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Impact of chelators on the oxidative stability of whey protein isolate-stabilized oil-in-water emulsions containing ω-3 fatty acids

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Abstract

The oxidative stability of oil-in-water emulsions can be improved by engineering the surface of the emulsion droplets to decrease transition metal–lipid interactions. This can be accomplished in protein-stabilized emulsions where the pH is less than the p*I* of the protein and the emulsion droplets are cationic. While these emulsions have high oxidative stability, little is known about whether metal chelators can be used to further decrease lipid oxidation. Therefore the purpose of this research was to investigate the impact of ethylenediamine tetraacetic acid (EDTA), citrate and polyphosphates on the physical and oxidative stability of algae oil-in-water emulsions, stabilized by whey protein isolate. Chelators were added to emulsions at concentrations ranging from 1 to 100 μM and lipid oxidation was monitored with lipid hydroperoxides and headspace propanal. The oxidative stability of whey protein isolate-stabilized emulsions, at both pH 3.0 and 7.0, was improved by EDTA at concentrations $\geqslant 1$ μM. Neither citrate nor polyphosphates were effective at inhibiting lipid oxidation at the concentrations tested. None of the chelators tested had any impact on the physical stability of the emulsions. These results indicate that the oxidative stability of whey-protein isolate-stabilized oil-in-water emulsion can be increased by EDTA without having any impact on physical stability.

Keywords: Whey protein; Emulsions; Lipid oxidation; EDTA; Citrate; Polyphosphate; Chelators; ω-3 fatty acids

1. Introduction

Proteins are highly functional ingredients that are critical to the quality of numerous food products. The major functional properties of proteins include gelation, viscosity enhancement, water binding and stabilization of dispersions. Proteins are very effective at stabilizing oil-in-water emulsions (Dickinson, 1994). During food processing operations, such as homogenization, proteins can be absorbed on the surface of newly formed oil droplets. The protein at the interface of the oil droplet increases the stability of oil-in-water emulsion by lowering interfacial tension, forming protective membranes around the lipid and imparting an electrical charge at the emulsion droplet surface when the pH is not equivalent or close to the p*I* of the interfacial protein (Dickinson, 1997).

Numerous epidemiological, clinical, animal and in situ experiments have shown the health benefits of ω-3 fatty acids (for review see Simopoulos, 1999). These benefits have included decrease risk of coronary heart disease, immune response disorders and mental illness, as well as benefits to infants and pregnant women. The consumption of ω-3 fatty acids in developing countries has decreased over the past several decades suggesting that many populations would benefit from the development of ω-3 fortified foods (Simopoulos, 1999). However, the development of these products is limited by the high susceptibility of ω-3 fatty acids to oxidative rancidity that leads to the formation of off-flavours and potentially toxic compounds.

Transition metals are found in all foods since they are common constituents of raw food materials, water, ingredients and packaging materials. Reactive transition metals decompose hydroperoxides through a redox

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cycling pathway that generates free radicals (Halliwell & Gutteridge, 1990). The formation of free radicals by interactions between lipid hydroperoxides and transition metals is important in the promotion of lipid oxidation in both model system studies and foods. In a Tween 20-stabilized salmon oil emulsion, lipid oxidation was inhibited by both ethylenediamine tetraacetic acid (EDTA) (50 μM) and transferrin (31 μM) even though no exogenous metals were added to the system (Mancuso, McClements, & Decker, 1999). The ability of transferrin to inhibit oxidation suggests that iron was the main prooxidant in these emulsions, since transferrin has a strong preference for the chelation of iron over other transition metals. Iron-promoted lipid oxidation can also be decreased in systems where iron and lipid hydroperoxides are in different physical locations. This is the case in protein-stabilized oil-in-water emulsion when the pH is below the p*I* of the interfacial protein and the positive charge of the emulsion droplet electrostatically repels iron and decreases its interactions with lipid hydroperoxides (Donnelly, Decker, & McClements, 1998; Hu, McClements, & Decker, 2003a, 2003b).

While research has been conducted on the utilization of proteins to produce cationic, oxidatively-stable oil-in-water emulsions at low pH, little is known about whether metal chelators could be used to further reduce lipid oxidation in these emulsion systems. Therefore, the objective of this study was to evaluate the impact of chelators on the oxidative stability of algal oil-in-water emulsions stabilized with whey protein isolate at pH 3.0 and 7.0, where the emulsion droplets are cationic and anionic, respectively.

2. Materials and methods

2.1. Materials

Algae oil was obtained commercially from Martek Biosciences, Boulder, Colorado, USA. Sodium hexametaphosphate, sodium citrate, sodium tripolyphosphate, EDTA, imidazole, sodium acetate, ferrous sulfate, cumene hydroperoxide, and propanal were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Whey protein isolate (WPI) was obtained from Davisco Food International Inc. (Eden Praire, MN, USA) and was used without further purification. The protein content of WPI, as indicated by the manufacturer, was 97.6%. All other reagents were of analytical grade or purer.

2.2. Preparation and characterization of emulsions

An oil-in-water emulsion was prepared using 5.0 wt% algae oil and 95% aqueous phase containing acetate-imidazole buffer (5 mM each, pH 3.0 or 7.0) and 0.2% WPI. Oil-in-water emulsions were made by blending the lipid and aqueous phases for 2 min using a hand-held homogenizer (M 133/1281-Biospec Products, Inc. Bartlesville, UK). The coarse emulsion was then homogenized three times at 5000 psi through a high-pressure value, two-stage APV Lab 1000 homogenizer (Albertslund, Denmark). Chelators were added after preparation of the emulsions. The particle size distribution ($d_{3,2}$) of the emulsions was measured using a Coulter LS 230 laser light scattering instrument (Coulter Cooperation, Miami, FL, USA). Droplet size distributions were measured, after homogenization, to monitor emulsion stability. Emulsion droplet charge (zeta potential, ξ) was measured by directly injecting diluted (1:1000) oil-in-water emulsions into the measurement chamber of a ZEM 5003 Zetamaster (Malvern Instruments, Worchester, UK). The ξ-potential measurements are reported as the averages of two separate injections, with five readings made per injection.

2.3. Measurement of lipid oxidation

To monitor lipid hydroperoxide formation during storage, emulsions (5 ml) were placed in lightly sealed screw-cap test tubes and allowed to oxidize at 37 °C in the dark. Lipid hydroperoxides (Nuchi, Hernandez, McClements, & Decker, 2002) were measured by mixing 0.3 ml of emulsion with 1.5 ml of isooctane/2-propanol (3:1, v/v) by vortexing (10 s, three times) and isolation of the organic solvent phase by centrifugation at 1000*g* for 2 min. The organic solvent phase (200 μl) was added to 2.8 ml of methanol/1-butanol (2:1, v/v), followed by 15 μl of 3.97 M ammonium thiocyanate and 15 μl of ferrous iron solution (prepared by mixing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$). The absorbance of the solution was measured at 510 nm, 20 min after addition of the iron. Hydroperoxide concentrations were determined using a standard curve made from cumene hydroperoxide.

For headspace analysis, emulsion samples (1 ml) were placed into 10 ml headspace vials and sealed with poly (tetrafluoroethylene) butyl rubber septa. Headspace propanal was determined using a Shimadzu 17A Gas Chromatograph equipped with a Hewlett Packard 19395A headspace sampler (Chaiyasit, Silvestre, McClements, & Decker, 2000). The headspace conditions were as follows: sample temperature, 40 °C; sample loop and transfer line temperature, 110 °C; pressurization, 10 s; venting, 10 s; injection, 1.0 min. The aldehydes were separated isothermally at 70 °C on a HP methyl silicone (DB-1) fused silica capillary column (50 m, 0.31 mm i.d., 1.03 μm film thickness). The splitless injector temperature was 180 °C, and the eluted compounds were detected with a flame ionization detector at 200 °C. Concentrations

were determined from peak areas using a standard curve made from authentic propanal.

2.4. Statistical analysis

All measurements were made on triplicate samples and reported as means ± standard deviations. The general linear model procedure (Snedecor & Cochran, 1989) was used to test for significance ($p \leqslant 0.05$). Duncan's multiple range test was used to separate means (Snedecor & Cochran, 1989).

3. Results and discussion

Citric acid, polyphosphates and EDTA are three of the primary chelators used in food products (Lindsay, 1996). The ability of all three of these chelators to inhibit lipid oxidation in whey protein isolate-stabilized algal oil emulsions was tested at pH 3.0 where the emulsion droplets would be cationic and at pH 7.0 where the emulsion droplets are anionic. The chelators were tested at concentrations ranging from 0 to 100 μM since EDTA has previously been shown to inhibit lipid oxidation in emulsions at these concentrations (Mancuso et al., 1999). At pH 3.0, sodium citrate did not decrease the formation of lipid hydroperoxides or headspace propanal at any of the concentrations tested (Fig. 1(a) and (b)). In the presence of 100 μM citrate, lipid hydroperoxides were significantly higher at 48, 96 and 144 h of storage ($p < 0.05$) and headspace propanal tended to be greater (although not statistically significant) than the no-citrate control emulsion at 102 and 146 h, suggesting that the citric acid could be prooxidative. Addition of EDTA to the whey protein isolate-stabilized algal oil emulsions at pH 3.0 resulted in inhibition of lipid hydroperoxide and headspace propanal formation at all concentrations tested (Fig. 2(a) and (b)). EDTA at 1.0 μM had less hydroperoxides and propanal than the controls after 24 and 123 h, respectively. Emulsions with 1.0 μM EDTA had higher lipid hydroperoxide concentrations that the 10 and 100 μM EDTA treatments at 123 and 172 h. A similar trend was observed in formation of headspace propanal, with 1.0 μM EDTA being less effective than 10 and 100 μM after 47 h of incubation. In a comparison of citrate, EDTA, sodium

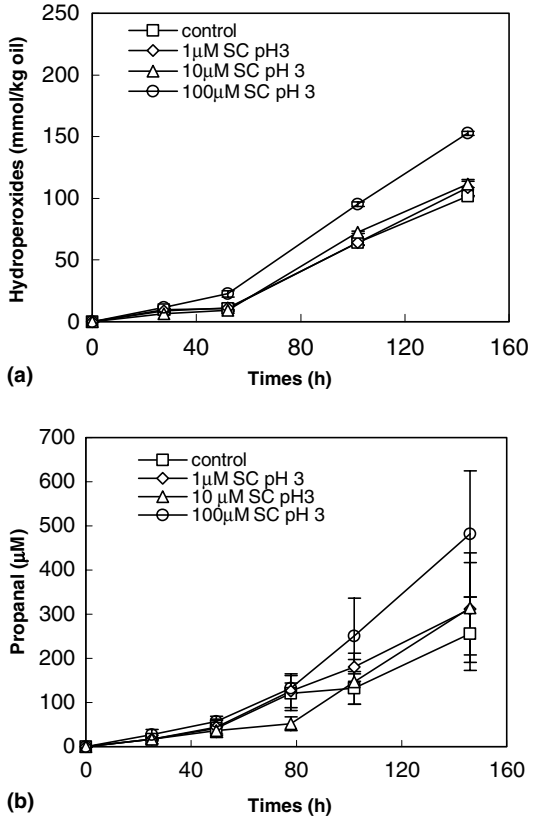

Fig. 1. The impact of sodium citrate (SC; 1–100 μM) on the formation of lipid hydroperoxides (a) and headspace propanal (b) in whey protein-stabilized algae oil-in-water emulsions at pH 3.0. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

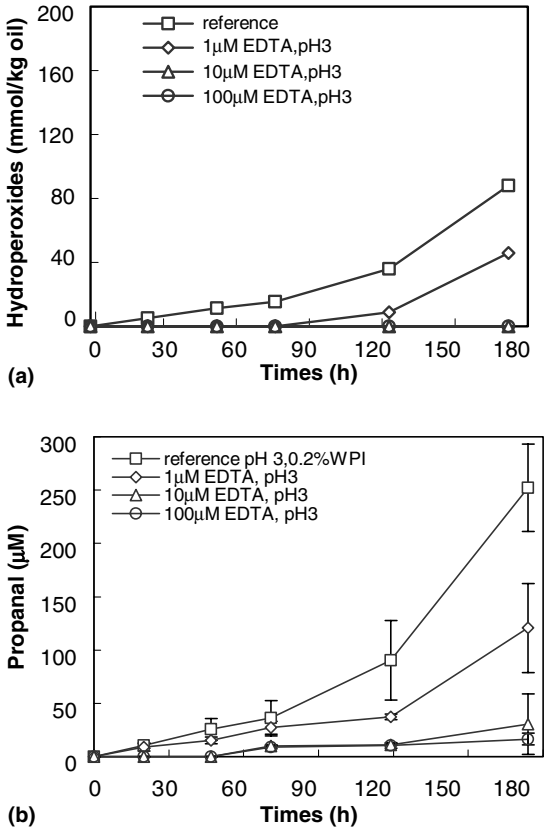

Fig. 2. The impact of ethylenediamine tetraacetic acid (EDTA; 1–100 μM) on the formation of lipid hydroperoxides (a) and headspace propanal (b) in whey protein-stabilized algae oil-in-water emulsions at pH 3.0. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

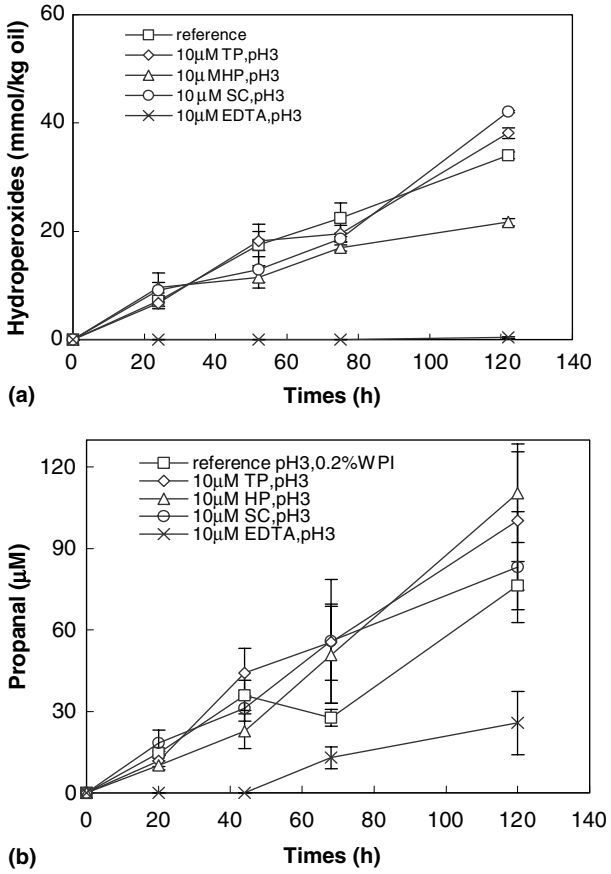

Fig. 3. The impact of 10 μM sodium tripolyphosphate (TP), hexametaphosphate (HP), sodium citrate (SC), ethylenediamine tetraacetic acid (EDTA) on the formation of lipid hydroperoxides (a) and headspace propanal (b) in whey protein-stabilized algae oil-in-water emulsions at pH 3.0. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

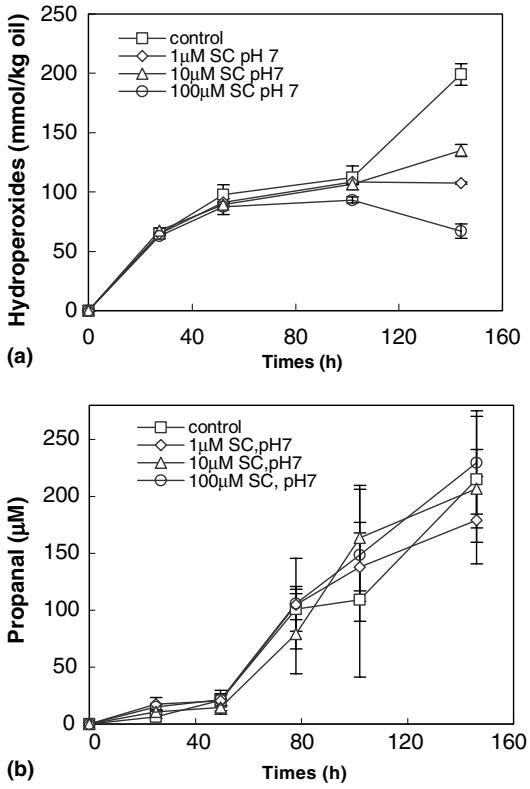

Fig. 4. The impact of sodium citrate (SC; 1–100 μM) on the formation of lipid hydroperoxides (a) and headspace propanal (b) in whey protein-stabilized algae oil-in-water emulsions at pH 7.0. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

tripolyphosphate and hexametaphosphate at 10 μM in the whey protein isolate-stabilized algal oil emulsion at pH 3.0, EDTA was again the only chelator to show consistent inhibition of lipid oxidation, as determined by both lipid hydroperoxides and headspace propanal (Fig. 3(a) and (b)).

At pH 7.0, the impacts of citrate and EDTA on lipid hydroperoxide and headspace propanal formation were similar to those at pH 3.0. In the presence of citrate, lipid hydroperoxide concentrations were not different from the control until the later stages of incubation (144 h) when all three concentrations decreased hydroperoxide concentrations (Fig. 4(a)). Citrate had no impact on headspace propanal concentrations at pH 7.0 during the entire storage period (Fig. 4(b)). All three concentrations of EDTA tested inhibited both lipid hydroperoxide and propanal formation at all storage times >24 h. At 1.0 μM EDTA, lipid hydroperoxides and headspace propanal were greater than 10 and 100 μM EDTA after 48 and 120 h, respectively (Fig. 5(a) and (b)). Tripolyphosphate and hexametaphosphate (10 μM) had no impact on hydroperoxide concentrations until after 75 h of incubation, when hydroperoxides unexplainably decreased to zero (Fig. 6(a)). Tripolyphosphate and hexametaphosphate (10 μM) had no consistent impact on headspace propanal formation (Fig. 6(b)).

A potential concern about the use of chelators with protein-stabilized emulsions is that the chelators could cause physical destabilization of the emulsions through association with the surface of the emulsion droplet, resulting in an alteration of charge. This would be especially true at low pH where the protein-stabilized emulsion droplets are cationic and thus the binding of the anionic chelators to the proteins could decrease charge and could thus promote droplet aggregation. The ability of the chelators to bind to the emulsion droplets was evaluated by measuring the zeta potential of the emulsion droplets in the presence and absence of the chelators.

Table 1 shows that none of the chelators (10 μM) had any impact on the emulsion droplet charge at either pH 3.0 or 7.0. Higher concentrations of citrate and EDTA (100 μM) were also found not to impact droplet charge (data not shown). In addition, no change in emulsion particle size or visual evidence of creaming was observed during storage (data not shown), further indicating that

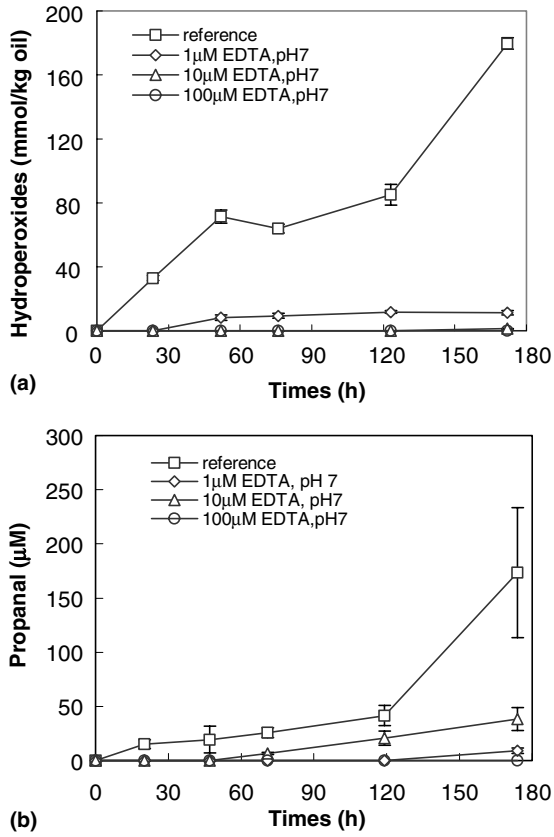

Fig. 5. The impact of ethylenediamine tetraacetic acid (EDTA; 1–100 μM) on the formation of lipid hydroperoxides (a) and headspace propanal (b) in whey protein-stabilized algae oil-in-water emulsions at pH 7.0. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

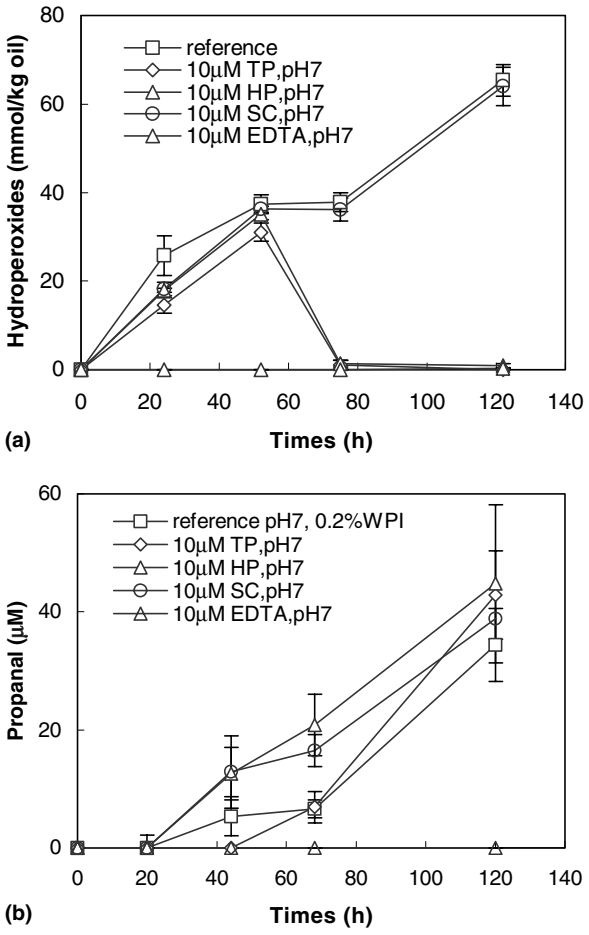

Fig. 6. The impact of 10 μM sodium tripolyphosphate (TP), hexametaphosphate (HP), sodium citrate (SC) and ethylenediamine tetraacetic acid (EDTA) on the formation of lipid hydroperoxides (a) and headspace propanal (b) in whey protein-stabilized algae oil-in-water emulsions at pH 7.0. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

the chelators did not impact the physical stability of the WPI-stabilized emulsions.

Transition metals, and in particular iron, are major prooxidants in oil-in-water emulsions due to their ability to decompose lipid hydroperoxides into free radicals (Decker & McClements, 2001). Because of the pro-oxidant role of metals, chelators can be an effective mechanism to increase the oxidative stability of unsaturated fatty acids. Chelators can inhibit lipid oxidation by sterically hindering metal–hydroperoxide interaction, altering metal redox potential, decreasing metal solubility, preventing metal redox cycling, and altering the physical location of metals (Decker, 2002). In the whey protein isolate-stabilized algal oil emulsions used in this study, the only chelator that substantially inhibited lipid oxidation at the concentrations tested was EDTA. EDTA has also been found to enhance the oxidative stability of ω-3 fatty acids in mayonnaise (Jacobsen et al., 2001) and lecithin-stabilized oil-in-water emulsions (Frankel, Satue-Gracia, Meyer, & German, 2002). The effectiveness of EDTA is dependent on iron concentrations, with EDTA being effective at EDTA:Fe ratios of $\geqslant$1 (Mahoney & Graf, 1986). Oxidation was only partially inhibited by 1.0 μM EDTA, suggesting that this EDTA concentration was not sufficient to completely inactivate iron. EDTA at 10.0 μM was more effective at inhibiting lipid oxidation and increasing EDTA to 100 μM did not result in additional

Table 1
The influence of 10 μM sodium tripolyphosphate (TP), hexametaphosphate (HP), sodium citrate (SC) and ethylenediamine tetraacetic acid (EDTA) on the surface charge of whey protein-stabilized algae oil-in-water emulsion droplets at pH 3.0 and 7.0 (units of zeta potential:mV)

	Control	10 μM TP	10 μM HP	10 μM SC	10 μM EDTA
pH 7.0	−54.3 ± 0.5	−54.0 ± 0.4	−54.9 ± 0.5	−53.9 ± 0.4	−54.7 ± 0.2
pH 3.0	49.0 ± 0.3	49.4 ± 0.4	47.9 ± 0.5	50.5 ± 0.7	49.2 ± 0.1

protection, suggesting that the EDTA:iron ratio was greater than 1 in the presence of 10 μM EDTA. The increased antioxidant activity of EDTA compared to citrate could be due to several factors. The ferric iron-binding constant of EDTA is 1.3×10^{25} M, while citric acid is 1.5×10^{11} M (Smith & Martell, 2003), indicating that EDTA is a much more effective iron chelator. In addition, EDTA is more effective than citrate at decreasing iron-lipid interactions in protein-stabilized emulsions through its ability to increase the transfer of iron out of emulsion droplets into the aqueous phase of oil-in-water emulsions (Cho, Alamed, McClements, & Decker, 2003). Finally, even though citrate binds iron, citrate-iron chelates have been shown to be able to promote lipid oxidation, indicating that the bound iron is still prooxidative (Mahoney & Graf, 1986). Why EDTA is more effective than the polyphosphates is less clear. EDTA and tripolyphosphate decrease the amount of iron association with oil-in-water emulsion droplets in a similar manner (Cho et al., 2003). The iron binding constants of tripoly- and hexametaphosphate are not available (Smith & Martell, 2003), so it is possible that EDTA has a higher binding constant and thus is more antioxidative or that the EDTA-bound iron is less chemically reactive than polyphosphate-bound iron.

These data indicate that, of the major chelators approved for food use, only EDTA was capable of further enhancing the oxidation stability of algae oil-in-water emulsions stabilized with whey protein isolate. An EDTA concentration of 10 μM resulted in maximal protection, suggesting that the EDTA:iron ratio was greater than 1 under these conditions. EDTA, as well as the other chelators did not alter the surface charge of the emulsion droplets or physical stability of the whey protein-stabilized emulsion, indicating that EDTA could be used to inhibit oxidation without causing emulsion destabilization.

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