Novel emulsions–based technological approaches for the protection of omega–3 polyunsaturated fatty acids against oxidation processes – A comprehensive review

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Highlights

- Omega-3 Polyunsaturated Fatty Acids need to be protected against lipid oxidation
- Different PUFA-Based Lipid Emulsions and other novel systems have been developed
- The main factors that impact the lipid oxidation rate are comprehensively reviewed

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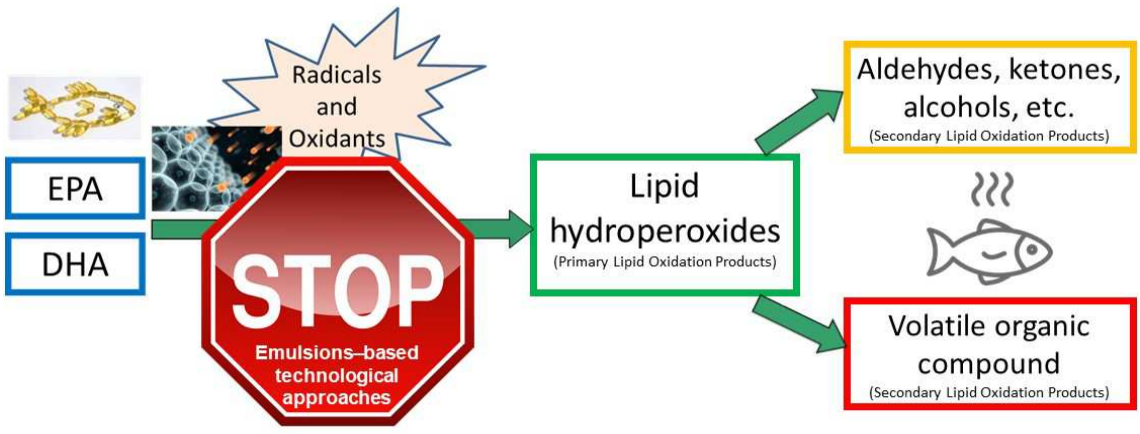
Abstract

Over recent decades, the therapeutic properties and health beneficial effects of omega-3 polyunsaturated fatty acids (omega-3 PUFAs) have been identified. These also contain a number of double bonds which make them highly reactive and, as a consequence, they are susceptible to oxidation. This is one of the main limitations when incorporating them into food matrices. This review article presents the state-of-the-art on the preparation of simple or multiple omega-3 PUFA-based lipid emulsions and other novel systems that have been developed, such as self-assembling systems or solid lipid nanoparticles. Furthermore, the main factors that impact lipid oxidation rate are comprehensively reviewed, highlighting the importance of proteins for increasing the physical stability of food emulsions. Currently, there are several works focused on simple emulsions enriched with omega-3 PUFAs that seek the definition of strategies to allow the control of lipid oxidation. Multiple emulsions and other novel systems are beginning to be considered as a possible alternative to conventional emulsions. This knowledge can be used to facilitate selection of the most appropriate system for the food industry.

Graphical abstract



Keywords: Bioactive lipid; Omega-3; PUFA; Emulsion; Stability; Lipid oxidation

1. Therapeutic properties of dietary omega-3 polyunsaturated fatty acids

Thanks to their beneficial effects on health, lipids, in particular polyunsaturated fatty acids, are amongst the bioactive compounds (functional ingredients) to receive greatest attention, both in qualitative and quantitative terms. This is because of their interesting potential for design and development of healthier products in food industry (Jiménez-Colmenero, 2013b). Among the different polyunsaturated fatty acids, those of the omega-3 series – comprising α-linolenic acid (ALA; 18:3 ω-3), eicosapentaenoic acid (EPA; 20:5 ω-3) and docosahexaenoic acid (DHA; 22:6 ω-3) – stand out because of their important roles in health promotion and disease risk reduction (Shahidi & Ambigaipalan, 2018).

During the last four decades, hundreds of studies have reported the potential therapeutic effects of omega-3 polyunsaturated fatty acids (omega-3 PUFAs) in the prevention of cardiovascular diseases (atrial fibrillation), circulatory system disorders (atherosclerosis, inflammation, thrombosis) and heart failure (sudden cardiac death) (Bucher, Hengstler, Schindler, & Meier, 2002; Marik & Varon, 2009; Filion et al., 2010; Musa-Veloso et al., 2011; Maki, Palacios, Bell, & Toth, 2017; Elagizi et al., 2018).

Although greatest attention has been focused on their effects on the cardiovascular system, other possible physiological effects and/or therapeutic properties are currently being explored. Over the past decade a large amount of research comprised of experimental and epidemiological studies, have been carried out to explore the different health benefits of omega-3 PUFAs. For example, higher fetal omega-3 PUFAs levels have been found to be related to better cognitive and neurological development in

newborns (Dijck-Brouwer et al., 2005; Harris & Baack, 2015). This seems to be related to the singular structural properties of the DHA molecule, which appear to provide optimal conditions for a wide range of cell membrane functions, especially in grey matter (Bradbury, 2011). Other studies have also associated a lower intake of omega-3 PUFAs with an increased risk of dementia and with age-related cognitive decline, especially in the case of Alzheimer's disease, although some authors have not obtained conclusive results in this respect (Cole, Ma, & Frautschy, 2009; Cederholm & Palmblad, 2010). Thus, omega-3 PUFAs are essential fatty acids necessary from conception, throughout pregnancy, during infancy and, doubtless, throughout life.

In contrast, recent studies have found important evidence that omega-3 PUFAs contribute to the reduction of inflammation levels in both healthy individuals and in people exhibiting features of metabolic syndrome (Robinson & Mazurak, 2013; Li, Huang, Zheng, Wu, & Li, 2014; Serhan & Levy, 2018). Precisely, due to their hypolipemic and anti-inflammatory effects, omega-3 PUFAs could exert beneficial effects when treating diseases such as rheumatoid arthritis – a chronic inflammatory autoimmune disease. Indeed, evidence has been found of a moderate benefit of omega-3 PUFAs on "joint swelling and pain, duration of morning stiffness, global assessments of pain and disease activity" (Miles & Calder, 2012). Although the role of omega-3 PUFAs in the control of chronic diseases is somewhat controversial, important studies demonstrate that consumption of diets rich in omega-3 PUFAs exert beneficial effects in the prevention of diseases such as metabolic syndrome, type 2 diabetes and obesity. In these cases, omega-3 PUFAs activate peroxisome proliferator-activated alpha receptors (PPARα) by stimulating lipid oxidation and decreasing insulin resistance and hepatic steatosis (Lalia & Lanza, 2016). Omega-3 PUFAs have also demonstrated beneficial effects in relation to other types of ailments, for instance reducing the

frequency, severity, and duration of migraines (Maghsoumi-Norouzabad, Mansoori, Abed, & Shishehbor, 2018). Moreover, these PUFAs have long been studied for their therapeutic potential in the context of autism, attention-deficit/hyperactivity disorder, dyslexia, and other developmental disabilities, where it has been concluded that omega-3 PUFAs offer a promising approach to complement standard treatments (Richardson, 2006). In addition, there is some evidence that omega-3 PUFAs impact upon mental health (Perica & Delaš, 2011) and that EPA and DHA act as anti-depressive agents which causes structural changes in the brain, including a reduction in the lateral ventricular volume and a reduction in the neuronal PL turnover. Several epidemiological studies have associated a higher intake of fish with a lower risk of depression, whilst others studies report that EPA is more effective than DHA in the treatment of this disease (Nemets, Nemets, Apter, Bracha, & Belmaker, 2006).

Furthermore, researchers have hypothesized that increased consumption of omega-3 PUFAs might reduce the risk of cancer due to their anti-inflammatory effects and their potential to inhibit cell growth factors. Several clinical studies have shown that suppression of nuclear factor-κB, modulation of cyclooxygenase (COX) activity, activation of AMPK/SIRT1, and up-regulation of novel anti-inflammatory lipid mediators such as protectins, maresins, and resolvins, are the main mechanisms of the antineoplastic effect of omega-3 PUFAs (Greene, Huang, Serhan, & Panigrahy, 2011; Huerta-Yépez, Tirado-Rodriguez, & Hankinson, 2016; Sulciner et al., 2018). In patients who already have cancer, some research papers suggest that certain omega-3 PUFAs, alone or in combination with chemotherapeutic drugs, exert tumoricidal actions and improve the cytotoxic action of anticancer agents specifically on drug-resistant tumour cells (Das & Madhavi, 2011). Omega-3 PUFAs have also been shown to affect several types of cancer such as breast, colon, colorectal, lung, ovarian, pancreatic, prostate, skin

and stomach cancers (Gerber, 2009, 2012; Shahidi & Ambigaipalan, 2018). In addition to this, omega-3 PUFAs have been reported to improve the tolerability and efficacy of chemotherapy, as well as improving quality of life (Mocellin, Camargo, Fabre, & Trindade, 2017). In patients with cancer cachexia, supplementation with omega-3 PUFAs is associated with improved biological, clinical, functional and quality of life parameters (Colomer et al., 2007; Werner et al., 2017).

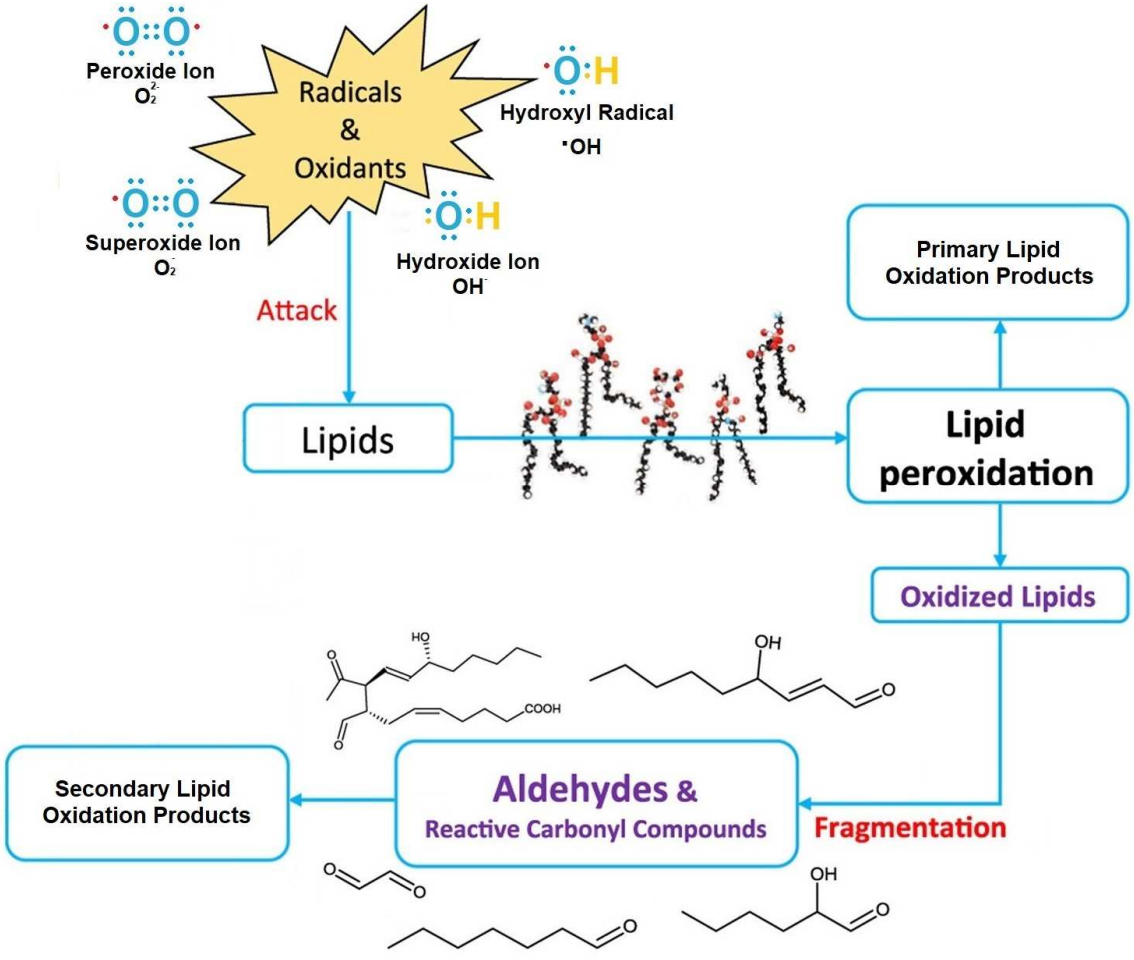
In summary, the key therapeutic properties of omega-3 PUFAs make them stand out as important components of a well-balanced diet. Despite this, the enrichment of food products with these types of ingredients is not a simple task as many of them are highly susceptible to oxidation and may, therefore, lose their therapeutic properties. Therefore, the ongoing search for strategies which enable control of lipid oxidation is of paramount importance and will be reviewed in the present paper.

2. Lipid oxidation of omega-3 polyunsaturated fatty acids

The term *lipid oxidation* refers to a highly complex series of chemical reactions which take place between unsaturated lipids and oxygen, and ultimately leads to the formation of a complex mixture of reaction products, including aldehydes and ketones, alcohols and hydrocarbons (Figure 1). Lipid oxidation is one of the most important and common mechanisms of (chemical) instability in food products containing lipids (McClements, 2015). It is also the most important cause of deterioration and quality loss in PUFA-based lipid products (Coupland & McClements, 1996). As a result, lipid oxidation is a huge problem for the food industry since it impairs appearance, taste, texture, nutritional profile and shelf-life. It also promotes undesirable “off-flavours” (rancid smell), potentially toxic reaction products and, ultimately, leads to unstable food

products (it could even lead to the physical instability of certain emulsions containing PUFAs) (Kargar, 2014; McClements, 2015; Coupland & McClements, 1996). In summary, lipid oxidation presents a serious handicap for the food industry, which is therefore committed to investigating novel techniques for avoiding or, at least, delaying its occurrence in food products.

Figure 1. Lipid Oxidation Process.



Although lipid hydroperoxides are the primary lipid oxidation products, they are not the primary cause of off-flavour and rancidity in food products. Lipid hydroperoxides are unstable and they can easily decompose into a wide variety of volatile and non-volatile low-molecular-weight compounds (known as secondary lipid oxidation products), such as aldehydes, ketones, alcohols, and hydrocarbon compounds.

These secondary lipid oxidation products are more stable than lipid hydroperoxides and are responsible for (fishy) off-flavours and off-odours (Kargar, 2014). Environmental factors such as light, heat and oxygen concentration, and factors intrinsic to the system itself, such as the chemical structure of lipids, and the presence of antioxidants or pro-oxidants drastically influence the rate of lipid oxidation. This undesirable process is so influential that extensive research has been promoted in this area over recent years (McClements, 2015).

The food industry has been looking for a feasible and reliable formulation to include these marine omega-3 rich oils in food matrices. Emulsions, in their different variants (simple, multiple, etc.), are among the most promising technological processes used to design and develop functional foods of this type (Fustier, Taherian, & Ramaswamy, 2010; Kargar, 2014).

3. Different systems for the vehiculization and protection of omega-3 polyunsaturated fatty acids against lipid oxidation

Over recent decades, research efforts have been devoted towards developing different stable aqueous systems for better protection and improved vehiculization of omega-3 PUFAs against lipid oxidation. Amongst all of these developed systems, the various forms of emulsion systems stand out for their importance. Below are some examples of various types of these systems tested, including simple and multiple emulsions, liposomes, self-assembling emulsions and solid lipid nanoparticles emulsions.

3.1. PUFA-based lipid emulsions

In this review, the term "PUFA-based lipid emulsions (PUFAs BLEs)" refers to those emulsions containing a dispersed phase enriched by PUFAs. PUFAs BLEs have been classified into two broad groups as a function of the number of liquid immiscible phases included: *simple* emulsions and *multiple* emulsions (Pal, 2011). Next, some of the other systems used will be briefly discussed.

3.1.1. Simple PUFA-based lipid emulsions

In recent years, different systems have been proposed for the delivery of hydrophilic and hydrophobic bioactive compounds in foods and beverages (Flaiz et al., 2016). In this context, O/W emulsions have been widely employed in the food industry for the protection and release of hydrophobic compounds, including antioxidants and omega-3 fatty acids (Lee et al., 2006; McClements, Decker, & Park, 2009).

One of the main advantages of simple PUFAs BLEs is that they allow the incorporation of an oil phase enriched in omega-3 PUFAs and an aqueous phase, into the same product. In combining two different phases, they permit the easy incorporation of both oil-soluble compounds and water-soluble compounds. For example, Sugasini and Lokesh (2017) studied the design of a PUFAs enriched-phospholipid based nanoemulsion containing curcumin (an oil-soluble bioactive compound). They reported increased bioavailability with respect to free curcumin and also found that this nanoemulsion improved DHA serum levels. In addition, since the phase that occurs at a greater sensory intensity also makes up the external phase of the simple emulsion, these systems allow off-flavours and off-odours of fish oil to be partially masked by incorporating flavorings, sweeteners and masking agents in the external aqueous phase (Jeyakumari, Janarthanan, Chouksey, & Venkateshwarlu, 2016). When considering

their main limitations, it should be noted that simple PUFAs BLEs are not thermodynamically stable and, therefore, have an expiration date that varies as a function of the quality of the emulsion design. This being said, physical instability will always be a factor that must be taken into account as its presence in these types of systems is unavoidable. As a consequence, a large effort has been dedicated to studying the different mechanisms that lead to the eventual breakdown of a simple emulsion: gravitational separation (creaming/sedimentation), flocculation, coalescence, partial coalescence and Ostwald ripening. To this end, other studies such as that conducted by Dey, Banerjee, Chatterjee, and Dhar (2018), have studied the design of omega-3 PUFA-enriched biocompatible nanoemulsions. Furthermore, addition of certain amounts of emulsifier is essential for formation of the emulsion itself. In most cases and depending on the emulsifier used, this produces a rather unpleasant taste and can sometimes modify the physical properties of the emulsion. Finally, formation of the system also requires it to be subjected to aggressive external conditions. This is another important limitation since it usually leads to considerable increases in the temperature of the system which can degrade thermolabile components, accelerate oxidation reactions and so on.

Many examples of simple PUFAs BLEs can be found in the scientific literature. Traditionally, the vast majority of studies have used fish oil as a source of omega-3 PUFAs when making their simple PUFAs BLEs (García-Moreno, Guadix, Guadix, & Jacobsen, 2016). However, over recent years, the scientific community has been trying to find alternative sources of fish oil to incorporate omega-3 into products. As a result, more and more studies on simple PUFAs BLEs are appearing in which other sources of omega-3 are being used (algal, chia seed, flaxseed, canola or perilla seed oils, for example) (Cofrades et al., 2011). Further, different emulsifiers have also been tested to

make simple PUFAs BLEs: lecithin (Santhanam, Lekshmi, Chouksey, Tripathi, & Gudipati, 2015), Tween 80 (Uluata, McClements, & Decker, 2015), citrem (Ghelichi, Sørensen, García-Moreno, Hajfathalian, & Jacobsen, 2017), sodium caseinate (Karadağ et al., 2017), whey protein (Owens, Griffin, Khouryieh, & Williams, 2018), amongst others. Simple PUFAs BLEs can be found both with and without added antioxidants (Mbatia, Kaki, Mattiasson, Mulaa, & Adlercreutz, 2011), and both with and without added stabilizers and thickeners (Chivero, Gohtani, Yoshii, & Nakamura, 2015), etc. Finally, different emulsification methods have been used to make simple PUFAs BLEs: stirring (He et al., 2017), high-speed shearing (principally the rotor-stator machine) (Kristinova, Mozuraityte, Aaneby, Storrø, & Rustad, 2014), high-pressure homogenization (Komaiko, Sastrosubroto, & McClements, 2016), microfluidization (Horn, Nielsen, Jensen, Horsewell, & Jacobsen, 2012), (ultra)sonication (Pazos, Alonso, Sánchez, & Medina, 2008) or combinations of the aforementioned methods (Gadeyne et al., 2015), when performing testing in different operating conditions. Table 1 briefly summarizes some phases compositional information published during the last decade for a selection of simple PUFAs BLEs, alongside some examples of previous compilations.

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Table 1. Phases compositional information for a selection of simple PUFAs BLEs.

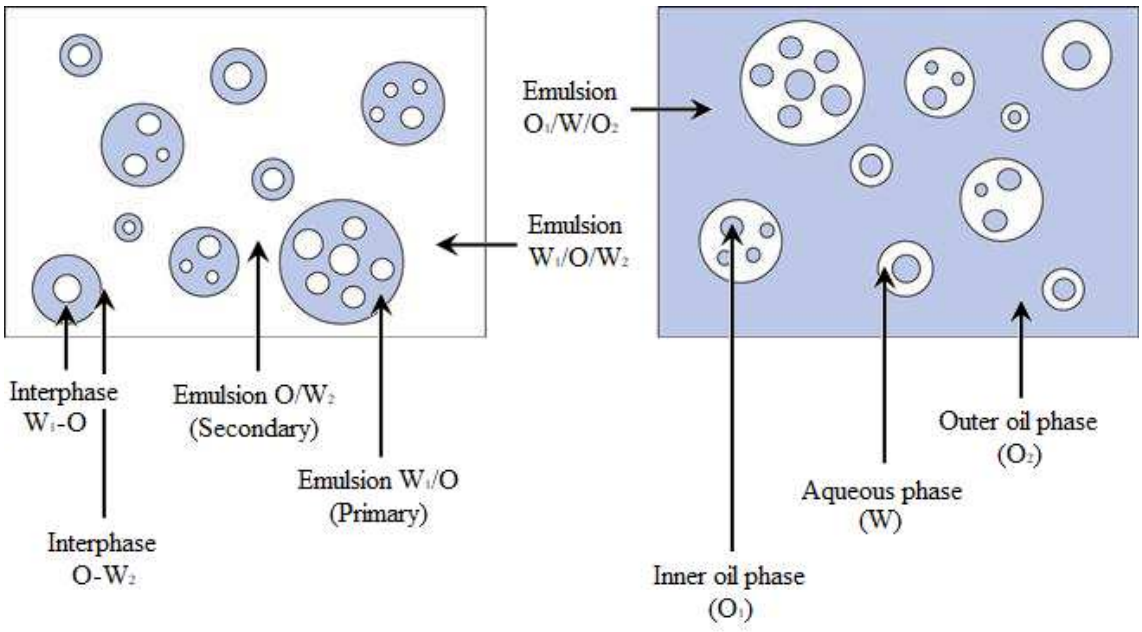
Type	Dispersed phase	Continuous phase	Emulsification method	Reference
O/W	Flaxseed oil, O:W = 12.5:87.5	Distilled water containing different amounts of whey protein concentrate (WPC-80), sodium caseinate, lactose and ascorbyl palmitate as antioxidant	Low-pressure homogenization (20 LPH, pressure = 69 bar) followed by high-pressure homogenization (20 LPH, pressure = 241.31 bar)	Goyal et al. (2015)
O/W	Flaxseed oil and fish oil, O:W = 10:90	Water containing 0-3 % (w/w) sodium caseinate, 0-0.3 % (w/w) oat β-glucan and 0.04 % (w/w) sodium azide	Pre-emulsification with a Polytron homogenizer at 14000 rpm for 2 min followed by high-pressure homogenization with a Panda 2K homogenizer (2-stages, pressure = 80 and 8 MPa)	Liu, Singh, Wayman, Hwang, & Fhaner (2015)
O/W	Menhaden oil, O:W = 20:80	Deionized water containing 2 % (w/v) whey protein isolate, 0-0.3 % (w/v) gums (xanthan gum, guar gum and enzymatic modified guar gum) and 0.05 % sodium azide	Rotor-stator homogenization using PowerGen 500 at 30000 rpm for 5 min followed by ultrasound homogenization using VWR sonicator for 1 min	Chityala, Khouryieh, Williams, & Conte (2016)
O/W	Fish oil, O:W = 10-50:90-50	Aqueous solution composed of buffer solution (5 mM sodium phosphate, pH 7.0) and emulsifier (Tween 80, rhamnolipids or quillaja saponins) keeping the emulsifier-to-oil ratio fixed at 1:10	High-pressure homogenization using Microfluidics PureNano (pressure = 13 kpsi)	Liu et al. (2016)
O/W	Fish oil containing Span 80, O:W = 5:95	Water containing Tween 80 (different emulsifier-to-oil ratios 0.5-1.5)	Pre-emulsification with a Heidolph Silent Crusher at 20000 rpm for 5 min followed by ultrasound homogenization with a Hielscher UP 200H for 10 min	Nejadmansouri, Hosseini, Niakosari, Yousefi, & Golmakani (2016)
O/W	Corn oil, MCT, fish oil or lemon oil, O:W = 10:90	Aqueous solution composed by buffer solution (10 mM sodium phosphate, pH 7.0) and 0.1-5.0 wt.% polysaccharide (gum arabic, corn fiber gum or beet pectin)	Pre-emulsification using a high shear mixer for 2 min followed by high-pressure homogenization using an air-driven microfluidizer (3-stages, pressure = 62-130 MPa)	Bai, Huan, Li, & McClements (2017)
O/W	Fish oil, O:W = 5:95	Aqueous solution composed by buffer solution (5 mM potassium phosphate, pH 7.0) and 2.5 wt.% β-cyclodextrin or octenyl succinic-modified β-cyclodextrin	Pre-emulsification with an Ultra-Turrax at 24000 rpm for 3 min followed by high-pressure homogenization with a Nano DeBEE homogenizer (3-stages, pressure = 50 MPa)	Cheng et al. (2017)
O/W	Fish oil or Miglyol 812 N mixed with rosemary extract (10 wt.%), O:W = 10:90	Aqueous solution composed by buffer solution (10 mM sodium phosphate, pH 5.0), 2 % (w/w) Tween 80	Pre-emulsification with an Ultra-Turrax T10 at 24000 rpm for 5 min followed by high-pressure homogenization with an EmulsiFlex-C3 homogenizer (5-stages, pressure = 1500 bar)	Erdmann, Lautenschlaeger, Zeeb, Gibis, & Weiss (2017)
O/W	Algae oil, O:W = 10:90	Aqueous solution composed of buffer solution (10 mM sodium phosphate, pH 7) and 0.25-5 % (w/w) protein concentrate (pea, lentil and faba bean)	Pre-emulsification with a M133/1281-0 mixer at 10000 rpm for 2 min followed by high-pressure homogenization with a PureNano Microfluidizer	Gumus, Decker, & McClements (2017)

			(3-stages, pressure = 10000 psi)	
O/W	Weighed amounts of fish oil and carrier oil (MCT, lemon oil or thyme oil), O:W = Not described	Aqueous solution composed by buffer solution (0.8 % citric acid, 0.08 % sodium benzoate, pH 3.0) and 1.5 wt.% Tween 80	Pre-emulsification with a Bamix ESGE Ltd mixer for 2 min followed by high-pressure homogenization with a Microfluidics M-110P (5-stages, pressure = 20000 psi)	Walker, Gumus, Decker, & McClements (2017)
O/W	Fish oil, O:W = 1:99	Aqueous solution composed by buffer solution (5 mM phosphate, pH 7.0), 0.3 wt.% protein (hydrolyzed rice glutelin), 0.1 wt.% polysaccharide (pectin or xanthan gum) and 0.005 wt.% sodium azide	Pre-emulsification with a M133/1281-0 mixer for 2 min followed by high-pressure homogenization with a M110Y Microfluidizer (3-stages, pressure = 12000 psi)	Xu, Liu, Luo, Liu, & McClements (2017)
O/W	Fish oil, O:W = 10:90	Water containing 1 % (w/w) thiol-modified β-lactoglobulin fibrils, 0-0.5 % (w/w) chitosan and 15 % (w/w) maltodextrin	Pre-emulsification with a Silverson L4R homogenizer at 7500 rpm for 5 min followed by high-pressure homogenization with a Panda 2K homogenizer (3-stages, pressure = 750 bar)	Chang et al. (2018)
O/W	Chia seed oil, O:W = 5:95	Aqueous solution composed of buffer solution (98 mM acetic acid, 2 mM sodium acetate, pH 3.0), 1 wt.% emulsifier (phosphatidylcholine-enriched lecithin or deoiled lecithin), 0.2 wt.% powdered chitosan, 20 wt.% maltodextrin, 0.0012 wt.% nisine and 0.1 wt.% potassium sorbate	Pre-emulsification with an Ultra-Turrax T25 at 10000 rpm for 2 min followed by high-pressure homogenization with a Panda 2K (4-stages, pressure = 600 bar)	Julio, Copado, Diehl, Ixtaina, & Tomás (2018)
O/W	Menhaden oil, O:W = 10:90	Deionized water containing 2 % (w/v) whey protein isolate, 0.1 % (w/v) polysaccharides (xanthan gum or locust bean gum) and 0.04 % (w/v) sodium azide	Rotor-stator homogenization using PowerGen 500 homogenizer at 30000 rpm for 6 min	Owens, Griffin, Khouryieh, & Williams (2018)
O/W	Cod liver oil, O:W = 50-70:50-30	Distilled water containing different amounts of sodium caseinate (CAS) and phosphatidylcholine (PC) (total CAS + PC content = 1.4, 2.1 and 2.8 % (w/w); ratio of CAS to PC = 0.4, 1.2 and 2.0 (w/w)) and 0.05 % (w/w) sodium azide	Pre-emulsification with a Stephan Universal mixer at 1200 rpm for 3 min followed by an emulsification for additional 2 min × 2 min under reduced pressure (approximately 40 kPa)	Yesiltas, García-Moreno, Sørensen, Akoh, & Jacobsen (2019)

3.1.2. Multiple PUFA-based lipid emulsions

The use of multiple PUFAs BLEs within the food sector may offer interesting possibilities. *Multiple* PUFAs BLEs (also called *double emulsions*, *duplex emulsions* or *emulsions of emulsions*) are multi-compartmentalized systems, characterized by the coexistence of two simple emulsions: water-in-oil (W/O) and oil-in-water (O/W), in which the droplets of the dispersed phase are smaller and more equally dispersed (Garti, 1997). This singular structure poses a number of advantages: it provides a potentially useful strategy for producing reduced fat and low-calorie products, it prevents oxidation and enhances the sensorial properties of foods, it masks flavours, and controls and protects the delivery of sensitive ingredients during the processing and preservation of food products, or even the action of certain enzymatic activity after ingestion. These PUFA-based lipid systems can also be used in food, taking advantage of the external aqueous phase that is more acceptable in terms of palatability (Dickinson, 2011; Kukizaki & Goto, 2007).

Figure 2. Structure of water-in-oil-in-water and oil-in-water-in-oil PUFAs BLEs.



There are two main types of multiple PUFAs BLEs. The most common form is water-in-oil-in-water (W/O/W) PUFAs BLEs, although oil-in-water-in-oil (O/W/O) PUFAs BLEs can also be employed in some applications. Water-in-oil-in-water PUFAs BLEs consists of tiny water droplets (W1) dispersed inside fat droplets – PUFA enriched oils – (O), which are, in turn, dispersed in a continuous aqueous phase (W2) (Figure 2). As a result, a system (W1/O/W2) consisting of three phases is formed, two aqueous phases (one outer and another inner, normally with different compositions) and a lipid phase enriched in PUFAs. The lipid phase is located between the aqueous phases and the two are separated from each other by two types of interfaces which are stabilized by means of emulsifiers: one hydrophobic (or lipophilic), designed to stabilize the interface of the internal emulsion (W1/O) and another hydrophilic, to stabilize the outer interface of the PUFA-enriched fat globules (for W1/O/W2 PUFAs BLE) (Jiménez-Colmenero, 2013a).

As already mentioned, multiple PUFAs BLEs have potential advantages over simple PUFAs BLEs. These include release systems for bioactive compounds, although these are generally more appropriate for encapsulation, vehiculization, protection, and delivery of hydrophilic compounds (McClements, Decker, & Weiss, 2007). Due to their characteristics, including the capacity to hold and protect certain components and control their release from one phase to another, these PUFAs BLEs have been used as a means of microencapsulation in food and clinical nutrition (encapsulation of essential fatty acids and omega-3 polyunsaturated fatty acids), pharmacology (carriers for anticancer agents and other active ingredients), cosmetics (improving the application of creams with encapsulated compounds) and in other industrial applications (Benichou, Aserin, & Garti, 2004; Kukizaki & Goto, 2007; Muschiolik, 2007). Multiple PUFAs BLEs offer a promising means of preparing micro- and nano- capsules (solids or

semisolids) which can contain specific lipophilic and hydrophilic compounds (Benichou, Aserin, & Garti, 2004). Despite this potential, there are still few examples of multiple PUFAs BLEs currently used in food products. The main reason is that multiple PUFAs BLEs are highly susceptible to breakage during storage or when subjected to extreme environmental conditions often found in the food industry. The stability of simple PUFAs BLE is more simply determined than that of multiple PUFAs BLEs (Jiao, Rhodes, & Burgess, 2002). Several instability mechanisms are responsible for the breakdown of multiple PUFAs BLE. Some of these are similar to those of found in simple PUFAs BLEs, whilst others are exclusive to multiple PUFAs BLEs (for example, the non-equilibrium between the internal and external aqueous phases could produce destabilization of the system) (Jiao, Rhodes, & Burgess, 2002).

In the scientific literature, there are some examples of multiple PUFAs BLEs. However, due to the complexity of these types of emulsions, investigation into them has only been initiated recently and, consequently, only a small number of studies are identified. Comunian, Ravanfar, Dando, Favaro-Trindade, and Abbaspourrad (2017) and Flaiz et al. (2016) reviewed the most recent studies on W/O/W multiple PUFAs BLEs, using echium seed oil and perilla oil as a source of omega-3. Table 2 contains the compositional information for a selection of multiple PUFAs BLEs published during the last decade, alongside relevant examples taken from previous research.

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Table 2. Phases compositional information on a selection of multiple PUFAs BLEs.

Type	Inner phase	Middle phase	Primary emulsification method	Continuous phase	Secondary emulsification method	Reference
O/W/O	Cod liver oil stabilized with tocopherols; O1:W = 20:80	Distilled water containing 6 wt.% sodium caseinate	Rotor-stator homogenization using Ultra-Turrax T18 at 20000 rpm for 10 min	[*Dispersed phase: 50 wt.% primary emulsion and 50 wt.% lactose monohydrate (14 wt.%)*] Extra virgin olive oil containing 50 wt.% polyglycerol polyricinoleate (PGPR); Dispersed phase:O2 = 62.5:37.5	Stir using a magnetic stirrer	Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Ruiz, & Antequera (2015); Jiménez-Martín, Antequera, Gharsallaoui, Ruiz, & Pérez-Palacios (2016)
W/O/W	Water containing 0.50 mg/mL phenolic compound (sinapic acid or rutin); W1:O = 1:2	Echium seed oil containing 0.5 wt.% polyglycerol ricinoleic acid (PGPR 90)	Rotor-stator homogenization using Ultra-Turrax at 12000 rpm for 4 min	Aqueous solution composed by 7.5 wt.% polymers (gelatin and arabic gum, ratio 1:1); (W1/O):W2 = 50-100:50-0	Rotor-stator homogenization using Ultra-Turrax at 10000 rpm for 3 min	Comunian, Boillon, Thomazini, Nogueira, & Favaro-Trindade (2016)
W/O/W	Distilled water containing 0.584 % (w/v) NaCl, 0.04 % (w/v) sodium azide and 0.375 % (w/v) hydroxytyrosol; W1:O = 20:80	Perilla oil containing 6 % (w/w) PGPR	Pre-emulsification with a Thermomix food processor TM-31 at 3250 rpm for 15 min followed by high-pressure homogenization with a Panda Plus 1000 homogenizer (2-stages, pressure = 55 and 7 MPa)	Distilled water containing 0.5 % (w/v) sodium caseinate, 0.584 % (w/v) NaCl and 0.04 % (w/v) sodium azide; (W1/O):W2 = 40:60	Pre-emulsification with a Thermomix food processor TM-31 at 700 rpm followed by high-pressure homogenization with a Panda Plus 1000 homogenizer (2-stages, pressure = 15 and 3 MPa)	Flaiz et al. (2016)
O/W/O	Echium oil with and without added antioxidant (500 and 800 ppm quercetin)	Sodium alginate aqueous solution with and without added antioxidant (0.025 and 0.050 g/g sinapic acid)	Microfluidic homogenization or gelation homogenization	Corn oil containing 2 % (w/w) soy lecithin	Microfluidic homogenization or gelation homogenization	Comunian, Ravanfar, Dando, Favaro-Trindade, & Abbaspourrad (2017)
W/O/W	Distilled water containing 0.584 % (w/v) NaCl, 0.125 % (w/v)	Perilla oil containing 6 % (w/w) PGPR	High-pressure homogenization with a Panda Plus 2000 homogenizer (2-stages, pressure	Distilled water containing 0.5 % (w/v) sodium caseinate, 0.584 % (w/v) NaCl and 0.04	High-pressure homogenization with a Panda Plus 2000 homogenizer (2-stages, pressure	Freire, Bou, Cofrades, & Jiménez-Colmenero (2017)

	hydroxytyrosol and 0.04 % (w/v) sodium azide; W1:O = 20:80		= 7 and 55 MPa)	% (w/v) sodium azide; (W1/O):W2 = 40:60	= 3.5 and 15 MPa)	
W/O/W	Distilled water containing 0.584 % (w/v) NaCl and 0.375 % (w/v) hydroxytyrosol; W1:O = 20:80	Perilla oil containing 6 % (w/w) PGPR	Pre-emulsification with a Thermomix food processor at setting 6 for 15 min followed by high-pressure homogenization with a Panda Plus 2000 homogenizer (2-stages, pressure = 55 and 7 MPa)	Distilled water containing 0.584 % (w/v) NaCl and 0.04 % (w/v) sodium azide; (W1/O):W2 = 40:60	Pre-emulsification with a Thermomix food processor at setting 3 followed by high-pressure homogenization with a Panda Plus 2000 homogenizer (2-stages, pressure = 15 and 3 MPa)	Cofrades et al. (2017)
W/O/W	Water containing 2 - 225 mg/kg gallic acid and NaCl to prevent diffusion phenomena; W1:O = 20:80	Blend of olive, linseed and fish oils (70:20:10) containing 6 % (w/w) PGPR	Pre-emulsification with a Thermomix food processor at 3250 rpm for 5 min followed by high-pressure homogenization with a Panda Plus 2000 homogenizer (2-stages, pressure = 7977 and 1015 psi)	Water containing 2 - 225 mg/kg quercetin, NaCl to prevent diffusion phenomena and 0.5 % (w/w) sodium caseinate; (W1/O):W2 = 40:60	Pre-emulsification with a Thermomix food processor at 700 rpm for 5 min followed by high-pressure homogenization with a Panda Plus 2000 homogenizer (2-stages, pressure = 2175 and 435 psi)	Silva et al. (2018)
W/O/W	Deionized water containing 0.1 % (w/w) fish protein hydrolysate, 0.033 % (w/w) NaCl and 0.033 % (w/w) vitamin B_{12}; W1:O = 30:70	Fish liver oil containing 6 to 10 % (w/w) PGPR	Rotor-stator homogenization using Ultra-Turrax T25 at 20000 rpm for 5 min	Aqueous solutions prepared by adding different whey protein concentrate(40 % w/w)/inulin(3 % w/w) weight ratios, included 1:1, 1.608:1, 2.5:1, 3.39:1 and 4:1; (W1/O):W2 = 2:5	Rotor-stator homogenization using Ultra-Turrax T25 at 10000 rpm for 3 min	Jamshidi, Shabanpour, Pourashouri, & Raeisi (2019)

3.2. PUFA-based lipid self-assembling systems

A self-assembling system could be defined as a dynamic system composed of different interacting components from which, without the need for external intervention, a completely spontaneous large-scale structure emerges by virtue of the local interactions taking place within its own constituents. The large-scale emergence of this structure does not take place by accident. In fact, if the system were reset to its initial state, the aforementioned structures would likely reappear. Amongst the advantages of the system, it should be noted that the way in which it is formed minimizes the total energy of the system and it is, therefore, usually a reasonably stable system. Nevertheless, it is difficult to find ingredients with self-assembling properties, which limits its practical application. This is the reason why there are very few existing studies on PUFA-based lipid self-assembling systems; however, some will be presented here. Zheng, Liu, Wang, and Baoyindugurong (2011) were the first (to the author's knowledge) to develop self-assembling fish oil microemulsions using exclusively food-grade ingredients and to study their physical properties. From the results obtained, the authors proposed these self-assembling systems as interesting and promising ways to deliver and release fish oil. Some years later, Calligaris, Ignat, Biasutti, Innocente, and Nicoli (2015) synthesized saturated monoglyceride-based self-assembly structures and explored the feasibility of using these structures for the incorporation of omega-3 PUFAs into the production of fortified cheese. More recently, Yaghmur, Al-Hosayni, Amenitsch, and Salentinig (2017) and Shao, Bor, Al-Hosayni, Salentinig, and Yaghmur (2018) studied the structural characteristics of self-assemblies based on differently synthesized omega-3 PUFA monoglycerides following excessive exposure to water. They showed that their unique structural properties held promise for future drug and functional food delivery applications.

3.3. PUFA-based lipid liposomes

Liposomes (or *lipid vesicles*) are spherical structures composed of at least one lipid bilayer that enclose a number of aqueous or liquid compartments. Due to their particular structure, liposomes have some interesting properties with great potential. Furthermore, different liposomes have characteristics that can be adapted for various applications, especially in the food and pharmaceutical industries. Thus, liposomes have been extensively studied in recent decades as model membranes and drug and nutrient delivery systems, including omega-3 PUFAs. Some of the latest work on the encapsulation of omega-3 PUFAs using liposomes was developed by Ghorbanzade, Jafari, Akhavan, and Hadavi (2017) who effectively encapsulated nano-liposomes containing fish oil. Further, Rasti, Erfanian, and Selamat (2017) prepared nano-liposomes using omega-3 PUFAs and soybean-PLs as liposomal ingredients under previously optimized preparation conditions.

3.4. PUFA-based solid lipid nanoparticles

Colloidal particles between 10 and 1000 nm in size are known by the name of nanoparticles. When these nanoparticles are composed of solid lipids, they are referred to as solid lipid nanoparticles (SLNs). Among their main advantages, one that stands out is the chemical protection they provide to the incorporated bioactive compounds, controlling their release for several weeks and improving the bioavailability of the enclosed drug. In recent years, this type of system has become increasingly important, emerging as a possible alternative to encapsulate omega-3 PUFAs and protect them against lipid oxidation. As proof of this we must highlight the works of Salminen,

Helgason, Kristinsson, Kristbergsson, and Weiss (2017) and Yang and Ciftci (2017). The former prepared solid lipid nanoparticles using different ratios of tristearin as the carrier lipid and fish oil as the incorporated liquid lipid. The latter developed hollow solid lipid nanoparticles formed from fully hydrogenated soybean oil in order to encapsulate fish oil. Both authors concluded that the oxidative stability of fish oil encapsulated in these types of systems increased significantly when compared to the free fish oil.

3.5. Nanostructured PUFA-based lipid carriers

Nanostructured lipid carriers (NLCs) are delivery systems in which partially crystallized lipid particles –with sizes smaller than 100 nm– are dispersed in an aqueous phase containing emulsifiers. If the partially-crystallized lipid is enriched with PUFAs, it creates a useful system for the release of these fatty acids in the body. The main difference between this type of delivery system and that of SLNs is that NLCs combine solid lipid and liquid lipid, i.e. oil, in order to enhance their drug-loading capacity. These systems offer some advantages in comparison to other colloidal carriers: NLCs may improve consumer acceptability, functionality, safety, shelf-life and nutritional value of food systems, improve bioavailability and increase stability of many bioactive compounds, and provide controlled release of encapsulated materials. Similarly to SLNs, this type of delivery system has become increasingly important in recent years as it combines the advantages of other lipid nanocarriers, whilst avoiding some of their disadvantages. As a result, NLCs offer a potential alternative for encapsulating omega-3 PUFAs and protecting them against lipid oxidation. Huang, Wang, Li, Xia, and Xia (2017) encapsulated omega-3 PUFA and quercetin-enriched linseed oil into NLCs using a high pressure homogenization method and found a lower lipid oxidation than seen in a

conventional linseed oil emulsion. In addition, research on stability has produced positive outcome. Azizi, Kierulf, Connie Lee, and Abbaspourrad (2018) investigated the role of different lipid carriers in echium oil encapsulation. Both studies suggest that NLCs could be a promising vehicle for delivery of hydrophobic bioactive compounds and omega-3 PUFAs within the food industry.

4. Prevention of lipid oxidation in omega-3 PUFAs BLEs

Despite the different systems designed and developed for the vehiculization and protection of omega-3 PUFAs, lipid oxidation remains as a major problem and one of the limiting factors when incorporating these compounds within the food industry. Thus, for several years the scientific community has explored different options to prevent or, at least, slow down omega-3 PUFAs lipid oxidation, especially when they are contained within BLEs.

Many studies have shown that the ingredients that make up the aqueous phase – and the possible interactions taking place between them – have an important impact on lipid oxidation. Depending on their nature, chemical properties and the environmental conditions in which they are found, these ingredients can act as antioxidants or pro-oxidants. Careful selection of the composition of the aqueous phase will, therefore, be a determining factor of the control of omega-3 PUFA lipid oxidation.

When considering all of these ingredients, the importance of proteins which are frequently used to improve the physical stability of food emulsions should be emphasized. The proteins dissolved in the aqueous phase are electrically charged as a function of the pH of the medium and, consequently, attract or repel metal ions, catalysing oxidation reactions. In addition, certain proteins can act as free radical

scavengers, transition metal chelators, activating pro-oxidant compounds or, simply, they can have antioxidant properties (as is the case of caseins, for example) (McClements & Decker, 2000). For all of these reasons, a large number of studies exist within the scientific literature targeting the design and development of protein-stabilized O/W emulsions which incorporate functional lipids that are susceptible to oxidation (e.g., omega-3 polyunsaturated fatty acids). Djordjevic, Kim, McClements, and Decker (2004) and Cho, Decker, and McClements (2010) have demonstrated that the oxidative stability of polyunsaturated lipids can be enhanced by integrating them into oil droplets with protein coatings. Djordjevic, McClements, and Decker (2004) concluded that protein-stabilized O/W emulsions with a pH below the isoelectric point of the protein, allows it to positively charge the emulsion droplets and slow lipid oxidation by diminishing iron-lipid interactions. Thus, by controlling the pH of the medium, we can positively and negatively load proteins and, in this way, affect both the physical and chemical stability of PUFAs BLEs. As a result of these findings, many studies on different protein-stabilized O/W emulsions have emerged in recent years. O'Dwyer, O'Beirne, Eidhin, and O'Kennedy (2013) evaluated the impact of sodium caseinate concentration on the chemical stability of O/W emulsions. Both primary and secondary lipid oxidation products of emulsions were found to decrease as sodium caseinate concentration increased. Similarly, sodium caseinate decreased as microfluidization pressure increased. This finding was attributed to the apparent antioxidant effect of sodium caseinate, which interacts with metal ions and scavenges the free radicals present in the aqueous phase. Similar conclusions were drawn by Liu, Singh, Wayman, Hwang, and Fhaner (2015), who developed a physically stable PUFAs BLE using sodium caseinate dispersions and β-glucan rich oat products. These authors found that caseinate typically contributed to a reduction in the oxidation of omega-3 oils, though

425 there was no significant influence of β-glucan on oxidation. Sivapratha and Sarkar
426 (2017) also studied chemical stability and the impact of stress factors on flaxseed O/W
427 emulsions stabilized by a sodium alginate-sodium caseinate-chitosan interfacial
428 membrane. The results showed that the created membrane may be able to act as a
429 physical barrier that separates the lipid phase from pro-oxidants contained in the
430 aqueous phase. This demonstrates the possibilities of interfacial technology for
431 developing an emulsion system with the necessary properties. Not only proteins
432 influence lipid oxidation, protein hydrolysates and amino acids are also influential.
433 García-Moreno, Guadix, Guadix, and Jacobsen (2016) investigated the physicochemical
434 stability of fish O/W emulsions stabilized with fish protein hydrolysates (FPH). Sardine
435 hydrolysates with low degrees of hydrolysis (3 - 4 %) provided the most efficient
436 peptides for producing physically stable emulsions with a smaller droplet size. This
437 involved a greater protein adsorption at the interface enabling it to act as a physical
438 barrier against pro-oxidant compounds, which could also result in a greater oxidative
439 stability of these emulsions. These results demonstrate the possibilities of FPH as
440 alternative protein emulsifiers in the design of chemically stable fish O/W emulsions.
441 Similar results were obtained by Ghelichi, Sørensen, García-Moreno, Hajfathalian, and
442 Jacobsen (2017), who investigated the oxidative stability of O/W emulsions fortified
443 with common carp roe protein hydrolysates (CRPHs). These hydrolysates exhibited
444 antioxidant properties, radical scavenging and chelating activities, all of which slowed
445 lipid oxidation. All of these studies have shown that proteins and their hydrolysates,
446 which are two of the most common ingredients in omega-3 PUFAs BLEs, significantly
447 influence the chemical stability of emulsions. Thus, prior to designing a new emulsion,
448 a detailed study is necessary for selecting the most appropriate protein content for
449 reducing lipid oxidation.

Although proteins have potentially been the most studied ingredient, the type and concentration of emulsifier also has a very important role within lipid oxidation. These emulsifiers, which may have an ionic character or not, are amphiphilic in nature and can bind to both polar substances and nonpolar substances, interact with antioxidants or with pro-oxidants and, therefore, impede lipid oxidation. Fomuso, Corredig, and Akoh (2002) investigated the impact of different emulsifiers – comprising Tween 20, lecithin, whey protein isolate, mono-/diacylglycerols, and sucrose fatty acid ester – on the oxidative stability of fish oil-based structured lipid emulsions. These researchers demonstrated that the concentration and type of emulsifier influenced oxidation rate. Higher emulsifier concentrations usually showed a lower oxidation rate than lower concentrations, which in this case was attributed to a higher concentration of emulsifier creating a thicker interface that acted as a semipermeable barrier against the pro-oxidant compounds catalysing oxidation reactions. Further, the chemical structure of the emulsifier such as its ionic character, also influences permeability and, resultantly, oxidation rate. Chen, Rao, Ding, McClements, and Decker (2016) studied the role of different emulsifiers on lipid oxidation. These authors found that polyglycerol polyricinoleate (PGPR) promoted the oxidation of emulsion while several lecithins (defatted soybean lecithin (PC 75) or defatted lyso-lecithin (Lyso-PC)) showed a protective effect on omega-3 enriched oil. These authors associated the greater emulsifying capacity of PGPR to its worse performance protecting against oxidation. Its greater emulsifying capacity produced oil droplets of a smaller size and a greater interfacial surface area. This greater interfacial area led to the oil being more exposed to the pro-oxidants, ultimately leading to greater lipid oxidation. On the other hand, lecithins, with their lower emulsifying capacity and, in addition, a certain amount of antioxidant added, revealed a better oxidative response. Therefore, the choice of the

475 emulsifier not only influences the physical stability of the emulsion but, by acting
476 directly on the oil-water interface, it is another decisive ingredient for the chemical
477 stability of omega-3 PUFAs BLEs.

478 Another possibility for slowing lipid oxidation in BLEs is to add antioxidants or
479 scavenging pro-oxidants. Many investigations have demonstrated that transition metals
480 (pro-oxidant compounds) are mainly found in the water phase, whilst hydroperoxides
481 are located at the oil/water interface due to the fact that hydroperoxides are surface-
482 active compounds. Copper or iron are some of the most abundant pro-oxidant
483 compounds which may be found in packaging materials, food ingredients and water.
484 Thus, lipid oxidation occurs at the droplet interface, where the pro-oxidant ions of the
485 aqueous phase come into close contact with the lipid hydroperoxide located at the
486 droplet surface (Kargar, 2014; McClements, 2015; Waraho, Mcclements, & Decker,
487 2011). Consequently, some studies are on track to evaluate the effect of iron
488 encapsulation within the interior aqueous phase of W/O/W emulsions on lipid oxidation
489 (Choi, Decker, & McClements, 2009). Results obtained suggest that no significant
490 changes to lipid droplet size in multiple emulsions occurred during storage. This
491 suggests that the emulsions were resistance to flocculation and coalescence of lipid
492 droplets, and internal water expulsion/diffusion. Multiple emulsions containing
493 encapsulated iron did foster lipid oxidation when added to fish oil emulsions. Curiously,
494 multiple emulsions in the absence of added iron seem to be highly effective at delaying
495 lipid oxidation in fish oil emulsions. This may be due to their influence on the
496 redistribution of pro-oxidants ions and reaction products in the system. Another possible
497 alternative for delaying lipid oxidation is the addition of compounds with antioxidant
498 properties. Chen, Mcclements, and Decker (2010) studied the antioxidant ability of
499 selected polysaccharides (high-methoxyl and low-methoxyl pectin and others) in the

continuous phase of a fish O/W emulsion. None of these polysaccharides showed any effect on the physical properties of the emulsions; however, they reduced the formation of primary and secondary lipid oxidation products. The authors attributed the lowering of lipid oxidation to the ability of these polysaccharides to bind free radicals together through the chelating effect of pro-oxidative metals. Hence, these results suggest that the addition of anionic polysaccharides to the continuous phase of O/W PUFAs BLEs could be employed to enhance the chemical stability of O/W emulsions and in so doing, prolong their shelf-lifetime. Natural extracts have also been tested as a source of antioxidants. Karadağ et al. (2017) explored the capacity of Icelandic brown algae *Fucus vesiculosus* extracts incorporated into O/W emulsions to protect the lipid phase against lipid oxidation. These extracts, rich in polyphenolic compounds, were found to improve the oxidative stability of the omega-3 PUFAs emulsions, and thus could provide a different natural source of new effective antioxidant compounds. Recent studies have evaluated the efficacies of lipophilized phenolic compounds as potential antioxidants in PUFAs BLEs and they have obtained promising results. According to the cut-off effect hypothesis, the antioxidant efficacy increases with the increment of alkyl chain length until a threshold is reached. This threshold is the optimal chain length to obtain the highest efficacy of the antioxidants. To study the effect of alkyl chain unsaturation on the antioxidant activities, Pande and Akoh (2016) tested the antioxidant potential of three tyrosol-based phenolipids and they found that tyrosyl esters exhibited lower antioxidant activity than tyrosol whereas the addition of an alkyl chain enhanced the antioxidant efficiency of tyrosol in O/W emulsions.

Finally, it must always be kept in mind that the emulsification conditions, the characteristics of the medium, and the environmental conditions surrounding the emulsions also have a decisive influence on its chemical stability, regardless of the

ingredients present in the omega-3 PUFAs BLE. These will both affect the properties of the interface, its greater or lesser antioxidant capacity, and so on. For example, the emulsification method used influences the characteristics of the emulsion, such as droplet size, and this can affect the oxidative stability of the emulsion. So, Horn, Nielsen, Jensen, Horsewell, and Jacobsen (2012) studied the impact of homogenization equipment (microfluidizer vs. two-stage valve homogenizer) on oxidative stability of fish oil-in-water emulsions prepared with two different milk proteins: sodium caseinate and whey proteins. Emulsions were prepared at pH 7 with similar droplet sizes. Results showed that the oxidative stability of emulsions prepared with sodium caseinate was not influenced by the type of homogenizer used. Whereas, the type of homogenization equipment significantly influenced lipid oxidation when whey protein was used as emulsifier, with the microfluidizer resulting in lower levels of oxidation. They suggested that these results are related to the different distribution of protein components between the interface and the aqueous phase due to the different droplet disruption patterns in the two equipments. So, the microfluidizer produced a change in the protein composition of the interface compared to that obtained when the valve homogenizer was used. Regarding the characteristics of the medium, one of the critical factors is the pH. Owens, Griffin, Khouryieh, and Williams (2018) investigated the impact of the pH of the medium on the physicochemical stability of whey protein isolated-stabilized fish O/W emulsions containing xanthan (XG)-locust bean gum (LBG) mixtures. The results obtained suggest that the net electrical charge of the protein-coated droplets can be modified according to PH. This then acts on the electrostatic interactions of the protein-polysaccharide complex to reduce lipid oxidation. In addition, they demonstrated the ability of xanthan (an anionic polysaccharide) to chelate transition metal ions at negatively charged sites, thereby

preventing them from coming into close contact with the lipid phase. The presence or absence of light also influence lipid oxidation since light is necessary for the initial stage of oxidation reactions. The concentration of available oxygen represents another decisive factor as oxidation is not possible in the absence of oxygen. Finally, the storage temperature of the emulsion is clearly important. Klinkesorn and Geraldine (2012) evaluated the impact of the storage temperature on the oxidation rate of omega-3 PUFAs BLEs during storage. The authors hypothesized regarding the kinetic reaction and evaluated the value of the kinetic constant as a function of the storage temperature. They identified that the kinetic constant was dependent upon the temperature, with greater lipid oxidation occurring at higher storage temperatures.

Table 3 presents a selection of important studies on the chemical instability of PUFAs BLEs due to lipid oxidation.

Table 3. Selection of important studies on the chemical instability of PUFAs BLEs.

Type	Dispersed phase	Continuous phase	Emulsification method	Aim	Reference
O/W	Flaxseed oil and fish oil, O:W = 10:90	Water containing 0-3 % (w/w) sodium caseinate, 0-0.3 % (w/w) oat β-glucan and 0.04 % (w/w) sodium azide	Pre-emulsification with a Polytron homogenizer at 14000 rpm for 2 min followed by high-pressure homogenization with a Panda 2K homogenizer (2-stages, pressure = 80 and 8 MPa)	To study the effects of protein and polysaccharide content on lipid oxidation	Liu, Singh, Wayman, Hwang, & Fhaner (2015)
W/O	Double distilled water containing antioxidants and/or metal chelators, W:O = 2:98	Algae oil containing 0.15 wt.% emulsifier (PGPR, PC75, PC50, lyso-PC and MAG-DAG)	Pre-emulsification with a M133/1281-0 blender for 2 min followed by high-pressure homogenization with a M-110 L Microfluidizer Processor (3-stages, pressure = 68 MPa)	To evaluate the impact of antioxidants and emulsifiers on lipid oxidation	Chen, Rao, Ding, McClements, & Decker (2016)
O/W	Fish oil, O:W = 5:95	Distilled water containing 2 wt.% fish protein hydrolysates (sardine hydrolysates or small-spotted catshark hydrolysates)	Pre-emulsification with an Ystral mixer at 16000 rpm for 3 min followed by high-pressure homogenization with a M110L Microfluidics (3-stages, pressure = 9000 psi)	To investigate the effects of fish protein hydrolysates on lipid oxidation	García-Moreno, Guadix, Guadix, & Jacobsen (2016)
O/W	Cod liver oil, O:W = 5:95	Distilled water containing 1 % (w/w) citrem and 2 mg/mL protein (common carp roe protein hydrolysate)	Pre-emulsification with an Ultra-Turrax T1500 at 16000 rpm for 3 min followed by high-pressure homogenization with a Panda 2K (3-stages, pressure = 250 bar)	To examine the effects of carp roe protein hydrolysate on lipid oxidation	Ghelichi, Sørensen, García-Moreno, Hajfathalian, & Jacobsen (2017)
O/W	Fish oil, O:W = 70:30	Water containing 10 % (w/v) sodium caseinate and 0.5-1 % (w/v) seaweed extracts	Rotor-stator homogenization using Ultra-Turrax at 20000 rpm	To study the ability of *Fucus vesiculosus* extracts to inhibit lipid oxidation	Karadağ et al. (2017)
O/W	Flaxseed oil, O:W = 1:99	Aqueous solution composed of buffer solution (5 mM sodium acetate, pH 3.0), 0.4 % (w/v) sodium caseinate, 0.25 % (w/v) sodium alginate, 0.05-0.4 % (w/v) chitosan and 0.01 % (w/v) sodium azide	Pre-emulsification with a blender followed by ultrasound homogenization with a QSonica 700 at 50 % amplitude for 5 min	To investigate the effect of stress factors on the lipid oxidation of multilayer protein-stabilized emulsions	Sivapratha & Sarkar (2017)
O/W	Walnut oil, O:W = 5:95	Aqueous solution composed of phosphate buffer (5 mM, pH 7.0), 1.5 % (w/w) lecithin and 0, 1.0 or 1.6 % (w/w) NaCl or KCl	Pre-emulsification with a F6/10 blender followed by high-pressure homogenization with a HP-4L homogenizer (3-stages, pressure = 9000 psi)	To understand the impact of salts on lipid oxidation of lecithin-stabilized oil-in-water emulsions	Cui, Fan, Sun, Zhu, & Yi (2018)
O/W	Menhaden oil, O:W = 10:90	Deionized water containing 2 % (w/v) whey protein isolate, 0.1 % (w/v) polysaccharides	Rotor-stator homogenization using PowerGen 500 homogenizer at 30000 rpm for 6 min	To study the impact of pH on the lipid oxidation of emulsions	Owens, Griffin, Khouryieh, &

		(locust bean gum or xanthan gum) and 0.04 % (w/v) sodium azide		containing protein-polysaccharides mixtures	Williams (2018)
O/W	Tuna oil containing 0.7 mmol α-tocopherol and/or eugenol/kg of emulsion, O:W = 30:70	Ultrapure water containing 1 % (w/w) whey proteins or 0.5 % (w/w) Tween 80, 0.5 % (w/w) potassium sorbate and antioxidants. Emulsions were supplemented or not with guar gum (0 to 0.6 % (w/w))	Rotor-stator homogenization using Ultra-Turrax T25 at 10000 rpm for 5 min; ultrasound homogenization at 20 kHz for 20 min with alternating sonication/rest cycles (10 s sonication/10 s rest)	To understand the impact of compositional and structural parameters of emulsions rich in omega-3 on oxidative stability	Pernin, Bosc, Soto, Roux, & Maillard (2019)

5. Conclusions and future trends

Omega-3 polyunsaturated fatty acids are sensitive to oxidation. This is an undesirable process in the food industry as it leads to the deterioration of food. Understanding the mechanisms and factors by which this lipid oxidation occurs is key to its control, reduction and elimination. Within this context, a wide range of research studies on lipid oxidation have been published in the last decades, trying to shed light on this extremely complex process. However, extensive research is still needed to better understand this process in its entirety.

Currently, there is a wide variety of different systems available for the protection and vehiculization of bioactive lipids, with each presenting their own advantages and disadvantages. This review article tries to evaluate and update state-of-the-art research relating to use of the main simple and multiple omega-3 PUFAs BLEs as novel alternative encapsulation systems. Nowadays, simple oil-in-water emulsions are the most widely used systems for encapsulating omega-3 PUFAs. However, their capacity to encapsulate and protect PUFAs against lipid oxidation, one of the main problems posed to food manufacturers, is rather limited. As a result, the food industry requires alternative delivery methods. Other lipid-based delivery systems technologies such as multiple emulsions, solid lipid nanoparticle emulsions, liposomes, and self-assembling emulsions hold some advantages over traditional emulsions. However, they are not simple to prepare and can sometimes be even more unstable than simple emulsions due to the additional instability mechanisms they possess. In this sense, although multiple PUFAs BLEs have begun to be studied in recent years, there is still only a small number of studies available and none have been conducted on an industrial scale. Further research on lipid oxidation and multiple PUFAs BLEs is therefore necessary, in addition to exploring new types of emulsions capable of encapsulating omega-3 PUFAs.

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