Singlet Oxygen Oxidation of Lipids Resulting from Photochemical Sensitizers in the Presence of Antioxidants

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ABSTRACT: Singlet oxygen produced by photochemical sensitizers may play an important role in the oxidation of lipids in foods. Therefore, we studied the singlet oxygen oxidation of lipids and the scavenging ability of antioxidants. Singlet oxygen was generated using the photosensitizer rose bengal. The oxidation products of lipids and antioxidants were separated by high-performance liquid chromatography and monitored using post-column chemiluminescence and/or iodometric detection. The competitive reaction rates of various antioxidants and lipids were studied to elucidate the roles played by antioxidants in the prevention of food oxidation by singlet oxygen.
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KEY WORDS: Antioxidants, chemiluminescence, conjugated dienes, fatty acids, lipid hydroperoxides, photosensitizers.

Oxidative stress has a major role in aging and the etiology of diseases such as cancer and atherosclerosis. Oxidative changes in foods are important in terms of nutritional quality, flavor, odor, spoilage, and potential toxicity resulting from ingestion of oxidation reaction products. Consumers and the consequential industry preference for transparent packaging material together with the presence of photosensitizers, either natural components, added food colors or impurities, increase the likelihood of photochemical generation of singlet oxygen and subsequent lipid oxidation. Despite the many protective mechanisms such as enzymes and antioxidants in biological systems, reactive oxygen species can initiate oxidative reactions.

Rawls and Van Santen (1) have postulated that free radical initiation of lipid peroxidation may be due to impurities acting as photochemical initiators of singlet oxygen. Generation of activated oxygen species and some of their reactions in food systems has been extensively reviewed (2,3). Singlet oxygen oxidizes double bonds and initiates lipid oxidation by the ene-reaction to yield lipid hydroperoxides (4,5). Decomposition of the hydroperoxides yields free radicals which in turn initiate new chain reactions. Additional studies are needed to determine the relationships of photosensitizers and antioxidants in order to improve the shelf life of foods and beverages, especially in transparent packages and containers. In this study, methyl linoleate was selected as a model lipid and oxidized by singlet oxygen with and without the presence of antioxidants. The lipid and antioxidant oxidation reaction products resulting from the generation of singlet oxygen using a photosensitizer were analyzed by high-performance liquid chromatography (HPLC) with specific detection of the hydroperoxides by post-column chemiluminescence and iodometric techniques (6).

MATERIALS AND METHODS

Chemicals. All solvents were HPLC grade. Methanol and water were obtained from Burdick and Jackson (Muskegon, MI); 2-propanol and potassium iodide came from J.T. Baker Inc. (Phillipsburg, NJ). Methyl linoleate (ML), butylated hydroxy anisole (BHA), rose bengal (RB), and luminol and hemin were purchased from Sigma Chemical Co. (St. Louis, MO), butylated hydroxy toluene (BHT) from Aldrich (Milwaukee, WI), and *t*-butyl hydroquinone (TBHQ) from Eastman Kodak Company (Rochester, NY). LC-SAX solid-phase extraction columns (500 mg) were purchased from Supelco (Bellfonte, PA).

The luminol solution was prepared by mixing 1.25 g anhydrous sodium carbonate, 124 mg luminol, and 2.5 mg hemin in 1 L of water. The pH of the solution was adjusted to 11.5 with sodium hydroxide.

Generation of singlet oxygen. ML (10 mg), the respective antioxidants (10 mg), and RB (5 mg) were dissolved in 10 mL methanol and irradiated with a 330 watt quartz halogen light source. Periodically, 0.5 mL of this solution was pipetted from the irradiation vessel and passed through a SAX disposable column (solid-phase extraction, 3 mL). ML, antioxidants, and their respective hydroperoxides were eluted from the SAX column with 2 mL of methanol, while RB was retained.

Separation and detection of lipid hydroperoxides. A schematic diagram of the HPLC-chemiluminescence and HPLC-KI instrumentation is shown in Scheme 1. A Waters (Milford, MA) HPLC system was used with a Supelcosil LC-18-DB 250 × 4 mm, 5 μ column. A gradient mobile phase elu-

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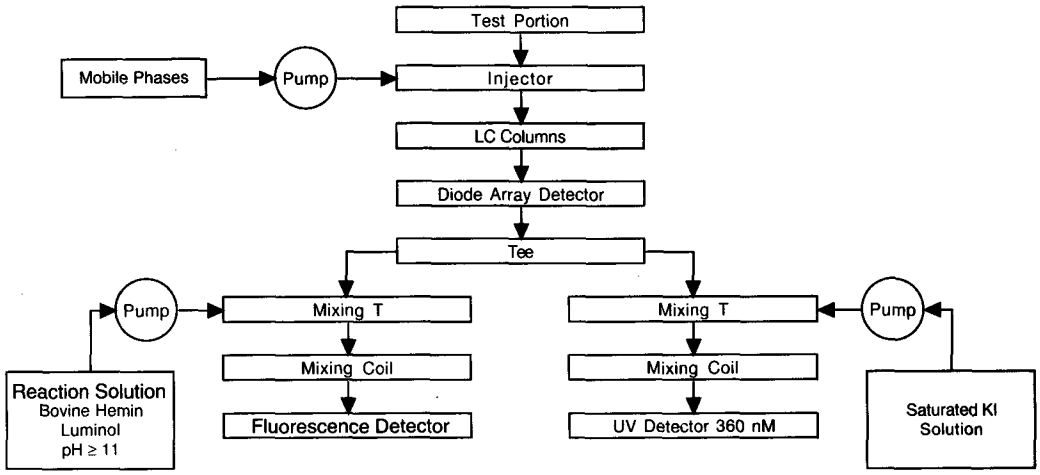


SCHEME 1

tion consisting initially of water/methanol/isopropanol (30:50:20, vol/vol/vol) and changing to 0:50:50 in 10 min was used. This final solvent was continued for 10 min. The flow rate was set at 1 mL/min. For ultraviolet (UV) detection a Waters 991 photodiode array detector was used to detect ML (210 nm), BHA (280 nm), BHT (210 and 280 nm), TBHQ (250 nm), and their various conjugated dienes (220–240 nm). For the post-column reactions, the HPLC eluent exiting from the photodiode array detector was split. One stream mixed with luminol solution flowing at 1 mL/min (6) in a 100 μL mixing loop at room temperature and the other stream with potassium iodide solution, 0.5% in methanol, flowing at 1 mL/min with a 3-mL reaction loop operated at 80°C. The chemiluminescence measurements were made using a Kratos FS 950 Fluromat Fluorescence detector (without excitation). For the iodometric measurements, a Kratos (Ramsey, NJ) spectroflow model 783 detection monitoring at 360 nm was used (6).

RESULTS AND DISCUSSION

The antioxidants BHT, BHA, and TBHQ were studied in the presence of a model lipid, ML. Singlet oxygen was generated by irradiating the solution with added RB. Figure 1 shows the HPLC chromatograms with UV detection of the three systems: ML-BHA, ML-BHT, and ML-TBHQ. Additional peaks in each chromatogram support the hypothesis that hydroperoxides of the ML and the added antioxidants are present because these peaks not only exhibit absorption around the conjugated diene maxima of 234 nm but also give positive responses with both the chemiluminescence (Fig. 2) and KI (Fig. 3) post-column detection. However, the antioxidants and some of their oxidized products gave negative peaks with KI detection. Since these may interfere with other positive peaks, for quantitation, only chemiluminescence and UV data were used.

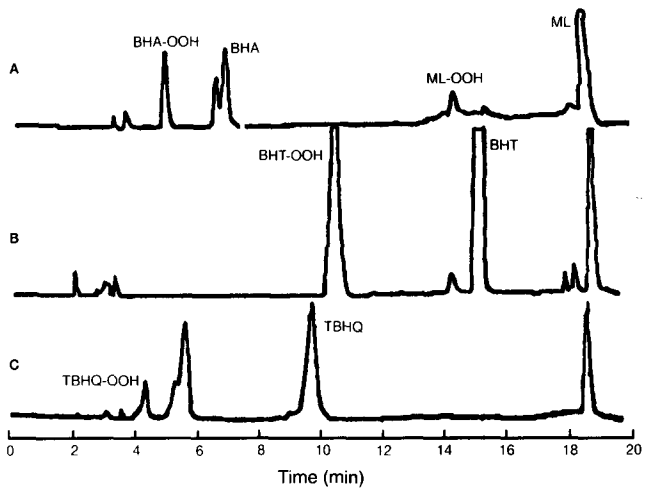


FIG. 1. High-performance liquid chromatography chromatograms of singlet oxygen-generated oxidation of methyl linoleate (ML) with diode array detection in the presence of (A) butylated hydroxyanisole (BHA), (B), butylated hydroxytoluene (BHT), and (C), *t*-butyl hydroquinone (TBHQ).

The chemical structure for BHT hydroperoxide intermediate is shown in Scheme 2 (7). BHA and TBHQ may form similar hydroperoxide intermediates; however, unlike the hydroperoxide of BHT which is stable, the hydroperoxides of BHA and TBHQ have not been isolated. At various time intervals, samples were withdrawn for injection onto an HPLC. The frequency of these intervals and the rate of the oxidation were determined by the time required for the completion of the analysis. The results for the decay of ML and antioxidants and the production of their respective hydroperoxides are shown in Figures 4, 5, and 6. In all cases, antioxidants react much faster than the lipid.

In order to compare the effectiveness of various antioxidants, a composite graph, showing decrease of ML during

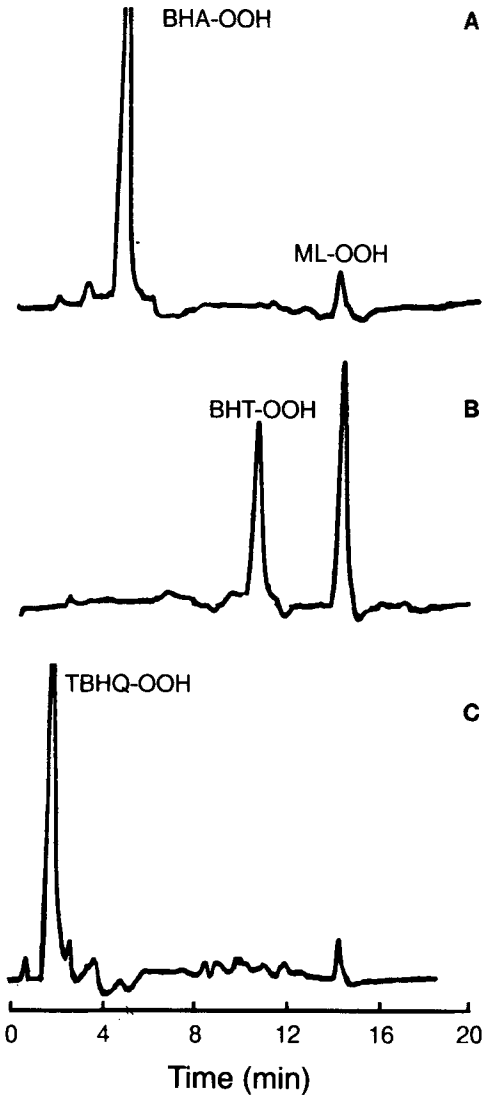


FIG. 2. High-performance liquid chromatography chromatograms of singlet oxygen-generated oxidation of ML with chemiluminescence detection in the presence of (A) BHA, (B) BHT, and (C) TBHQ. See Figure 1 for abbreviations.

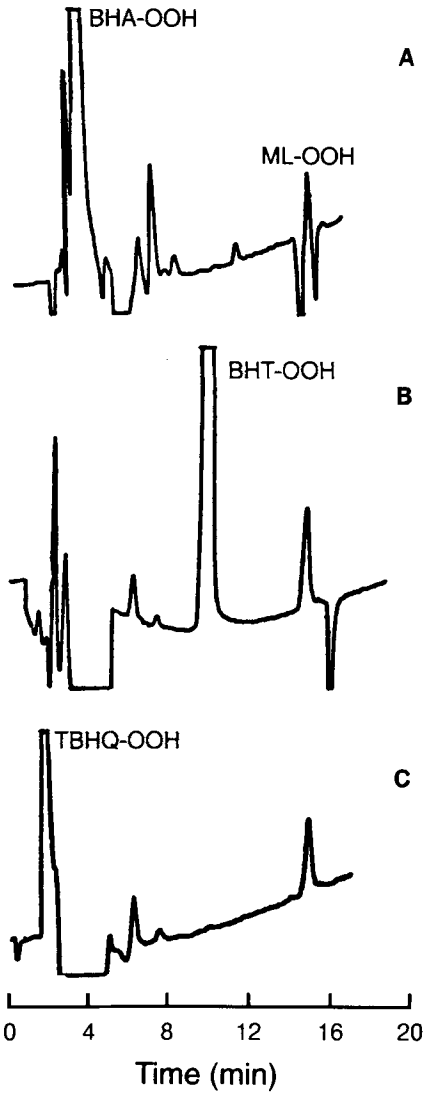
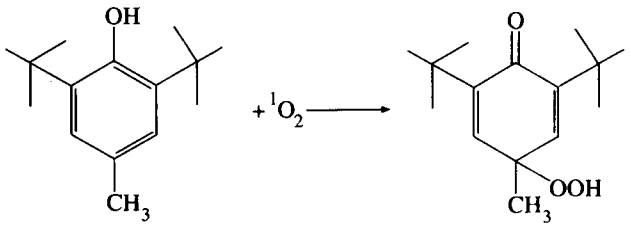


FIG. 3. High-performance liquid chromatography chromatograms of singlet oxygen-generated oxidation of ML with potassium iodide detection in the presence of (A) BHA, (B) BHT and (C) TBHQ. See Figure 1 for abbreviations.



SCHEME 2

the continuous creation of singlet oxygen, is shown in Figure 7. This graph shows that very little protection is provided by the antioxidants against singlet oxygen oxidation of lipids, even at relatively high concentrations of antioxidants. This finding is in agreement with the results of a previous study of the comparative rates of reaction between linoleate and alkylperoxy radicals and singlet oxygen. This study revealed that phenolic antioxidants do not effectively protect fats from oxidation by singlet oxygen (3,8). Many naturally occurring sensitizers such as chlorophyll b, myoglobin b, and riboflavin, are found in food (9). In addition, certain color additives are found to generate singlet oxygen under visible light exposure (10). Therefore, more efficient singlet oxygen scavengers that can be added to foods or changes in food wrap composition may be required for protection against single oxygen oxidation of lipids.

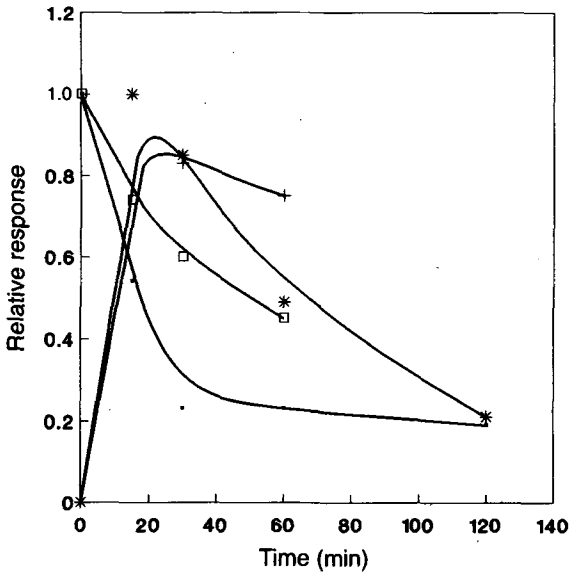


FIG. 4. Decay of ML and BHA and production of their respective hydroperoxides in the presence of singlet oxygen. See Figure 1 for abbreviations. ■ BHA; + ML-OOH; * BHA-OOH; □ ML.

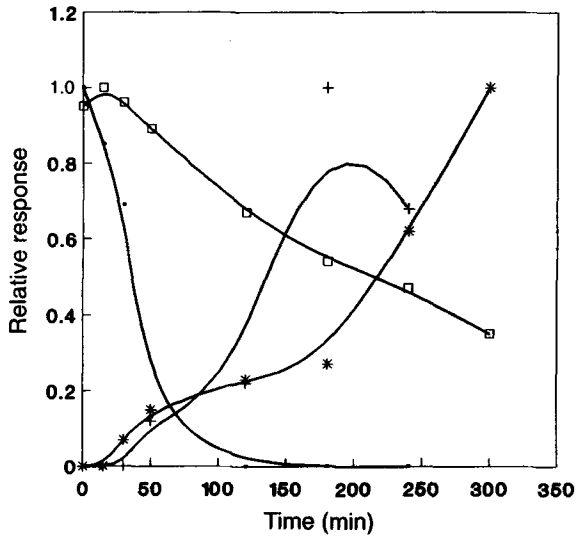


FIG. 6. Decay of ML and TBHQ and production of their respective hydroperoxides in the presence of singlet oxygen. See Figure 1 for abbreviations. ■ TBHQ; * TBHQ-OOH; + ML-OOH; □ ML.

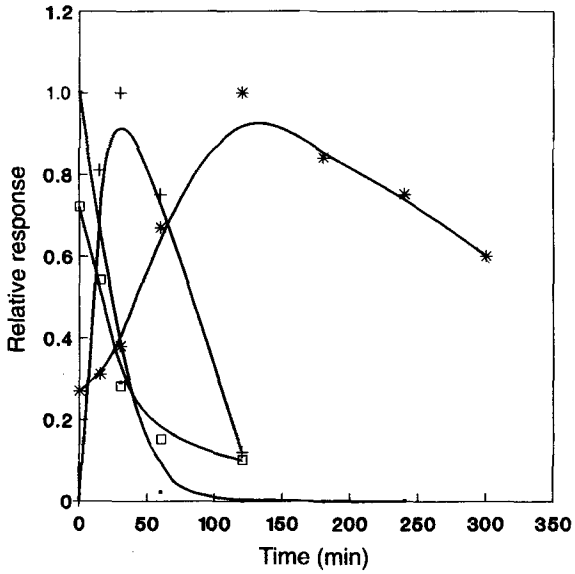


FIG. 5. Decay of ML and BHT and production of their respective hydroperoxides in the presence of singlet oxygen. See Figure 1 for abbreviations. ■ BHT, + ML-OOH; * BHT-OOH; □ ML.

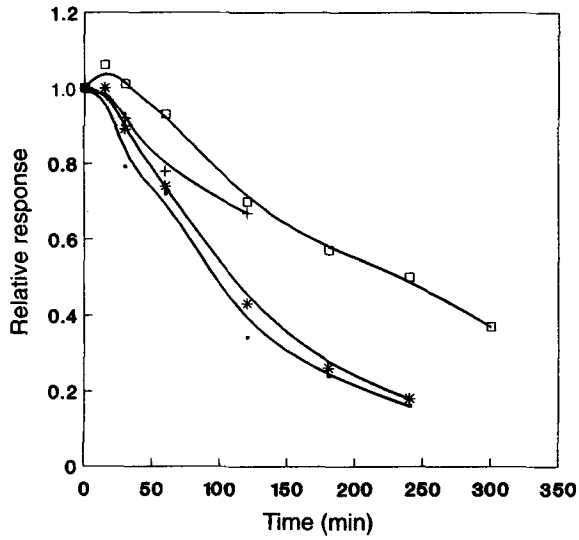


FIG. 7. Decay of ML with singlet oxygen-generated oxidation in the presence of BHA, BHT, and TBHQ. See Figure 1 for abbreviations. ■ No antioxidant; * + BHT; ++ BHA; □ + TBHQ.

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