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The development of vegetarian omega-3 oil in water nanoemulsions suitable for integration into functional food products

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A B S T R A C T

Global trends show that habitual omega-3 intakes are short of recommended guidelines, particularly among vegetarians and vegans. The potential health implications of low long chain omega-3 polyunsaturated fatty acid (LCω3PUFA) intakes coupled with concerns about sustainability of fish stocks call for innovative approaches to provide food based solutions to this problem. Nanoemulsions are systems with extremely small droplet sizes that could provide a solution while improving the bioavailability of LCω3PUFA. Oil in water nanoemulsion systems were successfully created using ultrasound with oil loads of up to 50% (w/w) using vegetarian LCω3PUFA oils (flaxseed and algae). Nanoemulsions of 50% (w/w) with mean droplet size measurements of 192 (flaxseed) and 182 nm (algae) using combinations of the emulsifiers Tween 40 and lecithin were prepared.

This technique could be applied to create vegetarian LCω3PUFA nanoemulsions suitable for integration into enriched functional food products with the potential to increase LCω3PUFA intake and bioavailability.

1. 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 140 g 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

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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 500 nm 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 80 nm, 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. 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

Table 1 – Fatty acid composition of flaxseed oil and DHA-S™ algae oil.

Fatty acid	Flaxseed oil (g/100 g)	DHA-S™ algal oil (g/100 g)
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**