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Impact of Extra Virgin Olive Oil and Ethylenediaminetetraacetic Acid (EDTA) on the Oxidative Stability of Fish Oil Emulsions and Spray-Dried Microcapsules Stabilized by Sugar Beet Pectin

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ABSTRACT: The influence of EDTA on lipid oxidation in sugar beet pectin-stabilized oil-in-water emulsions (pH 6, 15% oil, wet basis), prepared from fish oil (FO) and fish oil−extra virgin olive oil (FO−EVOO) (1:1 w/w), as well as the spray-dried microcapsules (50% oil, dry basis) prepared from these emulsions, was investigated. Under accelerated conditions (80 °C, 5 bar oxygen pressure) the oxidative stability was significantly ($P < 0.05$) higher for FO and FO−EVOO formulated with EDTA, in comparison to corresponding emulsions and spray-dried microcapsules formulated without EDTA. The EDTA effect was greater in emulsions than in spray-dried microcapsules, with the greatest protective effect obtained in FO−EVOO emulsions. EDTA enhanced the oxidative stability of the spray-dried microcapsules during ambient storage (~25 °C, $a_w = 0.5$), as demonstrated by their lower concentration of headspace volatile oxidation products, propanal and hexanal. These results show that the addition of EDTA is an effective strategy to maximize the oxidative stability of both FO emulsions and spray-dried microcapsules in which sugar beet pectin is used as the encapsulant material.

KEYWORDS: *EDTA, extra virgin olive oil, emulsion, omega-3, oxidation, spray drying, sugar beet pectin*

1. INTRODUCTION

Extra virgin olive oil (EVOO) obtained by mechanical cold-pressing of ripe olive fruits (*Olea europea* L.) is recognized for its health benefits. In particular, it lowers the incidence of diseases associated with oxidative stress (e.g., certain cancers, cardiovascular diseases, and atherosclerosis).[1] The importance of EVOO is mainly attributed to a high proportion of monounsaturated fatty acids (FAs), namely, oleic acid (C18:1ω-9), and an abundance of natural antioxidants, including tocopherols, carotenoids, flavonoids, and phenolic compounds.[2] Several classes of phenolic compounds have been identified in EVOO, including secoiridoids (aglyconic derivatives of oleuropein), phenylethyl alcohols (tyrosol and hydroxytyrosol), lignans, and phenolic acids (hydroxycinnamic and hydroxybenzoic acid derivatives).[2,3] The phenolic compounds have a strong natural antioxidant activity, attributed to both their radical-scavenging and metal-chelating properties, which contribute to the excellent oxidative stability of EVOO.[3,4]

Dietary omega-3 (ω-3) polyunsaturated fatty acids (PUFAs) are associated with various health benefits, including a decreased risk of cardiovascular diseases, inflammatory diseases, mental illnesses, and certain cancers.[5] Fish oil (FO) is an important dietary source of the ω-3 PUFAs eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6w-3). However, ω-3 FAs are extremely susceptible to oxidation, resulting in a rapid decrease in palatability, nutritional quality, and shelf life of foods into which they are directly incorporated. Microencapsulation of FO prior to its incorporation into food offers the possibility to improve its oxidative stability.[6]

Microencapsulation of lipophilic food ingredients typically involves spray-drying an oil-in-water emulsion, stabilized by a suitable emulsifier. Sugar beet (*Beta vulgaris* L.) pectin (SBP) displays excellent emulsifying activity,[7,8] is an established food ingredient, and shows potential as an encapsulant material.[9−11] However, commercial SBP extracts contain relatively high concentrations of metal ions, which catalyze lipid oxidation.[10,11] Therefore, oil-in-water emulsions prepared using SBP require the presence of an antioxidant to improve their oxidative stability.

In a previous study, we showed that spray-dried microcapsules containing a 1:1 blend of FO−EVOO prepared from emulsions (pH 3) had oxidative stability similar to that of FO spray-dried microcapsules when stored under ambient conditions, despite endogenous antioxidants present in the olive oil.[12] When commercial SBP with its high concentrations of metal ions is used, it may be expected that there would be benefits from the incorporation of metal-chelating agents to overcome the catalytic effects of metal ions on oil oxidation. The efficacy of the chelating agents is dependent on the type of chelating agent used and also the food system to which it is added.[13] The addition of metal-chelating agents to overcome the prooxidant behavior of metal ions associated with SBP has not yet been investigated. Ethylenediaminetetraacetic acid (EDTA), a multidentate ligand, has been shown to be an effective

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inhibitor of metal-catalyzed lipid oxidation in oil-in-water emulsions.[14] EDTA acts as an antioxidant by sequestering the metal ions and preventing their interaction with lipid hydroperoxides.[15]

The objective of this study was to investigate the protective effects of EDTA and EVOO on lipid oxidation in SBP-stabilized FO-in-water emulsions, as well as the spray-dried microcapsules made from these emulsions. Our previous study was conducted in emulsions at pH 3.[12] In this study we chose pH 6, because increasing the pH from 3 would make the drying of the emulsions in commercial practice more attractive; drying of fluids at pH <5 is not desirable because it exposes the spray-dryer to corrosion. Furthermore, it is reasonable to expect that the protective effects against metal ion-induced lipid oxidation will be most apparent at acidic pH due to increased iron solubility at lower pH.[16] The oxidative stabilities of the emulsions and spray-dried microcapsules, as assessed by the induction period for oxidation and rate of oxygen consumption post-induction, were measured under accelerated conditions. Additionally, the oxidative stability of spray-dried microcapsules was assessed during long-term storage under ambient conditions, where changes in the concentration of headspace volatiles (hexanal and propanal) and FA composition were used as indicators of lipid oxidation.

2. MATERIALS AND METHODS

2.1. Materials. FO (Hi-DHA 25N), with a peroxide value of 1.4 mequiv O_2 kg^{-1} and a total ω-3 content of 35% (26% DHA and 6% EPA) according to the manufacturer's specifications, was obtained from Nu-Mega Ingredients, Altona North, VIC 3025, Australia. The specification sheet from the supplier indicated that the FO contained the antioxidant tocopherol (2.1 g kg^{-1}). As previously determined, the FO contained 21.09% palmitic acid (C16:0), 13.51% oleic acid (C18:1), 6.64% EPA, and 26.54% DHA.[12]

EVOO (Premium Harvest), having a peroxide value of <15 mequiv O_2 kg^{-1} according to the manufacturer's specifications, was procured from Boundary Bend Marketing Pty Ltd., Lara, VIC 3212, Australia. The EVOO used was primarily made up of 6.99% palmitic acid (C16:0) and 79.54% oleic acid (C18:1).[12] The total phenolic content (expressed as caffeic acid equivalents) of the EVOO used was 99.3 ± 2.55 mg kg^{-1}.[12]

SBP (GENU pectin type BETA, of ≥60 kDa and degree of acetylation of 23.8% according to the manufacturer's specifications) was kindly donated by CP Kelco, Cheltenham, VIC 3192, Australia. The SBP used was previously determined to have 4.9 ± 0.05% w/w protein.[12] The iron (310 ppm) and copper (10 ppm) contents in SBP were determined by Dairy Technical Services (DTS Food Laboratories, South Kensington, VIC 3031, Australia). Dried glucose syrup (DGS) (with dextrose equivalent of 26−30 according to the manufacturer's specifications) was obtained from Manildra (Manildra Harwood Sugars, Harwood Refinery, Harwood Island, NSW 2465, Australia). FO and EVOO were stored under nitrogen in dark bottles at 4 °C (up to 6 months) or at −20 °C (6−24 months). All other chemicals were of analytical grade.

2.2. Preparation and Drying of Emulsions. Emulsions (30% total solids; 2% SBP:13% DGS:15% oil) containing FO or FO−EVOO (1:1) were prepared as previously described[12] except that the emulsions were adjusted from pH 3 (their natural pH) to pH 6 with 1 M NaOH. When EDTA was used, it was added to the emulsions at a level of 0.05% w/v. The level of EDTA per 100 g of emulsion (1.3×10^{-4} mol) was in excess of the levels of Fe (0.1×10^{-4} mol) and Cu (0.003×10^{-4} mol) contributed from the SBP component in the emulsions. This ensures that all of the metal ions were complexed by EDTA.

The homogenized emulsions were spray-dried using a Drytec laboratory spray-dryer (Tonbridge, U.K.) with a twin fluid nozzle at 2.5 bar atomizing pressure. The oil-in-water emulsions were heated to 60 °C prior to atomization. The inlet and outlet air temperatures of the spray-dryer were 180 and 80 °C, respectively. Two independent manufacturing trials were carried out, yielding a total of eight spray-dried microcapsules. The emulsions and microcapsules prepared were characterized using the procedures described in sections 2.3−2.5.

2.3. Characterization of Emulsions: Particle Size and Zeta Potential. The particle size distributions of the emulsions were determined by laser light scattering (Mastersizer 2000G, Malvern Instruments Ltd., Worcestershire, U.K.) using standard optical parameters as previously described.[12] Results are given in surface-weighted diameter, $d_{3,2} = (\Sigma n_i d_i^3/\Sigma n_i d_i^2)$.

The zeta potential (ξ) on the oil droplets at 22 °C was determined using a Nano Zetasizer (Malvern Instruments Ltd.). The emulsion (7 μL) was diluted in water (20 mL, pH 6) and the zeta potential measured over the pH range of 1−6. The pH was adjusted using a titrator (Autotitrator-MPT-2, Malvern Instruments Ltd.).

2.4. Characterization of the Spray-Dried Microcapsules. The moisture contents of the spray-dried microcapsules (4 g) were determined (MA30, Sartorius Mechatronics, Gottingen, Germany) at 80 °C. The a_w was determined (Aqua-Lab Water Activity Meter, series 3, Decagon Devices Inc.) at 25 °C. The particle size of the spray-dried microcapsules was measured after reconstitution of the powders in water. A powder dispersion (10% w/v) was stirred at 70 °C for 3 h and then rested for 1 h at room temperature (~25 °C) prior to particle size measurements.[12]

The total oil contents of the microcapsules were determined according to the Schmid−Bondzyndki−Ratzlaff method (AS 2300.1.3).[17] The solvent-extractable fat was estimated using a method based on that of Pisecky[18] except that petroleum ether replaced carbon tetrachloride, as previously described.[12] The microencapsulation efficiency (ME) was calculated as follows:

$$\text{ME}\ (\%) = 100 - (\%\text{solvent-extractable oil}/\%\ \text{total oil}) \times 100 \quad (1)$$

2.5. Oxidative Stability. The oxidative stability of the bulk oils, emulsions, and spray-dried microcapsules was measured under accelerated conditions. The oxidative stability of the spray-dried microcapsules was also assessed during long-term storage at ambient conditions.

2.5.1. Under Accelerated Conditions. The oxidative stability of the bulk oils, emulsions, and spray-dried microcapsules (equivalent to 4 g of total oil) was assessed at 80 °C under oxygen pressure (5 bar) in an ML Oxipres apparatus (DK-8270, Mikrolab Aarhus A/S, Højbjerg, Denmark) installed with Paralog software. The induction period (IP) was determined. The slope (−mbar h^{-1}) was the oxidation rate.

2.5.2. Under Ambient Storage Conditions. The spray-dried microcapsules (20 g) were stored for 0, 1, 2, and 3 months in transparent, stoppered, oxygen-permeable plastic containers (100 mL volume, 10.8 cm length × 4 cm width) under ambient conditions (~25 °C, a_w = 0.5). At each time point the contained samples were transferred to frozen storage (−18 °C), prior to analysis of FA composition of the extracted oil and headspace volatiles of the spray-dried microcapsules.

The FA composition of oil extracted with isopropanol/hexane under mild conditions was determined by gas chromatography (GC), as previously described.[12]

Propanal and hexanal analyses were performed on 2 g of the microcapsules that were placed in 10 mL headspace vials, sealed, and equilibrated at 60 °C for 10 min in a water bath. Headspace propanal and hexanal concentrations were measured in 1 mL of the headspace using GC, as previously described.[12] Quantification of propanal and hexanal was determined from peak areas using propanal (R^2 = 0.9724) and hexanal (R^2 = 0.9580) standard curves covering the range of the respective volatile contents found in the headspace of the microcapsules and were respectively prepared from propanal and hexanal in water.

2.6. Statistics. All analytical determinations were carried out in duplicate with at least two measurements within each run. The results were reported as the mean ± standard deviation (SD) of these measurements. Analysis of variance (ANOVA) and least significant

difference comparisons between sample means were performed using SPSS 18 (SPSS Inc., Chicago, IL).

3. RESULTS AND DISCUSSION

3.1. Properties of Emulsions and Spray-Dried Microcapsules. *3.1.1. Emulsions.* The average oil droplet size ($d_{3,2}$) of all emulsions was 0.35 ± 0.00 μm. This result was similar to that previously obtained for the emulsions at pH 3.[12] Furthermore, this result concurred with observations reported by others, showing SBP is an effective emulsifier for stabilization of oils.[8,10]

The SBP-stabilized oil droplets had an isoelectric point (p*I*) of 1.55. Thus, the droplets were more negatively charged at pH 6 (ξ-potential = −41.5 ± 2.12 mV) than pH 3 (ξ-potential = −14.0 ± 1.41 mV). The less negative charge at pH 3 is expected because of the protonation of the carboxyl groups on pectin as the pH is decreased.

3.1.2. Spray-Dried Microcapsules. After manufacture, the moisture contents and a_w of all spray-dried microcapsules were 2.9−3.0% and 0.29−0.30, respectively. The measured total oil content of all spray-dried microcapsules was 49.5%. ME was 90% for all spray-dried microcapsules, which was similar to that obtained by Drusch.[9] The nonencapsulated oil (10%), which is primarily located on the surface and within pores and cracks within the microcapsules, was similar irrespective of the formulation. Whereas surface fat has been associated with increased oxidation, it cannot be used to predict the shelf life of microencapsulated oils.[19] However, a high ME is important for oils rich in PUFAs as the volatile secondary lipid oxidation products of these oils (e.g., propanal) have a low sensory threshold, which may limit the shelf life of the final product. The similar ME between spray-dried microcapsules prepared from emulsions at pH 6 (this work) and that found previously for SBP microcapsules of similar gross oil composition spray-dried from pH 3 emulsions[12] shows that the encapsulation efficiency of the SBP was not affected by adjustment of pH in the range 3−6, suggesting that the increased negative charge of SBP pectin as pH is raised does not compromise the emulsifying potential of the pectin or its ability to form robust films on drying. The average oil droplet size ($d_{3,2}$) of spray-dried microcapsules reconstituted in water was ∼0.8−0.9 μm. Propanal was not detected in any of the microcapsules prior to storage.

The properties of the spray-dried microcapsules changed during storage. The moisture content increased to 3.5% after 3 months of storage and the a_w to 0.5, due to equilibration with the ambient humidity (a_w ∼ 0.5). The a_w of the environment is higher than the ideal a_w range of 0.2−0.4 for lipid oxidation storage stability reported elsewhere.[20] The particle size distribution of the reconstituted dispersions of the spray-dried microcapsules increased on storage (Figure 1). These results are similar to those obtained previously for FO and FO−EVOO spray-dried microcapsules prepared from pH 3 emulsions.[12] These findings were ascribed to destabilization of the emulsion droplets during drying and to aggregate formation on storage of the spray-dried microcapsules.[12] The reconstituted microcapsules containing the oil blends (FO−EVOO and FO−EVOO−EDTA) showed a larger particle size distribution on reconstitution than the FO-only formulations after 3 months of storage. This result may be attributed to differences in the composition and structure of the interfaces due to the surface-active molecules in the olive oil, including mono- and diglycerides, fatty acids, peptides, phospholipids, and phenolic compounds, at the time of drying. The physical and chemical properties of

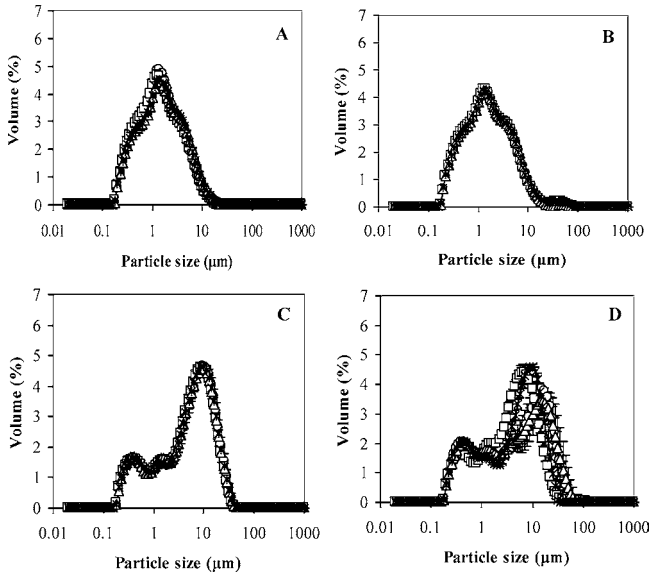

Figure 1. Particle size distributions of fish oil and fish oil−extra virgin olive oil spray-dried microcapsules in water after storage for (A) 0, (B) 1, (C) 2, and (D) 3 months under ambient conditions (∼25 °C, a_w = 0.5): (□) FO; (∗) FO with added EDTA; (○) FO−EVOO; (△) FO−EVOO with added EDTA. Each value is the mean of triplicate measurements ± SD from two independent manufacturing trials.

the interface can influence the density and thickness of the interfacial layer. The increase in moisture content and a_w of the microcapsules with increased storage duration may have affected the mobility of the interfacial components present in the microencapsulated FO−EVOO, resulting in partial coalescence of the oil droplets within the matrix on prolonged storage.

3.2. Oxidative Stability of Emulsions under Accelerated Conditions. FO and FO−EVOO emulsions (pH 6) had similar IP values. However, oxygen consumption was higher for FO−EVOO than for FO emulsions, indicating that incorporation of olive oil did not protect the FO emulsions from oxidation (Table 1). Minor components in the commercial oils, such as phospholipids, phenolic compounds, and free FAs, can potentially affect lipid oxidation rates. The

Table 1. Oxipres Data Showing Oxidative Stability of Fish Oil and Fish Oil−Extra Virgin Olive Oil Emulsions (pH 6) and Corresponding Spray-Dried Microcapsules Exposed to 80 °C and Oxygen Pressure of 5 bar[a]

sample	induction period (h)	slope (mbar h^{-1})
Emulsions		
FO	3.2 ± 0.28 a	−24.0 ± 7.07 ab
FO with EDTA	13.9 ± 0.14 b	−16.5 ± 2.12 ab
FO−EVOO	3.9 ± 0.28 a	−31.5 ± 3.54 a
FO−EVOO with EDTA	23.7 ± 0.49 c	−11.5 ± 4.95 c
Spray-Dried Microcapsules		
FO	7.4 ± 0.21 a	−480.5 ± 4.95 a
FO with EDTA	10.0 ± 0.28 b	−421.5 ± 6.36 b
FO−EVOO	12.7 ± 0.14 c	−97.0 ± 16.97 c
FO−EVOO with EDTA	19.7 ± 1.06 d	−131.5 ± 3.54 d

[a]Each value is the mean of duplicate measurements ± SD from two independent manufacturing trials. Means within columns followed by different letters are significantly different (P < 0.05).

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effects of minor components on oxidation depend on how the components partition in the oil and water phase and their location at the interface.[21] Interfacial phospholipids produce an emulsion droplet, which hastens lipid oxidation.[22] Free FAs also display prooxidant activity.[23] The physicochemical properties of surface-active agents can affect packing of emulsifiers at the oil−water interface, which may influence the diffusion of free radicals, prooxidants, and oxygen through the interfacial layer.[24] The incorporation of EDTA significantly ($P < 0.05$) increased the IP and lowered the oxygen consumption for FO and FO−EVOO emulsions (Table 1). EDTA was more effective at arresting oxidation in emulsions containing EVOO. Studies have demonstrated that EDTA removes metal ions from the surface of emulsion droplets.[16,25]

The oxidation of lipids in emulsion systems is initiated at the oil droplet interface. Numerous variables (e.g., pH, a_w, type of emulsifier, oxygen availability, oil droplet size, and thickness and composition of the interface) can influence the rate and extent of lipid oxidation and the efficacy of different antioxidants in emulsion-based systems.[14,25−27] It has also become evident that the antioxidant activity of phenolic compounds in oil-in-water emulsions depends on several parameters, such as their structure (i.e., number and position of hydroxyl groups), polarity, location (i.e., water, oil, interface), and radical-scavenging and metal-chelating attributes, as well as electron/hydrogen-donating capacity.[28]

Our results confirm the complex interplay between pH, antioxidants (i.e., phenolic antioxidants in olive oil, EDTA), and prooxidants (e.g., Fe and Cu) regarding oxidation in emulsion-based systems. It is not possible to ascribe the relative influence of each of the interactions between the emulsifier used (i.e., SBP), metal ions, antioxidants, and prooxidants to the observed oxidation behavior of the emulsions. However, it is instructive to consider how these factors affect oxidation. The effects of pH on oxidative stability of FO and FO−EVOO emulsions may be obtained by comparing the results of this work to those of previous work on pH 3 FO and FO−EVOO emulsions having the same gross compositions.[12] The IPs of the pH 6 FO and FO−EVOO emulsions (3.2−3.9 h) (Table 1) were shorter than those of pH 3 FO and FO−EVOO emulsions of the same gross composition (4.6−4.7 h).[12] However, oxygen consumption for the corresponding emulsions did not follow a consistent trend: the values were 24.0 and 31.5 mbar h^{-1} for FO and FO−EVOO pH 6 emulsions, respectively (Table 1) and 38.0 and 27.0 mbar h^{-1} for FO and FO−EVOO pH 3 emulsions, respectively.[12] The pH can influence lipid oxidation by affecting the solubility of metal ions, as well as the complexation of metal ions by biopolymer encapsulants having ionizable functional groups. The pH can also alter the dissociation of the functional groups of antioxidants and thereby their partitioning between the oil and bulk phase or into micelles.

A number of counteracting effects on oxidative stability come into play when the pH is changed. Among these, the charge of the emulsifier is an important factor in lipid oxidation due to electrostatic interactions with the positively charged metal ions. When the pH is decreased from 6 to 3, SBP loses some of its negative charge as confirmed by ξ measurement, above. Hu et al.[29] demonstrated that oxidation in salmon oil-in-water emulsions stabilized by whey proteins was retarded at pH < p*I*, due to increased repulsion of metal ions from the positively charged interface. Consideration of this factor alone suggests that at pH 6 the metal ions would be more concentrated at the droplet surface and therefore facilitate oxidation. This may explain in part the longer IP values for FO and FO−EVOO emulsions at pH 3[12] than at pH 6 (this work). Another factor that possibly contributed to the higher oxidative stability of the emulsions at pH 3, compared to pH 6, is the increased partitioning of antioxidants (e.g., phenolics with carboxylic acid groups such as caffeic and vanillic acid that are present in EVOO; tocopherols present in FO used) into the oil phase at lower pH. Previous work has demonstrated that when phenolics are retained in the emulsion droplet, they are more effective antioxidants.[30]

In contrast, other studies have shown that low pH exerts a pro-oxidative effect in oil-in-water emulsions.[22,26] The increased solubility of iron was suggested to be partly responsible for the rapid oxidation of emulsions at low pH. In the absence of EDTA, metal ions, such as iron and copper, have been shown to decrease the antioxidant effectiveness of EVOO phenolics in oil-in-water emulsions.[31−33] These studies showed that in specific circumstances (e.g., pH <6), the ability of the phenolic compounds to act as antioxidants by chelating metal ions was less effective due to their enhanced ability to reduce metal ions back to their most prooxidant state. However, in the current findings (Table 1) it appears that the potential prooxidant activity of phenolics in the presence of iron and copper (associated with SBP) was not a major factor influencing the relative rates of oxidation of FO−EVOO emulsions at either pH 3 or 6.

The results showed that EDTA had a significant ($P < 0.05$) protective effect on FO and FO−EVOO emulsions at pH 6 (Table 1). The protection is attributed to the chelation of metal ions (Fe and Cu) by EDTA. The protective effect of EDTA in SBP-stabilized emulsions suggests that the metal ions were a major contributor to the oxidation of these emulsions. It was noted that the protective effect of EDTA was more pronounced in FO−EVOO than in FO emulsions. Although phenolic components of olive oil typically show antioxidative behavior in metal ion-free systems, they may become prooxidant in the presence of ferric and copper ions,[32,33] although the pH also has an effect (above). In emulsions that contain EDTA at concentrations in excess of the metal ions, as was the case in this study, the higher chelating power of EDTA compared with the complexing power of the phenolics in EVOO and functional side groups of SBP results in the unavailability of metal ions for complexation with these other components. Under conditions when the phenolics do not complex with metal ions (e.g., Fe and Cu), the phenolic compounds act as antioxidants. This is consistent with the larger protective effect of EDTA in FO−EVOO than in FO emulsions. Incorporation of EDTA resulted in a 6-fold increase in IP and a 2.7-fold decrease in oxygen consumption in FO−EVOO emulsions compared to a 4.3-fold increase in IP and a 1.5-fold decrease in oxygen consumption in FO emulsions (Table 1).

3.3. Oxidative Stability of Spray-Dried Microcapsules under Accelerated Conditions. In the case of spray-dried microcapsules made from pH 6 emulsions, the FO spray-dried microcapsules (with and without added EDTA) had lower oxidative stability than the FO−EVOO spray-dried microcapsules (with and without EDTA). The incorporation of EDTA improved the oxidative stability of the FO, as indicated by the shorter IP and higher oxygen consumption of FO spray-dried microcapsules without EDTA. Interestingly, although EDTA addition increased the IP of FO−EVOO spray-dried microcapsules, it increased oxygen consumption (Table 1). The FO and FO−EVOO spray-dried microcapsules prepared without

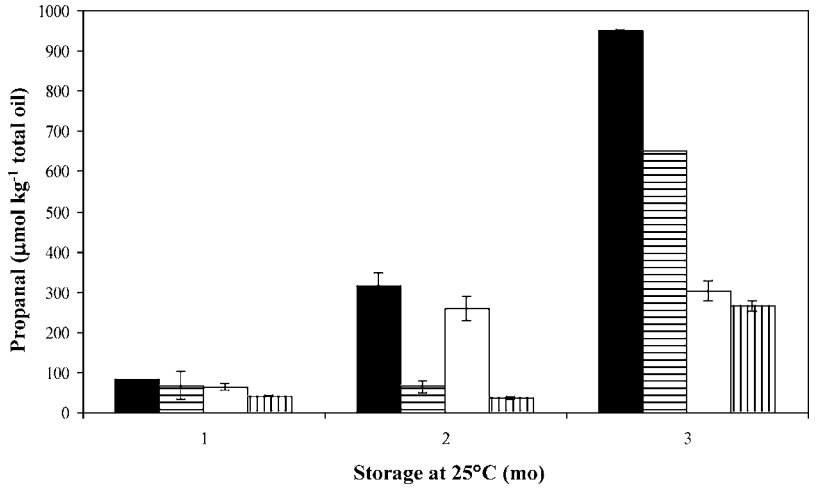

Figure 2. Headspace propanal in fish oil and fish oil−extra virgin olive oil spray-dried microcapsules during storage (1−3 months) under ambient conditions (~25 °C, a_w = 0.5): (solid bars) FO; (horizontally striped bars) FO with EDTA; (white bars) FO−EVOO; (vertically striped bars) FO−EVOO with EDTA. Each value is the mean of duplicate values ± SD from two independent manufacturing trials;

addition of EDTA had longer induction periods compared to their respective emulsions. However, the FO and FO−EVOO spray-dried microcapsules with added EDTA had lower oxidative stability than the corresponding emulsions with EDTA, showing that EDTA was more effective at retarding oxidation in emulsions than in spray-dried microcapsules (Table 1).

The IP values for FO and FO−EVOO spray-dried microcapsules (7.4 and 12.7 h, respectively) obtained from spray-drying pH 6 emulsions (Table 1) were similar to those of spray-dried microcapsules prepared from corresponding pH 3 FO and FO−EVOO emulsions (6.95 and 11.95 h, respectively).[12] However, after the IP, the oxygen consumption was much higher for spray-dried microcapsules from pH 3 FO and FO−EVOO emulsions (2350 and 792 mbar h^{-1})[12] compared to that for spray-dried microcapsules made from pH 6 emulsions (480 and 97 mbar h^{-1}, respectively). This shows that changing the pH of emulsions prior to drying results in altered partitioning of metal ions and phenolic components. Differences in the physical location of key components within an emulsion prior to drying might be expected to influence the location of these components in the spray-dried microcapsule, such that they may be trapped in different positions within the spray-dried microcapsule particle. This possibly accounts for the differences in oxidative stability of spray-dried microcapsule obtained by drying emulsions from different pH values. However, irrespective of the pH of the emulsions prior to drying, a beneficial effect of partial substitution of FO with olive oil was observed under conditions that promoted accelerated oxidation.

The addition of EDTA resulted in a 4.3-fold increase in IP and a 1.5-fold decrease in oxygen consumption in FO emulsions but only a 1.4-fold increase in IP and a 1.1-fold decrease in oxygen consumption for FO spray-dried microcapsules (Table 1). The corresponding values for FO−EVOO were a 6-fold increase in IP and a 2.7-fold decrease in oxygen consumption in FO−EVOO emulsions compared to a 1.6-fold increase in IP but, surprisingly, a 1.4-fold increase in oxygen consumption for FO−EVOO spray-dried microcapsules (Table 1). EDTA was more protective in emulsions because EDTA and the pro-oxidative metal ions originally associated with SBP would be co-located in the aqueous phase of the oil-in-water emulsions as EDTA-metal ion complexes. The polar EVOO phenolics would also be present in the aqueous phase. Therefore, it is likely that, in the case of FO and FO−EVOO emulsions with added EDTA, the shared location and high level of contact between the prooxidants and antioxidants in the aqueous phase of the liquid emulsions were effective at preventing lipid oxidation. This is in contrast to the spray-dried microcapsule state, where components are trapped within a glassy matrix and may be isolated from one another.

3.4. Oxidative Stability of Spray-Dried Microcapsules under Ambient Storage Conditions. *3.4.1. Headspace Analysis.* Propanal was not detected in any of spray-dried microcapsules immediately after manufacture. During storage at ambient conditions the concentration of propanal generally increased with increased storage duration. The extent of oxidation was less in spray-dried microcapsules that had been formulated with EDTA than in those prepared without EDTA, for equivalent oil compositions (Figure 2).

Hexanal was detected in stored spray-dried FO microcapsules (3.4 and 14.2 μmol kg^{-1} oil after 2 and 3 months of storage, respectively) and FO−EVOO microcapsules (11.4 and 12.2 μmol kg^{-1} oil after 2 and 3 months of storage, respectively). At 3 months of storage, hexanal levels were lower in FO−EVOO microcapsules when EDTA was present (4.86 μmol kg^{-1} oil) but higher in FO microcapsules formulated with EDTA (34 μmol kg^{-1} oil), compared to corresponding spray-dried microcapsules without EDTA The relatively late evolution and low concentrations of hexanal, in comparison to propanal, could be expected as monounsaturated FAs are approximately 10−40-fold less susceptible to oxidation than PUFAs,[34] and this was confirmed in the FA composition analysis (Table 2). Interestingly, spray-dried FO microcapsules with EDTA produced the highest amount of hexanal on storage. EDTA has been found to have a prooxidant effect on the production of particular volatiles, including hexanal, in ω-3-enriched oil-in-water emulsions stabilized by lecithin and caseinate at pH 7.[22] Mahoney and Graf[15] suggested that the EDTA−metal ion complex could act catalytically in the presence of these emulsifiers due to the availability of a free coordination site that is available for redox reactions. This could explain the increased amount of headspace hexanal found in the FO−EDTA formulation in comparison to the other formulations.

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Table 2. Fatty Acid Composition (Percent) of Fish Oil and Fish Oil−Extra Virgin Olive Oil Spray-Dried Microcapsules during Storage (0−3 Months) under Ambient Conditions (~25 °C, a_w = 0.5)[a]

	storage time							
fatty acid	0 months	1 month	2 months	3 months	0 months	1 month	2 months	3 months
	FO				FO with Added EDTA			
C16:0	19.10 ± 0.57	19.48 ± 0.65	20.37 ± 0.11	20.16 ± 0.38	19.50 ± 0.66	19.66 ± 0.35	20.13 ± 0.50	19.71 ± 0.44
C18:1	17.38 ± 2.29	16.95 ± 2.88	18.16 ± 2.70	18.74 ± 2.29	14.71 ± 0.72	13.56 ± 0.28	15.06 ± 0.85	15.36 ± 1.93
C20:5ω-3	5.82 ± 0.06	5.64 ± 0.02	5.36 ± 0.05	5.08 ± 0.06	6.06 ± 0.24	6.44 ± 0.33	5.89 ± 0.21	5.48 ± 0.35
C22:6ω-3	23.28 ± 0.15	22.30 ± 0.37	20.43 ± 0.60	18.82 ± 0.18	24.13 ± 0.18	25.05 ± 0.33	22.52 ± 0.16	20.57 ± 1.33
DHA:EPA	4.00	3.95	3.81	3.70	3.98	3.89	3.82	3.76
others	34.42 ± 1.66	34.98 ± 1.85	35.84 ± 1.94	37.20 ± 2.00	35.52 ± 0.36	35.30 ± 0.27	36.65 ± 0.29	38.88 ± 1.90
	FO−EVOO				FO−EVOO with Added EDTA			
C16:0	15.76 ± 0.23	15.86 ± 0.13	16.21 ± 0.21	16.25 ± 0.08	15.30 ± 1.02	16.31 ± 0.25	16.39 ± 0.55	16.56 ± 0.78
C18:1	40.26 ± 1.21	41.18 ± 1.54	42.19 ± 0.99	42.10 ± 0.90	35.88 ± 2.09	40.40 ± 0.68	39.80 ± 0.37	40.70 ± 2.01
C20:5ω-3	3.17 ± 0.28	3.04 ± 0.26	2.84 ± 0.15	2.84 ± 0.12	3.69 ± 0.08	3.22 ± 0.24	3.22 ± 0.14	3.19 ± 0.04
C22:6ω-3	12.52 ± 0.68	11.88 ± 0.61	10.77 ± 0.08	10.53 ± 0.38	14.39 ± 0.25	12.66 ± 0.68	12.60 ± 0.03	12.11 ± 0.11
DHA:EPA	3.95	3.91	3.79	3.71	3.90	3.93	3.91	3.80
others	28.30 ± 0.09	28.04 ± 0.54	27.99 ± 0.55	28.28 ± 0.96	30.74 ± 2.78	27.99 ± 0.51	27.99 ± 0.33	27.44 ± 2.86

[a]Each value is the mean of duplicate measurements ± SD from two independent microcapsule manufacturing trials.

A comparison of the propanal data for 50% oil microcapsules made by spray-drying pH 6 emulsions (this work) with that obtained for 50% oil spray-dried microcapsules prepared from pH 3 emulsions[12] showed that propanal values after 3 months of storage were similar for FO microcapsules dried from emulsions at pH 6 (950.0 μmol kg^{-1} total oil) and pH 3 (989.7 μmol kg^{-1} total oil). However, propanal values were higher for FO−EVOO spray-dried microcapsules made from emulsions at pH 3 (1115.5 μmol kg^{-1} total oil) compared to pH 6 (303.4 μmol kg^{-1} total oil). This shows the interaction between pH of the emulsion at the time of spray-drying and oil composition. Whereas the oxidative stability of the FO spray-dried microcapsules was not affected by the pH of the emulsion prior to drying, that of FO-EVOO spray-dried microcapsules was improved when the pH of the emulsions was raised from 3 to 6, prior to drying.

3.4.2. Fatty Acid Composition. The FA composition of the spray-dried microcapsules is provided in Table 2. As expected, the levels of EPA and DHA decreased on storage due to oxidation. Oxidation of the spray-dried microcapsules was affected by oil composition and the presence of EDTA (Table 2).

DHA was generally more sensitive to oxidation than EPA throughout the storage duration. The higher rate of DHA autoxidation in comparison to EPA may be due to DHA being more unsaturated. It could also be expected that DHA will outcompete EPA for any available oxygen as the initial content of DHA in the FO is higher than that of EPA. The presence of EDTA retarded the oxidation of EPA and DHA, indicating that the metal ions were partially responsible for PUFA oxidation in both FO and FO−EVOO spray-dried microcapsules.

In conclusion, we have demonstrated that the oxidative stability of SBP-stabilized emulsions and spray-dried microcapsules formulated with FO and a FO−EVOO blend were improved by the addition of EDTA. The incorporation of EDTA was more beneficial in emulsions compared to spray-dried microcapsules. Lipid oxidation and antioxidant mechanisms in multiphasic systems are complex phenomena. More research is required to understand the interplay between oxidation reactions and antioxidant reactivities in heterophasic systems.

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