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Oxygen permeability and oxidative stability of fish oil-loaded electrosprayed capsules measured by Electron Spin Resonance: effect of dextran and glucose syrup as main encapsulating materials

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Abstract:

The oxygen permeability and oxidative stability of fish oil-loaded electrosprayed capsules were studied by Electron Spin Resonance (ESR). Electrosprayed capsules with dextran as main biopolymer showed a significantly faster broadening (ΔH_{pp}) of 16-doxyl-stearate ESR spectrum when compared to glucose syrup capsules. This finding indicates a higher oxygen permeability of dextran capsules than glucose syrup capsules, which is explained by a reduced average free volume in the glucose syrup matrix than in the dextran shell. Moreover, glucose syrup capsules showed a significantly lower increase in the peak-to-peak amplitude of N-tert-butyl-α-phenylnitrone (PBN) ESR spectrum during storage when compared to dextran capsules. This implies a higher oxidative stability of glucose syrup capsules than dextran capsules, which correlated well with the lower oxygen permeability of the former. These results indicated the importance of the oxygen barrier properties of the wall materials when encapsulating long chain omega-3 polyunsaturated fatty acids by electrospraying.

Keywords: lipid oxidation; omega-3; electrospraying; encapsulation; electron spin resonance

1. Introduction

Omega-3 polyunsaturated fatty acids (PUFAs), especially long chain PUFAs such as eicosapentaenoic (C20:5n-3, EPA) and docosahexaenoic (C22:6n-3, DHA) acids have beneficial effects on human health (Miles & Calder, 2012). These healthy PUFAs can be found in krill, crustaceans, fish and algae (Bailey, 2009). Unfortunately, people in general do not consume enough of these products, which leads to a lack of sufficient PUFAs in their diet (Bimbo, 2013) .

Therefore, it is interesting to look for possibilities to enrich common foods with omega-3 PUFAs. This is challenging because omega-3 PUFAs are highly prone to oxidation due to their high number of bis-allylic hydrogens (Ismail, Bannenberg, Rice, Schutt, & MacKay, 2016). Moreover, complex food matrices contain several prooxidants such as metal ions, which initiate lipid oxidation (Angelo, 1996). Thus, the protection of omega 3-PUFAs is required before they are incorporated into food matrices. For that, one of the strategies used is the encapsulation of omega-3 PUFAs in a biopolymer wall material, which prevents the attack of oxygen and prooxidants by creating a physical barrier (Jacobsen, García-Moreno, Mendes, Mateiu, & Chronakis, 2018).

The most common encapsulation techniques for omega-3 PUFAs are emulsification, which results in oil-in-water emulsions preferably used in water-based foods like dairy and beverages, and spray-drying, which leads to powdered encapsulates mainly employed in dry products (e.g. bread, infant formula) (Taneja & Singh, 2012). An alternative to spray-drying is electrospraying, which is an encapsulation technique that does not require heat to dry (e.g. it is carried out at room temperature). This reduces the degradation of thermo-sensitive bioactives such as omega-3 PUFAs during processing (García-Moreno et al., 2018; Torres-Giner, Martinez-Abad, Ocio, & Lagaron, 2010). Instead of heat, electrospraying uses a high-electrostatic field to dry the biopolymer emulsion with omega-3 PUFAs dispersed. In the moment that the electric field overcomes the surface tension of the droplet, a charged jet is ejected from the Taylor cone at the tip of the needle to a grounded collector. Due to varicose instability, the jet is destabilized into droplets, which are further disrupted into fine droplets because of repulsion electrostatic forces. This allows the evaporation of the solvent, leading to dry

nano-microcapsules in the collector (García-Moreno, Chronakis, & Jacobsen, 2018; Drosou, Krokida, & Biliaderis, 2017).

Previous studies have reported the encapsulation of omega-3 PUFAs by electrospraying (García-Moreno, Chronakis, & Jacobsen, 2018; Busolo, Torres-Giner, Prieto, & Lagaron, 2018). Recently, we have reported the potential of dextran and glucose syrup as main biopolymers (in combination with whey protein and pullulan) for the production of fish oil-loaded electrosprayed capsules (García-Moreno et al., 2018). Our results indicated that, besides the higher content of non-encapsulated oil for glucose syrup capsules when compared to dextran capsules, glucose syrup capsules loaded with fish oil had higher oxidative stability than fish oil-loaded dextran capsules (as shown by the content of hydroperoxides and secondary volatile oxidation products). This finding led us to hypothesize that the enhanced oxidative stability of fish oil-loaded glucose syrup capsules when compared to dextran capsules was due to a lower oxygen diffusivity in the former capsules (García-Moreno et al., 2018).

Electron Spin Resonance (ESR) is a common technique employed to determine oxygen permeability of oil-loaded capsules by using an oxygen sensitive spin probe, which is dissolved in the lipid phase (Svagan et al., 2016). ESR-based oximetry makes use of stable nitroxide free radicals (spin labels), which possess an unpaired electron and has the ability to interact with another molecule (e.g. a paramagnetic molecule such as oxygen). Hence, the interaction between spin probe and oxygen lead to a broadening of the ESR signal of the spin probe through Heisenberg spin exchange, which is proportional to the concentration of oxygen (Andersen, Risbo, Andersen, & Skibsted, 2000; Svagan et al., 2016).

ESR has been used to measure oxygen permeability of different types of encapsulates (e.g. freeze-dried or spray-dried capsules) (Andersen, Risbo, Andersen, & Skibsted, 2000; Orlien, Andersen, Sinkko, & Skibsted, 2000) but not on electrosprayed capsules. It is noteworthy that permeability of oxygen/air through the wall material in electrosprayed capsules may play a more important role on oxidative stability when compared to other type of encapsulates. For instance, electrosprayed capsules (< 5 μm) have a significantly reduced size compared to spray-dried capsules (5-50 μm) (Jacobsen et al., 2018). This implies that electrosprayed capsules present a significant increase in specific surface area as well

as a reduced wall material thickness (e.g. for the same fish oil load) when compared to spray-dried capsules. The latter makes encapsulates produced by electrospraying more easily permeable than capsules produced by spray-drying, highlighting the importance of measuring oxygen permeability in electrosprayed capsules.

In addition, ESR spin trapping, which is based on the formation of stable radicals (spin adducts) due to the reaction of free radicals and spin probes, has been widely employed to evaluate early stages of lipid oxidation in different food systems (e.g. oil-in-water emulsions and beer) (Andersen & Skibsted, 2008; Frederiksen, Festersen & Andersen, 2008; Velasco, Andersen, & Skibsted, 2005).

In the light of the above, this study aimed at evaluating the oxygen permeability and oxidative stability of fish oil-loaded electrosprayed capsules by using ESR. To the best of the authors' knowledge, this is the first work studying the influence of the oxygen barrier properties of shell materials on autooxidation of lipids encapsulated by electrospraying. Particularly, in this study we investigated the effect of dextran or glucose syrup as main encapsulating materials on both oxygen permeability and oxidative stability of electrosprayed capsules loaded with fish oil.

2. Materials and Methods

2.1 Materials

Glucose syrup (DE38, C*Dry 1934) was provided by Cargill Germany GmbH (Krefeld, Germany). Dextran (Molecular weight 70 kDa) was kindly provided by Pharmacosmos A/S (Holbaek, Denmark). Pullulan (molecular weight 200 kDa) was provided by Hayashibara Co., Ltd. (Okayama, Japan). Whey Powder Concentrate (WPC) was provided by ARLA Food Ingredients (Viby, Denmark). MCT oil, under the commercial name MIGLYOL, was kindly provided by IOI Oleo GmbH (Witten, Germany). Fish oil with a content of 9.3 wt.% EPA and 10.9 wt.% DHA was delivered by Maritex A/S (Sortland, Norway). The peroxide value (PV) of the fish oil was 0.4±0.1 meq/kg oil. Citrem (esters of citric acid without antioxidants), with a PV of 2.3±0.1 meq/kg oil, was provided by Danisco (Copenhagen, Denmark). ESR probes 16-DOXYL-stearic acid (DSA) and N-tert-Butyl-α-phenylnitrone (PBN) were purchased from

Sigma Aldrich (Søborg, Denmark). Oxygen (≥ 99.5%) and nitrogen (≥ 99.999%) gasses were supplied by Air Liquide (Taastrup, Denmark)

2.2 Preparation of emulsions for electrospraying containing the ESR probes

Biopolymer solutions were prepared as described in García-Moreno et al. (2018). In brief, WPC (0.5 wt. %), pullulan (4 wt. %) and glucose syrup (15 wt. %) or pullulan (1 wt.%) and dextran (15 wt. %) were dissolved in distilled water by stirring overnight at 500 rpm. To the biopolymers solutions, oil and Citrem (20 wt.% and 0.5 wt.% with respect to biopolymers weight, respectively) were added and dispersed at 17,500 rpm using an Ultraturrax T-25 homogenizer (IKA, Staufen, Germany). The oil and Citrem were added during the first minute and the total dispersion time was of five minutes. After this, the formed coarse emulsion was passed 3 times through a Microfluidizer (M110L Microfluidics, Newton, MA, USA) at 9,000 psi as described in García-Moreno et al. (2018).

For determining oxygen permeability, DSA (a lipophilic probe) was added as hexadecane solution (concentration of 25 mg/mL) to MCT oil in order to have 10 μM of DSA in the oil phase of the emulsion. MCT oil is a stable oil that will not react with oxygen or the probe. On the contrary, DSA probe is sensitive to paramagnetic substances like oxygen (Andersen, Risbo, Andersen, & Skibsted, 2000). Thus, when oxygen diffuses through the biopolymer shell, it will interact with DSA probe and broaden the ESR signal of DSA, quantified as the peak-to-peak width, ΔH_{pp} (Svagan et al. 2016; Hatcher & Plachy, 1993).

To investigate oxidative stability, N-tert-Butyl-α-phenylnitrone (PBN) was added as an ethanol solution (concentration of 50 mg/mL) to fish oil in order to have 30 mM of PBN in the oil phase of the emulsion. PBN is a lipophilic spin trap, which reacts with free radicals derived from lipid oxidation to form spin adducts. The amount of spin adducts, which is proportional to the free radicals formed, was quantified by using the peak-to-peak amplitude of the ESR spectrum (Velasco et al., 2005).

2.3 Droplet size distribution of electrospraying emulsions

The droplet size distribution of emulsions for electrospraying containing MCT oil was analyzed by laser diffraction using a Mastersizer 2000 (Malvern Instruments, Ltd., Worcestershire, UK). At a stirring rate

of 3000 rpm, the emulsions were diluted until an obscuration of 12% was reached. The refractive indices of water (1.330) and sunflower oil (1.469) were applied as dispersant and particle (García-Moreno et al., 2018). Results were given in volume-weighted mean diameter ($D_{4,3}$) and 90% volume-percentile diameter ($D_{0,9}$). Measurements were conducted in triplicate.

2.4 **Electrospraying process**

The emulsions were electrosprayed in lab scale according to the method described by García-Moreno et al. (2018) at a flow rate of 0.003-0.010 mL/min with an applied voltage of 15-20 kV (depending on the main biopolymer used). The time required to collect 600 mg of capsules varied between 6 to 8 h depending on the applied flowrate and voltage, which were optimized to minimize dripping and avoid wet droplets in the collector. All experiments were conducted at 20°C±4°C and 17 to 46% relative humidity.

2.5 Morphology

The morphology of MCT oil-loaded electrosprayed capsules was investigated using a Scanning Electron Microscope (SEM) (Phenom Pro, Phenom-World B.V., Eindhoven, The Netherlands). After 5-10 minutes of electrospraying, a piece of approximately 0.5 x 0.5 cm electrosprayed aluminum foil, that was located on the collector and contained the sample, was placed on carbon tape and sputter coated with gold during 8 seconds at 40 mA by a Q150 Quorum Coater (Quorum Technologies Ltd, East Sussex, UK). The capsule diameter distribution was measured by using the open source processing program ImageJ (National Institutes of Mental Health, Bethesda, Maryland, USA). The capsules size distribution was obtained by measuring 100 random capsules.

2.6 ESR experiments

ESR spectra of capsules containing spin probe or spin trap were obtained using a Miniscope MS200 X-band ESR spectrometer (Magnettech GmbH, Berlin, Germany). All experiments were carried out at 20°C ± 4°C at 1 atm.

2.6.1 Determination of oxygen permeability

The capsules (200 mg) were placed in a quartz ESR tube (height: 18 cm, outer diameter: 0.5 cm) with a minimum sample height of 2.3 cm. The tube was closed at one end with a plug of glass wool. Thereafter, the capsules loaded in the tubes were washed two times with 5 mL of heptane to remove the non-encapsulated oil from the surface of the capsules as described by Andersen, Risbo, Andersen, & Skibsted, 2000. Washing the surface oil is important to prevent that the ESR measures the signal of DSA probe contained in the surface oil, which get immediately in contact with oxygen. To determine oxygen permeability, nitrogen (59.50 mL/min) was run through the capsules-loaded tubes for 20 minutes to displace oxygen. Thereafter, the nitrogen atmosphere was replaced with pure oxygen (50.49 mL/min). Oxygen was run through the capsules-loaded tube for 30 min and the line broadening of the ESR spectra was determined and calculated at several times as described by Svagan et al. (2016). After that, the atmosphere was changed back to pure nitrogen (59.50 mL/min) and the line narrowing was measured at several time points for 30 min. A modulation amplitude of 0.2 mT was used.

A calibration curve relating the line broadening with the concentration of oxygen (obtained by the oxygen partial pressure using Henry's law) was constructed by adding the DSA-hexadecane solution to MCT oil (having a concentration of DSA of 10 μM in the oil). A piece of filter paper (± 0.3 x 2.3 cm) was soaked in the oil and placed in the ESR tube with glass wool at the bottom. First, nitrogen was flushed for 2 minutes to obtain a 100% nitrogen atmosphere in the tube. Next, the calibration curve was obtained by exposing the filter paper soaked in the MCT oil containing the DSA probe to different oxygen/nitrogen gas compositions. Each point of the calibration curve was run in triplicate.

2.6.2 Determination of oxidative stability

After electrospraying, the capsules were placed in the ESR tubes under nitrogen atmosphere, covered with parafilm and aluminium foil and stored at -40°C. Before measuring oxidative stability, the capsules were thawed during 15 min at room temperature. The oxidative stability of the capsules was determined during 16 days storage at 50 °C. The capsules were measured at days 0, 1, 3, 6, 9 and 16. The tubes filled with the capsules containing fish oil and PBN probe were placed in the ESR equipment and were measured at a modulation amplitude of 0.2 mT. The distance between the highest and lowest

points of the second peak in the PBN-ESR spectra was measured. On each measurement day, an average of this distance from three replicates was calculated.

Additionally, the spin probe PBN was added to pure fish oil as the PBN-ethanol solution mentioned above to have a concentration of PBN of 30 mM in the oil. The ESR spectra of fish oil containing PBN was measured by soaking a filter paper in the oil and placing it in an ESR tube. The spectra of fish oil were measured at day 0 and after two days of storage at 50°C and were compared with the spectra of fish oil-loaded capsules. These results confirmed that the radicals produced and trapped by the PBN probe in the capsules were formed in the oil phase as a consequence of lipid oxidation (results not shown).

2.7 Statistical analysis

All measurements were conducted in triplicate and data were expressed as mean ± standard deviation. Statgraphics Centurion XV (Statistical Graphics Corp., Rockville, MD, USA) was used to analyze the data. First, the multiple sample comparison analysis was conducted to detect a significant difference between measurements of the same sample. Secondly, the two-tailed paired t-test was applied to compare the mean values between different biopolymers. Significant differences were attributed when $p < 0.05$.

3 Results and discussion

3.1 Characterization of electrospraying emulsions and electrosprayed capsules

Droplet size distribution of the emulsions and morphology of the electrosprayed capsules were determined in order to gain insight about the influence of the oil droplet size on capsule diameter as well as to determine the available contact surface between prooxidants and oil (Drusch & Berg, 2008; Jimenez, García, & Beristain, 2006).

Fig. 1 shows that the oil droplet size distribution of the electrospraying emulsions was monomodal and that the dextran emulsion had smaller droplets than the glucose syrup emulsion, as also confirmed by the significant differences in $D_{4.3}$ and $D_{0.9}$ ($p<0.05$). Dextran has a higher molecular weight than glucose

syrup (70 vs. 12.5 kDa), thus the higher viscosity of the dextran emulsion may have reduced flocculation and coalescence of the oil droplets by limiting their movement.

Fig. 2 shows the morphology and size distribution of the electrosprayed capsules. Overall, both capsules presented spherical shape without fibrils connecting the capsules. Although there were more glucose capsules below 1 μm than dextran capsules (Fig. 2), no significant differences were observed when comparing the average diameter of both type of capsules (1.26±0.57 μm for dextran and 1.39±0.52 μm for glucose syrup capsules), with more than 90% of dextran and glucose syrup capsules below 2 μm. It is noteworthy that a considerably smaller size for capsules produced in lab scale by electrospraying process was obtained when compared to capsules produced by high-throughput electrospraying process, which uses pressurized air to impel the solution into the electric field (García-Moreno et al., 2018). Significantly lower capsules diameter of capsules produced by lab-scale electrospraying implies a larger surface-to-volume ratio (e.g. increased contact area between lipids and prooxidants), which might be detrimental in terms of oxidative stability (García-Moreno et al. 2017). On the other hand, the release of the omega-3 fatty acids will be improved when having large specific area, which will favor the action of enzymes in the digestion system. Moreover, capsules with reduced size will be more easily dispersed into food matrices, having a minimum impact on food properties (e.g. texture) (Jacobsen et al. 2018).

3.2 Oxygen permeability

Oxygen permeability of wall materials plays a key role for the oxidative stability of encapsulated lipids (Drusch et al., 2009). This is of special importance in electrosprayed capsules, which have a reduced size (e.g. when compared to spray- or freeze-dried capsules) and, thus a larger surface area where the oxygen can diffuse through.

The ESR spectrum of DSA probe in MCT oil-loaded electrosprayed capsules showed a three-line ESR spectra due to the ^{14}N hyperfine coupling (Fig. 3). A broadening of the ESR line (ΔH_{pp}) together with a decrease in the intensity of the signals was observed when increasing time exposure of the capsules to pure oxygen atmosphere (Fig. 3). It is noteworthy that the spectrum of DSA in pure MCT oil also

presented a three-line ESR spectra, overlapping with the DSA spectra of encapsulated MCT oil (see Supplementary Material, Fig. S1). This indicates that DSA had a similar mobility and microenvironment in both systems (capsules and bulk oil), and then that DSA was located in the lipid phase of the capsules. Therefore, the increase of ΔH_{pp} for the ESR spectra of the capsules was due to the interaction of DSA spin probe and dissolved oxygen in the oil phase of the capsules, and it was used to determine the oxygen permeability of oil-loaded electrosprayed capsules.

Fig. 4a shows the evolution of the DSA ESR line width (ΔH_{pp}) in the capsules versus time upon exposure to oxygen or nitrogen atmosphere. The headspace of the capsule-loaded ESR tubes was first equilibrated with nitrogen and the gas environment was changed to oxygen at time zero. Both capsules presented an initial value of ΔH_{pp} of 0.2 mT. This relative high value is due to the modulation amplitude of 0.2 mT, which was applied to fast recording of spectra with good signal-to–noise ratios during the kinetic experiments.

It was observed that the line width of the ESR spectra for dextran capsules increased exponentially, reaching an asymptotic value of ΔH_{pp}= 0.293±0.007 mT after 2 min exposure to pure oxygen (Fig. 4a). According to the standard curve (Fig. 4b), ΔH_{pp} value of 0.293±0.007 mT indicates that the capsules were in equilibrium with the pure (100%) oxygen atmosphere after 2 min. On the other hand, the line width of ESR spectra for glucose syrup capsules increased linearly, and at a significantly lower rate, when compared to dextran capsules, reaching a plateau for ΔH_{pp}= 0.274±0.001 mT after 17.5 min (Fig. 4a). These results imply a higher oxygen permeability for dextran capsules than glucose syrup capsules. It should be noted that both dextran and glucose syrup capsules are in glassy state at room temperature since the glass transition temperature of glucose syrup capsules is 94.2 °C, whereas no Tg could be detected for dextran capsules below 200 °C (García-Moreno et al., 2018). Besides oxygen solubility within the carbohydrate matrices, oxygen diffusivity is mainly affected by average free volume in the glassy matrices (Drusch et al. 2009). Thus, from these results, it is clear that glucose syrup with a lower molecular weight than dextran (12.5 vs. 70 kDa) packed more densely within the shell leading to a reduced free volume, which decreased oxygen diffusivity. Likewise, Drusch et al.

(2009) reported an increase in free volume elements, which drastically affected oxygen diffusivity, for fish oil-loaded microcapsules produced by spray-drying when using carbohydrates with higher molecular weight.

After replacing the oxygen atmosphere by pure nitrogen (at t=30 min), the broadening of DSA ESR line (ΔH_{pp}) decreased exponentially for both types of electrosprayed capsules. As expected, the permeability of glucose syrup capsules was also lower for nitrogen gas when compared to dextran capsules, reaching both capsules asymptotic values of ΔH_{pp} of 0.203±0.006 mT after 12.5 and 0.5 min under nitrogen atmosphere respectively (Fig. 4a). It should be mentioned that the different rate of change for the increase (under oxygen) or decrease (under nitrogen) of ΔH_{pp} for a particular type of capsule is explained by the heterogeneity of the capsules (e.g. encapsulated oil droplets containing the DSA spin probe were placed at different distances from the wall material). The later determines the interaction of the probe with the gases and then the ESR line width (Andersen et al. 2000). For instance, shorter times were observed to reach the asymptotic values of ΔH_{pp} under nitrogen when compared to oxygen atmosphere (Fig. 4a). This is attributed to the fact that the line width of the recorded average ESR-spectra is dominated by the sharpness and high intensity of ESR spectrum from oil droplets located closer to the wall (e.g. which interact first with nitrogen) (Andersen et al. 2000; Svagan et al., 2016).

Overall, both dextran and glucose syrup electrosprayed capsules showed lower oxygen barrier properties when compared to hexadecane-loaded nanocellulose capsules with similar size (1.66±0.35 μm) and prepared via direct miniemulsion polymerization. This can be attributed to the high oxygen barrier properties reported for nanocellulose (Svagan et al. 2016).

3.3 Oxidative stability

ESR spin trapping, which is based on the reaction of radicals with diamagnetic molecules (spin probes such as PBN) to form more stable radicals (spin adducts) (Velasco et al., 2005; Zhou & Elias, 2012), was used to determine the oxidative stability of fish oil-loaded electrosprayed capsules.

The ESR spectra of PBN-adducts formed in fish oil loaded-electrosprayed capsules (due to the reaction of lipid radicals and PBN spin probe) consisted of three broad lines (see Supplementary Material, Fig.

S2). This correlated well with the ESR spectra of PBN-adducts formed in bulk fish oil, having the typical coupling for nitroxyl radicals due to the nitrogen nucleus. An additional coupling to hydrogen in PBN spin adducts is often not resolved in triglyceride systems (Velasco et al. 2005).

The peak-to-peak amplitude of the middle-field line of the ESR spectra of PBN-adducts was determined in order to monitor lipid oxidation during storage. Higher intensity for the peak-to-peak amplitude implies higher formation of PBN-spin adducts, which correlates proportionally with the concentration of radicals derived from lipid oxidation (e.g. peroxyl and alkoxyl radicals) (Andersen & Skibsted, 2008). Fig. 5 shows the evolution of the peak-to-peak amplitude in the ESR spectra of PBN-adducts during storage of fish oil-loaded capsules at 50 °C. Interestingly, PBN-adduct spectra were not detected at time zero for both types of capsules (see also Supplementary Material, Fig. S2), despite a reasonable concentration of lipid hydrogen peroxides (5-10 meq/kg oil) were found in fish oil-loaded capsules after production (García-Moreno et al., 2018) indicating a certain level of oxidation during the electrospraying.

In Fig. 5, a lag-phase of one day was clearly observed (with no significant increase in the peak-to-peak amplitude) for glucose syrup capules. In contrast, no lag.phase was detected for dextran capsules. Moreover, a significantly higher increase in the peak-to-peak amplitude for fish oil-loaded electrosprayed capsules produced with dextran as main biopolymer was observed when compared to fish oil-loaded electrosprayed capsules containing glucose as main shell material (Fig. 5). Therefore, these results indicated a significantly higher oxidative stability for glucose syrup capsules than dextran capsules, which correlated well with its lower oxygen permeability. Similarly, in our previous study we reported a lower formation of primary and secondary oxidation products in fish oil-loaded electrosprayed glucose syrup capsules during storage at room temperature than in dextran capsules, although dextran capsules had higher encapsulation efficiency (García-Moreno et al., 2018). Thus, taken altogether, these results confirm that oxygen diffusivity through the glassy matrix of fish oil-loaded electrosprayed capsules influences drastically their oxidative stability. Likewise, Drusch et al.

(2009) reported that glassy matrices composed of high molecular weight carbohydrates led to fish oil-loaded microcapsules with lower oxidative stability.

Finally, it should be also noted that the peak-to-peak amplitude decreased after 6 days storage for both capsules (Fig. 5). This is attributed to the reaction of spin adducts with new radicals to form diamagnetic species (Qian, Wang, Schafer, & Buettner, 2000). Hence, although ESR spin trapping is an adequate technique to study early stages of lipid oxidation, it cannot be used to evaluate advanced stages of lipid degradation where high concentrations of lipid radicals exist (Velasco et al. 2005). In any case, it is worthy to mention that ESR-spin trapping is a reliable method to investigate oxidative stability of oil-loaded electrosprayed capsules, requiring a low amount of sample. Nonetheless, complementary analyses are also required in order to identify specific oxidation products (e.g. volatiles) which lead to different non-desired off-flavours (e.g. rancid, metallic, fishy and others).

4 Conclusion

This study investigated, by using ESR, the oxygen permeability of fish oil-loaded electrosprayed capsules produced with dextran or glucose syrup as main biopolymers and their influence on the oxidative stability of the capsules. Electrosprayed capsules produced with glucose syrup as the main wall material were significantly less oxygen permeable than capsules containing dextran as the main biopolymer. This finding was attributed to the lower molecular weight of glucose syrup compared to dextran, which decreased the free volume in the capsule shell limiting oxygen diffusivity. Moreover, ESR results indicated that glucose syrup capsules were more oxidative stable than dextran capsules. Overall, these results denote the significant influence of oxygen diffusivity on the oxidative stability of fish oil-loaded electrosprayed capsules. In addition, this work demonstrated that ESR is a reliable method to measure the oxygen permeability and oxidative stability of electrosprayed nano-microcapsules loaded with lipophilic bioactives.

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Conflict of interest

The authors have declared no conflict of interest.

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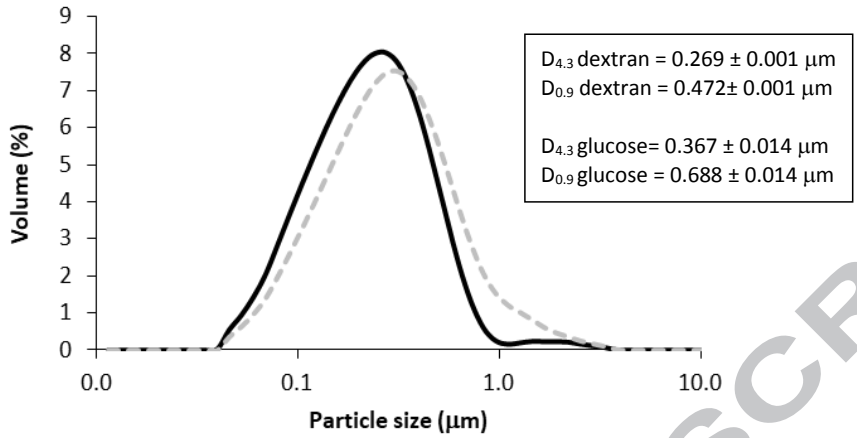

Figure 1. Droplet size distribution, volume-weighted mean diameter ($D_{4,3}$) and 90% volume-percentile diameter ($D_{0,9}$) of the dextran (-) and glucose syrup (- -) electrospraying emulsions

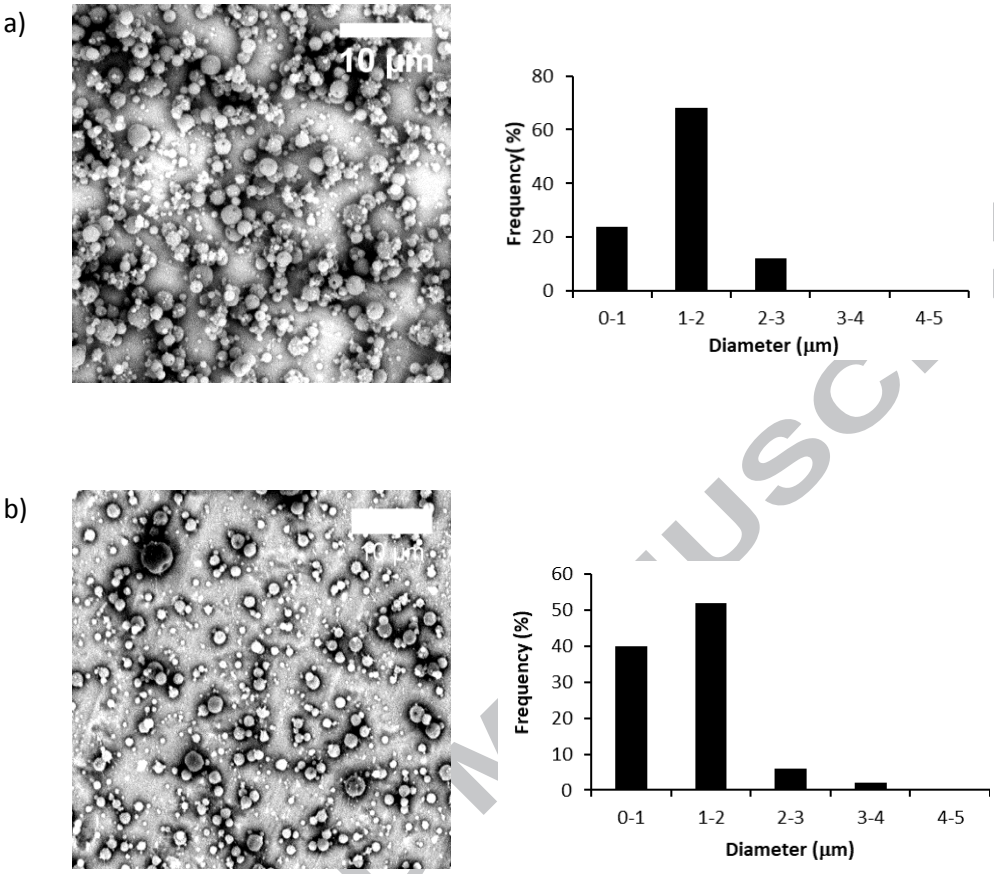

Figure 2. SEM images and capsule diameter distribution of MCT oil-loaded electrosprayed capsules produced with dextran (a) or glucose syrup (b) as main biopolymers. Scale bar, showed as a white bar in both figures, is of 10 µm.

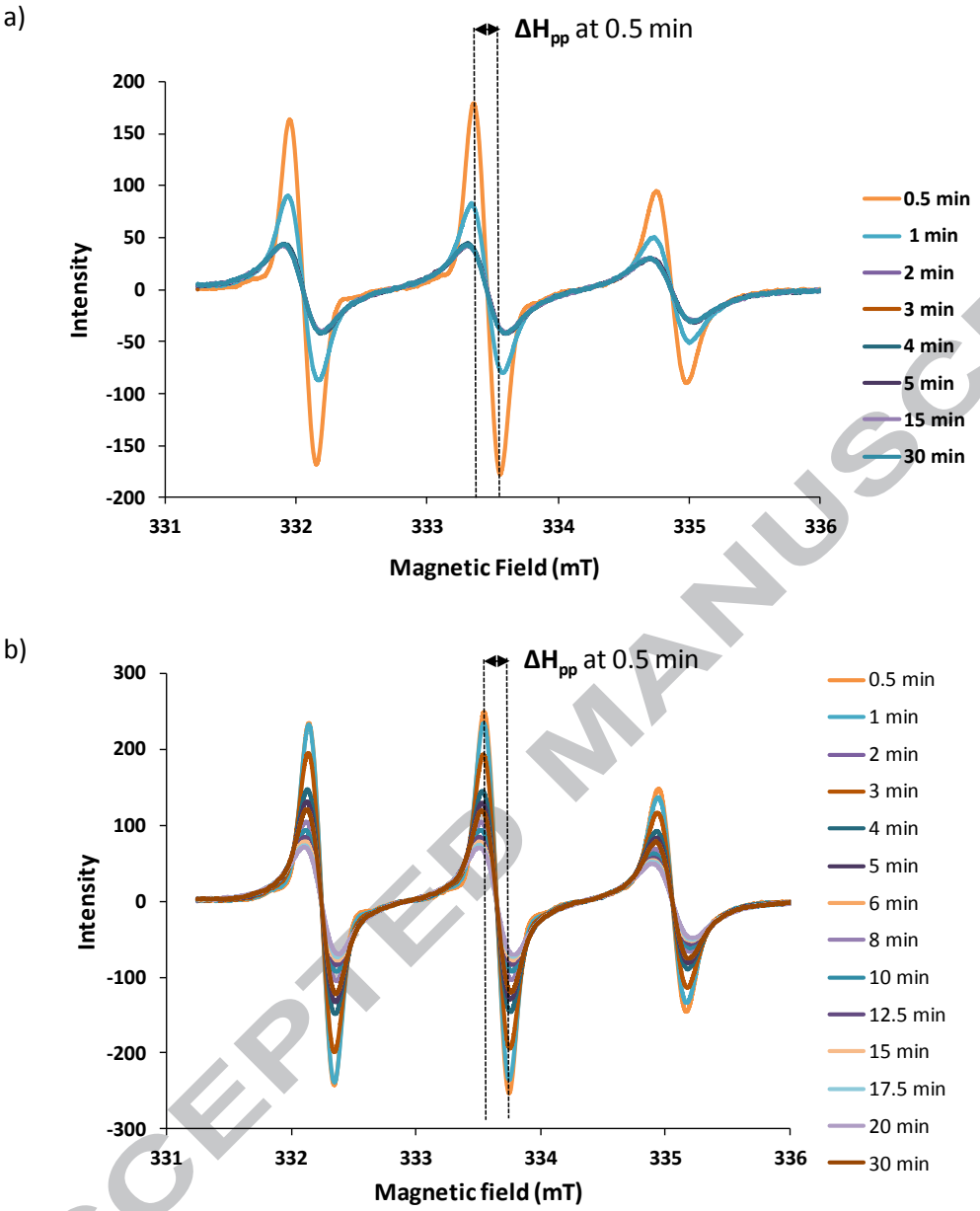

Figure 3. Broadening of ESR spectra of biopolymer capsules filled with MCT oil and DSA probe when changing from pure nitrogen to pure oxygen atmosphere: a) dextran, and b) glucose syrup capsules.

No all the ESR spectra obtained from dextran capsules are shown since they overlapped after 2 min.

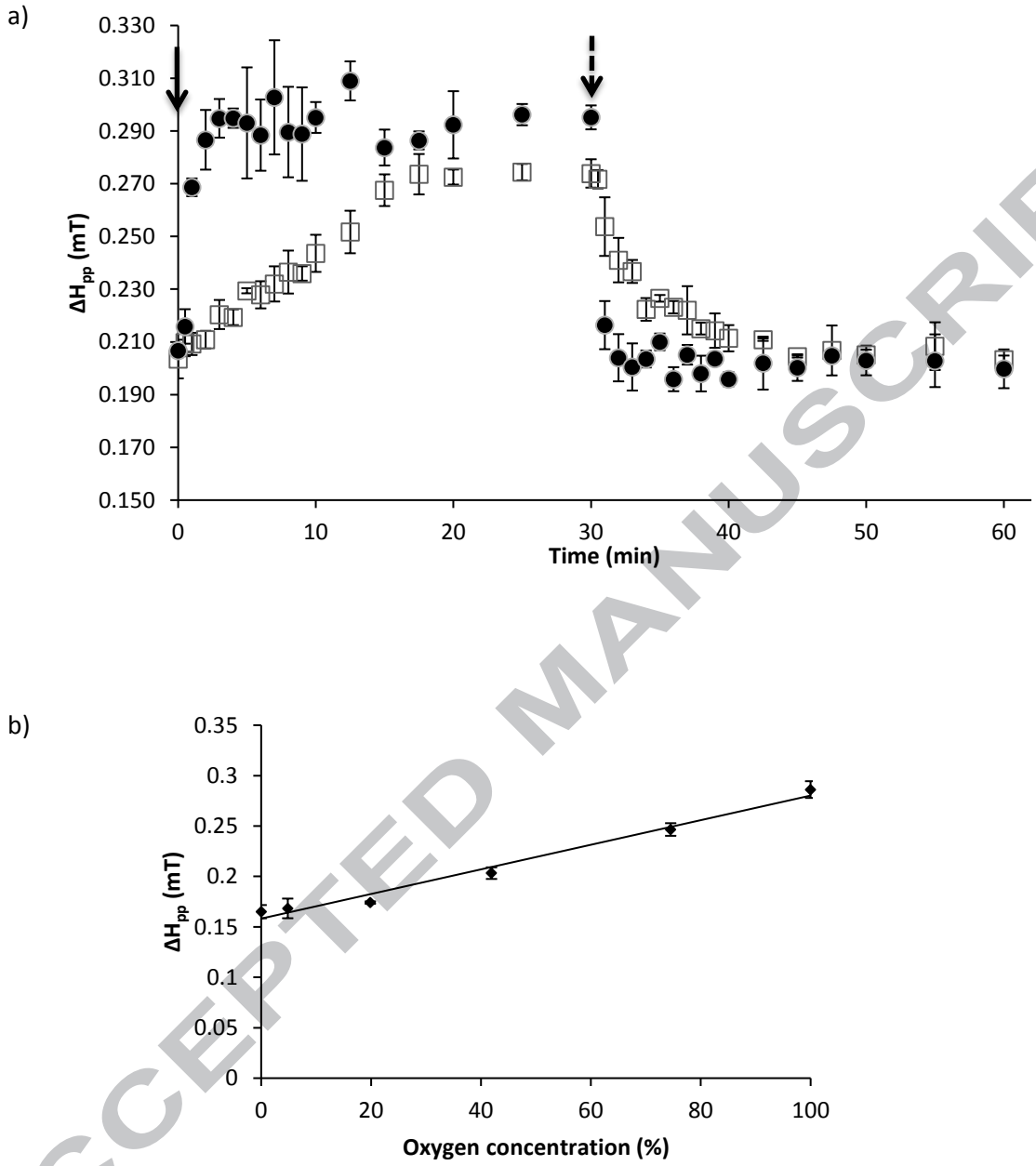

Figure 4. a) Evolution of the oxygen concentration (broadening of ΔH_{pp}) with time measured for dextran (●) and glucose syrup (□) capsules containing spin probe DSA/MCT oil. The black arrow marks when the atmosphere is change from pure nitrogen to pure oxygen. The striped arrow marks when the atmosphere is changed back from pure oxygen to pure nitrogen. b) The line width (ΔH_{pp}) as function of different oxygen concentrations for DSA in pure MCT oil. The solid line is the least-squares fit to the experimental data.

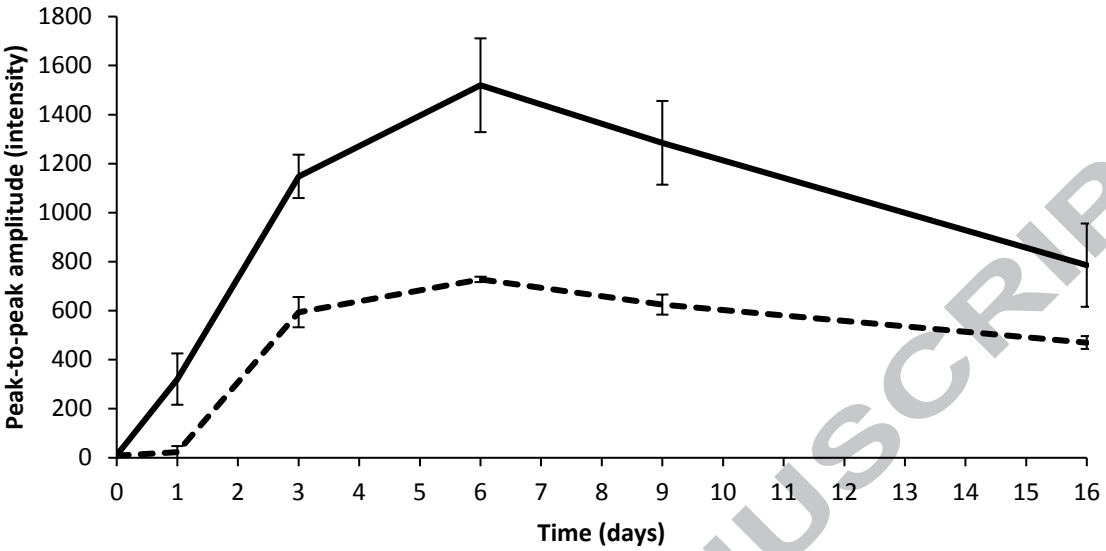

Figure 5. Evolution of the oxidative stability of electrosprayed dextran capsules (–) and glucose syrup capsules (---) loaded with fish oil. The peak-to-peak amplitude of the second peak in the PBN ESR spectrum was used.

Highlights

- Electrosprayed oil-loaded capsules were analyzed by Electron Spin Resonance
- Oxygen permeability and oxidative stability of the capsules were measured
- Electron Spin Resonance was an effective way to measure both properties
- Dextran capsules showed higher oxygen permeability than glucose syrup capsules
- Oxygen permeability significantly influenced oxidative stability of the capsules