Investigating the Relationship between Antioxidants and Fatty Acid Degradation Using a Combination Approach of GC-FID and Square-Wave Voltammetry

Katelyn A. Keene, Robert M. Ruddy, and Matthew J. Fhaner*®

University of Michigan at Flint, 303 E. Kearsley Street, 566A Murchie Science Building, Flint, Michigan 48502-1950, United States

Supporting Information

ABSTRACT: Analytical methodology for direct investigation of antioxidant systems continues to be a pressing research area. Consumer demand for natural products requires an increase in natural antioxidants; thus, fast, high-throughput, and cost-effective screening methods are in demand. In this study, square-wave voltammetry and gas chromatography with flame ionization detection (GC-FID) were used in conjunction to monitor antioxidant and fatty acid degradation, respectively, during the accelerated degradation of an omega-3 fatty acid sample. Butylated hydroxytoluene, sesamol, and rosemary extract were investigated as antioxidants. It was determined that voltammetry could be used to monitor the reduction in oxidation current, which provides a direct assessment method for the reduction of native antioxidant concentration throughout the accelerated degradation. Furthermore, results showed that voltammetry could be used to monitor fatty acid degradation similarly to the fatty acid methyl ester analysis routinely performed using gas chromatography separation. Both voltammetry and GC-FID methods reached similar conclusions about antioxidant quality and efficiency for omega-3 fatty acid protection.

INTRODUCTION

In recent years, the study of omega-3 (ω-3) fatty acids has greatly increased. This is largely due to their positive health benefits, including the reduction of cardiovascular-related deaths, prenatal brain development, and having potential implications in inflammation control.1−3 This interest has sparked a boom in ω-3 fatty acids being incorporated into a wide variety of consumer products, including direct supplementation, as functional foods, and in cosmetics.4−8 As ω-3 fatty acid consumer intake has increased, a need for effective antioxidants has become vital. Although intake of ω-3 fatty acids may provide health benefits, there is also evidence that consumption of oxidized ω-3 fatty acids may have undesirable consequences beyond foul smells and tastes and may negatively impact overall health.9−11 Esterbauer et al. found that oxidized ω-3 fatty acids reduced growth and increased inflammation markers in animals.12 There are few studies directly investigating human subjects and impacts of oxidized ω-3 fatty acids; however, the studies in the literature suggest that there are no significant adverse health effects over short-term monitoring.13 Without more complete long-term studies on the effects of oxidized ω-3 fatty acids, along with contradictory evidence found in the literature, it remains prudent to ensure that ω-3 fatty acids that reach public consumption are well protected from oxidation with high-efficiency antioxidants.

Another trend in consumer interest is a higher demand for more “clean labels” from their products.14 To this extent, interest in natural antioxidants has increased in recent years partly due to this demand for products with reduced amounts of synthetic molecules. This has led to a variety of studies investigating natural antioxidant species. One such species that has shown promise as a natural antioxidant is sesamol. Hwang et al. determined that sesamol provided improved protection of ω-3 fatty acids compared to butylated hydroxytoluene (BHT), an established synthetic antioxidant currently used in consumer products.15 Sesamol was also determined to provide protection that was comparable to a leading natural antioxidant, rosemary extract (RE).

The work of Hwang et al. provided insights related to lipid degradation in the presence of natural antioxidants; however, no direct analysis of the antioxidant degradation was performed. This is a critical connection to make for functional food researchers, as real-time monitoring of antioxidants during accelerated degradation studies would provide a more complete understanding of the relationship between antioxidant concentration and fatty acid protection. Although it is known that some antioxidant species such as TBHQ and...
sesamol can continue to provide protection after degrading to products such as dimers or radicals, direct monitoring of primary antioxidant levels could provide insights toward better characterization and understanding of established and novel antioxidants used in commercial products.16–18

In the current work, a novel application of electrochemistry is proposed to bridge the gap in knowledge between antioxidant depletion and fatty acid degradation. Electrochemistry has been widely used in the investigation of antioxidant species due to the low cost, rapid analysis times, low consumable requirements, and the direct analysis of electroactive species.19–21 Voltammetric techniques have been used to investigate prominent antioxidant species such as vitamin E (tocopherol).22,23 Our group previously investigated incorporating voltammetry into routine applications. Specifically, voltammetry was compared to high-performance liquid chromatography (HPLC) to monitor the removal of tocopherols.19 Voltammetric analysis of antioxidants is poised to be a critical tool for continued investigation of antioxidant systems, which could help solve complex issues within functional food research. These methods provide a direct analysis of antioxidant species, in comparison with spectroscopic methods such as ABTS or DPPH radical scavenging methods.

In this study, square-wave voltammetry is used in conjunction with gas chromatography coupled to a flame ionization detector (GC-FID) to elucidate a potential cause and effect relationship between antioxidant levels and fatty acid composition. Square-wave voltammetry was selected due to its background suppression, faster scan times, and improved sensitivity relative to differential pulse voltammetry.21 BHT, sesamol, and RE were added to commercial fish oil that was stripped of any supplemented antioxidants. Samples were subjected to an accelerated degradation protocol and monitored to track the changes in both fatty acid composition and antioxidant oxidation current. Individual antioxidants were investigated to determine their unique electrochemical behavior, including oxidation potential, limit of detection, and lower limit of quantitation. Additionally, antioxidant- and control-treated fish oil samples were subjected to a time-course study. Oxidative currents for the antioxidants, along with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were recorded in conjunction with fatty acid methyl ester (FAME) analysis of the fatty acids in each sample. Results support the ability of electrochemistry to be used as a real-time tool to monitor fatty acid degradation alongside relative antioxidant concentrations; thus providing a rapid and accessible alternative to GC-FID analysis.

### RESULTS

#### Determining Figures of Merit for Antioxidants

There has been recent interest in utilizing electrochemistry for the characterization of antioxidant systems, including BHT and sesamol.24–27 However, there are few, if any, direct measurements on figures of merits, and none to the knowledge of the authors in the presence of polyunsaturated fatty acid (PUFA) samples. Before engaging in quantitative work, the oxidation potentials of each antioxidant were first determined using 1 mM concentrations. The voltammograms are shown in Figure 1. Each antioxidant has a slightly different oxidation potential versus an Ag/AgCl reference electrode, with sesamol oxidizing at 0.804 V, RE oxidizing at 1.01 V, and BHT oxidizing at 1.16 V. Sesamol was observed to have a narrower peak when compared to RE and BHT. This suggests that sesamol is readily able to donate electrons, while yielding little current from the reverse process through reduction of the recently oxidized sesamol.27 This is supported by the fact that sesamol forms dimers, which are also readily oxidizable.28 For RE, the smaller oxidation current and broader peak are likely a result of competing reversible oxidation–reduction currents due to the different species that comprise rosemary extract. It has been shown that both carnosic and rosmarinic acid, which are the primary components of rosemary extract, produce reversible electrochemical properties when subjected to anodic and cathodic currents.29,30 BHT also shows a smaller current and a wider peak than sesamol, along with a broader decay slope, which could be partially due to the steric hindrance of BHT and sesamol.

After qualitative analysis of the antioxidants, figures of merit were investigated. Figure 2 provides an overview of the calibration curves for sesamol, BHT, and RE. Due to the low sensitivity of RE, additional concentrations were added at 50

![Figure 1](image1.png)

**Figure 1.** Overlay of voltammograms for sesamol (solid line), BHT (dotted line), and RE (dashed line) all at 1 mM concentrations. Potential was scanned versus a silver–silver/chloride reference electrode.

![Figure 2](image2.png)

**Figure 2.** Calibration curves produced from the addition of BHT (black circles), sesamol (white circles), and RE (black triangles) to solvent system spiked with stripped fish oil. Best-fit lines are provided using linear regression with the equations provided for each species.
treatments. The control oil, which was stripped of background-subtracted voltammetric signals for all four antioxidants, produced one oxidation peak near 0.6 V vs Ag/AgCl; however, the standard did not have any overlap with other peaks. The oxidation peak observed within the stripped fish oil has been described previously by Lanz et al.; however, there was only a supposition made that individual peaks were due to EPA and DHA individually. To better understand how EPA and DHA were oxidized, standards were investigated for their oxidation potentials.

Figure 4A provides overlays of 0.25 M EPA and 0.17 M DHA standards in the solvent system only. These concentrations mimic the concentration of each fatty acid in PUFA supplements used in the study. Both EPA and DHA show an oxidation peak near 1.19 V vs Ag/AgCl. EPA exhibits a second oxidation peak near 0.6 V vs Ag/AgCl; however, the standard was only guaranteed to be 95% pure, so there may have been a contaminant present. Figure 4B details a stripped fish oil sample spiked with EPA initially, followed by DHA. Addition of the EPA appears to cause an increase in both oxidation peaks; however, subsequent addition of DHA further increases the oxidation peak at 1.19 V. The combined data suggest that the fatty acid oxidation peak near 1.19 V is a combined oxidation current from EPA and DHA, whereas the fatty acid peak near 1.03 V is from a different species.

Validating the two native oxidation peaks exhibited by stripped fish oil allowed for the time-course overlays for each treatment to be fully examined, which are shown in Figure 5. Figure 5A shows the control treatment, for which only the oxidation of fish oil is observed. It was noted that the oxidation current magnitude (peak current) decreased with time. Oxidation current is a measure of the maximum analyte flux to the electrode surface. The peak current is related to the analyte concentration through the Cottrell factor shown in eq 1.

\[
\Delta i = \frac{nFAD_{1/2}C_0}{\pi^{1/2}t^{1/2}}
\]

The Cottrell factor relates the concentration of a species proportionally to the change in current. This relationship is assumed for linear sweep voltammetry but also holds true for square-wave voltammetric currents, which provide roughly 93% of expected signal generated for normal pulse voltammetry conditions. Thus, the decrease in current observed for the control treatment is related to a decrease in total nonoxidized fatty acids.

Figure 5B–D displays the day 0, 3, 7, and 10 background-subtracted voltammograms for BHT, sesamol, and RE. BHT is oxidized at 1.16 V vs Ag/AgCl, as shown in Figure 1. Therefore, the oxidation current for BHT overlaps with the fish oil oxidation and cannot be directly resolved. Sesamol and RE treatments are detailed in Figure 5C,D. Sesamol’s oxidation
peak is fully resolved from the sample peaks, whereas RE is adequately resolved at higher concentrations. Upon degradation, RE signal begins to overlap with the sample oxidation peaks as the concentration of reduced RE decreases. Figure 5 shows that all treatments, except for day 10 of the control treatment, show a decrease in observed oxidation current for the fish oil oxidation peaks from day 0 to day 10. After day 10, all oxidation peaks are indistinguishable from the background.
and RE (white circles). Statistical differences for data within a given time point are noted with appropriate symbols. (C) Oxidation current percentages for fatty acid peak 2 (EPA/DHA peak) are overlaid for stripped fish oil only (control; black triangles), sesamol (black circles), and RE (white circles). Statistical differences for data within a given time point are noted with appropriate symbols.

Figure 6. Changes in peak oxidation currents normalized to day 0 initial values. (A) BHT (black circles), sesamol (white circles), and RE (black triangles) oxidation current percentages are overlaid from day 0 to day 10. All oxidation current percentages decrease with time. (B) Oxidation current percentages for fatty acid peak 1 are overlaid for stripped fish oil only (control; white triangles), BHT (black circles), sesamol (white circles), and RE (black triangles). Statistical differences for data within a given time point are noted with appropriate symbols. (C) Oxidation current percentages for fatty acid peak 2 (EPA/DHA peak) are overlaid for stripped fish oil only (control; black triangles), sesamol (black circles), and RE (white circles). Statistical differences for data within a given time point are noted with appropriate symbols.

noise and can no longer be detected. It was observed among all treatments that the samples became more solid over time as they oxidized. The control treatments were the first to show these signs. They exhibited viscous properties by day 5 and became a solid gel by day 10. This most likely resulted in an increased sample resistance, which expressed itself as a higher overall current in day 10 as compared to previous time points. The antioxidant-treated samples did not show solidification until day 10. At this point, only BHT showed signs of morphology change, whereas sesamol and RE remained unchanged until later time points.

To further analyze the time-course dependency of oxidation currents, the background-subtracted current signals were converted to a percentage of day 0 values. In Figure 6A, the current percentage was plotted from day 0 to 10 for each antioxidant peak to investigate the rate of antioxidant depletion. BHT was included in an effort to provide full context to the data; however, it bears little significance since the oxidation current for BHT is a combination of the antioxidant, EPA, and DHA currents. A general trend is seen that the oxidation current of each species decreases with time, which is indicative of antioxidant concentration decreasing. The peak currents of both RE and sesamol fall to roughly 10% of their initial values by day 10, which suggests that they are being readily used to inhibit lipid oxidation. BHT has a higher peak current percentage than the other antioxidants on day 10; however, this can be attributed to the combination of signals observed at the potential where BHT is oxidized. Studies were undertaken to determine if adsorption may have contributed to the decrease in antioxidant signal over time. It was determined that adsorption had little to no impact on the oxidation current decline, as presented in Figures 2 and 3 of the Supporting Information section.

Figure 6B,C details the peak currents of the two oxidation peaks observed in fish oil. Again, current signals were converted to a percentage of their day 0 value for individual treatments. It was determined that the second oxidation peak in fish oil is due to a combination of EPA and DHA; however, the first oxidation peak observed from the fish oil sample remains unknown. Although unidentified, the initial oxidation peak provides a unique platform for monitoring lipid degradation in all treatments, as it has a lower amount of overlap between antioxidant signals when compared with the second oxidation peak. In Figure 6B, the control treatment’s peak current percentage decreased rapidly and is roughly 10% of the total current observed on day 0 by day 3. The trend observed for the first fatty acid oxidation peak is very similar for sesamol and BHT, with a 50% decrease observed by day 3. This decline continues for BHT to 40% on day 7 and 20% on day 10. However, sesamol preserves the oxidation peak through day 7, maintaining 50% of the day 0 value. By day 10, conservation of the oxidation peak ends, and the sesamol-treated sample begins showing further reduction in the fatty acid peak oxidation. RE also shows a decrease in oxidation current over the initial days of the study, dropping to roughly 60% of the day 0 value by day 5. However, RE does not show any further decrease and maintains a 60−70% value for the fatty acid oxidation peak through day 10. The data support RE as the antioxidant that preserved the fish oil oxidation signal most effectively, followed by sesamol and BHT respectively.

The second fatty acid peak, which will be referred to as the EPA/DHA peak, was also analyzed similar to the first peak and is provided in Figure 6C. Due to the overlap, BHT was not included in the data presented, as there was no way to deconvolute the two peaks. The EPA/DHA peak provides a direct comparison point for the GC-FID studies that were also performed and have been historically used for monitoring fatty acids. Similar to Figure 6B, the control treatment for the EPA/DHA peak showed a decrease in signal compared to the antioxidant treatments. The control treatment decreased to 50% of the day 0 value on days 2 and 3 and further decreased in value from 20 to 30% of the day 0 values for days 5−10. Sesamol also had a decrease in EPA/DHA peak current percentage between days 0 and 3; however, it preserved a significantly larger signal compared to the control treatment for days 5 and 7. By day 10, the sesamol EPA/DHA peak current decreased to control treatment levels. RE-treated samples were the only samples to maintain a significantly elevated oxidation signal for the EPA/DHA peak compared to the control sample through day 10. It is important to note that the RE EPA/DHA peak current percentages were not statistically different from sesamol, except for day 10. This suggests that RE provided similar protection to sesamol.
through day 7 and continued protecting EPA and DHA through day 10, whereas sesamol did not.

GC-FID Analysis. To provide a comparison point to the electrochemical studies, GC-FID analysis was also performed on each sample. An overview of the peak area percentages for the GC-FID analysis of EPA and DHA is shown in Figure 7. For both EPA and DHA, the control treatment showed an immediate decrease in peak area from day 0 to day 1, with a continued decrease until the value reached a minimum on days 14 and 17. An oddity occurred with the control treatment on day 3, which yielded an apparent negative spike in peak areas along with probing the possibility of antioxidant depletion in situ capabilities. RE yielded the lowest analytical sensitivity, yet provided superior protection of EPA and DHA compared to sesamol and BHT. The figures of merit and methodology presented in this study provide an important characterization for a number of intensely studied antioxidants; however, continued investigation into metrics connecting electrochemical characterization and antioxidant capacity will help researchers identify new antioxidant candidates in a targeted way.

Antioxidant depletion was monitored via oxidation current percentages, as shown in Figure 6A. A direct decrease in oxidation current can be seen for all antioxidant species, which should relate to a depletion in concentration through eq 1. Although the correlation between a decrease in peak current and antioxidant concentration is evident, there remains varying possibilities for what causes the decrease in analytical signal. Nonoxidized antioxidant levels may decrease in response to other species than strictly fatty acid radicals. One of the most likely candidates is dissolved oxygen. Numerous studies report the effects of oxygen content on lipid degradation. Fujisaki et al. found that in high-oleic safflower oil, decreasing the oxygen content from 20 to 2% greatly decreased the oxidation rate of tocopherol. Although no direct comparison was found for dissolved oxygen content in bulk fish oil, values of 10.3 ppm for processed soybean oil and 5.42 mg/L for extra virgin olive oil provide a baseline for dissolved oxygen content in bulk oils. These values are significantly lower than the manufacturer values for EPA and DHA, which are 2.92 × 10° and 2.02 × 10° mg/L, respectively. Furthermore, Min and Wen reported that dissolved oxygen content in soybean oil was completely consumed within 48 h of an accelerated oxidation reaction.

Figure 7. Peak area percentages are overlaid for stripped fish oil only (control; white triangles), BHT (black circles), sesamol (white circles), and RE (black triangles) for both EPA (A) and DHA (B). Statistical differences for data within a given time point are noted with appropriate symbols.
decrease in antioxidant current is related to dissolved oxygen and fatty acid degradation. However, the final days of heating would result in antioxidant levels being primarily related to interactions with degraded fatty acids.

With potential limitations in mind, an exciting finding in this work was the relative effectiveness in which antioxidant protections of EPA and DHA could be visualized and determined using both electrochemistry and GC-FID methods. Figure 6B shows that little statistical difference exists between antioxidant treatment peak currents through day 7, which is consistent with the GC-FID analysis results outlined in Figure 7. In both the electrochemistry and GC-FID analyses, it was concluded that RE preserved the signal for fatty acids more strongly than sesamol, which in turn was found to preserve signal more strongly than BHT. Both techniques also were able to identify day 10 as a key time point in the separation of each antioxidant, along with the superior performance of RE. Although GC-FID FAME analysis provides superior LOD and LLOQ for fatty acids, similar conclusions were reached for antioxidant effectiveness using both methods.57

This work provides new paths for exploration into real-time analysis of both antioxidant and fatty acid levels. By monitoring levels of antioxidants, along with fatty acid levels, electrochemistry provides a direct, quick, rugged, and transportable method for potentially assessing quality during the proposed shelf-life of a product. Furthermore, future work could include correlating sensory panel feedback and oxidation currents to determine if there is a threshold level that can be detected by consumers. These efforts may allow for relatively inexpensive and fast analysis of products suspected of having unacceptable levels of omega-3 fatty acids through antioxidant or fatty acid analytical signal. Another area of opportunity for this work may be within the manufacturing or restaurant industries. Square-wave voltammetry could provide a more direct method for determining if a product has degraded in a manufacturing or a restaurant environment prior to foul taste or smells developing. Finally, using square-wave voltammetry, a library of voltammograms for varying fatty acids could be generated to build on current knowledge and characterization tools of these systems. These areas of research provide the potential to yield fast, inexpensive, and rugged methods to provide higher quality products to consumers in an effort to preserve the functional food aspects, along with health and well-being qualities of a given product.

MATERIALS
Commercial ω-3 fish oil purified from sardines and anchovies and supplemented with tocopherol was obtained from Nordic Naturals (YE-1022/10686077, Watsonville, CA). Aluminum oxide (199974-1 KG, Sigma-Aldrich, St. Louis, MO), sand (S25S16B, Fisher Science, Waltham, MA), Pyrex glass wool (3950, Corning Inc., Corning, NY), and hexanes (H302-4, Fisher Science) were used for stripping the fish oil. Guardian Rosemary Extract 08 (Danisco), butylated hydroxytoluene (V218405-Sample-K, Sigma-Aldrich), and sesamol (S3003-25g-A, Sigma-Aldrich) were used as standards for the figures of merit investigation. The electrochemical system was comprised of a CH Instruments 660E SN: A3192, using a three-electrode configuration consisting of a platinum counter electrode (CH1115, CH Instruments, TX), silver−silver/chloride reference electrode (CH1111), and a glassy carbon working electrode (CH1104). The solvent system used for the electrochemical studies was 1:2 v/v benzene−methanol (benzene − BX220-5, EMD Millipore, Germany; methanol − A412-4, Fisher Chemical). It had a final concentration of 0.12 M sulfuric acid (A300-500, Fisher Scientific), which is an optimized solvent system reported by Litescu et al. For FAME analysis, Hach tubes were sealed using sealing tape (ContainerSeal, Diversified Biotech, MA), and methyl esterification was performed using 3N methanolic HCl (33050-U, Sigma-Aldrich). Extraction was performed with UltraPure DI water and hexanes (L-18419, Fisher Scientific). EPA and DHA were obtained from Larodan (Larodan, Sweden; EPA − 10-2005 at 95% purity; DHA − 10-2206 at 97% purity).

METHODS
Antioxidant Stripping from Commercial Fish Oil. Antioxidants were removed from commercial fish oil, as previously described. Briefly, commercial fish oil was passed through an aluminum oxide column using hexanes as a mobile phase. Fish oil was collected and spun on a rotovap under vacuum to remove excess hexanes. Stripped fish oil was tested using electrochemistry to determine if supplemented antioxidants were removed successfully. Stripped fish oil was stored at −80 °C under argon until needed for further experimentation.

Accelerated Degradation of Control and Treated Fish Oil Samples. Freshly stripped fish oil was divided into four batches. Three batches were supplemented with 0.84 mM of the antioxidants BHT, sesamol, and RE. The fourth batch was used with no further augmentation and served as a control treatment. Each batch of fish oil was distributed into 2 g aliquots and stored in 20 mL scintillation vials. Enough scintillation vials were prepared for each batch, such that each time point of the study was represented in triplicate, for a total of 30 individual vials prepared for every treatment. The vials were stored at 40 °C with their caps loosely fitting. Data were collected on days 0, 1, 2, 3, 5, 7, 10, 14, and 17. On day 17, all samples exhibited gel-like structural qualities, so further testing was canceled.

Antioxidant Figures of Merit Determination. Antioxidant standards were created in varying concentrations between 1 and 1000 μM. Each standard contained 750 μL of stripped fish oil, an appropriate amount of antioxidant standard for the given final concentration, and enough solvent such that every sample had a final volume of 8.25 mL. Each standard concentration was prepared freshly in triplicate directly before testing to provide a consistent amount of time from preparation to analysis for every sample. Prior to testing a new concentration, the glassy carbon electrode was polished for 30 s in an alumina slurry, followed by a 10 min soak in hexanes, then another 10 min in the solvent. Finally, three voltammograms were collected as background traces before introducing the electrode to a new standard concentration. Each standard voltammogram was background subtracted, and the oxidation current was plotted against the antioxidant concentration to generate a calibration curve. All voltammograms were collected using a CH Instruments potentiostat, a glassy carbon working electrode, silver−silver/chloride (Ag/AgCl) reference electrode, and a platinum counter electrode. Data were generated using square-wave voltammetry. The potential was scanned from 0.3 to 1.5 V (vs Ag/AgCl) at a frequency of 2 Hz in increments of 0.004 V and an amplitude.
of 0.005 V. The quiet time prior to each scan was 2 s, and the sensitivity was set to $10^{-5}$ A/V. These parameters are equivalent to a scan rate of 8 mV/s. Limits of detection (LOD) and lower limits of quantitation (LLOQ) for each species were determined using the following equations:

$$\text{Limit of detection} = \frac{3s}{m}$$  \hspace{1cm} (2)

$$\text{Lower limit of quantitation} = \frac{10s}{m}$$  \hspace{1cm} (3)

In both eqs 2 and 3, “s” is the standard deviation of the lowest observable change in background signal with the addition of a standard.

**Square-Wave Voltammetric Analysis of Control and Treated Fish Oil Samples.** Electrochemical measurements were carried out using an electrode configuration and voltammetric parameters identical to those described in the Figures of Merit section above. Each day, the working electrode was polished using a polishing kit (CHI1120, CH Instruments, TX). After polishing the working electrode, all electrodes were soaked in hexanes for 10 min, then in the solvent system for an additional 10 min. Three voltammograms were performed to condition the electrode. The fourth voltammogram collected was used as a background data file and was subtracted from all sample voltammograms. Three sample vials for each of the four treatments were removed from the oven. An amount of 750 µL was micropipetted from each sample vial into a Pyrex test tube (15 mm × 150 mm). Solvent system (7.5 mL) was added to each test tube. This process was repeated in duplicate for every sample vial, which yielded a total of six voltammetric traces collected for each treatment for every time point investigated. Directly before electrochemical recording, each test tube was vortexed for 15 s before transferring the entirety of the contents to a 10 mL electrochemical cell. The electrodes were inserted into the sample, and the voltammogram was collected. The electrochemical cell was cleaned with hexanes between each treatment.

**Gas Chromatography–Flame Ionization Detector Analysis of Fatty Acid Profile.** Each sample vial removed for a given time point was also analyzed using GC-FID. Each sample (20–25 mg) was placed into a Hach tube (10 mm × 100 mm). Methanolic HCl (1 mL, 3 N) was then added to each test tube. All test tubes were capped and sealed with elastic tape to prevent atmospheric gas exchange. Test tubes were placed on a heating block for 90 min at 90 °C. After heating, test tubes were placed under cold water for 30 s. Deionized water (1 mL) and 2 mL of hexanes were then added to each test tube. The test tubes were once again capped and vortexed for 60 s. The organic layer was transferred to target vials and placed in an autosampler of a Trace 1310 GC-FID, fitted with a Supelco SP-2380 column (30 m × 0.25 mm I.D. × 0.2 µm film thickness). Each sample (1 µL) was injected into a split inlet held at 300 °C with a split ratio of 50:1. Flow rate was held constant at 1 mL/min. An initial oven temperature of 130 °C was held for 2.5 min. After this, the temperature was increased at 4 °C/min until 140 °C, 6.75 °C/min to 150 °C, 2.5 °C/min to 180 °C, 3.75 °C/min to 203 °C and was held for 1 min. The oven was then increased at 4.75 °C/min to 220 °C, such that the total program took 30 min. The FID was held at 250 °C with a gas make up of 350 mL/min of air, 30 mL/min of nitrogen, and 35 mL/min of hydrogen.

**Statistical Analysis.** Statistical analysis for significance was performed using the SigmaPlot 13.0 software suite. Data sets were analyzed using a Bonferroni test with significance set to $p < 0.05$. For clarity purposes, the statistical differences between treatments were only reported within a time point to highlight the antioxidant properties of each treatment.

**Safety Considerations.** The solvent system used for the electrochemical work contains benzene, which is a known carcinogen, mutagen, and is flammable. Special attention to PPE was observed, and all works were performed in a fume hood.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02275.

Overlaid voltammograms provided to help visualize the peak currents detailed in Table 1 and Figure 1 of the manuscript; consecutive cyclic voltammetry traces collected at 10 mV/s for each antioxidant providing insights into potential adsorption impact on oxidation currents within an experiment; consecutive cyclic voltammetry traces collected before and after soaking electrodes in hexanes to determine if antioxidant adsorption was impacting our time-course data (PDF).

**AUTHOR INFORMATION**

**Corresponding Author**

*E-mail: fhaner@umflint.edu.*

**ORCID**

Matthew J. Fhaner: 0000-0003-3008-3639

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors would like to thank Dr. HongSik Hwang, Dr. Jill Moser, Dr. Greg Swain, and Dr. Cassie Fhaner for their comments and insights during revision. The authors would also like to thank the University of Michigan Research and Creative Activity Committee for providing funding for this project (U047589).

**ABBREVIATIONS**

ω-3, omega-3; BHT, butylated hydroxytoluene; RE, rosemary extract; HPLC, high-performance liquid chromatography; GC-FID, gas chromatography–flame ionization detection; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FAME, fatty acid methyl ester; Ag/AgCl, silver/silver chloride; LOD, limit of detection; LLOQ, lower limit of quantitation; PUFA, polyunsaturated fatty acid.

**REFERENCES**

(1) Albert, C. M.; Campos, H.; Stampfer, M. J.; Ridker, P. M.; Manson, J. E.; Willett, W. C.; Ma, J. Blood Levels of Long-Chain n-3 Fatty Acids and the Risk of Sudden Death. *N. Engl. J. Med.* 2002, 346, 1113–1118.

(2) Khan, A.; Khan, M. I.; Iqbal, Z.; Shah, Y.; Ahmad, L.; Watson, D. G. An Optimized and Validated RP-HPLC/UV Detection Method for Simultaneous Determination of All-Trans-Retinol (Vitamin A) and Tocopherol (Vitamin E) in Human Serum: Comparison of Different
Particulate Reversed-Phase HPLC Columns. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2010**, *878*, 2339–2347.

(3) Shoda, R.; Matsueda, K.; Yamato, S.; Umeda, N. Epidemiologic Analysis of Crohn Disease in Japan: Increased Dietary Intake of n-6 Polyunsaturated Fatty Acids and Animal Protein Relates to the Increased Incidence of Crohn Disease in Japan. *Am. J. Clin. Nutr.* **1996**, *63*, 741–745.

(4) Hu, F. B.; Bronner, L.; Willett, W. C.; Stampfer, M. J.; Rexrode, K. M.; Albert, C. M.; Hunter, D.; Manson, J. E. Fish and Omega-3 Fatty Acid Intake and Risk of Coronary Heart Disease in Women. *JAMA* **2002**, *287*, 1815–1821.

(5) Rubio-Rodriguez, N.; Beltran, S.; Jaime, I.; de Diego, S. M.; Sanz, M. T.; Carballido, J. R. Production of Omega-3 Polyunsaturated Fatty Acid Concentrates: A Review. *Innovative Food Sci. Emerging Technol.* **2010**, *11*, 1–12.

(10) Kanner, J. Dietary Advanced Lipid Oxidation Endproducts Are Risk Factors to Human Health. *Mol. Nutr. Food Res.* **2007**, *51*, 1094–1101.

(21) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; John Wiley, 2001.