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Effect of spray-drying with organic solvents on the encapsulation, release and stability of fish oil

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ABSTRACT

Fish-oil (FO) was encapsulated with hydroxypropylcelullose (HPC) by conventional spray-drying with water (FO-water) and solvent spray-drying with ethanol (FO-EtOH), methanol (FO-MeOH) and acetone (FO-Acet) in order to study the effect of the solvent on the encapsulation efficiency (EE), microparticle properties and stability of FO during storage at 40 °C. Results showed that FO-Acet presented the highest EE of FO (92.0%), followed by FO-EtOH (80.4%), FO-MeOH (75.0%) and FO-water (71.1%). A decrease of the dielectric constant increased the EE of FO, promoting triglyceride-polymer interactions instead of oil-in-water emulsion retention. FO release profile in aqueous model was similar for all FO-microparticles, releasing only the surface FO, according to Higuchi model. Oxidative stability of FO significantly improved by spray-drying with MeOH, both in surface and encapsulated oil fractions. In conclusion, encapsulation of FO by solvent spray-drying can be proposed as an alternative technology for encapsulation of hydrophobic molecules.

1. Introduction

Long-chain omega-3 polyunsaturated fatty acids (LCw3-PUFA), especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids are well-known for their health benefits (Yashodhara et al., 2009; Arab-Tehrany et al., 2012). The most important natural sources of EPA and DHA are marine organisms such as fish, seafood and algae. Other omega-3 sources are plant rich in α-linolenic acid (ALA) but, in the human body, the conversion of ALA into EPA and DHA is low (5–10% for EPA and 1–5% for DHA; Kralovec, Zhang, Zhang & Barrow, 2012).

Recommendations from the World Health and North Atlantic Treaty Organizations are 0.3–0.5 g/d of EPA + DHA. Nevertheless, the worldwide average consumption is below these recommendations (Arab-Tehrany et al., 2012). Therefore, healthy foods supplemented with LCw3-PUFA are gaining importance in the food market. However, one of the major drawbacks of oils containing a high amount of LCw3-PUFA, such as fish oils (FO), is their non-desirable off-flavours and high susceptibility to oxidation. Microencapsulation of FO with polymers (Desai & Park, 2005; Gharsallaoui, Rouaut, Chambin, Voilley, & Saurel, 2007) has been proposed as a strategy to retard lipid oxidation, improving the oil stability, prolonging its shelf life, limiting off-flavours and controlling release into food (Arab-Tehrany et al., 2012; Kralovec et al., 2012; Pothakamury & Barbosa-Cánovas, 1995). Recently, Bakry et al. (2015) have summarized the studies on microencapsulation of oils (marine, vegetable and essential oils), and Encina, Vergara, Giménez, Oyarzun-Ampuero, & Robert (2016) have reviewed the studies on conventional spray-drying for the microencapsulation of FO, which is the most common method for encapsulation of FO. Other methods for FO encapsulation have been also reported, such as freeze drying, gelation method, complex coacervation, inclusion complexation, emulsification and nanoencapsulation (Encina et al., 2016).

The conventional method of encapsulation of fish oil by spray drying has been undertaken by preparing o/w emulsions (Aghbashlo, Mobli, Madadlou, & Rafiee, 2013a; Drusch, 2007; Drusch, Serfert, Van Den Heuvel, & Schwarz, 2006), multilayer emulsion (Shaw, McClements, & Decker, 2007) or nano-emulsion of fish oil (Jafari, Assadpoor, Bhandari, & He, 2008). The disadvantages of feed emulsion preparation include the use of high shearing forces such as microfluidization, ultrasonication (von Staszewski, Pizones Ruiz-Henestrosa, & Pilosof, 2014; Ilyasoglu and Nehir, 2014), and high-pressure

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Table 1
Experimental design for the microencapsulation of fish oil by spray-drying.

Run/System	Ratio FO/HPC [X_1]	Gas inlet temperature (°C) [X_2]	X_1	X_2	FO-MeOH EE (%)	FO-EtOH EE (%)	FO-Acet EE (%)
1	1:1	80	−1	−1	51.9 ± 0.9	47.5 ± 3.3	28.5 ± 0.2
2	1:1	130	−1	+1	57.3 ± 1.0	55.9 ± 5.0	31.5 ± 5.5
3	1:4	80	+1	−1	83.1 ± 1.5	82.0 ± 2.1	95.8 ± 1.7
4	1:4	130	+1	+1	75.0 ± 1.5	89.5 ± 0.7	89.7 ± 1.1
5	1:2.5	75	0	−1.21	75.8 ± 1.4	75.5 ± 0.3	78.2 ± 1.7
6	1:2.5	135	0	+1.21	74.3 ± 1.3	78.0 ± 1.5	75.0 ± 2.0
7	1:0.7	105	−1.21	0	29.4 ± 0.5	40.8 ± 5.5	27.9 ± 4.6
8	1:4.3	105	+1.21	0	82.7 ± 1.5	87.3 ± 2.5	97.7 ± 2.7
9	1:2.5	105	0	0	81.8 ± 1.4	79.9 ± 6.5	79.7 ± 3.6
10	1:2.5	105	0	0	78.9 ± 1.3	71.9 ± 3.0	72.2 ± 4.7
11	1:2.5	105	0	0	72.7 ± 1.3	71.5 ± 0.1	85.2 ± 8.0
12	1:2.5	105	0	0	74.7 ± 1.4	74.0 ± 0.3	79.0 ± 1.8

FO: Fish oil; HPC: Hidroxypropyl cellulose; MeOH: methanol; EtOH: ethanol; Acet: Acetone; EE: Encapsulation efficiency.

homogenization, to diminish the droplet size and to increase the emulsion stability. Solvent spray-drying has been used for drug encapsulation by pharmaceutical industry, where hydrophobic molecules are dissolved in an organic solvent and dried in a closed loop mode at low temperatures using nitrogen as drying medium (Duan, Vogt, Li, Hayes, & Mansour, 2013; Encina et al., 2016). In the case of fish oil microencapsulation, solvent spray-drying could be as a strategy which would avoid the preparation of a fish oil-in-water emulsion and hence minimize lipid oxidation during spray-drying (Serfert, Drusch, Schmidt-Hansberg, Kind, & Schwarz, 2009). In this context, our group recently addressed microencapsulation of fish oil with ethanol as organic solvent (Encina et al., 2018). However, to the best of our knowledge, this is the first study focused on the effect of the type of organic solvent on the FO microparticles properties.

A high number of variables affect oxidation in microencapsulated oils, such as matrix components, drying procedure, water activity, oil globule size and lipid distribution (Márquez-Ruiz, Velasco, & Dobarganes, 2003). Specifically, lipid distribution between surface and encapsulated fractions and heterogeneity of oil globules encapsulated are key factors influencing oxidation in multiphase complex food systems such as microencapsulated oils. Therefore, it is essential to determine the oxidation state separately in surface and encapsulated fractions (Márquez-Ruiz, Velasco, & Dobarganes, 2000; Velasco, Dobarganes, & Márquez-Ruiz, 2000; Márquez-Ruiz, et al., 2003; Velasco, Marmesat, Dobarganes, & Márquez-Ruiz, 2006). The understanding of the supramolecular chemistry of lipid oxidation in multiphase complex food systems has advanced lately with the recognition of the role of micro or nanoemulsions wherein the oxidation and antioxidation sites are the interfaces between lipids and water (Budilarto & Kamal-Eldin, 2015; Ghnimi, Budilarto, & Kamal-Eldin, 2017). In this context, we proposed recently a new antioxidant protection strategy through formation of channels within microencapsulated antioxidants that will aid their diffusion to the lipid medium (Morelo, Márquez-Ruiz, Holgado, Giménez & Robert, 2017). Accordingly, Kamal-Eldin & Ghnimi suggested that such an engineered system could be used to encapsulate PUFA in combination with antioxidants and/or synergists (Kamal-Eldin & Ghnimi, 2017).

The oxidative stability of the microencapsulated FO has been usually evaluated by several methods such as peroxide value, anisidine value, volatile oxidation markers, thiobarbituric acid reactive substances (TBARS) and conjugated dienes (Encina et al., 2016). The measurement of the formation of different groups of oxidation compounds by a combination of adsorption and size exclusion chromatographies is an analytical approach that has been applied to microencapsulated oils and proven to be more complete and sensitive than other methods traditionally used such as peroxide value or TBARS (Márquez-Ruiz et al., 2000; Márquez-Ruiz et al., 2003; Márquez-Ruiz & Dobarganes, 2005).

The aim of this work was to study the influence of the solvent type used in spray-drying for fish oil microencapsulation on the encapsulation parameters, powder properties and fish oil release pattern in an aqueous model. Hydroxypropyl cellulose (HPC), a partially substituted poly(hydroxypropyl) ether of cellulose, was selected as encapsulating agent since this polymer is soluble in both water and organic solvents. Also, oxidative stability of microencapsulated fish oil was determined through separate analysis of surface and encapsulated oil fractions.

2. Material and methods

2.1. Materials

Fish oil (FO) was donated by Spes S.A. (Santiago, Chile). The major fatty acids were 18.3 ± 0.0% (EPA, C20:5 ω3), 11.7 ± 0.1% (DHA, C22:6 ω3), 15.5 ± 0.0% (C16:0), 8.8 ± 0.1% (C16:1 ω9 cis), 7.6 ± 0.1% (C18:1 ω9 cis) and 7.6 ± 0.0% (C14:0). The initial values of oxidation compounds and peroxide value were 5.7 ± 0.2% and 0.7 ± 0.03 mEq O_2/kg oil, respectively. Hydroxypropyl cellulose (KLUCEL®, HPC) was obtained from Laboratorios Saval S.A. (Santiago, Chile). Soy lecithin (Epikuron 145V) was supplied by Blumos Ltda. (Santiago, Chile). Ethanol, acetone, methanol, hexane, chloroform, diethyl ether, petroleum ether, anhydrous sodium sulfate and acetic acid were of analytical grade and were purchased from Merck (Santiago, Chile). Silica cartridges for solid-phase extraction (SPE) were Sep-Pack Vac Silica (6 cc/1g) columns were supplied by Waters (Milford, USA). Tricosanoic acid and monostearin (purity > 99%) were purchased from Nu-Check-Prep (Elysian, USA). Tetrahydrofuran (HPLC grade) was supplied by Merck (Santiago, Chile).

2.2. Methods

2.2.1. Preparation of fish oil microparticles by solvent spray-drying

FO microparticle systems with HPC in either ethanol (FO-EtOH), methanol (FO-MeOH) or acetone (FO-Acet) were performed using a central composite + star design with 12 runs (4 experimental points, 4 star points, and 4 central points) for each system (Table 1). The FO/HPC ratio (X_1) (1:0.7–1:4.3) and the air inlet temperature (X_2) (75–135 °C) were evaluated as independent variables. These experimental conditions were selected according to previous studies (Encina et al., 2016; Encina et al., 2018). FO encapsulation efficiency (EE) was the response variable. The data were fitted to a second-order regression model (Eq. (1)). All of the experiments were conducted randomly to avoid systematic bias.

$$EE = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon \quad (1)$$

where EE is the dependent variable predicted by the model (FO encapsulation efficiency); β0 the constant coefficient of the intercept; β_1

and β_2 the linear effects, β_{11} and β_{22} the quadratic effects, β_{12} the cross-product and ε the error term.

Non-significant terms ($p > 0.05$) were removed from the equation, but when the linear forms of FO/HPC ratio or inlet gas temperature were not significant, they were considered in the quadratic equation because they are fundamental elements of the mathematical model. The analysis of variance (ANOVA), test of lack of fit, and determination of regression coefficients were performed with the software Statgraphics (5.0 program, Manugistics Inc., Rockville, MA). The response surface methodology (RSM) was applied to determine the optimal conditions for each system studied, maximizing the FO encapsulation efficiency.

The microparticles were prepared as follows: HPC (1.7–10.8 g) was dispersed in ethanol, methanol or acetone (87–96 g) and stored at room temperature for 12 h in an orbital shaking (200 rpm) (JSSI-100C, JSR, Korea) to ensure complete solvation. Fish oil (2.5 g) in ethanol, methanol or acetone was added with constant stirring to the HPC solution and homogenized at 19,000 rpm for 3 min using a Polytron homogenizer (PT 2100, Kinematica AG, Switzerland). The resultant mixture was fed into the spray-dryer.

2.2.2. Preparation of fish oil microparticles by conventional spray drying

FO microparticles by conventional method (FO-water) were prepared according to optimal conditions (inlet air temperature of 200 °C and FO/HPC ratio of 1:4) (Encina et al., 2018). Briefly, lecithin (0.1 g) was dispersed in distilled water (30 g) at 40 °C, using a magnetic stirrer at 500 rpm for 20 min and then this dispersion was added to FO (2.5 g). HPC (10 g) was dispersed in distilled water (57.4 g) and stirred by 12 h in an orbital shaker at 200 rpm (JSSI-100C, JSR, Korea). Fish oil-in-water emulsion was added to the HPC solution and homogenized in a Polytron homogenizer (PT 2100, Kinematica AG, Switzerland) at 19,000 rpm for 3 min. The resultant mixture was fed into the spray-dryer.

2.2.3. Spray-drying

Conventional and solvent infeed were fed into laboratory scale spray-dryer (B-290 mini spray-dryer Büchi, co-current form, Switzerland). The spray-dryer was operated at an air inlet temperature of 200 °C for the conventional method, and ranging from 75 to 135 °C for the solvent method. In the case of solvent spray-drying the equipment was linked to a mobile unit inert loop B-295 (Büchi, Switzerland), using nitrogen as drying gas. The spray gas flow, gas flow rate (aspirator) and feed flow rate (peristaltic pump) were 600 L/h, 35 m^3/h and 1 mL/min, respectively. The powders were stored in plastic bottles at −18 °C for further analysis.

2.2.4. Characterization of the FO microparticles

Water activity (a_w) was determined by the dewpoint method using a Hygrolab 2 (Rotronic, USA) at 20 ± 0.3 °C. The moisture content was determined by using an infrared moisture analyzer (PMC50, Radwag, USA). Hygroscopicity was determined according to the procedure described by Cai and Corke (2000).

2.2.4.1. Encapsulation efficiency of fish oil. Surface fish oil determination: Surface oil was determined according to Drusch (2007) with some modifications. The microparticle powders (2 g) were dispersed in petroleum ether (20 mL), stirred for 1 min at room temperature and filtered with anhydrous sodium sulfate. The solvent was removed using a rotatory evaporator at 40 °C (R-100 Büchi, Switzerland) until constant weight.

Total fish oil determination: Microparticles were completely disrupted by the following procedure: microparticle powders (2 g) were dissolved in ethanol (20 mL). The oil extraction was performed with hexane (5 mL), and stirred for 1 min, sonicated for 5 min and centrifuged at 4000 rpm for 4 min. The supernatant (organic phase) was withdrawn and the extraction was repeated three times. The supernatants were joined and the solvent was removed using a rotatory evaporator (R-100 Büchi, Switzerland) at 40 °C until constant weight.

FO encapsulation efficiency (EE) was calculated using Eq. (2).

$$EE\ (\%) = \frac{Total\ fish\ oil - surface\ fish\ oil}{Total\ fish\ oil} \times 100 \quad (2)$$

2.2.4.2. Determination of EPA and DHA contents. Fatty acids methyl esters (FAME) were prepared according to AOAC (1990). FAMEs were analyzed using a gas chromatograph (7890B, Agilent Technologies, USA) equipped with a HP-88 fused-silica capillary column (100 m, 0.25 mm i.d., 0.20 μm film thickness, Agilent Technologies, USA) and a flame ionization detector (FID). The injector and detector temperature were maintained at 250 °C. The oven temperature was set at 180 °C for 32 min, and then increased to 230 °C at 20 °C/min, hold 32 min. The injection volume was 1 μL. EPA and DHA were quantified using tricosaenoic acid (C23:0) as internal standard. The results were expressed as mg EPA or DHA/g fish oil according to Eq. (3) (AOCS, 2007).

$$EPA\ or\ DHA\left(\frac{mg}{g}\right) = \frac{A_x \times W_{IS} \times CF_x}{A_{IS} \times W_S \times 1.04} \times 1000 \quad (3)$$

where A_x is the peak area of EPA or DHA; A_{IS} is the peak area of the internal standard; C_f is the theoretical detector correction factor (0.99 for EPA and 0.97 for DHA); W_{IS} is the weight of the internal standard added to the sample (mg); W_S is the weight of the sample (mg) and 1.04 is the factor necessary to express the results as mg EPA or DHA/g oil.

2.2.4.3. Morphology and particle size of the microparticles obtained under optimal conditions. The outer structures of the FO microparticles (FO-water, FO-EtOH, FO-MeOH and FO-Acet) obtained under optimal conditions were examined using scanning electron microscopy (SEM). The microparticles were coated with gold/palladium, using a Varian Vacuum Evaporator PS 10E and analysed using a LEO 1420VP SEM (LEO Electron Microscopy Ltd., Cambridge, UK) operated at 20 kV. The scanned images were collected digitally using the software EDS 7424 (Oxford Instruments, Oxford, UK).

Particle size and the size distribution were determined by light scattering using a laser diffraction particle size analyzer (LV 950-V2 Horiba, Kyoto, Japan). The microparticles were dispersed in re-circulating water, and the results were expressed as volume average diameter ($D_{4,3}$).

2.2.5. Release of fish oil from FO microparticles

Release of FO from microparticles obtained under optimal conditions (FO-water, FO-EtOH, FO-MeOH, FO-Acet) was evaluated in aqueous models (phosphate buffer solution pH 6.5 to simulate milk and acetate buffer solution pH 4.6 to simulate yogurt). FO-microparticles (5 g) in cellulose filter bags were placed into glass bottles (250 mL) containing either buffer pH 4.6 or pH 6.5 (100 mL), stoppered and stored at 30 °C ± 2 °C in an orbital shaker at 55 rpm (JSSI-100C, JSR, Korea) in the dark. Aliquots (15 mL) were removed (in triplicate) at specific time intervals and the fish oil released was extracted three times with hexane by liquid-liquid extraction. The initial volume was maintained by the addition of the corresponding buffer. The supernatants were joined and the solvent was removed using a rotatory evaporator (R-100 Büchi, Switzerland) at 40 °C until constant weight.

The data were fitted to Higuchi Kinetic model (Higuchi, 1963), according to Eq. (4):

$$\frac{M_t}{M_\infty} = k \times t^{1/2} \quad (4)$$

M_t is defined as the quantity of FO released at any time t, and M_∞ is the initial FO loading. The release rate constants (k) were obtained from the slope of a plot M_t/M_∞ versus $(time)^{1/2}$.

2.2.6. Storage stability assays of FO microparticles

Open Petri dishes (10 cm) with 2.5 g of FO microparticles obtained under optimal conditions (FO-water, FO-EtOH, FO-MeOH and FO-Acet) were stored at 40 ± 1 °C in a forced–air oven (BE 500, Memmert, Schwabach, Germany) in the dark. Petri dishes (in triplicate) were removed at 0, 18, 36 and 72 h to determine the formation and distribution of polar compounds in both surface and encapsulated FO. Additionally, samples were stored refrigerated at 5 ± 1 °C in the dark and analysed at 5 months.

2.2.6.1. Extraction of FO from microparticles during storage. Surface oil was extracted following the same procedure detailed in Section 2.2.4.1. For extraction of encapsulated oil from microparticles devoid of surface oil (2 g) were rubbed in a mortar with water (0.5 mL) to obtain a clotted mass. Then 2.5 g Na_2SO_4 were added and the mixture was rubbed again until a granular mass was obtained. The oil was extracted with diethyl ether (3 × 20 mL). After filtration through anhydrous Na_2SO_4, the solvent was removed using a rotatory evaporator (R-100, Büchi, Switzerland) at 40 °C and the sample dried until constant weight using a stream of nitrogen (Márquez-Ruiz et al., 2000).

2.2.6.2. Determination of polar compounds. The formation and distribution of polar compounds in both surface and encapsulated oil fractions of FO-microparticles (FO-water, FO-EtOH, FO-MeOH and FO-Acet) was evaluated by solid phase extraction (SPE) followed by high performance size exclusion chromatography (HPSEC) analysis. This methodology has been described in detail, including precision, accuracy and recovery data by Márquez-Ruiz, Jorge, Martín-Polvillo and Dobarganes (1996).

Solid phase extraction (SPE): extracted oil (50 mg) was dispersed in hexane (2 mL) and separated into two fractions by Sep-Pack Vac Silica columns (6 cc/1g), previously conditioned with 15 mL of petroleum ether:diethyl ether (90:10, v/v). The first fraction (unoxidized triacylglycerols) was eluted with 15 mL of petroleum ether: diethyl ether (90:10, v/v). The second fraction (containing triacylglycerol polymers, triacylglycerol dimers, oxidized triacylglycerol monomers, diacylglycerols, free fatty acids and polar unsaponifiable matter) was eluted with 25 mL of diethyl ether. For quantitative purpose, 1 mg of monostearin was used as internal standard. The separation efficiency was checked by thin layer chromatography, using a silica plate and hexane:diethyl ether:acetic acid (60:40:2) as elution solvent. Iodine vapour was used to reveal the spots on the plate.

High performance size exclusion chromatography (HPSEC): The separation and quantification of the polar compounds was performed by HPSEC, using a chromatograph equipped with a Waters 510 pump (Waters, Milford, USA), a Rheodyne 7725i injector (10 µL sample loop), and a refractive index detector (HP 1037 A, Agilent Technologies, Palo Alto, USA). The separation was performed on two 100 and 500 Å columns (25 cm × 0.77 cm i.d.) packed with porous, highly cross-linked styrene-divinylbenzene copolymers (film thickness 5 µm) (Agilent Technologies, Palo Alto, USA) connected in series, with tetrahydrofuran (1 mL/min) as the mobile phase. Quantitation of total polar compounds was achieved through the internal standard (monostearin) method and amounts of triacylglycerol polymers, triacylglycerol dimers, oxidized triacylglycerol monomers, diacylglycerols and free fatty acids were calculated from the individual peak areas and percentage of total polar compounds. The percentage of unoxidized triacylglycerols can be calculated by substracting the percentage of polar compounds from 100.

2.2.6.3. Polyene index. The polyene index was calculated according to Eq. (5):

$$\text{Polyene index} = \frac{[EPA] + [DHA]}{[Palmitic\ acid]} \times 100 \quad (5)$$

Where, concentration de EPA, DHA and palmitic acid were determined by gas chromatography after derivatization to fatty acid methyl esters with 2 N KOH in methanol, according to the IUPAC Standard Method (IUPAC, 1992). A gas chromatograph HP-6850 (Agilent Technologies, Palo Alto, USA) fitted with a HP Innowax fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) and a flame ionization detector was used. The column temperature was set at 180 °C for 2 min, then programmed from 180 °C to 230 °C at a rate of 3 °C/min and kept at 230 °C for 15 min.

2.2.7. Statistics

All the results were reported as mean ± standard deviation (SD). One-way ANOVA was applied to determine the statistical differences among systems, using Statgraphics, Version 7.0 (Manugistic Inc., Statistical Graphics Corporation, Rockville, MD).

3. Results and discussion

3.1. Optimization of the fish oil encapsulation by spray-drying

In this study, a central composite design was applied to evaluate the effect of the FO/HPC ratio (formulation variable) and the inlet gas temperature (process variable) on the encapsulation of FO by solvent spray-drying (FO-MeOH, FO-EtOH and FO-Acet). Table 1 shows the experimental design for the microencapsulation of FO by solvent spray-drying. The EE of FO was the response variable, since it represents the FO encapsulated within the microparticle, where the higher the EE, the lower the FO on the surface of the microparticles. The EE of FO ranged from 29.4 to 83.1% for FO-MeOH; 40.8 to 89.5% for FO-EtOH; and 27.9 to 97.7% for FO-Acet (Table 1), thus showing higher EE levels in FO-Acet. The FO recovery was high (close to 100%) for all the solvent spray-drying systems, thereby this variable was not considered as response variable.

RSM was used to optimize the response variable (EE), considering the linear, quadratic and cross-product forms of the independent variables (inlet gas temperature and FO/HPC ratio) at 95% confidence level. The analysis of variance (ANOVA, Supplementary material 1) for the EE of FO microparticle systems by spray-drying showed that the EE of FO was significantly affected by the linear form ($p < 0.05$) and quadratic form ($p < 0.05$) of FO/HPC ratio. In contrast, the linear, quadratic and cross-product forms of the inlet gas temperature were not significant in any of the three solvent spray-drying systems (FO-MeOH, FO-EtOH and FO-Acet). The models explained over 95% of the variability (R^2 adj. for d.f.) in EE for FO-MeOH, FO-EtOH and FO-Acet (Supplementary material 1), with residual values below 6.0. Moreover, the lack-of-fit was not significant, indicating that the mathematical model fitted well with the experimental data. The equations describing the effect of the independent variables on the EE of fish oil are the following:

$$EE\ (FO{-}MeOH) = 8.68362 + 47.3993{*}X_1{-}0.0732635{*}X_2{-}5.40762{*}X_1^2$$

$$EE\ (FO{-}EtOH) = 14.1104 + 27.7714{*}X_1 + 0.109264{*}X_2{-}3.16086{*}X_1^2$$

$$EE\ (FO{-}Acet) = -4.89508 + 49.1339{*}X_1{-}0.0402529{*}X_2{-}5.78613{*}X_1^2$$

Response surface plots are shown in Fig. 1(B.1–D.1). According to this figure, the higher the content of encapsulating agent the higher the EE of FO in all the systems, generally attributed to the rapid dry crust formation over the oil droplets surface (Gharsallaoui et al., 2007), leading to higher FO-polymer interaction. Although an increase in the EE of FO may be expected with the increase of the inlet gas temperature, the variation of the inlet gas temperature in the range studied did not affect the EE. In the case of encapsulation of FO, the inlet gas temperature may be a determining variable on FO oxidation, but not on EE. Response surface plots for the three FO microparticles systems showed a maximum EE included in the experimental domain and at the highest FO/HPC ratios.

Table 2 shows the optimal conditions and characterization of FO-

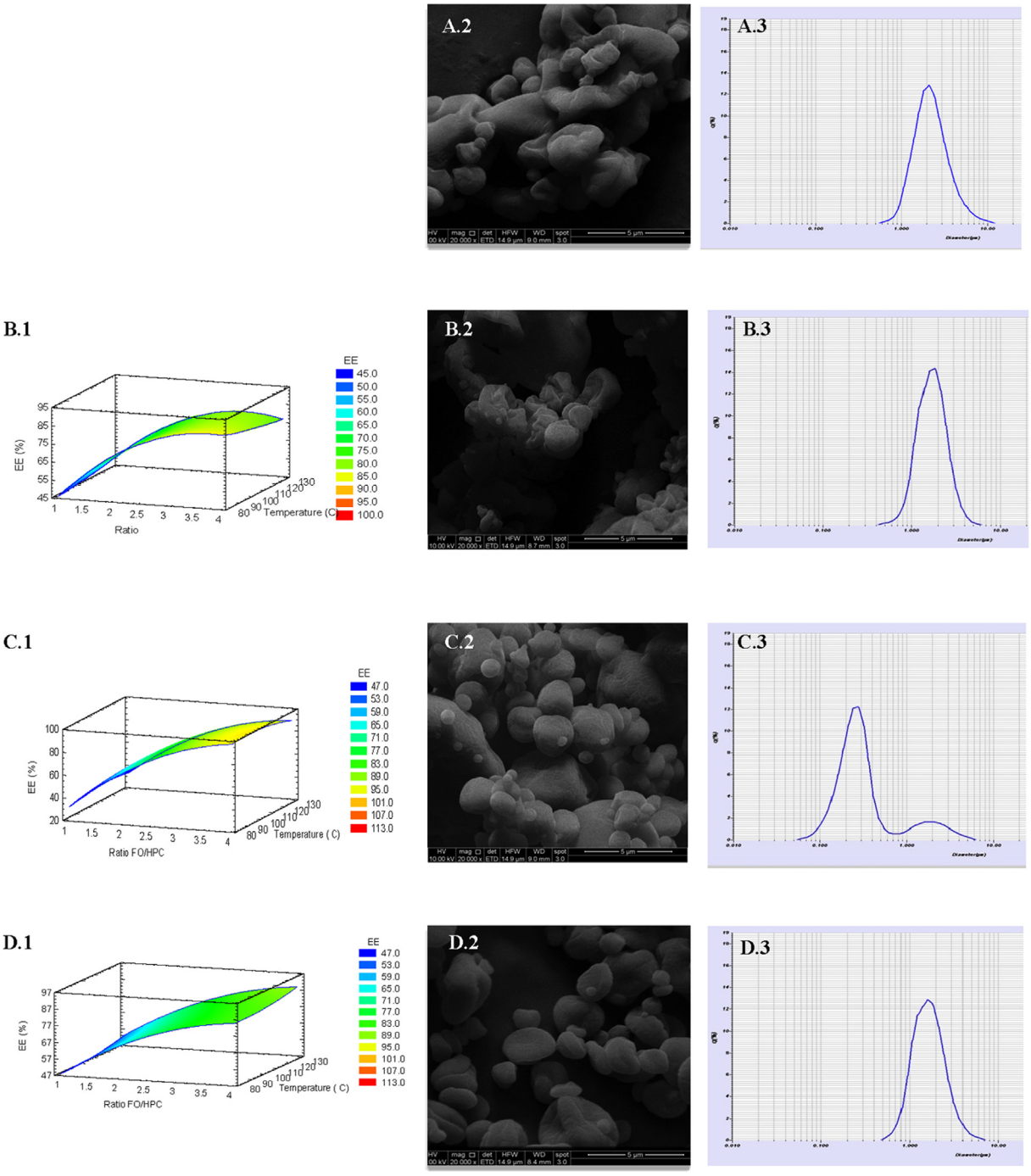

Fig. 1. Response surface graphs for FO-MeOH (B.1.), FO-EtOH (C.1) y FO-Acet (D.1); scanning electron microscopic photographs for FO-water (A.2), FO-MeOH (B.2), FO-EtOH (C.2) and FO-Acet (D.2); particle size distribution for FO-water (A.3), FO-MeOH (B.3), FO-EtOH (C.3) and FO-Acet (D.3).

HPC microparticles obtained by solvent and conventional (control) spray-drying. The optimal FO/HPC ratio was the same (1:4) for the encapsulated fish oil by solvent and conventional spray-drying methods (FO-water, FO-MeOH, FO-EtOH and FO-Acet), since all the systems have the same content of total solids. The range of FO/encapsulating agent ratio used for microencapsulation of fish oil by conventional spray-drying method is known to be from 1:1 to 1:6 (Encina et al., 2016) but, so far, the values for microencapsulation of FO by solvent spray-drying had not been reported.

The inlet gas temperature is an important variable in the microencapsulation of FO by spray-drying because the extent of oxidation reactions increases with temperature. The optimal inlet gas temperature was 200 °C for conventional spray-drying (FO-W), whereas this parameter was lower for FO-solvent systems: FO-EtOH (135 °C), FO-MeOH (79 °C) and FO-Acet (78 °C), showing that the optimal gas temperature was characteristic for each system. The optimal value obtained for conventional spray-drying is, as reported in the literature, in the range of 140–215 °C (Encina et al., 2016) but, to the best of our knowledge, no data had been provided so far using organic solvents for FO spray-drying encapsulation. Some criteria, such as boiling point, dielectric constant, and solubility of active and encapsulating agent, have been considered for the solvent selection in spray-drying process (Patel, Patel, Chakraborty, & Shukla, 2015). In this study, considering all the FO-systems obtained under optimal conditions, a high correlation

Table 2
Optimal conditions and characteristics of FO-water, FO-MeOH, FO-EtOH and FO-Acet microparticles.

Parameters	FO-water	FO-MeOH	FO-EtOH	FO-Acet
FO/HPC ratio	1:4	1:4	1:4	1:4
Gas inlet temperature (°C)	200	79	135	78
FO EE (%)	71.1 ± 1.0c	75.0 ± 1.4c	80.4 ± 0.6b	92.0 ± 0.5a
Total FO (mg/g)	198.4 ± 0.5a	200.0 ± 0.6a	200.0 ± 0.6a	200.0 ± 0.6a
Surface FO (mg/g)	57.3 ± 2.0a	50.0 ± 2.1ab	39.2 ± 5.4b	16.0 ± 1.1c
EPA (mg EPA/g microparticle)	24.7 ± 0.6a	25.1 ± 1.3a	25.4 ± 1.2a	24.8 ± 1.3a
DHA (mg DHA/g microparticle)	15.2 ± 0.9a	15.5 ± 1.5a	15.6 ± 0.8a	15.2 ± 1.0a
Moisture (%)	5.0 ± 0.1a	4.0 ± 0.1b	4.5 ± 0.2b	2.3 ± 0.2c
a_w	0.280 ± 0.004b	0.270 ± 0.01b	0.330 ± 0.001a	0.340 ± 0.006a
Hygroscopicity (g/100 g)	24.4 ± 0.4a	25.6 ± 0.4a	22.4 ± 0.6b	22.9 ± 0.4b
Particle size D(4.3) (μm)	2.43 ± 0.07a	1.79 ± 0.01b	0.53 ± 0.01c	1.77 ± 0.02b
FO release rate constant ($days^{0.5}$) at pH 4.6*	0.030 ± 0.003a	0.021 ± 0.003a	0.020 ± 0.002a	0.022 ± 0.002a
FO release rate constant ($days^{0.5}$) at pH 6.5*	0.029 ± 0.001a	0.024 ± 0.001a	0.027 ± 0.012a	0.017 ± 0.003a

FO: Fish oil; MeOH: methanol; EtOH: ethanol; Acet: Acetone; EE: encapsulation efficiency.
Different letters show significantly different between systems ($p \leq 0.005$).
* Data fitted to Higuchi model.

($R^2 = 0.965$) was found between the optimal inlet gas temperature and solvent boiling point (100 °C for water, 78 °C for ethanol, 65 °C for methanol, 56 °C for acetone). Therefore, the differences found in the optimal inlet gas temperature among FO-systems may be attributed to the different boiling point of the solvents used. Furthermore, this high correlation would allow us to predict the optimal inlet gas temperature from the boiling point of each solvent.

3.2. Characterization of fish oil microparticles obtained under optimal conditions

3.2.1. Encapsulation efficiency of fish oil

As shown in Table 2, in this study the highest EE of FO was obtained for FO-Acet (92.0%), the system with the lowest inlet gas temperature, followed by FO-EtOH (80.4%). Similar EE values ($p > 0.05$) were found in the case of water and methanol systems (71.1% and 75.0%, respectively). Differences in the EE of FO between solvent and conventional spray-drying methods could be explained by the mechanism for encapsulation of FO (Fig. 2). In solvent spray-drying, the triacylglycerol-polymer interaction can occur through hydrophobic interactions and Van der Waals forces, whereas in conventional spray-drying encapsulation would occur by oil-in-water emulsion retention. It is important to notice that when solvent spray-drying is used as encapsulation method, the oil-in-water emulsion preparation and the application of high shearing forces are avoided.

Another factor that may affect the EE of hydrophobic molecules is the dielectric constant of the medium. Therefore, hydrophobic molecules such as FO could increase its solubility by reducing the dielectric constant of the solvent (20.7 for acetone, 25.8 for ethanol, 32.7 for methanol and 81.1 for water) (Moribe et al., 2014), hence promoting triacylglycerol-polymer interactions and increasing the encapsulation efficiency of FO. Thus, in this study, a high correlation was found between EE obtained under optimal conditions and dielectric constant ($R^2 = 0.916$) for solvent FO-systems (FO-Acet, FO-MeOH, FO-EtOH). In spite of the high EE of FO for FO-Acet, some FO remained on the surface of the microparticle (non-encapsulated oil), since spray-drying is more an immobilization method rather than a true encapsulation method (de Vos, Faas, Spasojevic, & Sikkema, 2010). Surface FO is highly susceptible to oxidative reactions with environmental oxygen as well as to development of unpleasant flavors.

The total FO content in the FO-microparticle systems ranged from 198.4 to 200.0 mg/g (Table 2), within the wide range reported in the literature (108–119 mg/g, Patrick et al., 2013; 244-492 mg/g, Polavarapu, Oliver, Ajlouni, & Augustin, 2011) for conventional spray-drying. In this study, one gram of FO-microparticles provided around 40 mg of EPA + DHA, which is approximately 13–8% of the recommended daily dose (0.3–0.5 g of EPA + DHA per day).

FO-water microparticles (conventional spray-drying) showed significantly higher moisture content than those obtained by solvent spray-drying ($p < 0.05$) (Table 2). The water activity of the microparticles reached values ranging from 0.28 to 0.34, within the range of values reported in the literature for spray-drying powder to ensure microbiological stability. However, the hygroscopicity was similar for all the systems, suggesting that this parameter depends on the chemical

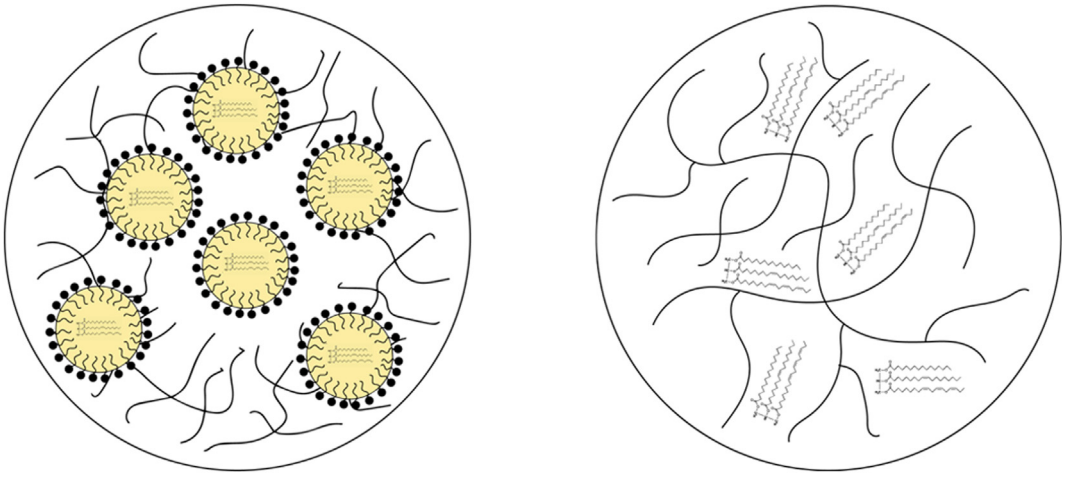

Fig. 2. Mechanism for encapsulation of FO by conventional and solvent spray-drying.

features of the encapsulating agent that was the same for all the systems.

3.2.2. Morphology and particle size of fish oil microparticles

Fig. 1(A.2–D.2), shows the SEM photographs and particle size distribution of FO microparticle powders (FO-water, FO-MeOH, FO-EtOH and FO-Acet) obtained under optimal conditions. FO-water and FO-solvent microparticles systems showed irregular and spherical shapes, with some shrinkage. The presence of agglomerations was more noticeable for FO-water microparticles due to higher moisture content. The formation of indented surfaces found in all the FO-systems during spray-drying is attributed to particle shrinkage that can take place both at high and low inlet gas temperatures. At high inlet temperatures (as in the case of FO-water), the rapid water evaporation and high pressure inside the particles produced shrinkage, whereas at low temperatures (as in the case of FO-solvent systems) solvent diffusion was slower and it took longer for the particles to shrink (Alamilla-Beltrán, Chanona-Pérez, Jiménez-Aparicio, & Gutiérrez-López, 2005). The distribution of particle size was unimodal for all the systems except for FO-EtOH (Fig. 1, A.3–D.3), which showed a bimodal distribution. The powder particle size ($D_{4,3}$) was higher in FO-water (2.43 μm) than in FO-solvent systems (1.79 μm for FO-MeOH, 0.53 μm for FO-EtOH, and 1.77 μm for FO-Acet) (Table 2). In the case of FO-water system, the retention of the emulsion led to a higher microparticle size than in FO-solvent systems, where the mechanism for FO encapsulation was based on triacylglycerol-encapsulating agent interactions. Aghbashlo, Mobli, Madadlou, and Rafiee (2013b) reported that the size of FO microparticles obtained by conventional spray-drying increased when the drying temperature increased, which was explained by the faster crust formation. However, this behavior was not found for the solvent spray-drying microparticles. This result suggests that the process variables in solvent spray-drying technology could affect microparticle properties in a different way with respect to conventional spray-drying, as was described for the encapsulation efficiency.

3.3. Release of FO from FO microparticles

The FO release profile was assayed in FO microparticles (FO-water, FO-EtOH, FO-MeOH and FO-Acet) obtained under optimal conditions, in aqueous models (pH 4.6 and 6.5) at 30 °C (Supplementary material 2). A gradual release of FO was observed, reaching values of 6.5–15% at 101 days. According to the EE of FO in all the systems (Table 2), the surface FO was in the range 8–28.9%. Therefore, the FO released corresponded to surface FO and release of FO within the microparticles (encapsulated FO) was not observed along the time period studied. This result was expected because the FO diffusivity inside the matrix decreases due to the low solubility of FO in the aqueous media, which could be used as a method to control the release.

Higuchi model was applied to explain the release mechanism of surface FO. The correlation coefficient (R^2 above 97% for all FO-microparticles and both aqueous models) showed a good fit to Higuchi model, showing that the release mechanism of FO was through Fickian diffusion for all the microparticle systems. No significant differences ($p > 0.05$) were found among FO release rate constants (Table 2) for FO-water, FO-EtOH, FO-MeOH and FO-Acet microparticles at each pH studied, showing that the release of surface FO was independent of the solvent used in the elaboration of the microparticles.

3.4. Stability of microencapsulated fish oil during storage

Table 3 shows the results obtained for polar compounds, including total level and distribution in different groups of oxidation compounds and hydrolysis products, in surface and encapsulated fractions of the oils extracted from initial microcapsules. Even though hydrolysis is not expected to occur in these dried systems devoid of lipase activity, values for diacylglycerols and free fatty acids were quantified in the same analysis to ascertain that oil-in-water emulsion preparation in the case of FO-water microparticles did not lead to hydrolytic changes as compared to FO-solvent systems. This method, based on a combination of adsorption and size exclusion chromatographies, has been widely used by Márquez-Ruiz et al. (2000) to evaluate oxidation in microencapsulated oils, as commented in the Introduction. Concomitant determination of oxidized triacylglycerol monomers, dimers and polymers provides a complete, quantitative picture of the oxidation state from initial to advanced stages of oxidation (Márquez-Ruiz & Dobarganes, 2005). While oxidized triacylglycerol monomers include hydroperoxides formed in the early stage of oxidation, dimers and polymers start increasing when oxidation is accelerated. However, other methods normally used provide partial information on the oxidation progress, as peroxide value which only detects primary oxidation compounds or volatiles such as hexanal, only formed at the end of the induction period (Márquez-Ruiz et al., 2003).

Values of oxidation compounds were in general low and close to those in the FO before spray-drying (0.4% triacylglycerol polymers, 1.4% triacylglycerol dimers and 3.9% oxidized triacylglycerol monomers). These results indicate that preparation either by water, ethanol, acetone or methanol led to similar levels of oxidation of FO. Still, statistical differences were obtained among surface and encapsulated oil fractions in total polar compounds, being slighter higher in the former, as it has been previously observed after preparation of microencapsulated fish oils (Velasco et al., 2000, 2006; Velasco, Holgado, Dobarganes & Márquez-Ruiz, 2009). Also, the sum of triacylglycerol dimers and polymers in surface oil was significantly lower in microcapsules prepared with solvents as compared to FO-water microparticles.

Fig. 3 shows time-course of formation of total polymerization compounds (triacylglycerol dimers plus polymers) during oxidation at 40 °C in the dark. These oxidation conditions have been previously found useful in studies on microencapsulated oils since oxidation is thus accelerated while keeping temperature below that leading to structural changes in the matrix components (Velasco, Dobarganes, & Márquez-Ruiz, 2003). As it is well-known, polymerization compounds increase rapidly in fish oils even at low temperature due to their considerable content in polyunsaturated fatty acids, which are highly susceptible to oxidation (Márquez-Ruiz et al., 2000; Velasco et al., 2006). Results showed the lowest oxidation for FO-MeOH microparticles and the highest for FO-water and FO-Acet microparticles, both in surface and in encapsulated oil fractions. Besides, results at 5 °C for 5 months confirmed the highest stability for FO-MeOH microparticles (6.2% polymerization compounds) as compared with FO-water, FO-EtOH and FO-Acet microparticles (10.5%, 8.3% and 12.4% polymerization compounds, respectively).

In addition, polyene index, which reflects the loss of omega-3 polyunsaturated fatty acids (EPA and DHA), was determined as indirect measurement of oxidative stability (Supplementary material 3) in FO microparticles at time 0 and 72 h of storage at 40 °C in the dark. Data were consistent with results presented in Fig. 3 and showed that oxidative stability of FO in microparticles was in the order: FO-MetOH ≥ FO-EtOH ≥ FO-Acet = FO-water. Overall, results showed that oxidative stability of FO improved when spray-drying with MetOH or EtOH instead of water but comparative oxidative behavior of FO-MetOH, FO-EtOH and FO-Acet microparticles cannot be explained by differences in EE, particle size or other microparticles characteristics evaluated in this study.

4. Conclusions

Spray-drying with organic solvents affected the FO encapsulation efficiency and FO stability in different ways. The encapsulation of FO with HPC by methanol and acetone spray-drying (FO-systems with lower dielectric constant) improved the encapsulation efficiency of FO (FO-EtOH, 80.4% and FO-Acet, 92.0%) with respect to conventional

Table 3
Polar compounds in oils extracted from FO-water, FO-EtOH, FO-Acet and FO-MeOH microparticles at the beginning of the storage.

Polar compounds (% on oil extracted)	FO-Water		FO-EtOH		FO-Acet		FO-MeOH	
	Surface oil	Encapsulated oil	Surface oil	Encapsulated oil	Surface oil	Encapsulated oil	Surface oil	Encapsulated oil
TG polymers	0.5 ± 0.06$^{1\ a}$	0.4 ± 0.01$^{1\ ABC}$	0.3 ± 0.06$^{1\ cd}$	0.5 ± 0.01$^{2\ A}$	0.1 ± 0.01$^{1\ e}$	0.4 ± 0.02$^{2\ AB}$	0.3 ± 0.02$^{1\ bc}$	0.5 ± 0.01$^{2\ A}$
TG dimers	1.6 ± 0.00$^{1\ a}$	1.4 ± 0.01$^{2\ C}$	1.2 ± 0.08$^{2\ b}$	1.7 ± 0.02$^{1\ A}$	0.8 ± 0.00$^{2\ c}$	1.6 ± 0.04$^{1\ B}$	1.2 ± 0.02$^{2\ b}$	1.6 ± 0.00$^{1\ AB}$
Oxidized TG monomers	4.8 ± 0.13$^{1\ cd}$	3.4 ± 0.01$^{2\ E}$	6.9 ± 0.13$^{2\ a}$	7.6 ± 0.08$^{1\ A}$	3.8 ± 0.03$^{2\ e}$	4.6 ± 0.00$^{1\ C}$	6.0 ± 0.12$^{1\ b}$	5.0 ± 0.13$^{2\ B}$
Diacylglycerols	3.0 ± 0.00$^{2\ d}$	4.1 ± 0.07$^{1\ A}$	6.6 ± 0.12$^{1\ b}$	2.5 ± 0.01$^{2\ D}$	9.9 ± 0.15$^{1\ a}$	3.0 ± 0.08$^{2\ BC}$	4.6 ± 0.08$^{1\ c}$	2.9 ± 0.04$^{2\ BC}$
Free fatty acids	0.7 ± 0.01$^{2\ d}$	1.1 ± 0.01$^{1\ A}$	1.0 ± 0.01$^{1\ b}$	0.9 ± 0.01$^{2\ C}$	1.2 ± 0.01$^{1\ a}$	1.0 ± 0.00$^{2\ BC}$	0.8 ± 0.04$^{2\ c}$	1.1 ± 0.02$^{1\ A}$
TG polymers + TG dimers	2.0 ± 0.06$^{1\ a}$	1.8 ± 0.00$^{2\ C}$	1.5 ± 0.02$^{2\ b}$	2.2 ± 0.03$^{1\ A}$	1.0 ± 0.01$^{2\ c}$	2.0 ± 0.06$^{1\ B}$	1.5 ± 0.03$^{2\ b}$	2.1 ± 0.01$^{1\ AB}$
Total polar compounds	10.5 ± 0.08$^{1\ c}$	10.3 ± 0.05$^{1\ C}$	16.0 ± 0.26$^{1\ a}$	13.3 ± 0.10$^{2\ A}$	15.8 ± 0.18$^{1\ a}$	10.6 ± 0.15$^{2\ BC}$	13.0 ± 0.13$^{1\ b}$	11.1 ± 0.20$^{2\ B}$

Different numbers in the same microparticle system mean significant differences between surface and encapsulated oil fractions (n = 3, $p \leq 0.05$).
Different lower-case letters in each row mean significant differences between surface oil fractions (n = 3, $p \leq 0.05$).
Different upper-case letters in each row mean significant differences between encapsulated oil fractions (n = 3, $p \leq 0.05$).
TG: triacylglycerol.
Unoxidized triacylglycerols (% on oil) = 100 − Total polar compounds (%).

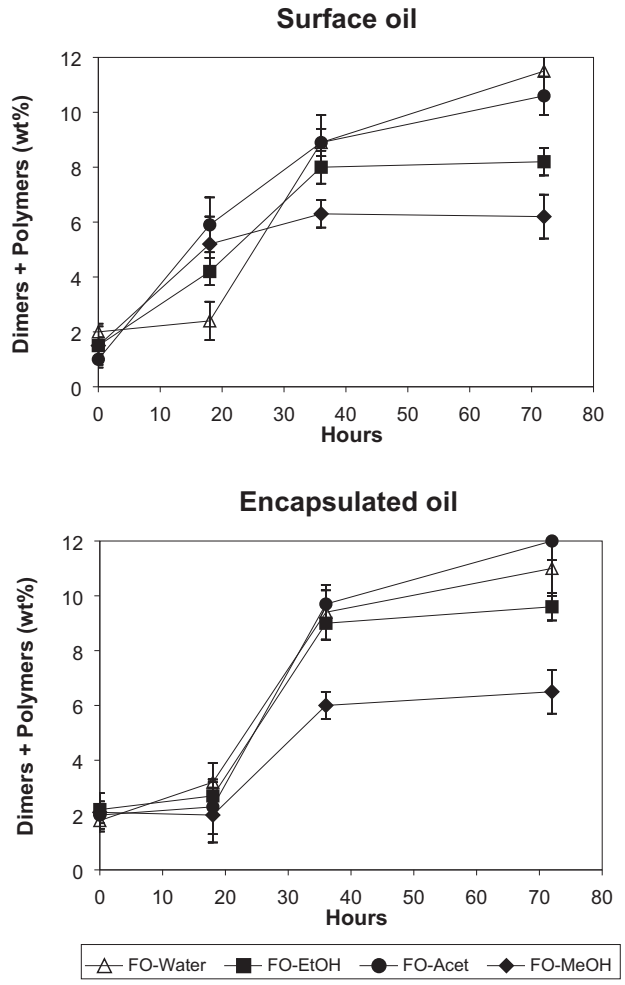

Fig. 3. Time-course of formation of triacylglycerol dimers and polymers in surface and encapsulated oil fractions of FO microparticles prepared with water (FO-water, triangles), ethanol (FO-EtOH, squares), acetone (FO-Acet, circles) and methanol (FO-MeOH, diamonds), stored at 40 °C in the dark. Values correspond to means of triplicate samples and error bars to standard deviations.

spray-drying (FO-water, 71.1%). However, the increase of EE was not in line with the oxidative stability of FO, since FO-Acet led to the highest encapsulation efficiency but lowest oxidative stability of FO. With respect to the release of FO in aqueous models (pH 4.6 and 6.5), similar results were found for all FO-microparticles, attributable only to the surface FO following a Fickian diffusion (Higuchi model). Oxidative stability of FO in FO-MetOH and FO-EtOH microparticles was higher than that for FO-water. Even though further studies are required to establish the mechanism involved and to explain the behavior of the different solvents used, results obtained in the present work show that solvent spray-drying has the potential to be an alternative technology for encapsulation of FO.

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Conflict of interest

This article represents the authors own work and we are not aware of any conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2018.05.026.

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