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Impact of iron encapsulation within the interior aqueous phase of water-in-oil-in-water emulsions on lipid oxidation

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ABSTRACT

Iron (Fe^{3+}) was encapsulated within the internal aqueous phase of water-in-oil-in-water (W/O/W) emulsions, and then the impact of this iron on the oxidative stability of fish oil droplets was examined. There was no significant change in lipid droplet diameter in the W/O/W emulsions during 7 days storage, suggesting that the emulsions were stable to lipid droplet flocculation and coalescence, and internal water diffusion/expulsion. The initial iron encapsulation (4 mg/100 g emulsion) within the internal aqueous phase of the water-in-oil (W/O) emulsions was high (>99.75%), although, a small amount leaked out over 7 days storage (≈10 μg/100 g emulsion). When W/O/W emulsions were mixed with fish oil droplets the thiobarbituric acid-reactive substances (TBARS) formed decreased (compared to fish oil droplets alone) by an amount that depended on iron concentration and location, i.e., no added iron < iron in external aqueous phase < iron in internal aqueous phase. These differences were attributed to the impact of W/O droplets on the concentration and location of iron and lipid oxidation reaction products within the system.

1. Introduction

Water-in-oil-in-water (W/O/W) emulsions consist of water droplets dispersed within oil droplets, which are themselves dispersed within an aqueous continuous phase (Benichou, Aserin, & Garti, 2004; Garti & Benichou, 2004; Khan, Talegaonkar, Iqbal, Ahmed, & Khar, 2006; Tedajo, Seiller, Prognon, & Grossiord, 2001; Vladisavljevic & Williams, 2005). W/O/W emulsions have a number of potential benefits over conventional oil-in-water (O/W) emulsions for certain applications, such as reduction of fat content, flavor masking, controlled or triggered release, and protection of labile ingredients (Benichou et al., 2004; Garti & Benichou, 2004; Garti & Bisperink, 1998; Muschiolik, 2007; Owusu, Zhu, & Dickinson, 1992). Many researchers have suggested that W/O/W emulsions can be used to encapsulate functional food components within the interior aqueous phase (Garti & Benichou, 2004; McClements, Decker, & Weiss, 2007; Muschiolik, 2007; Owusu et al., 1992). However, there are many challenges to their widespread application within the food industry because the internal water phase may migrate into the external aqueous phase, either due to expulsion of entire water droplets or due to diffusion of water molecules (Benichou et al., 2004; Garti, 1997a, 1997b). Previously, our laboratory developed W/O/W emulsions that were specifically designed to have improved resistance to internal water droplet expulsion and diffusion (Surh, Vladisavljevic, Mun, & McClements, 2007). This was achieved by preparing W/O/W emulsions with an internal aqueous phase that contained a concentrated whey protein isolate solution. In this study, we used similar systems to encapsulate iron within the internal aqueous phase of W/O/W emulsions.

Iron is one of the major pro-oxidants in foods because of its high chemical reactivity and abundance (Cho, Alamed, McClements, & Decker, 2003). In order to promote lipid oxidation in emulsions iron must come into close proximity with the lipid substrate (McClements & Decker, 2000). If iron can be prevented from coming into contact with the lipid substrate, then the stability of the lipid phase to oxidation can be greatly increased. It is for this reason that water-soluble chelators (such as ethylenediaminetetraacetic acid (EDTA), citrates, and some proteins) can inhibit lipid oxidation in O/W emulsions by binding aqueous phase iron and preventing it from accessing emulsified lipid substrates (Mahoney & Graf, 1986; McClements & Decker, 2000). In this study, we investigated an alternative strategy of physically isolating iron from an emulsified oxidatively unstable lipid substrate. The iron was encapsulated within the internal aqueous phase of a W/O/W emulsion prepared with a lipid phase that is relatively stable to lipid oxidation, i.e., corn oil (Fig. 1). We hypothesised that if this W/O/W emulsion was then mixed with an O/W emulsion containing oxidatively unstable lipid droplets (e.g., fish oil) the encapsulated iron should be physically isolated from these droplets and therefore reduce the tendency for oxidation to occur. This kind of delivery system may be useful for preparing food products that are high in both polyunsaturated lipids and iron.

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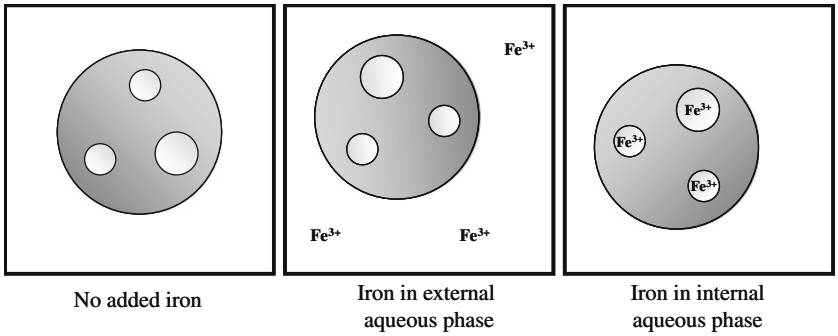

Fig. 1. Schematic representation of W/O/W emulsions containing no iron, iron encapsulated within the internal aqueous phase or iron present in the external aqueous phase.

2. Materials and methods

2.1. Emulsion preparation

2.1.1. Preparation of W/O emulsions

An oil phase was prepared by dispersing 8 wt% polyglycerol polyricinoleate (PGPR, 4150, Palsgaard, Morristown, NJ) into corn oil and heating to 50 °C. This PGPR concentration was selected because previous studies have shown that it is capable of forming W/O emulsions containing small water droplets with a narrow size distribution (Su, Flanagan, Hemar, & Singh, 2006; Surh et al., 2007). An aqueous phase was prepared by dispersing 15 wt% of whey protein isolate (WPI, BiPRO lot JE 259-7-440, Davisco Foods International, Le Sueur, MN) powder into 20 mM phosphate buffer solution (pH 7.0) containing 0.02 wt% sodium azide and either 0 or 0.1 wt% iron and stirring for at least 2 h at room temperature. The aqueous phase (20 wt%, 50 °C) was dispersed gradually into the oil phase (80 wt%, 50 °C) under agitation with a magnetic stirrer and then blended together using a high-speed blender (Tissu-Tearor, Biospec Products, Bartlesville, OK) for 2 min, followed by homogenisation with five passes through a micro-fluidizer (110L, Microfluidics, Newton, MA) at 42 ψ. After homogenisation, the emulsions were cooled to room temperature. The water droplets within this W/O emulsion were characterised by optical microscopy and dynamic light scattering (see below).

2.1.2. Preparation of W/O/W emulsions

A 20% W/O emulsion, prepared as described above, was homogenised with 80 wt% of aqueous surfactant solution (0.5 wt% Tween 20, 20 mM phosphate buffer, 0.02 wt% sodium azide, pH 7.0) using a membrane homogenizer. The W/O emulsions and aqueous surfactant solution were first premixed for 5 min using a stirring bar followed by five passes through a membrane homogenizer at 100 kPa (14.5 ψ) (MG-20-5, Kiyomoto Iron Works, Miyazaki, Japan). The pressure vessel was filled with 200 mL of coarse emulsion, and the required driving pressure was built up with compressed air using a pressure regulator (RPG101-120, Omega engineering, Stamford, CT). When the emulsion had passed through the membrane tube, it was collected into a beaker. The experiments were carried out at room temperature. The membrane used was a SPG (10 mm outer diameter × 0.8 mm wall thickness, mean pore size 8.0 μm) supplied from SPG Technology (Miyazaki, Japan). The emulsions were stored at room temperature for 24 h before being analysed. The final iron concentration in the W/O/W emulsions containing iron was 4 mg/100 g emulsion. After preparation the properties of the emulsions were analysed during storage for 1 week at room temperature.

2.1.3. Preparation of fish O/W emulsions

An O/W emulsion containing fish oil (an oxidatively unstable lipid phase) was also prepared by membrane homogenisation. This emulsion was prepared by passing a pre-mix of 16 wt% fish oil and 84 wt% of aqueous surfactant solution (0.5 wt% Tween 20, 20 mM phosphate buffer, 0.02 wt% sodium azide, pH 7.0) through the same membrane homogenizer using the same operating conditions as for the W/O/W emulsions described above.

2.1.4. Preparation of mixed W/O/W and fish O/W emulsions

The purpose of these experiments was to identify the impact of W/O/W emulsions containing encapsulated or non-encapsulated iron on the oxidative stability of O/W emulsions containing fish oil. Three different types of mixed emulsions were prepared by mixing different ratios of buffer solution (with or without iron), W/O/W emulsions (with or without encapsulated iron), and fish O/W emulsions:

(i) *Iron free system:* in this system, no iron was added to the emulsions.
(ii) *Encapsulated iron system:* in this system the iron was encapsulated within the internal aqueous phase of the W/O droplets, i.e., isolated from the fish oil droplets.
(iii) *Non-encapsulated iron system:* in this system, the iron was present within the external aqueous phase of the emulsion i.e., in close contact with the fish oil droplets.

After preparation, the mixed emulsion systems were stored at 37 °C for 1 week and the particle size and oxidative stability were measured.

2.2. Particle size measurement

Mean droplet diameters of O/W and W/O/W emulsions were measured using a static light scattering instrument. To avoid multiple scattering effects, W/O/W emulsions were diluted to a droplet concentration of approximately ≈0.005 wt% using buffer solution at the pH of the sample (pH 7) and stirred continuously throughout the measurements to ensure the samples were homogeneous. The particle size distribution of the emulsions was then measured using a static light scattering instrument (Mastersizer, Malvern Instruments, Worcestershire, UK). Particle size was reported as volume-surface mean diameter, $d_{32} = (\sum n_i d_i^3 / \sum n_i d_i^2$, where n_i is the number of particles with diameter d_i) and volume-weighted mean diameter, $d_{43} = (\sum n_i d_i^4 / \sum n_i d_i^3)$.

The mean particle sizes of W/O emulsions were measured using optical microscopy (see below) and dynamic light scattering. The W/O emulsions were diluted to a droplet concentration of ~0.5 wt% with hexadecane (refractive index = 1.434, viscosity = 3.13 mPa s at 25 °C) as a dispersant to avoid multiple scattering effects. The particle size of the emulsions was then measured at 25 °C using a dynamic light scattering instrument (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). This instrument measures the

rate of diffusion of particles via intensity fluctuations. Particle size was reported as the scattering intensity-weighted mean diameter, *z*-average.

2.3. Measurement of iron concentration

The aqueous phase of the emulsions was collected using a 3-mL disposable syringe. Due to their relatively large size the droplets in the emulsions formed a distinct cream layer at the top, which enabled the aqueous phase to be collected from the clear serum layer at the bottom. The removed aqueous phase was filtered through a 0.45-μm membrane filter (Millipore, Bedford, MA) to remove any residual oil droplets. Quantification of iron in the continuous phase of the W/O/W emulsions was determined spectrophotometrically as described previously (Diaz, Vattem, & Mahoney, 2002) with slight modification. A 0.5-mL aliquot of the filtered iron-containing sample was reduced with 0.5 mL of 10% hydroxylamine-hydrochloride in 0.25 N HCl for 15 min at room temperature, followed by the addition of 0.5 mL of 9.0 mM ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulphonic acid). After 10 min, the absorbance was measured at 562 nm, and concentrations were determined from a standard curve constructed using ferric chloride.

2.4. Optical microscopy

Emulsions were gently agitated in a glass beaker before analysis to ensure that they were homogeneous. A drop of emulsion was placed on a microscope slide and then covered with a cover slip. The microstructures of emulsions were then observed using a conventional optical microscope (ECLIPSE 80i, Nikon Instruments, Tempe, AZ) equipped with a CCD camera connected to digital image processing software (NIS-Elements Basic Research, Nikon Instruments). The microstructures of selected emulsions were also measured using a Laser Scanning Confocal Microscope (C1 Digital Eclipse, Nikon, Tokyo, Japan) with a 60× oil immersion objective lens. The protein phase in W/O emulsions was stained with a protein-specific fluorescent dye (fluorescamine, Molecular Probes, Eugene, OR), which was excited with 488-nm argon laser line. The fluorescence emitted from the sample was monitored using a fluorescence detector (515/30) with a pinhole size of 150 μm. The resulting images consisted of 512 × 512 pixels, with a pixel size of 414 nm, and a pixel dwell time of 61.44 μs. Images were recorded using image analysis software (Scion Image, Frederick, MD, USA). At least three pictures were taken of each sample.

2.5. Lipid oxidation measurements

Thiobarbituric acid-reactive substances (TBARS) were used as a measure of lipid oxidation reaction products, and were determined using a method based on one described previously (McDonald & Hultin, 1987). Previous studies of oxidation of polyunsaturated oils have shown a close correlation between TBARS and other secondary reaction product markers of lipid oxidation, such as hexanal and propanal (Khan & Shahidi, 2000; Wettasinghe & Shahidi, 1999; Zhong, Lall, & Shahidi, 2007). A TCA (trichloroacetic acid)–TBA–HCl solution was prepared by mixing 75 g trichloroacetic acid, 1.68 g TBA, 8.8 mL of 12 M HCl, and 414 g H_2O. One hundred millilitre of TCA–TBA–HCl solution was mixed with 3 mL of 2% butylated hydroxytoluene in ethanol and 4 mL of this solution was mixed with 2 mL of emulsion. After mixing, the mixture was centrifuged at 1000g for 15 min, and then 2 mL of solution from the bottom of the tube was transferred to a new tube. The collected solution was heated in a boiling water bath for 15 min, cooled down using tap water to room temperature for 10 min, and centrifuged at 1000*g* for 15 min. After 10 min, the absorbance was measured at 532 nm. Concentrations of TBARS were determined from standard curves prepared using 1,1,3,3-tetraethoxypropane.

3. Results and discussion

3.1. Properties of W/O emulsions

Initially, we prepared water-in-oil (W/O) emulsions by homogenising an aqueous phase (15 wt% WPI, 0 or 4 mg iron, pH 7) with an oil phase (6 wt% PGPR in corn oil) using a micro-fluidizer. When these emulsions were observed under a confocal fluorescent microscope after a protein dye (fluorescamine) had been added we observed many small ($d < 1$ μm) water droplets evenly dispersed throughout an oily continuous phase (data not shown). We could not reliably ascertain the size of the individual water droplets in the emulsions using this method since they were too small relative to the resolution of the microscope. For this reason, the emulsions were diluted with hexadecane oil and their particle size distribution was measured by dynamic light scattering. We observed a monomodal particle size distribution with a mean particle diameter (*z*-average) of 420 ± 40 nm.

3.2. Physical Stability of W/O/W Emulsions

After preparation, the physical stability of W/O/W emulsions with and without encapsulated iron was monitored during storage. There were no changes in the mean particle diameters (d_{32} or d_{43}) of the emulsions during 7 days of storage (Table 1), indicating that they were stable to water diffusion/expulsion, droplet coalescence, and Ostwald ripening throughout this period. For the no added iron and added iron W/O/W emulsions, the values of d_{43} were about 9.4 and 9.5 μm and the values of d_{32} were about 7.0 and 7.5 μm, respectively. These values are fairly close to the reported diameter of the pores in the membrane homogenizer ($d \approx 8$ μm). The particle size distributions of all the W/O/W emulsions were monomodal and did not change appreciably during storage (e.g., see Fig. 2 for the added iron W/O/W emulsions). Photographs taken by optical microscopy indicated that the emulsions consisted of relatively large oil droplets with some smaller water droplets inside (Fig. 3). Due to the relatively large diameter of the droplets in the W/O/W emulsions they were highly unstable to gravitational separation, and an optically opaque (white) layer of droplets was clearly visible on top of the emulsions after a few hours storage. In practical applications within the food industry, this type of gravitational separation may be retarded by reducing the size of the droplets, adding a thickening agent, or matching the density of the droplets to that of the surrounding aqueous phase (McClements, 2005). In this study, we wanted to use relatively large quasi-monodisperse droplets so that we could observe any changes in their dimensions during storage.

3.3. Efficiency and retention of iron encapsulation in W/O/W emulsions

The measured concentration of iron in the external aqueous phase of both W/O/W emulsions was initially around 10 μg/100 g emulsion, indicating that there was a small amount of endogenous iron present in these systems. This iron probably came from the water or other ingredients used to prepare the emulsions. For the emulsion containing added iron, this value was much lower than the total amount of iron present in the overall emulsion (4 mg/100 g emulsion), which indicates that the majority (>99.75%) of added iron initially remained encapsulated within the internal aqueous phase. Nevertheless, there was a significant increase in the iron concentration in the external aqueous phase of the W/O/

Table 1
Mean particle diameters (d_{43} and d_{32}, μm) of O/W (control) and W/O/W (with and without added iron) emulsions during storage at room temperature.

		0 day	1 day	2 days	3 days	4 days	5 days	6 days	7 days
Control (O/W)	d_{43}	10.47 ± 0.06	10.49 ± 0.04	10.54 ± 0.01	10.52 ± 0.01	10.50 ± 0.02	10.55 ± 0.02	10.45 ± 0.15	10.53 ± 0.06
	d_{32}	7.48 ± 0.08	7.44 ± 0.06	7.47 ± 0.01	7.45 ± 0.01	7.43 ± 0.01	7.45 ± 0.01	7.39 ± 0.10	7.43 ± 0.02
No added iron (W/O/W)	d_{43}	9.38 ± 0.02	9.42 ± 0.02	9.41 ± 0.04	9.45 ± 0.04	9.42 ± 0.02	9.45 ± 0.01	9.41 ± 0.03	9.41 ± 0.07
	d_{32}	6.89 ± 0.04	6.93 ± 0.02	6.93 ± 0.01	6.93 ± 0.06	6.91 ± 0.05	6.95 ± 0.01	6.90 ± 0.03	6.87 ± 0.04
Added iron (W/O/W)	d_{43}	9.26 ± 0.03	9.70 ± 0.16	9.76 ± 0.27	9.50 ± 0.13	9.37 ± 0.01	9.54 ± 0.42	9.70 ± 0.24	9.47 ± 0.09
	d_{32}	7.25 ± 0.01	7.41 ± 0.05	7.44 ± 0.01	7.42 ± 0.03	7.35 ± 0.00	7.28 ± 0.04	7.18 ± 0.07	7.20 ± 0.03

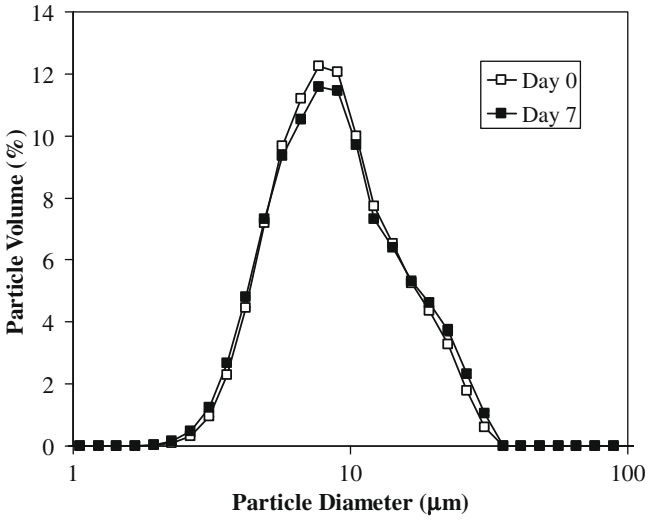

Fig. 2. Particle size distribution of W/O/W emulsion containing iron encapsulated within the internal aqueous phase after 0 and 7 days storage. The standard deviations of the measurements at each size class are less than the symbol size.

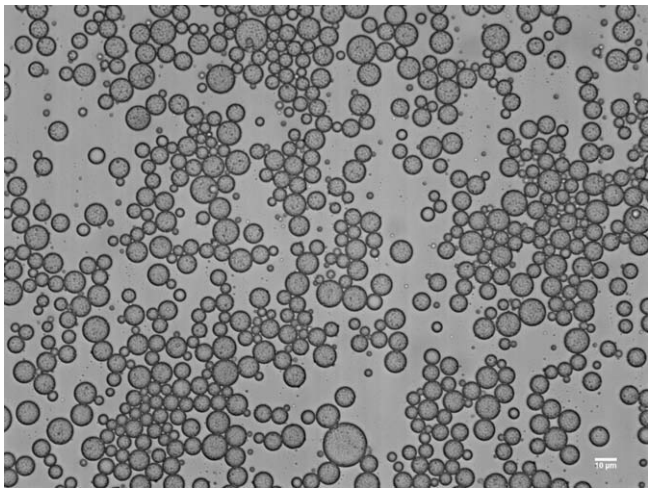

Fig. 3. Optical microscopy image of W/O/W emulsions containing encapsulated iron after 3 days of storage.

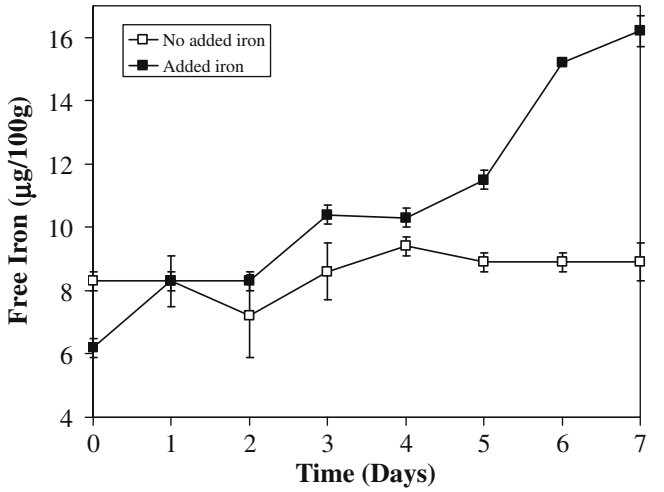

Fig. 4. Iron concentration in the external aqueous phase of W/O/W emulsions with or without encapsulated iron during storage.

W emulsion containing encapsulated iron during storage (e.g., from ≈6 to 16 μg/100 g emulsion from day 0 to day 7), indicating that a small amount of iron (≈10 μg/100 g emulsion) was released from the internal aqueous phase (Fig. 4). There are a number of possible physicochemical mechanisms that might be responsible for the observed increase of iron in the external aqueous phase over time, e.g., iron may move from the internal to external water phases by diffusing through the oil phase (either alone or as part of a reverse micelle) or by whole water droplets being expelled from the oil phase. We did not observe any significant decrease in the mean particle diameter of the W/O droplets during storage, which suggests that whole water droplets were not expelled. The volume of a sphere is related to the cube of its diameter ($V = \pi d^3/6$), so that the ratio of diameters of the initial W/O droplets and the final O droplets (assuming that all the water droplets were expelled) is given by $d_f/d_i = (V_f/V_i)^{1/3}$. The internal water phase initially comprised about 20% of the total volume of the W/O droplets, so that if all the water droplets were expelled from the oil droplets there should be a decrease in particle diameter to about 93% of the initial value, *i.e.*, $(d_f/d_i) = (V_f/V_i)^{1/3} = (0.8)^{1/3}$. This would correspond to a decrease in mean particle diameter (d_{43}) from about 9.3 to 8.6, which was not observed in this study (Table 1). This result suggests that the water droplets remained within the oil droplets, but that some of the iron still diffused out. It is known that iron has an appreciable solubility in oil and that water-soluble components may be incorporated within reverse micelles (McClements & Decker, 2000), and hence it is possible that some iron was transported across the oil phase by diffusion.

3.4. Oxidative stability of emulsified fish oil in the presence of W/O/W emulsions

Our original hypothesis was that if iron could be encapsulated within the internal aqueous phase of W/O/W emulsions (Fig. 1) prepared from a relatively oxidatively stable oil (such as corn oil), then the W/O droplets formed could be used as an iron delivery system that could be added to emulsions containing oxidatively unstable oils (such as fish oil) without promoting rapid lipid oxidation. We therefore measured the impact of adding W/O droplets with or without encapsulated iron on the oxidative stability of emulsified fish oil droplets. In this study, we used TBARS, a measure of secondary reaction products, to monitor the extent of lipid oxidation in the emulsions. Generally, one should use a

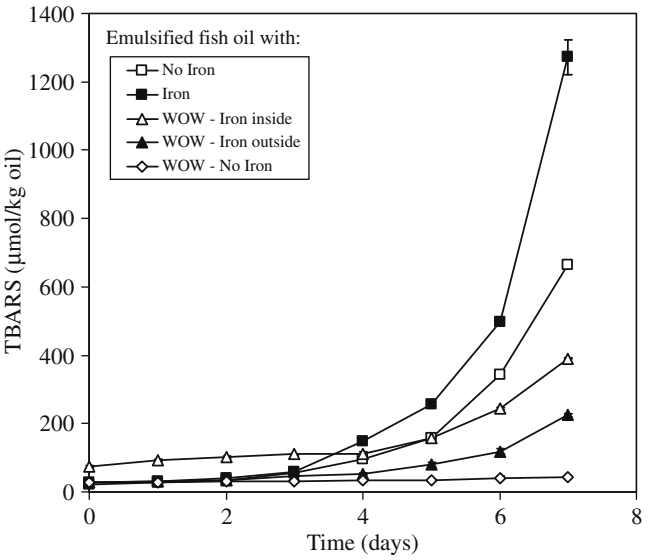

Fig. 5. Thiobarbituric acid-reactive substances (TBARS) concentrations in mixtures of W/O/W emulsion and fish oil emulsions. Annotation: "iron" and "no iron" = fish oil emulsion with and without added iron; W/O/W "no added iron", "iron inside", and "iron outside" = W/O/W emulsions containing no added iron, iron in the internal aqueous phase, and iron in the external aqueous phase.

combination of two or more different measurements of lipid oxidation products, such as loss of reactants, formation of primary oxidation products, or formation of secondary oxidation products to obtain a fuller picture of lipid oxidation (Frankel, 2005). Nevertheless, previous studies have found good correlations between TBARS and other secondary reaction product markers (specific aldehydes) of lipid oxidation in emulsions and bulk oils (Khan & Shahidi, 2000; Senanayake & Shahidi, 2007; Wettasinghe & Shahidi, 1999; Zhong et al., 2007), and so we only used TBARS as a marker in this study.

The TBARS produced (per unit mass of fish oil) was measured in a series of mixed emulsions containing fish oil droplets and W/O droplets: (i) no added iron; (ii) iron present within the internal aqueous phase; and (iii) iron present within the external aqueous phase (Fig. 5). For comparison, we also measured the oxidative stability of the fish oil emulsions alone in the absence and presence of the same iron concentration (Fig. 5). In the absence of W/O/W emulsions, the fish oil emulsions were relatively stable to lipid oxidation for 2–3 days (TBARS < 100 μmol/kg fish oil), but there was a notable increase in TBARS after this time, indicating that an appreciable amount of oxidation had occurred. The presence of additional iron in the emulsions caused an increase in the extent of lipid oxidation (Fig. 5), which is consistent with the fact that iron is known to accelerate oxidation. We also measured the change in TBARS of the W/O/W emulsions alone (i.e., without fish oil) from day 0 to 7, and found that the TBARS level increased from around 14 to 25 μg per kg oil in the presence of iron and from around 8 to 11 μg per kg oil. These TBARS values are much less than the values found for the emulsions containing fish oil (Fig. 5), which can be attributed to the fact that corn oil is more oxidatively stable than fish oil, and also that most of the secondary reaction products produced by corn oil oxidation are not detected by TBARS (McClements & Decker, 2007). To gain a more complete understanding of the impact of sample composition and structure on the extent of lipid oxidation in multiple emulsions, it would be useful in future studies to measure other markers of lipid oxidation as well as TBARS, such as hydroperoxides, conjugated dienes, hexanal or propanal.

The addition of W/O droplets to the fish oil emulsions tended to decrease the extent of lipid oxidation by an amount that depended on the concentration and location of the iron (Fig. 5). In the absence of added iron, the presence of W/O droplets greatly decreased the amount of lipid oxidation that was observed in the fish oil emulsions. A number of physicochemical phenomena can be postulated to account for this effect. It is possible that the presence of W/O droplets decreased the free iron concentration available to interact with the fish oil droplets (e.g., due to iron absorption to the surfaces or interior of W/O droplets), or that the W/O droplets incorporated some of the lipid oxidation pro-oxidants or reaction products thereby slowing down the initiation and propagation reactions within the fish oil droplets. Surprisingly, the mixed fish oil and W/O/W emulsions containing iron in the internal aqueous phase were less oxidatively stable (higher TBARS) than the mixed emulsions containing iron in the external aqueous phase (Fig. 5). We expected that the mixed emulsions with iron in the external aqueous phase would be much more unstable to oxidation since the iron could then come into direct contact with the emulsified fish oil. In contrast, we expected the mixed emulsions with iron in the internal aqueous phase to be more stable to oxidation since the iron would have been physically isolated from the emulsified fish oil. One possible explanation for this apparent discrepancy is that there had already been some oxidation of the corn oil in the W/O/W emulsions containing encapsulated iron prior to mixing it with the fish oil emulsions. Indeed, the initial TBAR measurements for this system were appreciably higher than that observed in the other W/O/W emulsion (Fig. 5). The reaction products from the corn oil may then have been able to initiate and propagate oxidation in the fish oil emulsions. In future studies, it would be interesting to examine the impact of iron encapsulation in W/O/W emulsions prepared from more oxidatively stable oils than corn oil (such as medium chain triglycerides) on lipid oxidation. Another possible explanation for this observed result is due to the creaming instability of these emulsions. The fish oil and W/O droplets are relatively large ($d \approx 10$ μm) and so they tend to cream rapidly and form a droplet-rich creamed layer on top of the emulsions. The density of the fish oil droplets is less than that of the W/O droplets and so they will tend to cream more rapidly. It is therefore possible that the fish oil droplets formed a separate layer on top of the mixed emulsions and that the W/O droplets formed another layer below this. Consequently, in the emulsions containing iron in the external aqueous phase, the W/O droplet layer may have prevented free iron present in the lower serum layer from coming into direct contact with the fish oil droplets. Conversely, in the emulsions containing iron in the internal aqueous phase, the W/O droplet layer would have been somewhat intermingled with the upper fish oil droplet layer, thereby potentially promoting oxidation due to the presence of reaction products from the corn oil and iron leakage from the W/O droplets.

Finally, we should note that we only measured the oxidative stability of the emulsions over a 7 day period. In practice, a food product containing polyunsaturated fatty acids would have to have a much longer shelf-life in order to be acceptable to consumers. Consequently, additional measures would be needed to retard oxidation in real emulsions, such as addition of antioxidants, incorporation of chelating agents, controlling oxygen content, or interfacial engineering (Frankel, 2005; McClements and Decker, 2000).

4. Conclusions

We have shown that iron can be encapsulated within the internal aqueous phase of W/O/W emulsions with a high loading efficiency (>99%), with only a small amount of iron leakage being observed over time. Nevertheless, W/O/W emulsions containing encapsulated iron did promote lipid oxidation when added to fish oil emulsions. Interestingly, W/O/W emulsions containing no added iron appeared to be highly effective at retarding lipid oxidation in

the fish oil emulsions, possibly by their impact on the distribution of pro-oxidants and reaction products in the system. In future studies it would be useful to prepare the W/O/W emulsions from a lipid phase that was completely stable to oxidation itself. In addition, if this approach was going to be used commercially by the food industry to encapsulate iron, it would be necessary to prepare multiple emulsions with good long term stability and resistance to environmental stresses, which would require careful choice of water-soluble and oil-soluble emulsifiers, particle size distributions and osmotic balance.

Acknowledgments

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References

Benichou, A., Aserin, A., & Garti, N. (2004). Double emulsions stabilized with hybrids of natural polymers for entrapment and slow release of active matters. *Advances in Colloid and Interface Science, 108–109*, 29–41.

Cho, Y. J., Alamed, J., McClements, D. J., & Decker, E. A. (2003). Ability of chelators to alter the physical location and prooxidant activity of iron in oil-in-water emulsions. *Journal of Food Science, 68*(6), 1952–1957.

Diaz, M., Vattem, D., & Mahoney, R. R. (2002). Production of dialysable and reduced iron by in vitro digestion of chicken muscle protein fractions. *Journal of the Science of Food and Agriculture, 82*(13), 1551–1555.

Frankel, E. N. (2005). *Lipid oxidation*. Bridgwater, UK: The Oily Press Lipid Library.

Garti, N. (1997a). Double emulsions – Scope, limitations and new achievements. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 123*, 233–246.

Garti, N. (1997b). Progress in stabilization and transport phenomena of double emulsions in food applications. *Food Science and Technology-Lebensmittel-Wissenschaft and Technologie, 30*(3), 222–235.

Garti, N., & Benichou, A. (2004). Recent developments in double emulsions for food applications. In S. E. Friberg, K. Larsson, & J. Sjoblom (Eds.), *Food emulsions*. New York: Marcel Dekker.

Garti, N., & Bisperink, C. (1998). Double emulsions: progress and applications. *Current Opinion in Colloid and Interface Science, 3*(6), 657–667.

Khan, M. A., & Shahidi, F. (2000). Oxidative stability of stripped and nonstripped borage and evening primrose oils and their emulsions in water. *Journal of the American Oil Chemists Society, 77*(9), 963–968.

Khan, A. Y., Talegaonkar, S., Iqbal, Z., Ahmed, F. J., & Khar, R. K. (2006). Multiple emulsions: An overview. *Current Drug Delivery, 3*(4), 429–443.

Mahoney, J. R., & Graf, E. (1986). Role of alpha-tocopherol, ascorbic-acid, citric-acid and EDTA as oxidants in model systems. *Journal of Food Science, 51*(5), 1293–1296.

McClements, D. J. (2005). *Food emulsions: Principles, practice, and techniques*. Boca Raton: CRC Press.

McClements, D. J., & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science, 65*(8), 1270–1282.

McClements, D. J., & Decker, E. A. (2007). Lipids. In S. Damodaran, K. L. Parkin, & O. R. Fennema (Eds.), *Food Chemistry* (pp. 155–216). Boca Raton, FL: CRC Press.

McClements, D. J., Decker, E. A., & Weiss, J. (2007). Emulsion-based delivery systems for lipophilioc bioactive components. *Journal of Food Science, 72*(8), R109–R124.

McDonald, R. E., & Hultin, H. O. (1987). Some characteristics of the enzymatic lipid-peroxidation system in the microsomal fraction of flounder skeletal-muscle. *Journal of Food Science, 52*(1), 15–21,27.

Muschiolik, G. (2007). Multiple emulsions for food use. *Current Opinion in Colloid and Interface Science, 12*(4–5), 213–220.

Owusu, R. K., Zhu, Q. H., & Dickinson, E. (1992). Controlled release of L-tryptophan and vitamin-B2 from model water oil–water multiple emulsions. *Food Hydrocolloids, 6*(5), 443–453.

Senanayake, S., & Shahidi, F. (2007). Measuring oxidative stability of structured lipids by proton nuclear magnetic resonance. *Journal of Food Lipids, 14*(3), 217–231.

Su, J. H., Flanagan, J., Hemar, Y., & Singh, H. (2006). Synergistic effects of polyglycerol ester of polyricinoleic acid and sodium caseinate on the stabilisation of water-oil-water emulsions. *Food Hydrocolloids, 20*(2–3), 261–268.

Surh, J., Vladisavljevic, G. T., Mun, S., & McClements, D. J. (2007). Preparation and characterization of water/oil and water/oil/water emulsions containing biopolymer-gelled water droplets. *Journal of Agricultural and Food Chemistry, 55*(1), 175–184.

Tedajo, G. M., Seiller, M., Prognon, P., & Grossiord, J. L. (2001). PH compartmented w/o/w multiple emulsion: a diffusion study. *Journal of Controlled Release, 75*(1–2), 45–53.

Vladisavljevic, G. T., & Williams, R. A. (2005). Recent developments in manufacturing emulsions and particulate products using membranes. *Advances in Colloid and Interface Science, 113*(1), 1–20.

Wettasinghe, M., & Shahidi, F. (1999). Evening primrose meal: A source of natural antioxidants and scavenger of hydrogen peroxide and oxygen-derived free radicals. *Journal of Agricultural and Food Chemistry, 47*(5), 1801–1812.

Zhong, Y., Lall, S. P., & Shahidi, F. (2007). Effects of oxidized dietary oil and vitamin e supplementation on lipid profile and oxidation of muscle and liver of juvenile Atlantic cod (*Gadus morhua*). *Journal of Agricultural and Food Chemistry, 55*(15), 6379–6386.