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

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Abstract

There is growing demand for functional food products enriched with long chain omega-3 fatty acids (LCω3PUFA). Nanoemulsions, systems with extremely small droplet sizes have been shown to increase LCω3PUFA bioavailability. However, nanoemulsion creation and processing methods may impact on the oxidative stability of these systems. The present systematic review collates information from studies that evaluated the oxidative stability of LCω3PUFA nanoemulsions suitable for use in functional foods. The systematic search identified seventeen articles published during the last 10 years. Researchers used a range surfactants and antioxidants to create systems which were evaluated from 7 to 100 days of storage.

Nanoemulsions were created using synthetic and natural emulsifiers, with natural sources offering equivalent or increased oxidative stability compared to synthetic sources, which is useful as consumers are demanding natural, cleaner label food products. Equivalent vegetarian sources of LCω3PUFA found in fish oils such as algal oils are promising as they provide direct sources without the need for conversion in the human metabolic pathway. Quillaja saponin is a promising natural emulsifier that can produce nanoemulsion systems with equivalent/increased oxidative stability in comparison to other emulsifiers. Further studies to evaluate the oxidative stability of quillaja saponin nanoemulsions combined with algal sources of LCω3PUFA are warranted.

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

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Introduction

There is increasing evidence in studies conducted over recent decades that numerous health benefits are associated with the consumption of long chain omega-3 (ω-3) polyunsaturated fatty acids (LCω3PUFA) throughout the human lifecycle (Bowen et al. 2016, Calder 2014, Simopoulos 2011). An adequate LCω3PUFA status is a key factor in the maintenance of health and may reduce the risk of chronic and inflammatory diseases (Deckelbaum and Torrejon 2012, Yates et al. 2014). Despite known health benefits, consumption of omega-3 fatty acids of which oily fish is the most abundant source (Lenihan-Geels and Bishop 2016) remains lower than recommended levels (table 1), with omega-3 intakes in Western regions being 5-fold lower than Japanese intakes (Bates et al. 2016, Meyer 2011, Meyer 2016, Papanikolaou et al. 2014). Supplementation may provide an alternative, however supplement use is not widespread and a collaborative strategy of food fortification, in addition to food sources (i.e., fish consumption) may need to be considered to achieve recommended intakes in Western populations (Bates, et al. 2016, Papanikolaou, et al. 2014). To address this problem there has recently been an emphasis on the incorporation of LCω3PUFA source oils into food products, which has led to increased interest from consumers and the food industry (Decker et al. 2012, Jacobsen, Nielsen, et al. 2013, Salvia-Trujillo et al. 2016).

Omega 3 source oils

Fish oils are currently the most prevalent source of the most beneficial LCω3PUFA which are eicosapentaenoic acid (20:5 ω-3; EPA) and docosahexaenoic acid (22:6 ω-3; DHA) (Lenihan-Geels and Bishop 2016). Fish oils contain a high concentration of LCω3PUFA's and have a vast number of different fatty acids contained in their triglycerides. The flesh of oily fish such as mackerel, salmon, sardines, anchovies and pilchards is rich in EPA and DHA (Bailey 2009). The use of fish oils as a LCω3PUFA source for supplementation and fortification is common place, however fish oil supplementation may be disliked due to commonly reported adverse effects including gastrointestinal upset, fishy aftertaste and gastric repetition (Fetterman and Zdanowicz 2009). Krill oil provides a rich source of EPA and DHA, however as with other marine based sources krill population numbers can fluctuate, therefore sustainability cannot be guaranteed (Lane and Derbyshire 2015, Surette 2013, Trivelpiece et al. 2011). Fish and krill based sources of LCω3PUFA are by their nature unsuitable for vegetarians and non-fish eaters who abstain from eating marine and fish sources for ethical reasons.

Further vegetarian sources are available in the form of flaxseed, echium seed, perilla seed, blackcurrant seed and algal oils. (Asif 2011, Linnamaa et al. 2010, Mir 2008). Flaxseed oil is currently the most significant vegetarian source of alpha- linolenic acid (18:3 ω-3; ALA). Also known as linseed oil it can contain up to 57 per cent ALA when cold pressed (Sharif et al. 2017). A considerable amount of research has examined supplementation and food enrichment with ALA rich oils, however conversion of ALA to its longer chain, more effective relatives EPA and DHA is limited in humans and alternative direct sources of EPA and DHA are available (Lane, Derbyshire, et al. 2014).

Micro-algae oils are a fairly recent advance within the food and nutraceutical industry. They are produced in tightly controlled, closed fermentation facilities or in the case of phototropic algae produced in photobioreactors or open raceways and are entirely free of animal products (Breivik 2007, Lenihan-Geels and Bishop 2016, Ryckebosch et al. 2012). Capable of providing large amounts of EPA and DHA algae are also the primary source of DHA in the food chain (Arterburn et al. 2006). Algae oils represent a sustainable LCω3PUFA source suitable for vegetarians, vegans and non-fish eaters (Lane, et al. 2014).

Food fortification

Functional foods provide an added health benefit over and above the food products nutritional value (Bigliardi and Galati 2013, Khan et al. 2013). In recent years the food industry has evolved, and there is an increased focus on innovative approaches in processing and the introduction of novel foods that may help to optimise health and wellbeing (Khan, et al. 2013). The use of LCω3PUFA source oils in functional foods may offer considerable health benefits, however it also gives rise to a number of challenges due to their low water solubility and poor chemical stability. The chemical structure of LCω3PUFA also makes them particularly susceptible to oxidation (Jacobsen 2010, Wang and Shahidi 2017). Oxidation occurs as the result of reactions with PUFA, free radicals and oxygen (Walker et al. 2015b). Lipid oxidation is a complex process which is influenced by many factors (Shahidi and Zhong 2010). Fatty acids with a high degree of unsaturation can be less stable to oxidation when incorporated into functional foods, which causes three main problems. Firstly, it gives rise to objectionable 'off' flavours. It also reduces the nutritional value of foods containing lipids (Wang and Shahidi 2017). Free radicals, which are formed during oxidation may cause the formation of atherosclerosis following ingestion posing a potential health risk to consumers (Jacobsen 2010).

Emulsions and nanoemulsions

In the case of LCω3PUFA, oil in water emulsion systems are commonly used in the food industry as delivery vehicles, particularly in foods with an aqueous base. An emulsion is a dispersion of two or more immiscible liquids consisting of a continuous phase and a disperse phase (Coultate 2009). There is some debate within the literature in relation to definitive nanoemulsion droplet size ranges. Solans and Solé (2012) state that nanoemulsions are emulsion systems with extremely small droplet sizes in the range of 20 to 500nm whereas McClements and Rao (2011) define nanoemulsions also referred to as a mini emulsions as a conventional emulsion that contains very small particles, with mean radii between about 10 to 100 nm. The incorporation of LCω3PUFA oils into functional foods using nanoemulsions has the potential to improve LCω3PUFA bioavailability (Lane, Li, et al. 2014). However this may also create further concerns in relation to oxidative stability due to small lipid droplet sizes and large droplet surface areas (Walker, et al. 2015b). Nanoemulsions can be created using high mechanical energy, high surfactant levels or combinations of both. Creation methods can normally be classed as high-energy and low-energy methods (Walker, et al. 2015b).

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

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

Lipid oxidation

The reaction mechanism and factors that influence oxidation reactions are different for emulsified fats and oils (lipids) than for bulk lipids (Hu and Jacobsen 2016). The interfacial membrane of an emulsion system is of importance in lipid oxidation as it represents the region where lipid and water soluble components are close enough to interact, potentially giving higher concentrations of lipid peroxides and other volatiles (Berton et al. 2011). Lipid oxidation in emulsions usually occurs at the oil in water interface when free radicals interact with PUFA's within the lipid droplets or when water soluble trace metal ions react with hydroperoxides located at the droplet interface (Jacobsen, Horn, et al. 2013, Walker, et al. 2015b). Most LC3PUFA oils contain trace levels of peroxides and foods suitable for enrichment can contain trace levels of transition metals so metal-catalysed breakdown of peroxides is considered to be one of the main quality issues for LC3PUFA enriched functional food products (Jacobsen, Horn, et al. 2013, Jacobsen, Sørensen, et al. 2013). The creation of nanosized lipid droplets in an aqueous continuous phase greatly increases the surface area of the lipid phase and therefore the susceptibility to oxidation. In addition, when system droplet ranges are smaller than the wavelength of light, the light waves are weakly scattered giving the system transparent or turbid appearance. The increased transmission of light waves through nanoemulsion systems may increase their susceptibility to light induced oxidation (Uluata et al. 2015). The susceptibility of oil droplets to lipid oxidation depends on whether the oxidation catalyst is electrostatically attracted to the interfacial membrane (McClements and Decker 2000, McClements and Rao 2011). If the oxidation catalysts are repulsed from the lipid water interface, lipid oxidation in emulsions can be lowered (Yi et al. 2014). The choice of homogenization equipment, emulsifier type and droplet size can also influence the oxidative stability of the resultant systems. The use of high-power ultrasound methods in the creation of nanoemulsion systems has been associated with increased oxidation reactions in lipids. (Pingret et al. 2012, Pingret et al. 2013). The use of microfluidization has been shown to result in decreased oxidation levels in comparison to high pressure valve homogenization when whey protein is used as an emulsifier (Horn et al. 2012)

The main focus of this article is to compare and contrast the findings of studies published during the last 10 years that have evaluated the oxidative stability of LCω3PUFA nanoemulsions suitable for functional food enrichment. The aim of the review is to evaluate some of the most recent key up to date papers in order fill a gap in the literature in relation to this topic and to inform future decisions and research into this promising area. This information should aid in the identification of safe, optimal components including types of oils and emulsifiers, processing and storage conditions to maintain the oxidative stability of LCω3PUFA nanoemulsions for use in in functional foods.

Methods

The aim of this review was to fill a gap in the literature by evaluating studies that focussed on the oxidative stability of LCω3PUFA nanoemulsions suitable for integration into food vehicles. A systematic literature search was conducted in accordance with the PRISMA checklist for systematic reviews and meta-analysis (Table 2) (Moher et al. 2009, Moher et al. 2015). Search engines PubMed, Science Direct, Google Scholar, and SCOPUS were used to identify English language, peer reviewed articles published over a 10-year period between January 2007 and January 2017.

Inclusion criteria

Search terms including nanoemulsion(s), nanotechnology, emulsions and foods, nutrients omega 3, ω3, LCω3PUFA, DHA, EPA, ALA, fish/ vegetable oils (e.g. salmon, tuna, carp, algae), or nut and seed oils (e.g. echium, walnut) identified a total of 1880 articles. These were then narrowed to 1420 articles with further inclusion of search terms: food vehicles, food delivery and functional foods.

Further inclusion criteria were that studies:

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

2) Encompassed nanoemulsion system creation methods such including high energy: ultrasound, ultrasonic, microfluidizer, high pressure valve and high speed homogenization and low energy: phase inversion; temperature and composition and spontaneous methods.

3) Examined the effect of different emulsifiers, hydrophilic lipophilic (HLB) balance and processing conditions including pH and Zeta potential. Including amongst others, emulsifiers lecithin, Tween products (all numbers), whey protein, caseinate, glycerol dioleate, Span products (all numbers), sucrose monolaurate, sodium steroyl, with HLB ranges from 1 to 20.

4) Examined the ability of antioxidants to retard or inhibit lipid oxidation in LC3PUFA oil in water nanoemulsions

Exclusion criteria

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

Results and Discussion

The literature search identified 17 key studies that have investigated the oxidative stability of LCω3PUFA nanoemulsion systems suitable for use as food enrichment vehicles. The 17 studies are summarised in Table 3.

Fish oil

As discussed previously, the chemical structure of LCω3PUFA makes them particularly susceptible to oxidation. Source oils with greater fatty acid chain length and higher numbers of double bonds generally demonstrate decreased oxidative stability. Relative susceptibility for DHA (22:6) is increased by 30 times in comparison to ALA (18:3) (Decker, et al. 2012). The majority of the studies examined in the review used fish oil as an enrichment vehicle, which may be due to its current ease of availability and high EPA and DHA content (Lenihan-Geels and Bishop 2016).

Rasti, Erfanian & Selamat (2017) evaluated the application, stability and suitability of fish oil in water nanoliposomes in bread and milk products. Nanoliposomes had significantly lower primary and secondary oxidation levels in comparison to microencapsulated and bulk fish oil ($P < 0.05$) and were found to be suitable as fortification vehicles in bread and milk. A further fish oil nanoliposome study was conducted by Ghorbanzade, et al (2017) with nanoliposomes incorporated into yoghurt. Peroxide value testing and sensory analysis established that liposomal structures were successful for the encapsulation of DHA and EPA, which remained stable during the 21-day storage period, nanoencapsulation was found to protect LCω3PUFA from deterioration by oxidation. Esquerdo et al (2015) created chitosan nanocapsules using a 15 per cent carp oil nanoemulsion. Peroxide values for the nanocapsules remained stable during storage while bulk oil peroxide values increased over a 45-day storage period. Salvia-Trujillo et al, (2016), Walker et al (2015a) and Belhaj et al (2010) investigated the oxidative stability of 10 per cent oil in water nanoemulsions using fish oil as the LCω3PUFA source. The authors investigated the addition of antioxidants and effects of different emulsifiers on the oxidative stability of the systems. The addition/presence of natural antioxidants such as lemon oil, marine lecithin, astaxanthin and sodium alginate was found to increase the oxidative stability of the resultant systems. Uluata et al (2015) investigated the oxidative stability of 1 per cent fish oil in water nanoemulsions, creating systems with droplet ranges under 100nm. A range of primary and secondary oxidation tests were used to evaluate synthetic and natural emulsifiers. Synthetic emulsifier Tween 80 was found to have significantly higher radical scavenging capacity ($P < 0.05$) and quillaja saponin was found to be an effective natural emulsifier due to its physical and oxidative stability.

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

Krill oil

Krill are small shrimp-like crustaceans that have particularly high content of EPA and DHA attributed to their diet, which is based on microalgae. Krill oil has recently emerged as a LCω3PUFA source oil and is similar to fish oil in terms of its EPA/DHA content although 30-65 per cent of the fatty acids are in phospholipid form which may increase bioavailability (Adarme-Vega et al. 2014, Lenihan-Geels and Bishop 2016). Two studies in the review used high-power methods to create nanoemulsions with droplet ranges <333nm. Wu et al, (2016) examined the physical and oxidative stability of 1 per cent krill oil in water nanoemulsions and the influences of antioxidant polarity with the addition of α-tocopherol and trolox antioxidants. The more polar trolox was found to be a more effective antioxidant for these systems than α-tocopherol.

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

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

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

Algal oil

Algal oil is derived from algae, which forms the foundation of the seafood chain. Most commercially produced algal oils are rich in DHA which is thought to be one of the most beneficial LCω3PUFA sources (Baker et al. 2016). However, DHA is particularly susceptible to oxidation due to its long carbon chain length and high number of double bonds. Three of the articles in the review examined the oxidative stability of algal oil nanoemulsions created using high-power methods. Karthik & Anandharamakrishnan (2016a) investigated the physiochemical stability and in-vitro digestibility of DHA nanoemulsions stabilised with Tween 40 (synthetic emulsifier), sodium caseinate and soy lecithin (natural emulsifiers) created using microfluidization. Significant differences were found in peroxide values of 10 per cent oil in water nanoemulsions stored over 20 days with soy lecithin stabilised systems significantly greater than Tween 40 systems ($P < 0.05$). There were no changes or differences in fatty acid profiles of the different systems which suggests that soy lecithin may be susceptible to oxidation reactions when processed using microfluidization. Tween 40 systems were found to be most stable in terms of primary oxidation and in-vitro digestibility. Additional work has also been completed to further evaluate algal oil nanoemulsions stabilised with Tween 40 created using high speed/pressure homogenisation. There were no significant differences in oxidative stability between systems created using high power or pressure homogenization. A combination of high speed/pressure homogenization was found to create better physical stability in 10 per cent systems stabilised with Tween 40 (Karthik and Anandharamakrishnan 2016b). Research to evaluate spray dried powders created from a 10 per cent algal oil nanoemulsion template was conducted by Chen et al, (2016). Spray dried algal oil powders were found to have excellent reconstructed behaviour during the 30 day trial. Enhanced oxidative stability was found in systems formed with β-sitosterol & γ-oryzanol phytosterols ($P < 0.05$). Spray dried powders also had lower levels of fishy off flavours which are associated with oxidised oils. Algal oils offer a potentially viable source of LCω3PUFA, which is sustainable and suitable for vegetarians and vegans. A review of 16 published clinical trials found that consumption of algal oil may be beneficial in cardiovascular risk factors and unlike fish oil, algal-DHA seldom caused gastrointestinal complaints such as fishy taste and eructation (Ryan et al. 2009). The studies identified in the review evaluated the oxidative stability of DHA oils, however more recently EPA/DHA algal oils have become available and these have been found offer similar benefits to fish oil for adults with hypertriglyceridemia (Maki et al. 2014). Research has yet to investigate the suitability of EPA/DHA oils in functional foods. Further work is therefore warranted to investigate integration of these oils into nanoemulsion systems with an additional focus on oxidative stability, which may be improved in comparison to algal DHA alone due to the shorter carbon chain and lower numbers of double bonds in EPA.

Flaxseed oil

Flaxseed oil is currently the most widely used source of vegetarian LCω3PUFA in supplementation and food enrichment (Lane, et al. 2014, Lenihan-Geels and Bishop 2016). Flaxseed oil contains up to 57 per cent ALA, which may have increased oxidative stability over its longer carbon chain counterparts EPA and DHA (Decker, et al. 2012, Sharif, et al. 2017). Two studies identified in the review evaluated the oxidative stability of 10 per cent flaxseed oil in water nanoemulsions created using microfluidization. Primary products were identified using peroxide value testing in both studies and the use of antioxidants eugenol and caseinate was found to significantly reduce the formation of peroxides ($P < 0.05$) (Chen et al. 2017, Sharif, et al. 2017). Analysis of secondary oxidation products was conducted using headspace analysis and thiobarbituric acid reactive substances tests, the use of eugenol and caseinate was also found to be significantly effective when compared to systems generated with no addition of antioxidants. Flaxseed nanoemulsions were found to have significantly increased total oxidation levels in comparison to bulk oil in both studies ($P < 0.05$), which suggests that nanoemulsion processing does have an effect on the oxidative stability of flaxseed oil.

Walnut oil

Walnut oil contains relatively low amounts of LCω3PUFA at around 10 per cent ALA (Zhao et al. 2004), longer chain EPA and DHA are not present which may give improved oxidative stability. Short term consumption of walnut oil has been found to significantly decrease total and LDL cholesterol ($P < 0.05$), walnuts may also have potential benefits on oxidative stress and inflammatory markers (Banel and Hu 2009). Limited research has been conducted to examine the physiochemical properties of walnut oil nanoemulsions with one study identified in the review. Emulsifying conditions were investigated including processing time and concentration ratio using 8, 6 and 4 per cent walnut oil-in-water nanoemulsion systems created using ultrasound. Loss of antioxidant activity testing over 35 days identified a quadratic effect of ultrasound treatment leading to significant losses of antioxidant activity ($P < 0.05$) (Homayoonfal et al. 2014).

Antioxidants

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

The effect of emulsion stability and pH

The zeta-potential of a conventional emulsion or nanoemulsion is the electrical potential at the “shear plane,” which is defined as the distance away from the droplet surface below which the counter-ions remain strongly attached to the droplet when it moves in an electrical field. Zeta potential is one of the fundamental parameters known to affect the physical stability of emulsion systems (McClements and Rao 2011). Zeta-potential and nanoemulsion oxidation stability was evaluated in 1% fish oil systems by Uluata et al (2015) over a 5 day storage period. The effect of pH was also determined over the range of 2 to 8. At pH 7 all lipid droplets were negatively charged. No significant change in particle size was noted in pH range 2 to 8. Of the four natural and synthetic emulsifiers used in the study quillaja saponin was found to create the most physically and chemically stable systems. The physical and oxidative stability of fish oil nanoemulsions was measured by Walker et al (2015a). The aqueous phase was buffered at pH 3.0 to simulate the aqueous phase of a beverage system. Neither particle size nor surfactant concentration had an impact on the oxidative stability of the systems over 14 days of storage. Further work to evaluate the effect of physical stability and pH ranges on the oxidative stability of nanoemulsions created with different LC3PUFA oils, emulsifiers and antioxidants is warranted to simulate conditions in food and beverage systems (Haahr and Jacobsen 2008).

Nanoemulsion production methods

The majority of studies identified used high power processing methods to create nanoemulsion systems. High power processing has become more commonplace in the creation of nanoemulsion systems in recent years, however interest in low-energy methods for some applications is increasing due to their simple production methods, lower costs and ability to create systems with smaller droplet size ranges than high-energy methods (Walker, et al. 2015b). Both methods have benefits and disadvantages. High-energy methods can be used to effectively create systems with narrow droplet ranges, however the necessary equipment can be expensive. Low energy methods are reasonably cheap in comparison, however high levels of surfactants are required to generate stable systems (Walker, et al. 2015b). One study in the review examined differences in the oxidative stability of fish oil in water nanoemulsion systems created using lower-power spontaneous emulsification compared to high-power microfluidization. Emulsions created using microfluidization were found to have higher levels secondary oxidation products in comparison to systems created using spontaneous emulsification with added iron over a 14-day storage period. The authors concluded that fabrication methods may have an impact on secondary oxidation products of nanoemulsions and that low energy methods can be used to produce fish oil nanoemulsions without the use of expensive equipment using high levels of synthetic surfactants (Walker, et al. 2015a). In addition to this study previous research has identified that ultrasound processing may cause degradation in edible oils with the increase of free radicals and oxidative products in sonicated oils when compared to untreated oils. Microfluidization and ultrasound were the commonly used processing methods identified in the review, with only the Walker et al, (2015a) study examining the effect of processing treatment on oxidative stability of nanoemulsions, further research in this area is therefore warranted.

Type of emulsifier

Emulsifiers are surface active substances that play a vital role in emulsion formation and stability (Ozturk and McClements 2016). The type of emulsifier used to create nanoemulsion systems can have a large impact on their oxidative stability with droplet size ranges and attraction to prooxidants in the continuous phase being key factors. Furthermore the oil/water ratio, emulsifier concentration and location of the emulsifier within the aqueous phase interface are all important factors that can influence the oxidation stability of resultant nanoemulsion systems (Jacobsen, Horn, et al. 2013, Jacobsen, Sørensen, et al. 2013). Studies identified in the review by Nejadmansouri et al, (2016) and Walker et al, (2015a) examined the influences of these factors and found droplet ranges affected oxidation stability when a high molecular weight emulsifier was utilized but there was no effect for a low weight molecular weight surfactants although different levels of energy were used to create the systems. Nejadmansouri et al, (2016) found droplet size ranges had a significant effect on TBARS in 1% fish oil in water nanoemulsions (*P* < 0.05) created with ultrasound when compared to conventional emulsions, both systems incorporated whey protein isolate. Proteins usually adsorb at the interface with the lipophilic groups in the oil disperse and the hydrophilic groups present in the aqueous continuous phase. Systems are stabilized through electrostatic repulsion arising from charged groups on the protein surface area (Genot et al. 2013, Nejadmansouri, et al. 2016). Conversely the study by Walker et al (2015a) found neither particle size nor surfactant concentration had an impact on the rate of oxidation in 10% fish oil nanoemulsions created using spontaneous emulsification and low molecular weight synthetic surfactant Tween 80 to stabilize the nanoemulsion systems.

Consumer demand is dictating that the food industry should substitute synthetic surfactants with more natural alternatives and there is considerable interest in food products formulated with natural ingredients to provide cleaner labels (Ozturk and McClements 2016, Román et al. 2017, Walker, et al. 2015b). Lecithin was the most prevalent natural emulsifier identified in the review with five studies analyzing the oxidative stability of systems created with lecithin from various sources. Uluata et al (2015) compared systems created using sunflower lecithin to various natural and synthetic emulsifiers and found that sunflower lecithin was less stable to oxidation under light exposure which may impact its use in delivery systems in food and pharmaceutical industries. The emulsifier quillaja saponin is a natural food-grade surfactant isolated from the bark of the *quillaja saponaria* molina tree (Yang et al. 2013), it can be used produce systems with increased oxidative stability particularly when additional antioxidants are utilized. Three of the studies in the review used quillaja saponin and high power methods to create nanoemulsion systems using fish oil, flaxseed and algae oil. Uluata et al (2015) analyzed 1 per cent fish oil ester systems over a 5 day storage period and found quillaja saponin was an effective emulsifier due to its physical and oxidative stability. F. Chen et al (2017) found the addition of sodium caseinate gave a significant protective effect ($P < 0.05$) for quillaja saponin stabilised flaxseed nanoemulsions in microgels stored over 14 days. X.- W. Chen et al (2016) found the addition of β-sitosterol & γ-oryzanol in the formation of 10 per cent algae oil and quillaja saponin nanoemulsions offered a significant protective effect over 30 days of storage ($P < 0.05$). Further research to fully evaluate the use of quillaja saponin as a natural surfactant in LCω3PUFA nanoemulsion enriched foods appears to be warranted.

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

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

Synthetic emulsifiers are still extensively used to create nanoemulsions. The review identified a that nonionic surfactants such as Span 80 and Tween 40 and 80 were widely used to create systems with lower droplet ranges and high physical stability than some of the available natural alternatives. Karthik et al (2016a) compared 10 per cent algal oil nanoemulsions created using natural soy lecithin and Tween 40. Refrigerated Tween 40 nanoemulsions exhibited lower lipid oxidation products and there was a significant difference in peroxide values between the Tween 40 and lecithin samples ($P<0.05$). Uluata et al (2015) compared the oxidative stability of nanoemulsions prepared with natural and synthetic surfactants over a 7 day storage period. Systems were created using 1 per cent fish oil with natural emulsifiers lecithin and quillaja saponin and synthetic emulsifiers Tween 80 and sodium dodecyl sulfate. Lecithin stabilised emulsions showed increased oxidation with light exposure and Tween 80 stabilised systems had significantly higher free radical scavenging capacity ($P<0.05$). Furthermore the nanoemulsions stabilised with quillaja saponin were found to offer a suitable alternative to synthetic emulsifiers due to their physical and oxidative stability. The authors concluded that quillaja saponin could be an outstanding natural emulsifier for LCω3PUFA ethyl ester nanoemulsions.

4.1 Recommendations

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

The effect of high/low processing methods has not been fully determined, further research is necessary to compare the oxidative stability of LCω3PUFA systems created with low-energy methods such as spontaneous emulsification to commonly used high-power methods.

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

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

Further research to determine primary and secondary oxidation products and the effects of natural and synthetic emulsifiers for LCω3PUFA nanoemulsions created using high and low processing methods is also warranted.

The review identified that use of nanoliposomes to encapsulate lecithin and fish oil nanoemulsions provides a promising solution with significantly improvements to primary and secondary oxidation stability. Further research should be conducted to evaluate oxidation stability of systems created with a variety of source oils incorporated into nanoliposomes.

5.1 Conclusions

There is considerable potential for LCω3PUFA functional foods that could act as alternative sources to oily fish. Ready formulated vegetarian sources of EPA and DHA such as algal oils are particularly promising as they provide direct sources of the more effective longer chain ω3 without the need for conversion in the metabolic pathway. Using nanotechnology to incorporate these source oils into foods offers increased bioavailability and, if processed under optimum conditions the oxidative stability of these systems may remain similar or be improved in comparison to unprocessed/bulk oils. Consumer demand dictates a clean label approach with considerable interest in the use of natural ingredients. The emulsifier quillaja saponin appears to be a particularly promising natural emulsifier that can produce systems with equivalent or increased oxidative stability in comparison to other natural and synthetic emulsifiers, particularly when additional antioxidants are used. Further studies to evaluate the oxidative stability quillaja saponin in combination with algal sources of EPA and DHA are warranted to enable the development of safe, clean label LCω3PUFA nanoemulsion enriched functional food products.

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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)

Table 2 – Summary of systematic review selection process

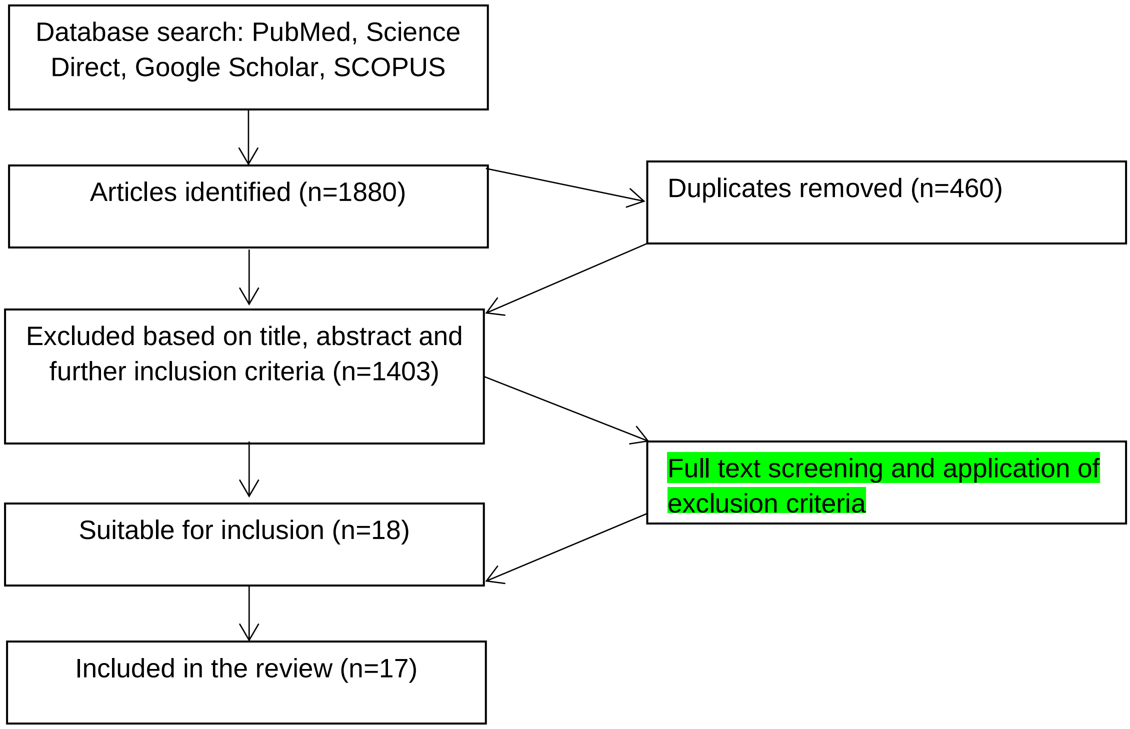

Table 3 – Results of the literature review

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 ω3 PUFAs incorporated nanoliposomes in food enrichment. Nanoliposomes compared to microencapsulated ω3 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 containing 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 ω3 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 EPA and 112.6-115.2mg DHA/100ml. Nanoliposomes can be used to fortify bread and milk.
Influence of OSA-starch on the physio chemical 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 131	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} D_{32} 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 Anandharamakrishnan 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 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 WPI-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 Anandharamakrishnan 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 30 ω-3 food oil) containing 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) containing 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 buffer 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 containing 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 (Nan structured lipo carriers) 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) (*X*1)	Lecithin surfactant (w/w) (*X*2) 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 physiochemical 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.