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Review

Conventional spray-drying and future trends for the microencapsulation of fish oil

Cristian Encina [a], Cristina Vergara [b], Begoña Giménez [c], Felipe Oyarzún-Ampuero [d], Paz Robert [a, *]

[a] Departamento Ciencia de los Alimentos y Tecnología Química, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. Santos Dumont 964, Independencia, Santiago, Chile
[b] Fundación Fraunhofer Chile Research, Av. Mariano Sánchez Fontecilla 310 piso 14, Las Condes, Santiago, Chile
[c] Departamento de Ciencia y Tecnología de Alimentos, Facultad Tecnológica, Universidad de Santiago de Chile. Ecuador 3769, Estación Central, Santiago, Chile
[d] Departamento de Ciencia y Tecnología Farmacéutica. Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. Santos Dumont 964, Independencia, Santiago, Chile

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ABSTRACT

Polyunsaturated fatty acids, especially long-chain polyunsaturated omega-3 fatty acids (LCω3-PUFA), are essential in human nutrition because they play an important role in humans and prevent several diseases. Fish oil is a natural source of LCω3-PUFA that can be incorporated into food products. One of the major drawbacks of oils containing a high amount of LCω3-PUFA, such as fish oils, is their high susceptibility to oxidation and unpleasant flavours. Microencapsulation of fish oil by spray-drying has been proposed as a strategy to retard lipid auto-oxidation, improving oil stability, prolonging its shelf life, limiting the development of off-flavours and controlling the release into food. The encapsulation of fish oil by conventional spray-drying has been performed by preparing fish oil-in-water emulsions (micro- or nano-sized) by applying high shearing forces.

The objective of this review is to compile the scientific research on the encapsulation of fish oil to discuss the main formulation and process variables that affect the physicochemical properties of the fish oil microparticles obtained by conventional spray-drying, the stability of fish oil during storage and the application of fish oil microparticles in food systems. An alternative strategy to conventional spray-drying (water-free spray-drying) is also proposed.

1. Nutritional value of fish oil

Long-chain omega-3 polyunsaturated fatty acids (LCω3-PUFA), especially eicosapentaenoic acid (EPA, C20:5 ω3) and docosahexaenoic acid (DHA, C22:6 ω3), are considered necessary because they exert a strong positive influence on human health (Arab-Tehrany et al., 2012; Valenzuela, Tapia, González, & Valenzuela, 2011). EPA and DHA are known for their health benefits, such as prevention of cardiovascular disease (reducing serum triglyceride levels) (Din, Newby, & Flapan, 2004) and some types of cancer (Larsson, Kumlin, Ingelman-Sundberg, & Wolk, 2004). In addition, other biological activities associated with the prevention of inflammatory and neurodegenerative diseases (rheumatoid arthritis, diabetes, allergies and Alzheimer's) have been reported (Yashodhara, Umakanth, Pappachan, Bhat, Kamath, & Choo, 2009; Valenzuela, Bascuñán, Valenzuela, & Chamorro, 2009). DHA also plays an important role in visual and neural development in infant nutrition (Arab-Tehrany et al., 2012; Kralovec, Zhang, Zhang, & Barrow, 2012; Lands, 2005; Rubio-Rodríguez, Beltrán, Jaime, de Diego, Sanz, & Rovira Carballido, 2010).

EPA and DHA can be obtained from omega-3 plant sources that are rich in alpha-linolenic acid (ALA, C18:3 ω3), such as certain seeds, including linseed and chia. However, in the human body, the conversion of ALA into EPA and DHA is low (5–10% for EPA and 1–5% for DHA) (Kralovec et al., 2012). Therefore, these fatty acids must be ingested from the diet (Lands, 2005; Valenzuela et al., 2011). The most important natural sources of EPA and DHA are marine organisms, such as fish, seafood and algae. The World

* Corresponding author.
E-mail address: proberts@uchile.cl (P. Robert).

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Health Organization and the North Atlantic Treaty Organization recommend consuming 0.3–0.5 g of EPA + DHA per day. The worldwide average consumption is less than these recommendations (Arab-Tehrany et al., 2012). In this context, healthy foods supplemented with LCω3-PUFA are gaining importance in the food market. The incorporation of fish oil into foods has limited success because of its low solubility in most food systems and its high susceptibility to oxidation (Aghbashlo, Mobli, Madadlou, & Rafiee, 2013a; Aghbashlo, Mobli, Madadlou, & Rafiee et al., 2012a; Aghbashlo, Mobli, Rafiee, & Madadlou, 2012b).

Fish oil is generally extracted from small cold-water fish species such as anchovy, sardine, herring, mackerel, menhaden or capelin, as well as from liver of lean fish species such as cod. Cold-press refined fish oil has been used for microencapsulation by spray-drying in most of the studies (Drusch, Serfert, Van Den Heuvel, & Schwarz, 2006; Drusch, 2007; Serfert, Drusch, & Schwarz, 2009b). In other studies concentrates of EPA and DHA in the form of ethyl esters or acylglycerides have been also microencapsulated (Rodrigues et al., 2011). LCω3-PUFAs (fish oil) are highly unsaturated molecules that may be degraded, leading to loss of sensory and nutritional value. There are two pathways to stabilize fish oil. The first is the addition of antioxidants into the bulk fish oil, but this pathway does not allow the removal of unpleasant fish oil flavours (Shahidi & Zhong, 2010). The second pathway is the microencapsulation of fish oil, which has been used to protect unsaturated fatty acids against oxidation and other unwanted reactions caused by environmental conditions (light, temperature, oxygen, humidity), thus increasing its shelf life. Moreover, encapsulation enables masking of the unpleasant fish oil flavours (Arab-Tehrany et al., 2012; Kralovec et al., 2012; Rubio-Rodríguez et al., 2010).

2. Fish oil encapsulation technologies

In recent years, encapsulation technologies have increased in importance in the food industry, particularly in the development of ingredients to design functional and/or healthy foods. However, due to its hydrophobicity, low oxidative stability and unpleasant flavour, the addition of fish oil to hydrophilic foods represents an important challenge.

Encapsulation is based on coating or entrapping solid, liquid or gaseous materials and can be used to protect, transport or control the release of active compounds (Desai & Park, 2005). The selection of an adequate encapsulating agent and the microencapsulation process determine the applicability of fish oil (Anwar & Kunz, 2011). Encapsulation of fish oil has been reported using physical, physico-chemical and chemical methods (Chatterjee & Judeh, 2016), such as freeze drying (Chung, Sanguansri, & Augustin, 2011; Heinzelmann, Velasco, & Márquez-Ruiz, 2000; Klaypradit & Huang, 2008; Márquez-Ruiz, Velasco, & Dobarganes, 2000; Velasco, Marmesat, Dobarganes, & Márquez-Ruiz, 2006), gelation (Cho, Shim, & Park, 2003), complex coacervation (Costa de Conto, Ferreira Grosso, & Guaraldo Gonçalves, 2013), inclusion complexation (Choi, Ruktanonchai, Min, Chun, & Soottitantawat, 2010), emulsification (Nasrin & Anal, 2014), nanoencapsulation (sodium caseinate-gum arabic complexes) (Ilyasoglu & Nehir El, 2014), electrospinning (García-Moreno et al., 2016; Moomand & Lim, 2014) and spray-drying (Morales-Medina, Tamm, Guadix, Guadix, & Drusch, 2016; Wang, Liu, Chen, & Selomulya, 2016; Patrick, Muhamyankaka, Denis, Ntsama, & Zhang, 2013; Aghbashlo et al., 2013a; Aghbashlo, Mobli, Madadlou, & Rafiee, 2013b; Aghbashlo, Mobli, Madadlou, & Rafiee, 2012a,b; Aghbashlo, Mobli, Madadlou, & Rafiee, 2012c; Kralovec et al., 2012; Anwar & Kunz, 2011; Polavarapu, Oliver, Ajlouni, & Augustin, 2011; Rodrigues et al., 2011; Wang, Tian, & Chen, 2011; Shen, Augustin, Sanguansri, & Cheng, 2010; Jafari, Assadpoor, Bhandari, & He, 2008; Drusch, 2007; Shaw, McClements, & Decker, 2007; Drusch et al., 2006; Kolanowski, Ziolkowski, Weißbrodt, Kunz, & Laufenberg, 2006; Hogan, O'Riordan, & O'Sullivan, 2003; Kagami et al., 2003; Keogh et al., 2001). The selection of the encapsulation method is governed by important variables, such as the desired size of the microparticles, the type of food to be developed and, if required, the controlled release of oil from microparticles in food or in the gastrointestinal tract. Table 1 shows the most important research related to fish oil microencapsulation by spray-drying.

3. Microencapsulation of fish oil by spray-drying

Spray-drying is a technique used for the encapsulation of active compounds and is simple, low-cost, reproducible and easy to scale up. Moreover, it is useful for encapsulating heat-sensitive materials (such as fish oil) because of its short drying times (5–30 s) (Desai & Park, 2005). This method produces powder microparticles with low water activity (a_w), simplifying transport, handling, and storage and ensuring microbiological quality (Gouin, 2004). However, encapsulation by spray-drying is considered an immobilization technology rather than a true encapsulation technology because some active compounds remain exposed on the microparticle surface (de Vos, Faas, Spasojevic, & Sikkema, 2010).

Spray-drying is a process for turning an infeed (solution or dispersion) into a dry powder in a single, continuous step. The infeed is atomized in the form of fine drops in a hot drying medium (air or nitrogen). When the small droplets make contact with the drying medium at high temperature, a fast evaporation of the solvent (mainly water) occurs and a powder is instantaneously produced (Gharsallaoui, Rouaut, Chambin, Voilley, & Saurel, 2007). The spray-drying process is typically performed with an aqueous feed solution, which limits the encapsulating agent and active compound to water-soluble compounds at an acceptable concentration (Gharsallaoui et al., 2007; Gouin, 2004). However, the encapsulation of lipophilic molecules, such as fish oil, requires a previous elaboration of oil-in-water emulsions (Aghbashlo et al., 2013b; Drusch, 2007; Drusch et al., 2006; Jafari et al., 2008; Keogh et al., 2001; Shaw et al., 2007). It has been demonstrated that the stability of feed emulsions in the spray-drying process plays an important role in the retention of fish oil (Drusch, 2007; Drusch et al., 2006; Keogh et al., 2001).

Different atomization devices can be used to atomize the feed emulsion, such as pressure nozzles (pressure energy) and rotary atomizers (centrifugal energy). When atomization of the feed solution occurs at higher atomization energies, smaller droplets are produced, increasing the heat and mass transfer between the atomized droplets and the drying medium due to a greater contacting surface (Gharsallaoui et al., 2007). Consequently, droplets are quickly dehydrated by the hot drying medium when the solvent (mainly water) rapidly evaporates in a heated chamber. Thus, the moisture evaporation from the droplets quickly forms a dry crust at their surface, and the residual moisture diffuses towards the outside of the particles. The dehydrated powdered particles are then transported to a cyclone separator for recovery. Drying is finished when the particle temperature equals the air temperature. This process takes a few seconds, and the feed liquid is converted into powdered particles (Gibbs, Kermasha, Alli, & Mulligan, 1999; Gouin, 2004; Mahdavi, Jafari, Ghorbani, & Assadpoor, 2014).

There are many spray-dryer designs available for various production levels, from the laboratory and pilot scale to the industrial scale. In addition, there are three mechanisms for the movement of sprayed air in the drying chamber, co-current, counter-current or mixed flow, depending on the direction of both the hot air and the feed atomization flow through the drying chamber (Masters, 1972).

The most common parameters employed in the characterization

Table 1
Main studies on fish oil microencapsulation by spray-drying.

Encapsulating agent	FO[a]/EA[b]	Temperature	Operational conditions	FO storage stability	Food applications	References
Glucose syrup	1:6	Inlet: 180 °C Oulet: 70 °C	Pressure: 4 bar Rotatory atomization: 22,000 rpm	20 °C/33% relative humidity/12 weeks Peroxide value	NR	Morales-Medina et al., 2016
Whey protein isolate	10:1/5:1/1:1/1:2	Inlet: 160 °C Oulet: NR	Spraying air flow rate: 900 L h^{-1}	40 °C/7 weeks Peroxide value	NR	Wang et al., 2016
Maltodextrin Fish gelatin κ carragenan	1:4	Inlet: 180 °C Outlet: 80 °C	NR	NR	NR	Mehrad, Shabanpour, Jafari, & Pourashouri, 2015
Skim milk powder	1:2	Inlet: 140–180 °C Outlet: NR	Aspirator rate: 55, 65, 75% Peristaltic pump rate: 5, 10, 15% Spraying air flow rate: 600, 800 L h^{-1}	Peroxide value	NR	Aghbashlo et al., 2013a
Skim milk powder Whey protein concentrate Whey protein isolate Milk protein concentrate Sodium caseinate	1:2	Inlet: 140–180 °C Outlet: NR	Aspirator rate: 65% Peristaltic pump rate: 10% Spraying air flow rate: 700 L h^{-1}	25 °C/4 weeks Peroxide value	NR	Aghbashlo et al., 2013b
Gum Arabic Carboxymethyl cellulose	NR	Inlet: 180 °C Outlet: 90 °C	NR	Peroxide value Anisidine value Total oxidation (TOTOX) Free fatty acids (FFA)	Fermented milk	Patrick et al., 2013
Skim milk powder Whey protein concentrate Whey protein isolate Milk protein concentrate Sodium caseinate	1:2	Inlet: 140–180 °C Outlet: 77.8–103.2 °C	Aspirator rate: 65% Peristaltic pump rate: 10% Spraying air flow rate: 700 L h^{-1}	NR	NR	Aghbashlo et al., 2012b
Skim milk powder	1:2.3/1:4/1:9	Inlet: 175 °C Outlet: NR	Aspirator rate: 65% Peristaltic pump rate: 10% Spraying air flow rate: 700 L h^{-1}	Peroxide value	NR	Aghbashlo et al., 2012c
Skim milk powder Maltodextrin Lactose Sucrose Tween 20	1:2	Inlet: 175 °C Outlet: 95–98 °C	Aspirator rate: 65% Peristaltic pump rate: 10% Spraying air flow rate: 700 L h^{-1}	Peroxide value	NR	Aghbashlo et al., 2012a
Soy fiber Maltodextrin Hydroxypropyl b-cyclodextrin n-OSA starch	1:4	Inlet: 180 °C Outlet: 85 °C	NR	21 °C/30%HR/8 weeks Peroxide value Headspace propanal	NR	Anwar & Kunz, 2011
Sugar beet pectin Glucose syrup	1:2–1:4	Inlet: 180 °C Outlet: 80 °C	NR	25 °C/3 months Headspace analysis Induction period	NR	Polavarapu et al., 2011
Arabic gum Tween 80	1:1.9–1:5.6	Inlet: 155–215 °C Outlet: NR	Aspirator rate: 90% Peristaltic pump rate: 25–58% Spraying air flow rate: 635–730 L h^{-1}	NR	NR	Rodrigues et al., 2011
Barley protein	1:1/1:2/2:1	Inlet: 120–180 °C Outlet: 60 °C	NR	40 °C/8 weeks Peroxide value	NR	Wang et al., 2011
Chitosan Glucose n-OSA starch	1:1.5–1:2.3	Inlet: 180 °C Outlet: 80 °C	NR	25 °C/4 weeks Induction period Oxidation volatile markers FAME analysis	NR	Shen et al., 2010
n-OSA starch Glucose syrup	1:1.5	Inlet air: 160, 210 °C Outlet air: 70, 90 °C Inlet N2: 180 °C Outlet N2: 70 °C	Air (NR) Spraying N2 flow rate: 37 m^3 h^{-1}	Inert conditions (20 °C/33%RH/56 days) Air conditions (20 °C/33%RH/35 days) Peroxide value Headspace propanal	NR	Serfert et al., 2009a
n-OSA starch	1:1.5	Inlet: 180 °C	NR	Air conditions (20 °C/33% RH/8 weeks)	NR	Serfert et al., 2009b

Table 1 (*continued*)

Encapsulating agent	FO[a]/EA[b]	Temperature	Operational conditions	FO storage stability	Food applications	References
Glucose syrup		Outlet: 70 °C		Peroxide value Headspace propanal		
Methylcellulose Calcium-gelatin casein Whey protein concentrate Maltodextrin Soy lecithin	1:2	Inlet: 160 °C Outlet: 78 °C	Feed flow: 20 mL min^{-1}	60°C/24 days	Bread manufacture	Davidov-Pardo et al., 2008
Maltodextrin n-OSA starch Whey protein concentrate	1:4	Inlet: 180 °C Outlet: 65 °C	Nozzle air pressure 310 kPa	NR	NR	Jafari et al., 2008
Sugar beet pectin Glucose syrup	1:1–1:2	Inlet: 170 °C Outlet: 70 °C	NR	NR	NR	Drusch, 2007
Chitosan Lecithin Corn syrup	1:0.4–1:4	Inlet: 180 °C Outlet: NR	Feed flow: 2.2 L h^{-1}	37 °C/33% RH Peroxide value Headspace propanal Thiobarbituric acid (TBARS)	NR	Shaw et al., 2007
n-OSA starch	1:1.5	Inlet: 170 °C	NR	20 °C/0–54.4% RH/8 weeks	NR	Drusch et al., 2006
Glucose syrup Trehalose		Outlet: 70 °C		Peroxide value Conjugated dienes Headspace propanal		
Methylcellulose Maltodextrin	1:1.5–1:3	Inlet: 160 °C Outlet: 65 °C	Feed flow: 15 min^{-1} Air pressure: 350 kPa	Room temperature/air Room temperature/ vacuum	NR	Kolanowski et al., 2006
Lecithin				5 °C/air 5 °C/vacuum Peroxide value		
Corn syrup Sodium caseinate Lecithin	1:1.5	Inlet: 210 °C Outlet: 95 °C	NR	30 °C/0–43% RH Peroxide value Thiobarbituric acid (TBARS)	NR	Baik et al., 2004
Maltodextrin (DE 5.5–38)	1:1/1:1.66/1:3	Inlet: 180 °C	Feed flow: 20 mL min^{-1}	4-30 °C	NR	Hogan et al., 2003
Sodium caseinate		Outlet: 95 °C		Anisidine value Peroxide value		
Calcium caseinate Sodium caseinate Skim milk powder Lactose	1:2	Inlet: 177 °C Outlet: 75 °C	NR	4 °C	NR	Keogh et al., 2001

[a] FO: Fish oil.
[b] EA Encapsulating agent.

of fish oil microparticles obtained by spray-drying are those related to the determination of the fish oil content in the microparticles. Usually, parameters such as the yield (powder recovery), fish oil retention and fish oil encapsulation efficiency (EE) are used to evaluate the microencapsulation process. The calculation of these parameters is detailed in the following equations (a–c):

$$\text{Yield } (\%) = \frac{\text{weight of powder after spray} - \text{drying (g)}}{\text{weight of solids in the feed solution (g)}} \times 100 \quad \text{(a)}$$

$$\text{Retention } (\%) = \frac{\text{fish oil in the powder (mg/g)}}{\text{fish oil in the feed solution (mg/g)}} \times 100 \quad \text{(b)}$$

$$\text{EE}\,(5) = \frac{\text{fish oil in the powder (g)} - \text{surface fish oil in the powder (g)}}{\text{fish oil in the powder (g)}} \times 100 \quad \text{(c)}$$

The yield is the ratio of the solids in the feed solution before spray-drying and the solids obtained after spray-drying. Fish oil retention corresponds to the relation between the fish oil in the feed solution (before spray-drying) and the fish oil in the microparticles (after spray-drying). EE represents the fish oil within the microparticles and is usually indirectly evaluated by assessing the surface and total fish oil. The surface fish oil from the microparticles is usually determined using a solvent (such as hexane), in which the coating material is insoluble. To determine total fish oil in the microparticles, they are first destroyed and then the fish oil is extracted with hexane.

4. Production of fish oil microparticles by spray-drying

To obtain fish oil microparticles with desirable characteristics by spray-drying, it is necessary to optimize the process and formulation variables, although in most cases, the conditions are determined through assays and error studies (Gharsallaoui et al., 2007). The effects of the process and formulation variables on the properties of fish oil microparticles, such as fish oil content, surface fish oil, EE, fish oil recovery, powder yield, moisture content, water activity, hygroscopicity, colour, particle size, powder morphology, true density, bulk density (tapped and untapped), solubility and glass transition temperature (Tg) (Drusch, 2007; Aghbashlo et al., 2012a,c) have been studied. However, there are few studies where experimental designs have been performed to optimize the encapsulation of fish oil. Most have been undertaken to optimize

the emulsion preparation (Drusch, 2007; Keogh et al., 2001; Aghbashlo et al., 2012c), and only a few have optimized the formulation and operational conditions for fish oil microparticle production. Encapsulation of DHA ethyl ester, applying a 2^4 central composite experimental design, was performed by Rodrigues et al. (2011). The DHA ethyl ester (DHAEE) percentage, inlet air temperature, atomizer gas flow rate and pump flow rate were the independent variables, whereas DHAEE content in the core material and the DHAEE content in the microcapsules were the dependent variables. In this context, statistical methodologies allow handling multiple responses simultaneously to obtain the best balance among the response variables. The application of the desirability methodology converts multiple responses into a single response, facilitating the interpretation of the results.

4.1. Formulation variables in the microencapsulation of fish oil by spray-drying

The emulsion (oil content, pH, type and content of emulsifiers, technology used to decrease droplet size) and the encapsulating agent (nature, content, and fish oil/encapsulating agent ratio) have been studied as variables that affect the properties of fish oil microparticles, such as the encapsulation efficiency, peroxide value, particle size, morphology, density, moisture content and hygroscopicity, and bulk density.

4.1.1. Infeed fish oil emulsions

The microencapsulation of fish oil by spray-drying requires a previous elaboration of oil-in-water emulsions, where natural or modified food-grade biopolymers, such as milk proteins, plant gums, modified starch and modified cellulose, are used as emulsifiers and encapsulating agents (Aghbashlo et al., 2013a; Hogan et al., 2003; Kolanowski et al., 2006; Rodrigues et al., 2011; Serfert, Drusch, Schmidt-Hansberg, Kind, & Schwarz, 2009a). Emulsification plays a key role in the preparation of microencapsulated oils by spray-drying because the behaviour of the product during the spraying and drying steps depends on the emulsion properties (Ré, 1998). Oil emulsions must be stable over a certain period prior to and during spray-drying, oil droplets should be less than 2 μm, and the emulsion viscosity should be low to prevent ballooning of the particles during the drying process (Drusch, 2007). Emulsion stability is usually evaluated by physical (Brownian flocculation, creaming, sedimentation flocculation and disproportionation) and chemical methods (primary and secondary oxidation of fatty acids) (Day, Xua, Hoobin, Burgar, & Augustin, 2007; Nasrin & Anal, 2014).

The emulsion droplet size has a critical effect on the encapsulation efficiency of fish oil by spray-drying because the reduction of the emulsion droplet size results in a lower amount of unencapsulated oil at the surface of the powder particles. This unencapsulated oil is prone to oxidation and also deteriorates the wettability and dispersability of the microparticles when they are reconstituted (Millqvist-Fureby, Elofsson, & Bergenstahl, 2001; Vega, Kim, Chen, & Ross, 2005). Furthermore, particle ballooning during drying leads to air inclusion in the particle and oxidation reactions (Drusch & Schwarz, 2006; Keogh et al., 2001).

4.1.1.1. Types of emulsifiers. Milk proteins and gelatin are the most commonly used proteins for the microencapsulation of functional food ingredients, such as fish oil, by spray-drying (Gharsallaoui et al., 2007). The two main classes of milk proteins, whey proteins and caseins, are widely used in the food industry because of their excellent surface activity and their ability to facilitate the formation and stabilization of oil-in-water emulsions (Dickinson, 1997). During emulsion formation, protein molecules are rapidly adsorbed at the newly formed oil-water interface, and the resulting steric-stabilizing layer prevents oil droplet recoalescence and provides physical stability to the emulsion (Vega & Ross, 2006). Caseins have a disordered structure, with a high content of proline, which impairs the formation of secondary structures, providing stability against denaturation, and contributes to their high surface activity (Fox, 2001). These milk proteins adsorb to the oil-water interface as flexible chains, whereas whey proteins form close-packed, globular protein monolayers (Dickinson, 1992). One of the main drawbacks regarding the use of whey proteins as emulsifiers for microencapsulation by spray-drying is their susceptibility to heat denaturation, which may result in aggregation of oil droplets and a decrease of the stability of the emulsion (Sliwinski et al., 2003). Milk proteins, such as whey proteins, caseins and skim milk powder (SMP), have been used as both emulsifiers and encapsulating agents for the microencapsulation of fish oil by spray-drying (Aghbashlo et al., 2013a,b; Aghbashlo et al., 2012a,b,c; Augustin et al., 2011; Jafari et al., 2008; Hogan et al., 2003; Keogh et al., 2001). Aghbashlo et al. (2012a) studied the effect of the encapsulating agent composition and the presence of Tween 20 on the properties of infeed fish oil emulsions and microcapsules generated by spray-drying. These authors reported that 10% fish oil emulsions prepared with 20% SMP as emulsifier showed smaller droplet size (1.41 μm, d_{32}) and higher viscosity than emulsions where SMP was replaced by 30% carbohydrate, such as maltodextrin, lactose or sucrose, whereas no differences in droplet size were found based on the type of carbohydrate (1.54–1.57 μm, d_{32}). However, the substitution of 30% of SMP by maltodextrin, sucrose or lactose altered the resulting microcapsule size after spray-drying.

Sugar beet pectin is obtained from sugar beet as a by-product during the extraction of sugar. The higher emulsifying ability in comparison with citrus or apple pomace pectin is related to the higher content of protein (10.4%) and acetyl groups, which reduce calcium-bridging flocculation (Leroix, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Thibault, 1988). According to Drusch (2007), a pectin content between 1 and 2% is sufficient to prepare a stable feed emulsion with up to 50% oil, with a median oil droplet size less than 2 μm and a maximum viscosity of 179 mPa. Polavarapu et al. (2011) investigated the physicochemical properties and oxidative stability of fish oil and fish-olive oil microcapsules obtained by spray-drying, using sugar beet pectin and glucose syrup as the encapsulating agent. They suggested that 2% w/w sugar beet pectin was sufficient to prepare stable feed fish oil emulsions for spray-drying with up to 15% w/w oil, in agreement with other previous studies (Drusch, 2007; Drusch, Serfert, Scampicchio, Schmidt-Hansberg, & Schwarz, 2007; Leroix et al., 2003). These fish oil emulsions, formulated with 2% sugar beet pectin and 7.5 or 15% fish oil, showed a non-Gaussian, largely unimodal distributions, with average droplet size ranging from 0.41 to 0.43 μm.

In recent years, the suitability of *n*-octenylsuccinate-derivatized (*n*-OSA) starch as an emulsifier and/or encapsulating agent for the microencapsulation of fish oil has been reported (Drusch & Schwarz, 2006; Drusch et al., 2006; Serfert et al., 2009b; Serfert et al., 2009a). Stable feed emulsions have been prepared with both low viscosity (132 mPa) and medium viscosity (340 mPa) *n*-OSA starch at 30% and 45% total solids (15% fish oil, 12% *n*-OSA starch and 3% glucose syrup and 9% fish oil, 4.5% *n*-OSA starch and 31.5% glucose syrup, respectively). A low pH (pH 4.5) was critical for emulsion stability in the case of emulsions based on low viscosity *n*-OSA starch at a high fish oil load (15%) (Drusch & Schwarz, 2006). Similarly, Serfert et al. (2009a) used four types of *n*-OSA starch with different average molecular weights and glucose syrup in a 1:5 ratio. The feed emulsions were prepared at 45% total solids (12% fish oil, 5.5% *n*-OSA starch and 27.5% glucose syrup) for the

microencapsulation of fish oil. The oil droplet size (50^{th} percentile) was not significantly influenced by the type of *n*-OSA starch or the viscosity of feed emulsions. In contrast, the size of the microcapsules increased with increasing average molecular weight of *n*-OSA starch.

Modified celluloses have also been used as emulsifiers and encapsulating agents. Most of these studies use methylcellulose combined with lecithin as an additional emulsifier (Davidov-Pardo, Roccia, Salgado, León, & Pedroza-Islas, 2008; Kolanowski, Laufenberg, & Kunz, 2004; Kolanowski et al., 2006). Kolanowski et al. (2004) reported that methylcellulose gave stable emulsions for at least 3 h after the homogenization process, sufficient for spray-drying; however, a tendency to aggregate and an increase in average droplet size were observed. According to Kolanowski et al. (2004), both methylcellulose and hydroxypropyl methylcellulose showed acceptable emulsifying properties to produce a stable emulsion for spray-drying. In the case of methylcellulose, lower air entrapment resulting in foaming during emulsification and smaller emulsion droplet size were reported.

The incorporation of surfactants in the infeed fish oil emulsion is rare in the literature, possibly due to the immediate feeding of the emulsion to the dryer and the stringent regulations for food additives in many countries. Rodrigues et al. (2011) used Tween 80 as an emulsifier (from 1 g Tween 80/1.5 g DHAEE to 1 g Tween 80/3.5 g DHAEE, corresponding to a 10% total solid content of all design trials), together with gum arabic, in the optimization of DHAEE microencapsulation by experimental design. However, the effect of Tween 80 on the droplet size was not addressed. Aghbashlo et al. (2012a) incorporated Tween 20 into the fish oil emulsion formula (1 g Tween 20/100 g fish oil) to evaluate its effect on the emulsion characteristics and the resulting microparticles obtained by spray-drying. A decrease in the droplet size was reported in all the formulations when SMP was used as the encapsulating agent. The incorporation of Tween 20 decrease the surface tension of the emulsion due to the alignment of surfactant molecules at the interface, and provides a mechanical barrier against coalescence and Ostwald ripening (Chidambaram & Burgess, 2000).

4.1.1.2. Preparation of infeed fish oil emulsions. The infeed emulsion preparation includes the use of high energy, such as high shearing forces, microfluidization, ultrasonication and high-pressure homogenization (Von Staszewski, Pizones Ruiz-Henestrosa, & Pilosof, 2014; Ilyasoglu & Nehir El, 2014), to diminish the droplet size and to increase the emulsion stability. Usually, the infeed fish oil emulsion is obtained by rotor-stator systems, followed in most cases by homogenization of the emulsion in a high-pressure homogenizer. Two-stage high-pressure homogenizers are typically used for the homogenization of fish oil emulsions, with pressures in the range of 50–500 bar and a varying number of passes (1–5 passes) (Augustin et al., 2011; Drusch et al., 2006; Hogan et al., 2003; Polavarapu et al., 2011; Serfert et al., 2009a,b; Shaw et al., 2007). Thus, the emulsification technique plays a key role in optimizing the encapsulation efficiency of food flavours and oils by spray-drying (Liu, Furuta, Yoshii, & Linko, 2000; Liu et al., 2001). The emulsion droplet size has a noticeable effect on the encapsulation efficiency (Jafari et al., 2008; Risch & Reineccius, 1988; Soottitantawat et al., 2005; Soottitantawat, Yoshii, Furuta Ohkawara, & Linko, 2003). The lower the droplet size, the higher the encapsulation efficiency and the lower the content of unencapsulated core material at the surface of the powder particles because small oil droplets will be enclosed more efficiently within the encapsulating agent of the microcapsules. Furthermore, emulsions with small droplet size will be more stable during the spray-drying process, which is also critical to achieving the optimum encapsulation efficiency (Jafari et al., 2008). In this study, two surface-active biopolymers (Hi Cap or whey protein concentrate, WPC) were used as emulsifiers to prepare fish oil infeed emulsions using three different emulsifying devices (rotor stator, microfluidizer and ultrasound) to investigate the influence of the emulsification system on the properties of fish oil microparticles. The feed emulsions were prepared with 50% total solids (20% fish oil, 20% emulsifier, 60% maltodextrin). Fish oil emulsions with both Hi Cap or WPC obtained by microfluidization at 60 MPa for one cycle had the lowest unencapsulated oil at the surface of particles and the highest encapsulation efficiency (over 80%) compared with the fish oil emulsion obtained with a rotor-stator system or a 24 KHz ultrasound probe. This result was attributed to the nano size range of the oil droplets of fish oil emulsions obtained by microfluidization (d_{43} 210–280 nm). In this study, emulsification technique was also reported to affect the molecular structure of surface-active compounds and their emulsification ability because emulsions with the same size obtained by two different techniques gave different encapsulation efficiencies (Jafari et al., 2008). Drusch (2007) studied the dependency of the oil droplet size and emulsion viscosity on the homogenization pressure (two-step homogenization with pressures 200/50 bar to 500/100 bar) and number of passes (2–4), among other variables. According to this study, homogenization pressure had a significant influence on the median oil droplet size, whereas the number of passes had no significant effect. In another study, the fish oil coarse emulsion was homogenized at single-stage pressures ranging from 15 to 50 MPa and 1 to 5 passes. The minimum oil droplet size before and after drying, together with the minimum surface fat, were achieved at the highest homogenization conditions (50 MPa × 5 passes) (Keogh et al., 2001).

In addition to the traditional oil-in-water feed emulsions, some alternatives, such as multiple or multilayered emulsions have also been successfully developed for fish oil microencapsulation by spray-drying (Jiménez-Martín, Antequera Rojas, Gharsallaoui, Carrascal, & Pérez-Palacios, 2016; Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Carrascal, & Antequera Rojas, 2015). Multiple oil-in-water-in-oil emulsions ($O_1/W/O_2$) were prepared with a hydrophilic emulsifier that stabilized the O_1/W interface and a hydrophobic emulsifier that stabilized the O_2/W interface. The encapsulated fish oil is in the inner oil phase (O_1) in these emulsions. In the case of multilayered emulsions, the fish oil is surrounded by multiple layers of coating material stabilized by electrical charges.

4.1.2. Encapsulating agents

An ideal encapsulating material for microencapsulation of fish oil should have emulsifying and film-forming properties. Additionally, it should have low viscosity at high solid contents, low hygroscopicity and low cost. However, biopolymers with all these properties are scarce.

There is a wide variety of research on the encapsulation of fish oil, making difficult its classification. In the opinion of the authors, two alternatives could be identified to simplify the classification.

1) A biopolymer with emulsifying and encapsulating properties in the same structure (e.g., gum arabic, modified starches and sugar beet pectin).
2) A biopolymer (protein, gum or modified polysaccharide) or a natural (lecithin) or synthetic surfactant as emulsifying agent, together with a bulk agent (low-molecular weight carbohydrate) or a biopolymer with film properties.

A variety of biopolymers have been described for the encapsulation of fish oil by conventional spray-drying (Table 1). Proteins such as sodium caseinate, calcium caseinate (Keogh et al., 2001), milk proteins (Keogh et al., 2001), SMP (Aghbashlo et al., 2012c),

whey protein concentrate (WPC) (Jafari et al., 2008), whey protein isolate (WPI), milk protein concentrate (MPC) (Aghbashlo et al., 2012b), barley protein (Wang et al., 2011) and soybean protein isolate (SPI) have been reported.

Proteins have emulsifying, gel and film-formation properties and are usually combined with carbohydrates, such as maltodextrin, starches, glucose syrup (Drusch, 2007; Drusch et al., 2006; Shaw et al., 2007), lactose or sucrose (Aghbashlo et al., 2012a; Keogh et al., 2001), trehalose (Drusch et al., 2006), sodium carboxymethyl cellulose (Patrick et al., 2013), methyl cellulose (Davidov-Pardo et al., 2008), soybean soluble polysaccharide (SSPS) (Anwar & Kunz, 2011), chitosan (Shaw et al., 2007) and sugar beet pectin (Drusch, 2007; Polavarapu et al., 2011). Carbohydrates cannot be used alone due to their poor interfacial properties, but some (lactose, sucrose, glucose, trehalose) have been incorporated because they change the glass transition temperature and accelerate the formation of a dry crust around the drying droplets (Aghbashlo et al., 2012a). Other biopolymers that consist of carbohydrates and covalently bonded protein moieties, such as gum arabic (Rodrigues et al., 2011) and sugar beet pectin (Drusch et al., 2007), have been used as emulsifying and encapsulating agents for fish oil microencapsulation. Other efforts have been undertaken by chemically modifying the carbohydrates to give them emulsifying properties, such as Capsul (Wang, Che, Selomuyla, & Dong Chen, 2014), OSA-starch (Hi-Cap) and hydroxypropyl-betacyclodextrin (Anwar & Kunz, 2011). The selection of encapsulating agent is important to design microparticles with specific properties (protection and/or controlled release).

Fish oil microparticles are often designed using low molecular weight carbohydrates (e.g., lactose, sucrose, glucose, and trehalose) as bulk or filler agents. The saccharides are added to decrease the glass transition temperature (Tg) of the feed emulsion, accelerating the crust formation and affecting the properties of the fish oil microparticles. Aghbashlo et al. (2012a) studied the effect of the bulk agent type on the fish oil microparticle properties. Fish oil microparticles with SMP and with 30% of the SMP replaced with maltodextrin, lactose or sucrose were obtained at an inlet air temperature of 175 °C. The substitution of 30% SMP with lactose and sucrose resulted in higher encapsulation efficiency, higher moisture content and larger particle size; these results were associated with the acceleration of the crust formation. In contrast, Drusch et al. (2006) did not find any effect of the bulk agent type (glucose syrup or trehalose) on the physicochemical properties (particle size, oil droplet size, and true density) of fish oil microparticles with n-octenylsuccinate-derivatized starch.

Keogh et al. (2001) used lactose as a filler agent and different types of casein as emulsifier (casein + lactose (SMP), sodium caseinate + lactose and calcium caseinate + lactose) as emulsifying agents in the encapsulation of fish oil. The experimental conditions were an inlet air temperature of 177 °C and a fish oil/total solid ratio of 1:3. The authors demonstrated that the type of casein affected the vacuole volume, achieving lower values for SMP. Fish oil double emulsions $O_1/W/O_2$ with lactose as the filler agent and sodium caseinate as the encapsulating agent were used as infeed emulsions for spray-drying, achieving a high EE for both EPA and DHA (93.93% and 84.62%, respectively). In the same study, multilayered fish oil emulsions were formulated with lecithin and chitosan. In this case, maltodextrin was added to the resultant emulsions as a bulk agent for spray-drying, and the EE for EPA and DHA was lower (approximately 60%) than in the microparticles produced from double emulsions (Jiménez-Martín et al., 2016).

In some studies, the encapsulating agents were mixtures of different biopolymers. For example, Patrick et al. (2013) encapsulated fish oil using gum arabic and sodium carboxymethyl cellulose at an inlet air temperature of 180 °C, obtaining one shell and multishell microparticles with encapsulation efficiencies of 75.2% and 82.8%, respectively. Anwar and Kunz (2011) also showed the effect of different biopolymer mixtures on the encapsulation efficiency in fish oil microparticles based on soybean soluble polysaccharides (SSPS) with MD or OSA-starch or betacyclodextrin (HPBCD) at an inlet air temperature of 180 °C. The SSPS + MD (1:5), SSPS + MD (1:6.5) and SSPS + OSA-starch (HiCap100) (1:6.5) systems reached higher encapsulation efficiency values than the SSPS + MD + HPBCD (1:5:1.5) system. Moreover, the authors suggested that the stability could be related to the morphology of the microparticles.

Wang et al. (2011) reported that the nature of the encapsulating agent influenced the morphology and encapsulation efficiency of fish oil microparticles based on barley protein fractions (B). Thus, fish oil microparticles with glutelin (BG), hordein (BH) and mixtures with glutelin/hordein ratios of 1:2 (BGH-1), 1:1 (BGH-2) and 2:1 (BGH-3) were obtained. BH and BGH-1 microparticles (systems with greater BH content) showed a porous surface structure. Porous microparticles can be formed during spray-drying through a ballooning mechanism as well as BH denaturation and loss of viscoelasticity. In contrast, systems with BG (BG, BGH-2 and BGH-3) formed crack-free microparticles, with a dense external structure and smooth surface. Moreover, BH microparticles had slightly lower fish oil encapsulation.

The Maillard reaction products produced by heat treatment of aqueous protein-carbohydrate mixtures were suitable for fish oil microencapsulation, irrespectively of the protein and carbohydrate source used in the formulation (Augustin, Sanguansri, & Bode, 2006). All heated sodium caseinate-glucose-glucose syrup mixtures gave high encapsulation efficiency. Furthermore, a lower susceptibility of fish oil powder to oxidation was reported by increasing the severity of the heat treatment and therefore the extent of the Maillard reaction. Maillard reaction products (MRPs) have been proven to exhibit strong antioxidant activity, and they have been reported to inhibit lipid oxidation, both in model systems and in food products (Vhangani & Van Wyk, 2016; Miranda, Rakovski, & Were, 2012; Kim et al., 2013). Mechanisms involved in MRP antioxidant activity include radical chain-breaking activity (Miranda et al., 2012), scavenging of reactive oxygen species, decomposing hydroperoxides and metal chelation, thus retarding the formation of primary and secondary oxidation products (Kim et al., 2013). In this context, Aghbashlo et al. (2013b) studied the encapsulation of fish oil using four encapsulating agents: SMP, WPI, WPI with milk protein concentrate (20%) (MPC) and WPI with sodium caseinate (20%). SMP had the highest EE and the lowest peroxide value for fish oil, which was attributed to the content of lactose, which induced the Maillard reaction and the formation of a tough skin.

Generally, the range of fish oil/encapsulating agent ratio used for microencapsulation of fish oil has been reported from 1:1 to 1:3. The fish oil/encapsulating agent ratio affects the surface fish oil (Drusch, 2007) and the encapsulation efficiency (Aghbashlo et al., 2012c; Kolanowski et al., 2006). Surface fish oil (non-encapsulated oil) is highly susceptible to oxidative reactions with environmental oxygen as well as to developing unpleasant flavours. Therefore, the focus of multiple studies has been minimization of these negative effects.

Drusch (2007) studied the encapsulation of fish oil with sugar beet pectin (emulsifying agent) and glucose syrup (bulk agent) at an inlet air temperature of 170 °C. In the case of microparticles formulated with fish oil contents of 20% or 50% (both with 2.2% sugar beet pectin), lower surface fish oil (0.4%) was achieved with the lowest fish oil content. Thus, the proportion of surface fish oil limits the maximum oil load of the microparticles.

Aghbashlo et al. (2012c) studied the effect of the emulsion

variables (aqueous phase content 70%–90%, oil concentration with respect to total solids 10%–30%, and emulsification time 5–15 min) on the encapsulation efficiency and the exergy efficiency of fish oil microparticles based on SMP. A face-centred full central composite design was applied. The oil proportion had the greatest effect on the encapsulation efficiency (76.81–95.07%), whereas the aqueous phase content had the greatest effect on the exergy efficiency. In contrast, Kolanowski et al. (2006) reported that the level of fish oil (1% and 2.6% in feed solution) did not show significant differences in the encapsulation efficiency (86.5% and 84.8%, respectively) in fish oil microparticles powders based on methylcellulose and maltodextrin.

4.2. Process variables studied in the microencapsulation of fish oil by spray-drying

Operating variables such as temperatures (feed temperature, inlet, outlet and ratio of inlet/outlet air temperatures), drying air mass flow rate (aspirator rate), air mass flow rate, feed mass flow rate (peristaltic pump rate) and type of atomization affect the product quality. Temperature is an important variable in the spray-drying of heat-sensitive materials such as fish oil because increasing the inlet air temperature increases the extent of oxidative reactions. However, in most studies there is no information to support the choice of the inlet air temperature. Furthermore, most lab-scale spray-dryers do not provide outlet temperature regulation, which may also affect the quality of the microcapsules. In this sense, low outlet temperatures give microparticles with higher moisture content, and the water present in the powder acts as a plasticizer, leading to a rubbery state, wherein physicochemical changes, such as caking, collapse, agglomeration, browning and oxidation, could occur. In contrast, when the outlet temperature is too high, the oxidative stability of the microencapsulated fish oil may be affected.

For example, Aghbashlo et al. (2013a) studied the influence of different drying conditions on fish oil microparticle characteristics. The microparticles were prepared with SMP (20%) at a fish/oil ratio of 1:2 under different operational conditions (air temperature, drying air mass flow rate, air mass flow rate and feed mass flow rate). The encapsulation efficiency increased when the drying temperature increased. Additionally, the particle size increased when the drying temperature, peristaltic rate and aspirator rate increased. Increases in both EE and particle size were explained by the faster crust formation. As expected, the moisture content decreased when the drying temperature and aspirator rate increased.

Wang et al. (2011) reported that the inlet air temperature is an important factor that influences the morphology of fish oil microparticles obtained with a glutelin/hordein ratio of 1:1 (BGH-2) and inlet air temperatures of 180 °C, 150 °C and 120 °C. The microparticles obtained at the lowest inlet temperature were prone to agglomerate due to the higher moisture content, which led to inter-particle bridge formation, causing caking and particle collapse.

The morphology of microparticles was analysed by scanning electron microscopy (SEM) and showed indented surfaces attributed to shrinkage of the particles during the drying process, which can occur at low or high inlet air temperatures. At low inlet air temperatures, there is less water diffusion, and the particles have more time to shrink. At high inlet air temperatures, rapid evaporation and high pressure inside the particles also produce shrinkage (Alamilla-Beltrán, Chanona-Pérez, Jiménez-Aparicio, & Gutiérrez-López, 2005). SEM microphotography of the fish oil powders has also been reported to be dependent on the type of encapsulating agent (Aghbashlo et al., 2012c; Davidov-Pardo et al., 2008; Drusch et al., 2006; Jafari et al., 2008).

5. Stability of microparticles during storage

The main focus of the microencapsulation of fish oil by conventional spray-drying has been the protection of fish oil from environmental conditions to prevent lipid oxidation and to extend its shelf-life. Although most of the studies have reported an improvement in fish oil stability when it is microencapsulated, there are some works where a lower fish oil stability from microparticles has been described when compared to bulk oil (Kolanowski et al., 2006). Studies addressing fish oil stability from microparticles can be found in the literature. However, conclusive results are difficult to obtain due to the different experimental storage conditions (light, temperature, oxygen, relative humidity, packaging systems), microparticle components (emulsifying type, fish oil/encapsulating agent ratio, type of encapsulating matrix) and the method used to evaluate lipid oxidation.

The peroxide value (PV) is a method used to determine the primary oxidation compounds, but it is only applicable when peroxide formation is more important than its decomposition (Wang et al., 2011). Secondary oxidation compounds (degradation of peroxide into secondary oxidation products, such as alcohols, ketones, aldehydes and volatile compounds) have been measured using the anisidine value (Patrick et al., 2013), thiobarbituric acid reactive compounds (Baik et al., 2004; Jiménez-Martín et al., 2015; Shaw et al., 2007), propanal content (Anwar & Kunz, 2011; Polavarapu et al., 2011; Shaw et al., 2007; Drusch et al., 2006) and sensory evaluation (Keogh et al., 2001). In studies focused on fish oil microencapsulation using other technologies, quantitative and concomitant determination of polar compounds has been applied to evaluate the oxidation of microencapsulated fish oils (Márquez-Ruiz et al., 2000). Determination of polymers was found to be very useful because of the rapid polymerization of highly polyunsaturated oils at room temperature (Velasco, Dobarganes, & Márquez-Ruiz, 2000). The use of these analytical approaches has enabled the ascertainment of the unequivocal discontinuous oxidation occurring in microencapsulated oils (Morales, Marmesat, Ruiz-Méndez, Márquez-Ruiz, & Velasco, 2015; Márquez-Ruiz, Velasco, & Dobarganes, 2003; Velasco et al., 2006).

A variety of works have studied the effect of the encapsulating system on the fish oil stability for microparticles at different storage conditions. Fish oil microparticles with different casein types (micellar casein as SMP, sodium caseinate + lactose and calcium caseinate + lactose) and with different packaging systems (vacuum + N_2 and vacuum) were stored at 4 °C. The fish oil stability of the microparticles was evaluated during storage in reconstituted powder following off-flavour development (sensory evaluation). Microparticles with dairy ingredients alone (SMP) as encapsulating agent showed low levels of off-flavours (Keogh et al., 2001). Anwar and Kunz (2011) studied fish oil microparticles with SSPS + MD (1:5), SSPS + MD (1:6.5) and SSPS + OSA-starch (HiCap100) (1:6.5) and SSPS + MD + HPBCD (1:5:1.5) stored at 21 °C and 30% RH for 8 weeks. The lowest fish oil protection was found for the SSPS + MD + HPBCD system, according to the PV and propanal determinations, due to non-inclusion complexes between HPBCD and fish oil. In another study, Drusch et al. (2006) evaluated the effect of relative humidity and type of bulk agent on the oxidative stability of encapsulated fish oil. Microparticles of fish oil with EmCap-Instant™ (a cold-water-dispersible modified starch) and either glucose syrup or trehalose stored at 0% and 54% relative humidity showed that the system with trehalose at the lowest humidity retarded fish oil oxidation, which was attributed to the binding properties of trehalose to dienes.

Kolanowski et al. (2006) studied the fish oil stability of

microparticles with methylcellulose and fish oil:total solids ratios of 1:3 and 1:2 during storage under different conditions: storage temperature (room and 5 °C) and packaging type (air or vacuum). The authors found that the encapsulation of fish oil did not significantly improve its oxidative stability with respect to bulk fish oil, as evaluated by peroxide value measurements, despite high encapsulation efficiency (over 80%). This behaviour was attributed to the higher surface area of the microparticles than bulk fish oil. In contrast, Wang et al. (2011) evaluated the peroxide value evolution for microencapsulated fish oil with barley proteins (BG, BGH-1, BGH-2 and BGH-3) under accelerated storage (40 °C). Encapsulation of fish oil for all systems showed a high protection with respect to bulk fish oil. As a consequence of surface fish oil oxidation, PV increased in all the microparticle systems until 3–4 days of storage and then decreased. The encapsulated fish oil (inside the microparticle) did not undergo oxidation because a further increase of PV was not detected during 8 weeks. However, the determination of PV should be carried out together with the determination of secondary oxidation products to evaluate the oxidative stability of the fish oil microparticles.

The type of infeed emulsion may affect the oxidative stability of fish oil from microparticles. Thus, fish oil microparticles obtained by spray-drying from multilayered fish oil emulsions showed lower TBARs values than those obtained from double emulsions when stored at 4 °C and 20 °C for one month (Jiménez-Martín et al., 2015).

In some studies, antioxidants from different sources have been added to the formulations of the microparticles due to the low fish oil oxidative stability (Keogh et al., 2001; Serfert et al., 2009b; Hogan et al., 2003; Kolanowski et al., 2006). Serfert et al. (2009b) studied the effect of a combination of antioxidants (α-tocopherol, δ-tocopherol, ascorbyl palmitate, lecithin, rosemary extract and citric acid esters from monoglycerides) on the oxidative stability of fish oil emulsions and microencapsulated fish oil (*n*-OSA:glucose syrup 1:5 and fish oil:encapsulating agent 1:1.5), measured by the PV and propanal content. The optimal antioxidant combination was dependent on the process stage of microencapsulation (dispersed oil in aqueous phase or dispersed oil in dry matrix). Keogh et al. (2001) used a mixture of ascorbic acid, lecithin and α-tocopherol as antioxidant, but they did not study the effect on fish oil oxidative stability. In another study, Hogan et al. (2003) reported that the addition of α-tocopherol to fish oil effectively delayed the onset of fish oil oxidation measured by PV during the storage of fish oil microparticles based on carbohydrates of different DE (fish oil:-encapsulating agent ranging from 1:1 to 1:3). Similarly, Kolanowski et al. (2006) studied the effect of α-tocopherol and lycopene on the oxidative stability of bulk fish oil and microencapsulated fish oil (methylcellulose:maltodextrin 2:1; fish oil:encapsulating agent 1:3 and 1:1.5), measured by PV, and found that these antioxidants had higher antioxidant activity in bulk fish oil.

6. Incorporation of fish oil microparticles into foods

Few studies have been performed on the incorporation of spray-dried fish oil microparticles into foods. This may be attributed to the fact that, in general, water-soluble biopolymers are used as encapsulating agents. Consequently, these biopolymers are dissolved when the microparticles are added to a liquid food, releasing the fish oil emulsion. Therefore, the fish oil is exposed to oxidative reactions and could negatively affect the sensory properties of the food.

Fish oil microparticles based on gum arabic and sodium carboxymethyl cellulose were incorporated into a probiotic fermented milk. The evaluation of the fatty acid profile in the final product showed a non-significant decrease in the EPA and DHA content during 28 day storage at 4 °C, indicating that these fish oil microparticles could be used as an ingredient in the development of functional foods (Patrick et al., 2013).

In another study, fish oil microparticles with methyl cellulose, calcium-gelatin casein and whey protein concentrate were used to enrich bread products at a concentration of 600 mg of fish oil for every 100 g of dough. The main conclusion was that the addition of methylcellulose fish oil microcapsules to bread products did not significantly modify the sensory and technological characteristics and led to an increase in nutritional value (Davidov-Pardo et al., 2008). Yep, Li, Mann, Bode, and Sinclair (2002) also enriched bread with a low level of tuna oil microcapsules and studied the nutritional influence of its consumption. A low dose of ω3 PUFA, consumed as bread enriched with microencapsulated tuna oil, was bioavailable and increased the ω3 PUFA levels in the plasma of human subjects. Unfortunately, these authors did not mention the methodology used to obtain the fish oil microcapsules.

LCω3-PUFA, including DHA and arachidonic acid (ARA), have been added to infant formula in an attempt to imitate the composition of mother's milk. Powdered infant formula is manufactured by two general types of processes, dry-blending and wet-mixing/spray-drying (Zink, 2003). Nagachinta and Akoh (2013) obtained structured lipids enriched with ARA and DHA by enzymatic esterification, which were microencapsulated in infant formulas using these two manufacturing methods. Two combinations of spray-drying inlet-outlet temperature were used (120 °C-70 °C and 180 °C-80 °C) when the infant formulas were prepared by wet-mixing and spray-drying, resulting in lower PV and TBARs values with the lowest inlet temperature (120 °C). Moreover, the dry-blending process yielded a powdered infant formula with the lowest PV and TBARs values, similar to those of commercial infant formula.

Table 2 shows several characteristics (company/product, technology/product description, matrix components omega-3 payload, kind of food able to be fortified with the powder) of various commercial fish oil microencapsulated powders produced around the world. According to the manufacturer's guidelines, the fish oil microparticles by spray-drying available in the market are generally intended for solid food categories such as bars, infant powder formulas, and baked products (muffins and bread among other). The components of these fish oil microparticles include gelatin, caseinate, corn starch, modified starch, whey protein, soy protein or gum arabic, and with or without starch granules, gelatin, sucrose, glucose syrup or corn syrup as coating agents. Other fish oil microparticles obtained by different encapsulation technologies are shown in Table 2, intended more specifically for liquid foods fortification, such as juice and salad dressing.

In recent years, a wide range of foods have been fortified with fish oil microparticles and launched onto the market, particularly in Europe, USA, Canada, Australia or New Zealand. Table 3 shows a representative sample of commercial food products with fish oil microparticles, their EPA and DHA content and the countries where they are sold. It is interesting to underline that it is not easy to relate the type of microencapsulated omega-3 powder with the final food product because the food product company rarely provides this information.

In terms of the dose of omega-3 (EPA + DHA) to be administered in foods products, each country/organization provide different guidelines to different populations (general population, at risk of cardiovascular disease and pregnant and lactating females, among others) being difficult to establish a common rule. For example, the National Health and Medical Research Council (Australian Nutrient Reference Values), the U.K. Department of Health, and the European Academy of Nutritional Science recommended a daily dose of EPA + DHA in the range of 190–200 mg, while the International Society for the Study of Fatty Acids and Lipids (ISSFAL)

Table 2
Commercial microencapsulated fish oils to fortify foods.

Company/*Product*	Technology/Product description	Matrix components	Payload (mg of omega-3/g powder)	Food able to be fortified	Source of information
Arjuna/*Omega-3 Powder ZP* (India)	Spray-drying	–	86 (minimum)	Capsules, tablets, infant food and in various food supplements	http://www.arjunanatural.com/products/omega-3/omega-3-powder/
BASF/*Dry n-3 18:12 and 5:25* (Germany)	Spray-drying	Gelatin (18:12) or Caseinate (5:25) coated with starch granules	88	**Dry n-3 18:12:** fortification of most food products. **Dry n-3 5:25:** fortification of basic food products, dairy-based powder products, baby foods and maternal nutrition products	http://www.basf.cl/sac/web/chile/es/function/conversions:/publish/content/chile/nutrition_health/documentos/nutricion_humana/acidos_grasos_poliinsaturados/fichas_tecnicas/dry_n3_18_12.pdf http://www.basf.cl/sac/web/chile/es/function/conversions:/publish/content/chile/nutrition_health/documentos/nutricion_humana/acidos_grasos_poliinsaturados/fichas_tecnicas/dry_n3_5_25.pdf
DSM/*Ropufa "10" n-3 INF and food* (Netherlands)	Spray-drying	Cornstarch-coated matrix of gelatin and sucrose	90	Infant formulas	http://www.dsm.com/corporate/media/informationcenter-news/2010/03/2010-03-12-dha-powder-infant-nutrition.html
The Wright Group/*Supercoat Omega-3* (USA)	Spray-drying	Modified starch and corn syrup, whey protein and gum arabic	100–150	Protein bar, muffin	http://www.thewrightgroup.net/resources/innovative-product-concepts/
National Starch-Omega Protein/*Novomega*	Spray-drying	Modified starch and soy protein	100	Baked and other goods	http://www.naturalproductsinsider.com/articles/2005/01/national-starch-food-innovation.aspx
Kievit/*Vana-Sana* (Netherlands)	Spray-dried by spraying on a moving belt	Caseinate, soy proteins, glucose syrup	190	Infant formulas as well as performance and life style products	https://www.kievit.com/en/markets-ingredients/ingredient/vana-sana-high-fat-nutritional-base-powders/
Nu-Mega/*Driphorm HiDHA Infant, Bake and 50* (Australia)	Spray-dried powder with Maillard products	**Infant:** Whey protein concentrate and dry glucose **Bake:** Strach, sodium caseinate and maltodextrin **50:** sodium caseinate and dry glucose	69–143	**Infant:** milk formulas (option for dairy white milk), other infant foods **Bake:** baking (bread, muffins, etc.) and other high temperature applications (ex. fish fillets) **50:** wide diversity of applications	https://www.google.cl/webhp?sourceid=chrome-instant&ion=1&espv=2&ie=UTF-8#q=driphorm%20bake
Wacker/*Omega Dry* (Germany)	Complexation in water followed by vacuum drying	γ-cyclodextrin	60	Cookies and bread	https://njaes.rutgers.edu/seafoodsafety/docs/karwe.ppt
Firmenich/*Duralife* (Suisse)	Melt injection	Matrix based on maltodextrin and sugar	–	Dry and dehydrated beverage applications (sweet and savoury, hot and cold), instant hot cereals, compressed tablets, fat fillings and powder mixes for cakes and deserts, baby food, soups and ready meals	http://www.nutraingredients.com/Suppliers2/Firmenich-makes-functional-debut-with-omega-3-technology
DSM/*MEG-3* (Netherlands)	Dried complex coacervates	Gelatin and polyphosphate	180	Breads, milk, nutrition bars, margarine, juice, chocolate, yogurt, and salad dressing	http://meg-3.com/about-meg-3/ingredient-info/
Denomega/*GAT 100 and pure arctic premium powder* (Norway)	Microspheres prepared via duplex emulsions and phase inversion	Calcium alginate	–	Bakery goods, energy bars, sport nutrition products, among others	http://www.denomega.com/About-us
KITII Corporation/*Calshell* (Japan)	Calcium carbonate precipitation	Calcium salt and surfactant	≤100	–	https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2005090534&recNum=1&maxRec=&office=&prevFilter=&sortOption=&queryString=&tab=PCTDescription
Morishita Jintan/*JINTAN* (Japan)	Submerged co-extrusion	Polisaccharides and glicerol	–	–	https://books.google.cl/books?id=wi3GFjTn5gcC&pg=PA171&lpg=PA171&dq=JINTAN+CAPSULES+FISH+OIL&source=bl&ots=JaYVH0s33G&sig=kX2IGRs9xTnrEerYqpoz81jTzco&hl=es-419&sa=X&ved=0ahUKEwiyh5eS2ITOAhUJIZAKHYLUCusQ6AEIKjAC#v=onepage&q=JINTAN%20CAPSULES%20FISH%20OIL&f=false
Stepans Lipid Nutrition/ *Marinol omega-3 D-40 powder and emulsion* (Canada)	Soft capsules, oil, powder, emulsion	Carbohydrate, proteins	195 (powder)	D-40 powder: infant products, medical foods, gummies and chews, food; emulsion: spoonable liquids, food	http://www.stepan.com/uploadedFiles/Literature_and_Downloads/General_Lit/Food,_Nutrition_and_Pharmaceutical/MarinolOmega3Triglycerides.pdf

Table 3
Commercial food products fortified with fish oil microparticles.

Food Category	Manufacturer	Product name and/or type	Country	Omega-3 content/ serving	Source of information
Baked goods	Wegmans	Whole wheat bread	USA	40 mg EPA + DHA/slice	http://www.wegmans.com/webapp/wcs/stores/servlet/ProductDisplay?langId=-1&storeId=10052&catalogId=10002&productId=363205
	Arnold Sales Company	Smart and Health Omega 3	USA	33 mg EPA/36 g	Whelan & Rust, 2006
	George Weston Foods	Tip Top Up	Australia, New Zealand	27 mg EPA + DHA/slice	Jin et al., 2008
	Warburton	Women's Bread	Europe	–	Jin et al., 2008
Nutritional bars	Zone Perfect Nutrition	ZonePerfect bars	USA	3 mg EPA + DHA/50 g	Jin et al., 2008
	Dr. Barry Sears	OmegaZone Nutrition bars	USA	96 mg EPA + DHA/55 g	Jin et al., 2008
	Light Heart LLC.	Chocolate orange bar, chocolate raspberry bar	USA	160 mg EPA + DHA/56 g	http://www.lightheartllc.com/id4.html
Milk and milk-based products	Organic Valley	Whole Omega-3 milk	USA	–	https://www.organicvalley.coop/products/milk/omega-3-milk/omega-3-whole-milk-ultra-pasteurized-half-gallon/
	Organic Valley	Omega-3 Reduced Fat Milk	USA	–	https://www.organicvalley.coop/products/milk/omega-3-milk/omega-3-reduced-fat-2-milk-ultra-pasteurized-half-gallon/
	Sadafco	Yogurt	Saudi Arabia	–	Jin et al., 2008
	President's Choice	Blue Menu Finess 0% MG yogurt	Canada	25 mg EPA + DHA/100 g	http://www.presidentschoice.ca/en_CA/products/productlisting/pc_blue_menu_finesse_0_m.f._yogurt_prod2040153.html
	President's Choice	Blue Menu Smoothie Frozen Dairy Dessert Bars	Canada	40 mg EPA + DHA/80 mL	http://www.presidentschoice.ca/en_CA/products/productlisting/pc_blue_menu_swirl_smoothie_orangeprod1520031.html
	OSM Grodzisk Mazowiecki	Mozzarella Cheese	Europe	–	http://www.osmgm.pl/#/pl/produkty/mozzarella/mozzarella_omega3_250g
	Conaprole	Ultra milk Omega-3	Uruguay	103 mg EPA + DHA/200 mL	http://www.conaprole.com.uy/leche/leche-ultra/omega-3-1005
Salad dressing	Ken's	Ken's Light Options	USA	2 g PUFA/30 g	http://www.kensfoods.com/proddetail.php?id=70
Nonmilk beverages	Natural Vitality	Kids Natural Calm Multi	USA	150 mg EPA + DHA/30 mL	http://naturalvitality.com/kids-natural-calm-multi/
	Inidan River Select, LLC.	Ultraslimming Grapefruit Juice	USA	32 mg EPA + DHA/240 mL	http://indianriverselect.com/our-juices/ultra-slimming-grapefruit-juice/
	Lassonde	Oasis Health break Omega 3 Juice	Canada	–	http://www.oasis.ca/en/products/oasis-pause-sante/#strawberry-kiwi-omega-3
	Lassonde	Oasis Orange Omega-3 Valencia Gold	Canada	–	http://www.oasis.ca/en/products/oasis-premium/#orange-omega-3-valencia-gold
	Tropicana	Pure Premium Healthy Heart Orange Juice	USA	50 mg EPA + DHA/240 mL	http://www.tropicana.com/products/pure-premium/healthy-heart/
	President's Choice	Blue Menu Oh Mega J Orange Juice	Canada	50 mg EPA + DHA/250 mL	http://www.presidentschoice.ca/en_CA/products/productlisting/pc_blue_menu_oh_mega_j_orange_juiceprod2040157.html
Processed meats	President's Choice	Blue Menu Chili Style Beans & Beef	Canada	40 mg EPA + DHA/415 g	http://www.presidentschoice.ca/en_CA/products/productlisting/pc_blue_menu_chili_style_beans_beefprod490186.html
	Hans Continental Smallgoods	Sliced Chicken	Australia, New Zealand	60 mg EPA + DHA/66.7 g	Jin et al., 2008
	Hans Continental Smallgoods	Strassburg	Australia, New Zealand	60 mg EPA + DHA/66.7 g	Jin et al., 2008
Sauces	President's Choice	Blue Menu Marinara Pasta Sauce	Canada	50 mg EPA + DHA/125 mL	http://www.presidentschoice.ca/en_CA/products/productlisting/pc_blue_menu_marinara_with_omega_3prod2040145.html
Spread	The J.M. Smucker Company	Jif Omega-3 Peanut Butter	USA	32 mg EPA + DHA/33 g	http://www.jif.com/products/creamy-omega-3-peanut-butter
	Meadow Lea-Hi	Omega Margarine	Australia, New Zealand	250 mg n-3/15 g	Jin et al., 2008
	President's Choice	Blue Menu Smooth Peanut Butter	Canada	30 mg EPA + DHA/15 g	http://www.presidentschoice.ca/en_CA/products/productlisting/pc_blue_menu_smooth_peanut_butterprod1520049.html
Infant Formula	Nutricia	Karicare	Australia, New Zealand	–	Jin et al., 2008
Candy	Oxford Nutrascience	Ellactiva, Cranberry chews	Europe	150 mg LCω3-PUFA/gummy	http://www.foodmanufacture.co.uk/NPD/Omega-3-chews-on-horizon
	Life Science Nutritionals	Adult Essential Gummies	Canada	25 mg EPA + DHA/gummy	http://adultessentials.com/en/product/omega-3
	Life Science Nutritionals	Ironkids Gummies	Canada	25 mg EPA + DHA/gummy	http://www.iron-kids.com/en/product/omega-3

recommended 650 mg. For people at risk of cardiovascular disease (CVD) the range of 500–1000 mg is recommended from the British Nutrition Foundation Task Force and from the American Heart Association (AHA). For pregnant and lactating females the National Institutes of Health (NIH, U.S.) recommended 300 mg, while to reduce triglyceride levels de AHA recommended the consume of oily fish twice a week (Garg, Wood, Singh & Moughan, 2006). According to the recommendations for EPA + DHA intake made by international organizations, none of these food products cover the recommended amount with a single serving.

7. Other fish oil encapsulation methods

Considering the potential applications of fish oil encapsulation, several alternative methods to conventional spray-drying have been reported (Table 4).

For example, Cho et al. (2003) encapsulated fish oil by double emulsification ($O_1/W/O_2$) followed by an enzymatic (induced by transglutaminase) and/or heat gelation method. Soy protein isolate was selected among the different wall materials tested (soluble wheat protein, whey protein isolate and sodium caseinate) because of its high emulsion activity and reactivity with transglutaminase. The microcapsules prepared by enzymatic gelation had high stability against oxygen and low water solubility, leading to a controlled release of fish oil in simulated gastric fluid. In a similar study, multiple emulsion technology followed by heat gelation was used to prepare fish oil microcapsules with succinic acid deamidated wheat gluten (Liao, Luo, Zhao, & Wang, 2012). Deamidated wheat gluten increased the oxidation stability of fish oil in the microcapsules and protected the payload from the gastric medium when the microcapsules were subjected to simulated gastric fluid.

Costa de Conto et al. (2013) studied the concentration effect of transglutaminase and wall materials (isolate soy protein/gum arabic), as well as the wall material:core ratio on the encapsulation of omega-3 ethyl ester by emulsification followed by complex coacervation. The only dependent variable capable of obtaining a mathematical model was the encapsulation process yield. Gan, Cheng, and Easa (2008) conducted a similar study, where fish oil microcapsules were prepared with soy protein isolate and ribose or sucrose, using a modified coacervation method followed by cross-linking treatments induced by transglutaminase. Fish oil microparticles containing ribose (260–280 μm) had a longer shelf-life than the other formulations. The authors attributed this effect to antioxidant Maillard cross-linking products, as well as the low gas permeability through the capsules.

Encapsulation of fish oil has also been performed by freeze-drying (Ilyasoglu & Nehir El, 2013; Chung et al., 2011; Klaypradit & Huang, 2008). In these studies, a fish oil emulsion was first prepared, using sodium caseinate or whey protein isolate as the emulsifying agent, and different emulsification techniques were tested. In addition, different coating materials, such as resistant starch, gum arabic, chitosan and maltodextrins, were evaluated. Chung et al. (2011) subjected the resultant freeze-dried fish oil microparticles to *in vitro* digestibility in a simulated gastrointestinal tract to understand the *in vitro* digestion mechanisms. In the study of Ilyasoglu and Nehir El (2014), the resultant fish oil nanoparticles were added to a fruit juice that was subjected to *in vitro* digestion to determine the bioaccessibility of EPA and DHA.

Choi et al. (2010) compared two formulations for fish oil encapsulation, one containing β-cyclodextrin (self-assembling aggregation) and the other containing polycaprolactone (emulsion-diffusion method). The authors studied fish oil release in de-ionized water, NaCl solutions and fish sauce and found that polycaprolactone microparticles more efficiently retarded the fish oil release in all continuous phase types.

Fish oil has also been encapsulated into nanofibres produced by electrospinning of zein-aqueous ethanol solutions (Moomand & Lim, 2014). At 30% fish oil loading, both ethanol-based and isopropanol-based zein fibres showed high fish oil EE values (91% and 96%, respectively). Furthermore, electrospun zein fibres provided a greater oxidative stability compared to non-encapsulated fish oil. In another study, fish oil was encapsulated in poly(vinyl alcohol) (PVA) nanofibres by emulsion electrospinning using whey protein isolate or fish protein hydrolysate as emulsifiers (García-Moreno et al., 2016). High fish oil EE was reported for fibres produced from 10.5% PVA-5% fish oil emulsion blend stabilized with WPI. However, the electrospun fibres showed poor oxidative stability in this case, with higher contents of hydroperoxides and

Table 4
Other fish oil encapsulation methods.

Techniques	Encapsulating agent	References
Freeze-drying	Sodium caseinate Arabic gum	Ilyasoglu & Nehir El, 2014
Emulsification Complex coacervation	Soy protein isolate Arabic gum	Costa de Conto et al., 2013
Double emulsification Heat gelation	Succinic acid deamidated wheat gluten	Liao et al., 2012
Freeze-drying	Sodium caseinate High amylose resistant starch	Chung et al., 2011
Self-assembling aggregation Emulsion-diffusion method	β-cyclodextrin Polycaprolactone	Choi et al., 2010
Coacervation Cross-linking by transglutaminase	Soy protein isolate Sucrose Ribose Sucrose	Gan et al., 2008
Freeze drying	Chitosan Maltodextrin Whey protein isolate	Klaypradit & Huang, 2008
Double emulsification Enzymatic/heat gelation	Wheat protein Whey protein isolate Isolated soy protein Sodium caseinate	Cho et al., 2003
Freeze-drying	Sodium caseinate Lactose	Heinzelmann et al., 2000
Freeze-drying	Sodium caseinate Lactose	Márquez-Ruiz et al., 2000

secondary oxidation products than a non-encapsulated fish oil emulsion.

8. Water-free spray-drying

Water is the most common solvent used in conventional spray-drying, limiting the type of encapsulating agents to those that are water soluble or at least water dispersible. Another drawback of this strategy is that a previously prepared infeed fish oil emulsion is required. This adds an additional stage to the procedure and requires the application of high energy (e.g., high shearing forces, microfluidization, ultrasonication and high-pressure homogenization) to decrease the droplet size. This step may also lead to the oxidation of fish oil (Serfert et al., 2009b), as well as increased cost of the formulation and additional critic points to consider in the microencapsulation process.

Water-free spray-drying is an advanced encapsulation technology, where hydrophobic molecules are dissolved in an organic solvent and dried in a closed loop mode at low temperatures using nitrogen as the drying medium (Duan, Vogt, Li, Hayes, & Mansour, 2013; Serfert et al., 2009a). Thus, fish oil oxidation during the encapsulation process should be avoided compared to the conventional method (higher temperatures and air as the drying medium). In this sense, Serfert et al. (2009a) prepared fish oil microparticles from fish oil-in-water emulsions by spray-drying using nitrogen. The authors reported high oxidative stability during storage compared to spray-dried emulsions using air. The physicochemical characteristics of spray-dried powder are significantly dependent on the selection of the solvent, the viscosity of the feed solution, the concentration of the solids in the feed solution, the feed rate and, to some extent, the surface tension of the solution (Patel, Patel, Chakraborty, & Shukla, 2015). For example, Patel et al. (2015) reported that lactose spray-dried with ethanol was a 100% crystalline powder, whereas lactose spray-dried with water was 100% amorphous. Thus, the solvent used in the spray-drying process affects the solid-state properties of the microparticles, their stability and their release behaviour. However, the effect of the solvent has not been studied in the encapsulation of bioactive compounds in food, opening an important research field.

Under normal operating conditions of the water-free spray-drying equipment, the solvents are condensed and could be reutilized, minimizing the concomitant environmental impact. The solvents commonly used in water-free spray-drying by the pharmaceutical industry include acetone, chloroform, methanol, methylene chloride, ethanol, dimethyl formamide (DMF), dimethyl sulfoxide (DMSO) and ethyl acetate (Patel et al., 2015). At the laboratory spray-drier scale, these organic solvents are recovered by a closed-loop cooling circuit, minimizing environmental pollution. The important criteria for the selection of the solvent in the water-free spray-drying process include the boiling point, solubility of the active compound and polymer and the dielectric constant. Additionally, the addition of a co-solvent can increase the solubility of hydrophobic molecules by reducing the dielectric constant of the mixture. Moreover, the toxicity of the solvents is an important criterion for the selection of the solvent. Class III solvents, those with less toxicity, include acetone, ethanol, DMSO and ethyl acetate (Patel et al., 2015). Among these solvents, ethanol is permitted in the food industry.

9. Conclusions and future trends

Spray-drying is widely used in industry to encapsulate a variety of molecules and has been fully scaled up by several ingredient supplier companies. Currently, spray-drying is the most commonly used method for the microencapsulation of fish oil, but the method required an o/w emulsion as infeed. Water-free spray-drying (using organic solvent) is a promising technology to encapsulate hydrophobic molecules, such as fish oil, that would avoid the need for emulsion preparation. Additionally, inert conditions (nitrogen as drying medium) minimize lipid oxidation during spray-drying. This technique has not yet been used in the food industry. However, the outcomes would the use of an alternative technique (water-free spray-drying) for the encapsulation of fish oil, extending the applicability beyond water-soluble polymers. In the case of water-free spray-drying, interactions between fish oil and the encapsulating agent may occur through hydrophobic interactions and van der Waals forces, whereas in conventional spray-drying, interactions may occur between the encapsulating agent and the emulsifier placed at the oil-water interface. This difference may affect the encapsulating efficiency, stability and release properties of fish oil.

The controlled release of fish oil from microparticles in food and in the gastrointestinal tract requires further investigation to understand the release mechanism in both systems. The release profile of fish oil would determine the applicability of fish oil microparticles in food systems, as well as the bioaccessibility and bioavailability of fish oil.

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