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Influence of heat processing and calcium ions on the ability of EDTA to inhibit lipid oxidation in oil-in-water emulsions containing omega-3 fatty acids

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Abstract

The nutritional benefits of ω-3 fatty acids make them excellent candidates as functional food ingredients if problems with oxidative rancidity can be overcome. Oil-in-water emulsions were prepared with 2% salmon oil, stabilized by 0.2% Brij 35 at pH 7. To determine the effects of heating (50–90 °C), ethylenediaminetetraacetic acid (EDTA), and calcium on the oxidative and physical stability of salmon oil-in-water emulsions, particle size, thiobarbituric acid reactive substances (TBARS), and lipid hydroperoxides were measured. The heat-processed emulsions showed no significant difference, in particle size, TBARS or hydroperoxides during storage, from unheated emulsions. Above 2.5 μM, EDTA dramatically decreased lipid oxidation in all samples. Addition of calcium to emulsions containing 7.5 μM EDTA significantly increased both TBARS and hydroperoxide formation when calcium concentrations were 2-fold greater than EDTA concentrations. These results indicate that heat-processed salmon oil-in-water emulsions with high physical and oxidative stability could be produced in the presence of EDTA.

Keywords: ω-3 fatty acids; Emulsion; Lipid oxidation; Antioxidants; EDTA

1. Introduction

The omega-3 (ω-3) fatty acids found in fish oil have been found to be clinically beneficial to health (Akoh & Min, 2002). The health benefits of dietary omega-3 fatty acids include reduced susceptibility to mental illness, protection against heart disease, and improved brain and eye function in infants (Innis, 1991; Simopoulous, 1991). Although ω-3 fatty acids have many health benefits, they are extremely sensitive to lipid oxidation, resulting in potential alteration in nutritional composition and food quality (Nuchi, McClements, & Decker, 2001). These fatty acids are subject to rapid and/or extensive oxidation and other chemical changes by exposure to air, light or heat during processing (Lytle, Lytle, Newmark, & Deschner, 1992). It is of great interest to food manufacturers to use ω-3 fatty acids, as physiologically functional ingredients, to improve the nutritional profile of food products; however, lipid oxidation limits the utilization of these oils in processed foods (Frankel, Satue-Gracia, Meyer, & German, 2002). The nutritional benefits of ω-3 fatty acids make them excellent candidates as functional food ingredients if problems of oxidative rancidity can be overcome.

Successful incorporation of ω-3 fatty acids into processed foods would most likely be in the form of lipid dispersions (Tong, Sasaki, McClements, & Decker,

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2000). Lipid dispersions that consist of oil dispersed in an aqueous phase in the form of small spherical droplets are referred to as oil-in-water emulsions (McClements & Decker, 2000). Oil-in-water emulsions consist of three distinct physical environments: the droplet's lipid core, the interfacial membrane, and an aqueous continuous phase. The differences in the physical environment of the lipids, and in the type and location of prooxidants and antioxidants, mean that there can be large differences in the rate and extent of lipid oxidation between bulk and emulsified oils (McClements & Decker 2000).

Lipid oxidation in oil-in-water emulsions has been extensively studied and it is believed that the interaction between lipid hydroperoxides located at the droplet surface and transition metals originating in the aqueous phase are the most common cause of oxidative instability (McClements & Decker, 2000). Incorporating antioxidants into foods is one of the most effective means of retarding lipid oxidation. In oil-in-water emulsions, the most successful type of antioxidant is one that chelates transition metal ions. A chelate is a complex that results from the combination of a metal ion and a multidentate ligand such that the ligand forms two or more bonds with the metal, resulting in a ring structure that includes the metal ion (Miller, 1996). Chelators that act as antioxidants can inhibit metal-catalyzed reactions by a variety of different mechanisms, including prevention of metal redox cycling, occupation of metal coordination sites, and steric hindrance of interactions between metals and lipid substrates (McClements & Decker 2000). Ethylenediaminetetraacetic acid (EDTA), a transition metal chelator, has been shown to dramatically retard lipid oxidation in salmon oil-in-water emulsions by removing iron from the droplet surface (Mei, Decker, & McClements, 1998). EDTA has been reported to be an inhibitor of lipid oxidation when the EDTA:iron ratio is greater than one. High concentrations of EDTA in relation to iron will inhibit lipid oxidation by surrounding the metal and preventing interaction with peroxides (Mahoney & Graf, 1986).

Many foods have relatively high concentrations of multivalent ions, other than iron, e.g., calcium. The effectiveness of EDTA as an antioxidant could be diminished by the presence of calcium, because it can compete with the iron for binding to EDTA. EDTA has a high calcium ion-binding constant, although it is less than that of iron ions. Relatively high concentrations of calcium may bind the EDTA in the system, leaving iron the opportunity to associate with the emulsion droplets, thus resulting in oxidation of the lipid and instability of the emulsion.

The objective of this paper is to determine the effects of heat processing and calcium ions on the ability of EDTA to inhibit lipid oxidation in Brij 35 stabilized salmon oil-in-water emulsions at pH 7.

2. Materials and methods

2.1. Materials

Salmon fillets were purchased at a local grocer (Stop & Shop, Hadley, MA). Brij 35 was acquired from Aldrich Chemical Company, Inc. (Milwaukee, WI). Ethylenediaminetetraacetic acid (EDTA), 2-thiobarbituric acid (TBA), ferrous sulfate, butylated hydroxytoluene, barium chloride, sodium acetate, and imidazole were obtained from Sigma Chemical Co. (St. Louis, MO). Trichloroacetic acid was purchased from Acros Organics (Morris Plains, NJ). All other chemicals and solvents were of reagent or HPLC grade and were obtained from Fisher Scientific (Suwanee, GA) or Sigma Chemical Company (St. Louis, MO).

2.2. Methods

2.2.1. Preparation of salmon oil

To obtain fresh salmon oil, salmon fillets were skinned, hand chopped into small pieces and minced in a food processor. The mince was then centrifuged at 10,000 rpm for 20 min at 5 °C in a Sorval superspeed RC2-B automatic refrigerated centrifuge (Newtown, CT). The liquid lipid layer was decanted, dispensed into capped glass test tubes (16 × 125 mm; Fisherbrand) and stored at −80 °C until use. The resulting salmon oil consisted of (99.5 ± 0.2%) triacylglycerol (Mei et al., 1998). The level of oxidation products initially in the oil was 0.32 mmol of lipid peroxide/kg of oil, as determined by a modification of the method of Shantha and Decker (1994) and 0.04 mmol of TBARS, as determined by the method of McDonald and Hultin (1987).

2.2.2. Preparation of emulsion

A course emulsion consisting of 2 wt% salmon oil, 0.2 wt% Brij 35 (a non-ionic surfactant), and 10 mM sodium acetate/imidazole buffer (pH 7) was made by homogenizing the lipid and aqueous phases for 2 min using a 2-speed hand held homogenizer (Biospec Products, Inc., Bartlesville, OK) at the highest speed setting. The coarse emulsion was passed three times through an APV-Gaulin model mini-lab 8.30H high-pressure valve homogenizer (APV Americas, Wilmington, MA) at 5000 psi. The final mean droplet diameter of the emulsion (d_{43}) was 1.1 ± 0.1 μm, as determined by laser light scattering (LA-900, Horiba Instruments, Irvine, CA and LS-230, Coulter Corp., Miami, FL).

For heat processing studies, a Brij 35 stabilized salmon oil-in-water emulsion was used in all experiments. The emulsion was separated into 30 ml allocations, which were heated in a water bath (NESlab GP-200, Fisher Scientific, Suwanee, GA) for a total of 10 min, and then immediately cooled in an ice bath for a total of 30 min.

2.2.3. Preparation of EDTA containing samples

EDTA solutions were made by dissolving EDTA in double-distilled water obtained from a water purification system (Barnstead NANOpure infinity ultra pure, Dubuque, Iowa). EDTA solutions of varying concentrations were added to 30 ml of emulsion at a volume of 100 μl and the emulsion was stirred for 1 min. EDTA was added to non-heated samples and to heated samples either prior to or after heat-processing.

2.2.4. Preparation of calcium components

Calcium chloride solutions were made by dissolving calcium chloride in double-distilled water. Calcium solutions of varying concentrations were added to 30 ml of emulsion at a volume of 100 μl and the emulsion was stirred for 1 min. Calcium was added to samples containing no EDTA and to samples containing 7.5 μM EDTA, all of which were non-heated samples.

2.2.5. Lipid oxidation measurements

Emulsions (10 ml) were placed in capped glass test tubes (16 × 125 mm; Fisherbrand) and incubated in the dark at 20 °C for 8 days. Controls contained the salmon oil emulsion only and were not heat-processed. Lipid hydroperoxides were determined daily using a method adapted from Shantha and Decker (1994). Emulsion (0.3 ml) was added to a mixture of 1.5 ml of isooctane/2-propanol (3:1 v/v), vortexed (10 s, 3 times), and the organic solvent phase was isolated 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 3.94 M ammonium thiocyanate and 15 μl of ferrous iron solution (prepared by adding equal amounts of 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$). After 20 min, the absorbance was measured at 510 nm using a UV–Vis scanning spectrophotometer (Shimadzu UV-2101PC UV-VIS, Kyoto, Japan). Hydroperoxide concentrations were determined, using a standard curve made from cumene hydroperoxide.

Thiobarbituric acid-reactive substances (TBARS) were determined daily, using the method of McDonald and Hultin (1987). Emulsion (0.05 ml) was combined with 0.95 ml of water and 2.0 ml of TBA reagent (15% w/v trichloroacetic acid and 0.375% w/v thiobarbituric acid in 0.25 M HCl mixed with 2% BHT in ethanol solution) in test tubes and placed in a boiling water bath for 15 min. The tubes were cooled to room temperature for 10 min and then centrifuged (1000*g*) for 15 min. The absorbance was measured at 532 nm. Concentrations of TBARS were determined from a standard curve prepared using 1,1,3,3-tetraethoxypropane.

2.2.6. Statistics

All experiments were conducted twice and measurements were performed on triplicate samples. Differences between means were determined with the least-squares means procedure at $p < 0.05$ (Snedecor & Cochran, 1989).

3. Results and discussion

3.1. Heat processing and holding time

The effect of holding temperature on the physical and oxidative stability of the emulsion was tested by heating 30 ml of emulsion in a water bath heated to 50, 70, and 90 °C, and holding for 10 min. A control was prepared that was not heated. The emulsions were then cooled to 20 °C and stored for 8 days in capped glass test tubes (16 × 125 mm; Fisherbrand) in the dark. The droplet size distribution was monomodal and the mean droplet diameter remained stable (d_{43} = 1.1 ± 0.1 μM) as a function of storage time, indicating that heat-processing had little effect on the physical stability of the emulsions. The effects of thermal processing on oxidative stability were initially screened using TBARS. Over 8 days, TBARS concentrations (Fig. 1) of all samples increased at similar rates. These results indicate that emulsions that were heat-processed at temperatures up to 90 °C for 10 min did not oxidize at faster rates during storage than the unheated control sample.

3.2. Influence of EDTA concentration on lipid oxidation

Various concentrations of EDTA (0–150 μM) were added to non-heated emulsions in order to determine the minimum amount of EDTA needed to retard lipid oxidation in the system. TBARS were used to initially screen the effects of EDTA concentration on oxidative stability. TBARS values showed that the control sample containing no EDTA oxidized at the fastest rate over 8 days (Fig. 2(a)). EDTA at 0.1 μM was not able to retard

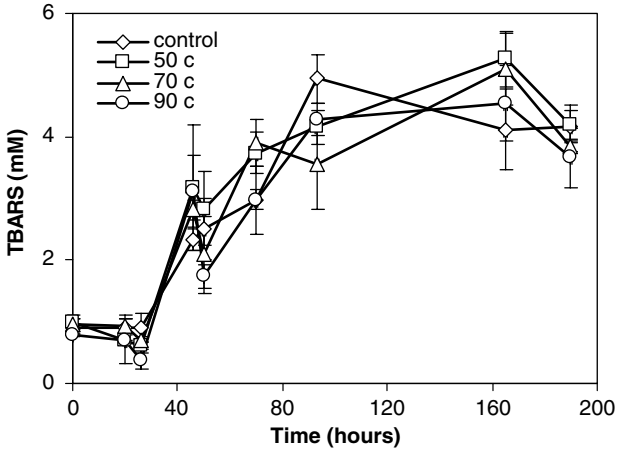

Fig. 1. TBARS concentration of salmon oil-in-water emulsions exposed to heat treatments of 50, 70, 90 °C and one control (not heated) stored at 20 °C and measured over 8 days.

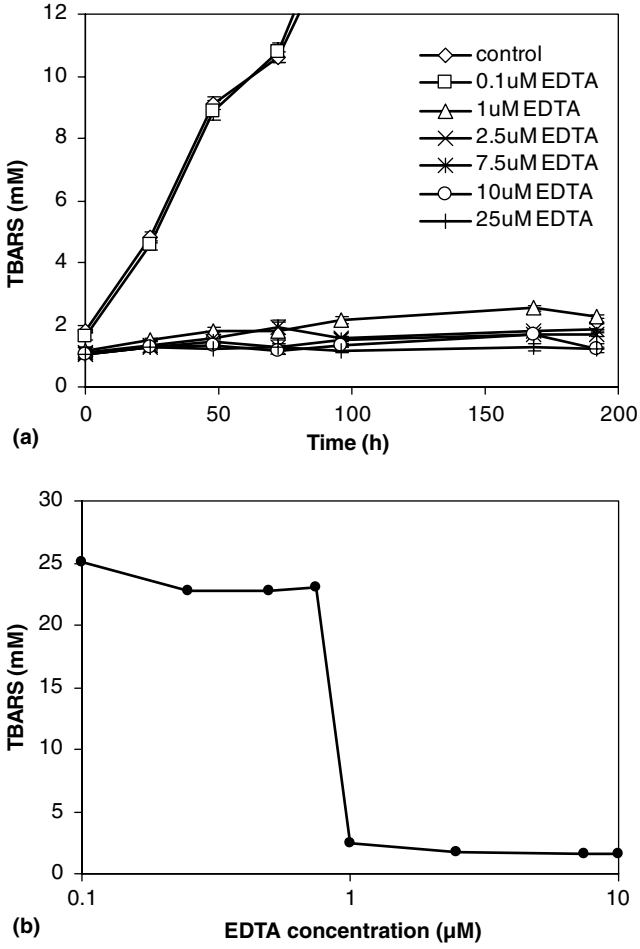

Fig. 2. TBARS concentration (a) of salmon oil-in-water emulsion stored at 20 °C and measured over 8 days. Samples contain varying amounts of EDTA as well as one control with no EDTA added. TBARS values comparison of EDTA concentration and its effect on lipid oxidation at day 7 (b).

lipid oxidation and this emulsion oxidized at the same rate as the control. Samples containing 1–25 μM EDTA successfully retarded lipid oxidation over 8 days, with ⩾2.5 μM EDTA being more effective at inhibiting oxidation than the sample containing 1.0 μM EDTA after 5 days of storage (Fig. 2(a)). As little as 1.0 μM EDTA was found to be sufficient for inhibiting oxidation after 7 days of storage (Fig. 2(b)). The ability of EDTA to completely inhibit oxidation suggests that the transition metals naturally present in the oil and/or water are promoting lipid oxidation (Cuvelier, Langunes-Galvez, & Berset, 2003). EDTA has been reported to promote oxidation by increasing both the solubility and the oxidation-reduction potential of iron when added at an EDTA to iron ratio of <1 (Mahoney & Graf, 1986). This prooxidant effect was not seen in the salmon oil-in-water emulsion used in this study. EDTA will act as an antioxidant at an EDTA to iron ratio of >1 (Mahoney & Graf, 1986). The results in Fig. 2(b) showing inhibition of lipid hydroperoxides at EDTA concentrations ⩾1.0 μM suggest that the iron level in the emulsion was between 0.1 and 1.0 μM.

3.3. Influence of adding EDTA before or after heating

Since samples heated to 50, 70, and 90 °C oxidized at similar rates (see Fig. 1), we chose to heat all samples at 90 °C for 10 min. EDTA at a concentration of 10 μM was added to samples either before or after heat processing, in order to determine if time of addition had any influence on chelating activity. TBARS (Fig. 3(a)) and lipid hydroperoxide (Fig. 3(b)) values illustrate that the control and the sample heated to 90 °C both oxidized, with the heated sample oxidizing at a similar rate to unheated emulsion, as seen in Fig. 1. EDTA (10 μM) was able to dramatically decrease lipid oxidation in the samples to which it was added. Samples where EDTA was added before heat treatment had slightly lower TBARS and hydroperoxide values than samples where

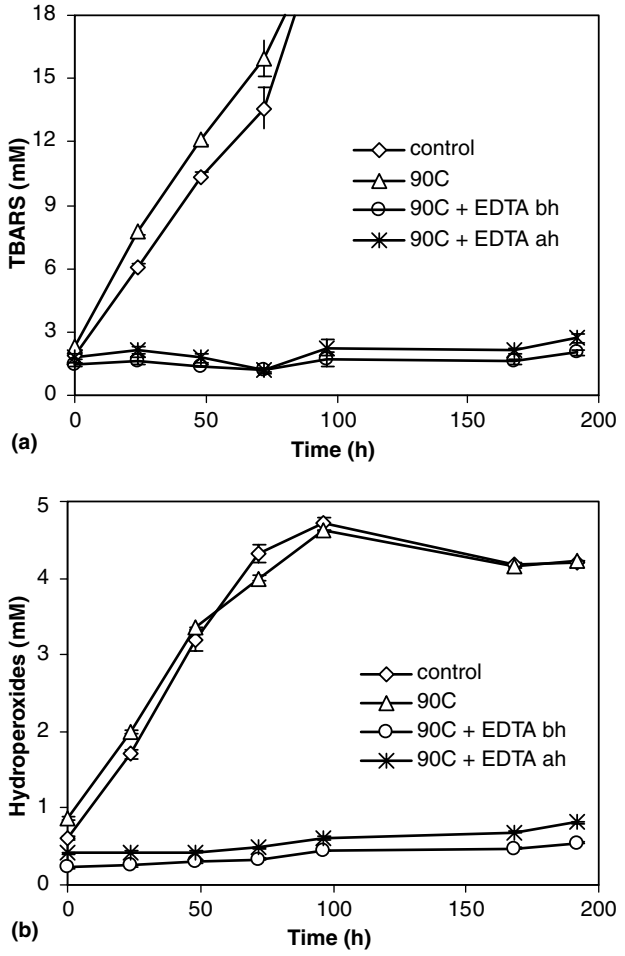

Fig. 3. TBARS concentration (a) and hydroperoxide concentration (b) of salmon oil-in-water emulsions heat-processed at 90 °C, stored at 20 °C and measured over 8 days. EDTA (7.5 μM) was added to samples before or after heat-processing.

EDTA was added after heating ($p \leqslant 0.05$). This experiment showed that addition of EDTA, either before or after heating, did not have a major impact on oxidation, but the best protection is obtained by adding it before thermal processing.

3.4. Influence of calcium on antioxidant effects of EDTA

It is possible that the effectiveness of EDTA as an antioxidant can be diminished in the presence of calcium. Calcium has a lower tendency to bind with EDTA than does iron but it may compete with iron for EDTA if it is present at a sufficiently high concentration. Therefore, when calcium is added to an emulsion, some unchelated, reactive iron could be left in the system, possibly overcoming the inhibitory effects of EDTA and leading to lipid oxidation. In this set of experiments, 7.5 μM EDTA was added to all samples (except the control) and calcium was added at concentrations ranging from 1.6 to 62.5 μM. Addition of calcium to emulsions was found to significantly ($p < 0.05$) increase both TBARS (Fig. 4(a)) and lipid hydroperoxide (Fig. 4(b)) formation after 3 days of storage when calcium concentrations were 2-fold greater than EDTA concentrations. EDTA-containing samples that contained less than 15.6 μM added calcium showed the same low levels of lipid oxidation, as in the absence of calcium.

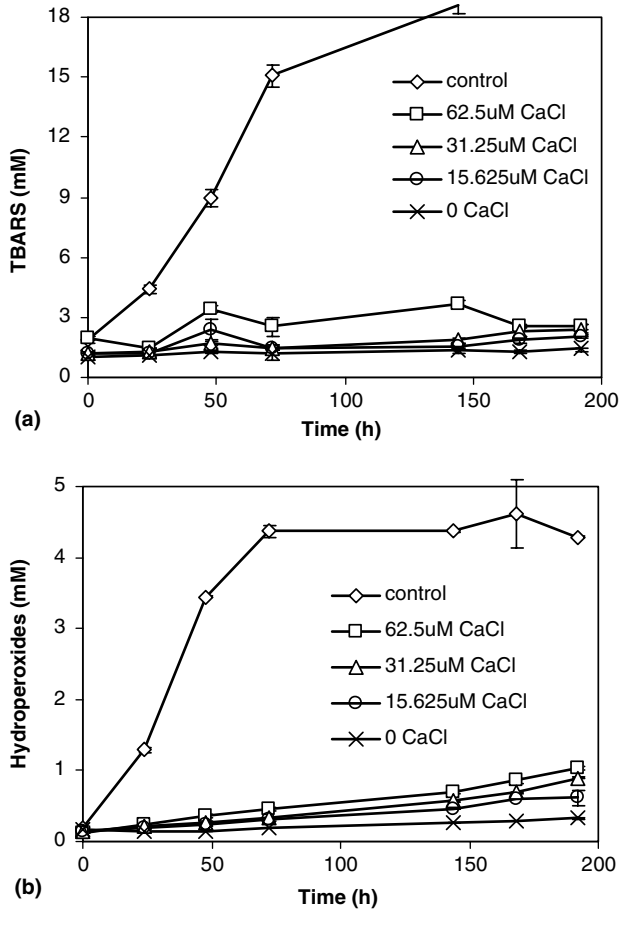

Fig. 4. TBARS concentration (a) and hydroperoxide concentration (b) of non-heated salmon oil-in-water emulsions stored at 20 °C and measured over 8 days. Samples contain 7.5 μM EDTA and varying amounts of CaCl, added after homogenization, as well as a control with no EDTA and no CaCl added.

4. Conclusions

We have examined the effects of heat-processing and calcium ions on the ability of EDTA to inhibit lipid oxidation in Brij 35-stabilized salmon oil-in-water emulsions at pH 7. Heat-processing had no effect on the physical or oxidative stability of emulsions in the absence of EDTA. EDTA, at a concentration of 2.5 μM, was able to almost completely inhibit oxidation. However, the addition of calcium at concentrations 2-fold higher than that of the concentration of EDTA resulted in higher oxidation values, presumably due to its ability to compete with the chelating agent and release iron. The addition of EDTA to samples before heat-processing had a greater effect on their overall ability to inhibit lipid oxidation than in samples where EDTA was added after heat-processing. These results indicate that heat-processed salmon oil-in-water emulsions, with high physical and oxidative stability, could be produced in the presence of EDTA. These emulsions could be an excellent source of oxidatively stable ω-3 fatty acids and could be used as functional food ingredients.

References

Akoh, C. C., & Min, D. B. (2002). In *Food lipids: chemistry, nutrition, and biotechnology* (2nd ed., revised and expanded, pp. 589–636). New York: Marcel Dekker, Inc..

Cuvelier, M. E., Langunes-Galvez, L., & Berset, C. (2003). Do antioxidants improve the oxidative stability of oil-in-water emulsions?. *Journal of the American Oil Chemists Society, 80*(11), 1001–1005.

Frankel, E. N., Satue-Gracia, T., Meyer, A. S., & German, J. B. (2002). Oxidative stability of fish and algae oils containing long-chain polyunsaturated fatty acids in bulk and in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 50*(7), 2094–2099.

Innis, S. M. (1991). Essential fatty acids in growth and development. *Progress in Lipid Research, 30*(1), 39–103.

Lytle, J. S., Lytle, T. F., Newmark, H. L., & Deschner, E. E. (1992). Stability of a commercially prepared fish oil (omega-3 fatty acid) laboratory rodent diet. *Nutrition and Cancer – An International Cancer, 17*(2), 187–194.

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

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.

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

Mei, L. Y., Decker, E. A., & McClements, D. J. (1998). Evidence of iron association with emulsion droplets and its impact on lipid oxidation. *Journal of Agricultural and Food Chemistry, 46*(12), 5072–5077.

Miller, D. D. (1996). Minerals. In O. R. Fennema (Ed.), *Food chemistry* (3rd ed., pp. 617–649). New York: Marcel Dekker.

Nuchi, C. D., McClements, D. J., & Decker, E. A. (2001). Impact of tween 20 hydroperoxides and iron on the oxidation of methyl linoleate and salmon oil dispersions. *Journal of Agricultural and Food Chemistry, 49*, 4912–4916.

Shantha, N. C., & Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of Agricultural and Food Chemistry, 77*, 421–424.

Simopoulous, A. P. (1991). Omega-3 fatty acids in health and disease and in growth and development. *American Journal of Clinical Nutrition, 54*(2), 438–463.

Snedecor, G. W., & Cochran, G. W. (1989). In *Statistical methods* (8th ed., pp. 343–438). Ames, IA: Iowa State University Press.

Tong, L. M., Sasaki, S., McClements, D. J., & Decker, E. A. (2000). Antioxidant activity of whey in a salmon oil emulsion. *Journal of Food Science, 65*(8), 1325–1329.