3PUFA	**52.00**	**38.17**

304

305

306 Table 2. - Final emulsion ingredient ratios

Sample	Flaxseed/algae oil (%)	Tween 40 (%)	Lecithin (%)	Lecithin: oil premix (g) (30:70)	Deionised water (%)
Lecithin (LE)	20	0	6	20	74
Tween 40 (TW)	20	6	0	0	74
Combined (TWLE)	7	3	3	10	74
Lecithin (LE)	50	0	6	20	44
Tween 40 (TW)	50	6	0	0	44
Combined (TWLE)	50	3	3	10	44
Lecithin (LE)	70	0	6	20	24
Tween 40 (TW)	70	6	0	0	24
Combined (TWLE)	70	3	3	10	24

307 Key: The lecithin premix is shown for completeness and was included as part of the lecithin
308 and oil. All ratios are displayed as percentage measures.

309

310 Table 3. - The effect of sonication time on droplet measurements d_{32} for 50% w/w samples

Min	Algae TW	Flax TW	Algae LE	Flax LE	Algae TWLE	Flax TWLE
0	1499	2213	2296	2888	3039	490
30 secs	261	283	1968	1569	1606	285
1	224	253	402	361	321	283
	219	228	347	331	283	268
2	238	227	319	313	267	270
	238	233	309	314	250	259
3	238	233	305	298	246	241
	224	235	319	306	245	244
4	233	225	316	318	271	265
	233	230	358	317	267	264
5	222	209	319	313	263	282
6	197	234	289	282	220	221
7	182	193	293	269	193	204
8	199	212	284	271	202	211
9	186	203	273	286	211	211
10	**182**	**203**	**258**	**264**	**189**	207
12		204		277		**192**
14		229		283		211
16		220		280		208
18		236		287		222
20		243		283		207

311 Key: Optimum mean d_{32} droplet measurements are underlined and emboldened. Statistical

312 analysis indicated no significant differences for type of emulsifier.

313

Table 4. - Temperature, emulsifier type, processing time and droplet measurement statistical analysis

Effect	**Parameters**	**Parametric test**	***P* value**	**Non-parametric test**	***P* value**
Type of emulsifier on temperature	50 % oil load LE, TW and TWLE emulsifiers	One-way ANOVA	>0.05 (NS)	Kruskal-Wallis	>0.05 (NS)
Type of emulsifier and processing time on d_{32}	50 % oil load LE, TW and TWLE emulsifiers	Two-factor repeated measures ANOVA	<0.001***	N/A	
Type of emulsifier on d_{32}	50 % oil load LE, TW and TWLE emulsifiers	One-way repeated measures ANOVA	=0.918	Friedman	<0.01**
Processing time on d_{32}	50 % oil load LE, TW and TWLE emulsifiers	One-way repeated measures ANOVA	<0.001***	Friedman	<0.001***
0 min			<0.001***		
2 min			>0.05 (NS)		
4 min			>0.05 (NS)		
6 min			>0.05 (NS)		
8 min		One-way ANOVA	>0.05 (NS)		
10 min			>0.05 (NS)		
12 min			>0.05 (NS)		
14 min			>0.05 (NS)		
16 min			>0.05 (NS)		
18 min			>0.05 (NS)		
20 min			>0.05 (NS)		
Oil loading on d_{32}	LE 20 and 50% oil	Paired *t*-test	=0.051(NS)	Wilcoxon	0.038*
	TW 20 and 50% oil	Paired *t*-test	<0.001***	Wilcoxon	<0.001***
	TWLE, 7 and 50% oil	Paired *t*-test	<0.05*	Wilcoxon	<0.001***

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