Antioxidant Properties of Evening Primrose Seed Extracts

A. E. Birch, G. P. Fenner, R. Watkins, and L. C. Boyd*

Department of Food Science, Box 7624, North Carolina State University, Raleigh, North Carolina 27695-7624

The antioxidant activity of extracts of evening primrose seeds (SE) and a commercially extracted filter cake (FC) were determined. The SE and FC were extracted with methanol/water (9:1) followed by evaporation and concentration. Extracts were tested in a bulk oil system and an oil-in-water emulsion using safflower oil as the major source of lipids. The antioxidant activity of the extracts was compared to that of a control and to that of butylated hydroxytoluene (BHT), singly, and in combination. Antioxidant activity was measured by the co-oxidation of β-carotene, an oxidative stability instrument, conjugated dienes, and headspace analysis of hexanal. The SE extract had greater antioxidant activity than the FC extract. The SE extract was more effective in controlling the oxidation in the oil-in-water model system than in the bulk oil system. The activity of SE was concentration dependent, and at higher concentrations the SE was as effective as BHT, but it required higher concentrations because of its lack of purity. Synergism between SE and BHT was demonstrated in both model systems.

Keywords: *Evening primrose; antioxidants; oxidation*

INTRODUCTION

Consumption of foods containing significant amounts of polyunsaturated fatty acids has increased the importance and use of antioxidants to prevent oxidation. The most widely used antioxidants are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, and tertiary butylhydroquinone (*1*). Antioxidants stabilize polyunsaturated fatty acids in foods by reacting with free radicals, chelating metal ions, and interrupting the propagation phase of lipid oxidation. Even though natural and synthetic antioxidants function similarly, questions have been raised concerning the safety of some commercial antioxidants because of model studies showing increased mutagenesis and carcinogenesis associated with some synthetic antioxidants (*2*−*4*).

Natural antioxidants (i.e., tocopherols, ascorbic acid, and flavonoids) have captured the interest of consumers and scientists of the medical and pharmaceutical industries because of their antitumor, anti-mutagenic, and anti-carcinogenic activities (*5*−*7*). The antioxidant properties of certain plant phenols are well established. Extracts from rosemary have been shown to retard the development of rancidity in lipid-containing foods (*7*−*9*). The compounds suggested to be responsible for over 90% of the antioxidant activity in rosemary were carnosol and carnosic acid (*8*, *9*). Plant phenolics constitute one of the major classes of natural antioxidants. These phenolics occur in all parts of the plant including fruits, vegetables, nuts, seeds, leaves, flower, roots, and barks (*10*). Many leguminous plants and spices such as soybeans, sesame seeds, and rosemary contain significant levels of flavonoids, whose antioxidant activities are comparable to those of many synthetic antioxidants (*6*, *11*). Therefore, extraction, characterization, and utilization of natural antioxidants are much needed.

Increased interest in plant flavonoids is due to their potent antioxidant activity and their application as biological response modifiers. A number of plant flavonoids have been shown to be anti-carcinogenic in several lipid model systems (*4*, *5*, *12*). Several flavonoids have been reported to quench active oxygen species and inhibit lipid oxidation in meats, fish oil, and lard (*1*, *7*, *13*−*15*). Flavonoids may act as primary and secondary antioxidants, depending on the types of compounds and the system.

Evening primrose oil (EPO) is a highly unsaturated oil containing a significant source of γ-linolenic acid (GLA). There is a growing demand for use of GLA in clinical and pharmaceutical applications as a very active essential fatty acid and the precursor of prostaglandin E_1 and its derivatives (*16*). As a number of inflammatory diseases, including rheumatoid arthritis, eczema, inflammatory bowel disease, and multiple sclerosis, are affected by prostaglandins, interest in the application of evening primrose oil containing GLA has increased. The objectives of this study were to determine the antioxidant activity of evening primrose seed and to compare its antioxidant activity with that of a synthetic antioxidant using various lipid model systems.

MATERIAL AND METHODS

Preparation of Evening Primrose Seed Extracts. Evening primrose seeds (SE; 50 g) and a commercially extracted filter cake (FC) were obtained from a commercial grower (Carolinian Virgin Oil, LLC, Stansburg, NC) and delipidized by the addition of petroleum ether and grinding for 10 min at high speed in a Tissumizer (Tekmar Co., Cincinnati, OH). Aliquots of the defatted seed and filter cake were ground in methanol/water (9:1) (Fisher Scientific, Pittsburgh, PA) using a tissue-to-solvent ratio of 1:10. The mixture was stirred for 24 h at room temperature. The SE and FC extracts were concentrated using a Buchi Rotavapor-R evaporator (Buchi, Switzerland) at 30 °C, and dried on a Virtis Model 6201 freeze dryer (The Virtis Co., Inc., Gardiner, NY) at −60

* To whom correspondence should be addressed. Phone: 919-513-2259. Fax: 919-515-7124. E-mail: leon_boyd@ncsu.edu.

10.1021/jf010542f CCC: $20.00
Published on Web 08/30/2001

°C. Approximately 25 mg of the dried extract was dissolved in methanol to give a concentration of 1 mg of extract/mL of solvent. Extracts were examined for their total antioxidant activities, and results were compared with those from a commercial antioxidant, butylated hydroxytoluene (BHT) (Sigma Chemical Co., St. Louis, MO).

Model Oil Systems. The antioxidant properties of SE and FC extracts and BHT were determined by their addition to antioxidant-free safflower oil (Sigma) in three different model systems. In each model system, extracts were added in a methanol solvent system.

Model System I. The first model system involved the use of safflower oil, SE, FC, and BHT. Treatments consisted of a control with no antioxidant, BHT at 200 ppm, and SE and FC at 150, 200, and 250 ppm per treatment on a weight/weight basis of the total lipid concentration. The antioxidant activity of all treatments was measured by an oxidative stability instrument (OSI) (Ominion, Inc., Rockland, MA) and the β-carotene assay. The β-carotene assay was carried out in linoleic acid, whereas the OSI measurements were conducted in antioxidant-free safflower oil.

Model System II. The second model system measured the antioxidant properties of SE and BHT in a bulk oil system at 60 °C. This model was designed to test the effects of extracts in a bulk oil system at a lower temperature which was more relevant to situations that might occur in a real food system. Treatments consisted of a control (i.e., with only safflower oil), BHT at 200 ppm, and SE at 500, 3520, and 8800 ppm. A synergistic effect was also examined with the combination of SE at 3520 ppm and BHT at 100 ppm. The antioxidative activity was measured by the production of hexanal and conjugated dienes.

Model System III. The third model consisted of an oil-in-water emulsion system. This model was designed to test the effectiveness of the extracts against BHT in a simulated mixed food system in which the hydrophilic and lipophilic properties of the extracts could be examined. Treatments were the same as in model system II and consisted of a control, BHT, and SE at varying concentrations. A 25-ml batch of a 10% percent safflower oil-in-water emulsion was made containing 2.5 g of oil, distilled water, 0.25 g of Tween 20 (Fisher), and the treatments. Emulsions were sonicated for 6 min. in an ice bath at high power using a Branson 1200 sonicator (Bransonic Inc., Shelton, CT). Emulsions were oxidized at 60 °C on a Pierce Reacti-Therm stirring/heating module (Pierce Chemical Company, Rockford, IL). Antioxidant activity of the treatments was evaluated by analyzing samples periodically for conjugated dienes and hexanal using gas liquid chromatography (GLC).

Measurement of Antioxidant Activity. *Antioxidant Index.* The antioxidant index was measured using the oxidative stability instrument which measures the change in conductivity vs time in hours with the inflection point of the graph representing the point of oxidation or the induction time of samples. The OSI was set at 100 °C, and oxygen was bubbled through the samples at 5 psi. The induction time was used to calculate the antioxidant index as the ratio of induction time of SE, FC, and BHT/control.

Beta-Carotene Assay. The co-oxidation of β-carotene in the presence of linoleic acid was used to determine the antioxidant activity of the SE and FC extracts against that of BHT and a control containing no antioxidants. The β-carotene assay was conducted as described by Hammerschmidt (*17*). In brief, this method involved the addition of test samples at varying concentrations to a solution containing β-carotene, linoleic acid, and Tween 40 (Fisher) in chloroform, followed by evaporation of the chloroform under a nitrogen flush. An aliquot of the emulsion containing each treatment was added to test tubes containing highly oxygenated distilled water. The oxygenated water was obtained by shaking distilled water in an open flask for 5 min immediately prior to the addition of each treatment. All treatments were incubated in aluminum-foil-covered test tubes at 60 °C in a Pierce Reacti-Therm stirring/heating module, and the absorbance was measured at 470 nm using a Gilford 250 UV−Vis spectrophotometer (Gilford Instrument Laboratory, Inc., Oberlin, OH). Absorbance values were taken immediately after preparation (i.e., time zero) and at 15-min intervals for 105 min. The relative decrease in absorbance of the treatments compared to that of the control was used as an indicator of antioxidant activity of treatments.

Measurement of Conjugated Dienes. Conjugated dienes measurement was conducted as described by Frankel et al. (*18*). An aliquot of the oil (0.1 g) was dissolved in 1 mL of cyclohexane. An aliquot (1.0 g) of oil-in-water emulsions was extracted with a 1:1 mixture of methanol and hexane. The methanol layer was washed twice with hexane. The combined hexane extracts were evaporated to dryness under nitrogen and redissolved in cyclohexane. Conjugated dienes were measured at 234 nm using a Gilford 250 UV−Vis spectrophotometer. The concentration was determined in ug/mL against a standard curve consisting of (+)-9-hydroxyoctadecadienoic acid (HODE) (Sigma).

Measurement of Hexanal by Static Headspace GC. Hexanal production was measured as reported by Frankel et al. (*19*) with modifications. Treatments containing oil (0.1 g) and emulsion (1 g) were measured into 6-mL headspace vials (Hewlett-Packard, Avondale, PA) and capped with silicone rubber Teflon caps using a crimper (Hewlett-Packard). Oil samples were heated at 100 °C for 10 min., and emulsion samples were heated at 80 °C for 10 min. After heating, a manual solid-phase micro-extractor containing 75 μm Carboxen PDMS (Supelco, Inc., Bellefonte, PA) was used to capture headspace volatiles that were injected directly into the GC as described in detail by Yang and Peppard (*20*) and Boyd et al. (*21*). The GC analyses were carried out on a Hewlett-Packard 5780 gas chromatograph (Hewlett-Packard, Avondale, PA). A capillary SPB-1 column (30 mm × 0.32 mm, 0.25 film thickness) (Supelco) was used. The GC conditions were as follows: helium linear gas velocity 20 cm/s and column head pressure 25 psi, splitless injector temperature of 180 °C, and detector temperature of 200 °C. Hexanal was determined by comparing retention time with that of a known hexanal standard (Sigma). Changes in hexanal peak area counts were measured over a period of 7 days.

Statistical Analyses. An analysis of variance (ANOVA) was used to determine significant differences between treatments for each of the assays performed using the SAS Statistical System (SAS, Inc., Cary, NC). Mean differences were determined by using Duncan's multiple range test for separation of means showing significant differences at $p < 0.05$. Each of the model oil systems (i.e., I, II, and III) was replicated twice and each assay was performed in duplicate (*22*).

RESULTS AND DISCUSSION

Results of model systems I, II, and III show similar patterns in that the evening primrose seed extracts and filter cake extracts contained significant antioxidant activity comparable to that of BHT in many respects.

Model System I. The SE extract was effective at preventing both primary oxidation products (conjugated dienes) and secondary oxidation products as noted by hexanal formation measurements and volatiles measured by OSI. Table 1 shows the results of model system I using both the OSI and the β-carotene assay. When compared with the control and the FC extract, the SE extract proved to be the most effective antioxidant. At a concentration of 150 ppm, the SE extract delayed the induction time of oxidation by 1 h longer than the control and, in general, delayed oxidation when the concentration was increased. The OSI time of the FC extract did not exceed that of the control. At 250 ppm, the antioxidant activity of the SE treatment was equivalent to that at 200 ppm of BHT.

Results of the β-carotene assay followed a trend similar to that of the OSI data. Treatments containing SE retained activity as long as the BHT treatment

Table 1. Effect of Evening Primrose Seed Extract (SE), Filter Cake (FC) Extract, and Butylated Hydroxytoluene (BHT) on Oxidative Stability of Safflower Oil[a]

sample ID	OSI time (hours)	antioxidant index[b]	β-carotene assay[c]
control (safflower oil)	4.73a	1.00a	0.178a
200 ppm BHT	7.28b	1.54b	0.388b
200 ppm FC	4.43a	0.94a	0.329b
150 ppm SE	5.68a	1.20a	ND[d]
250 ppm SE	7.00b	1.48b	0.453c

[a] In a column, numbers followed by the same letter were not significantly different at $p < 0.05$. [b] Antioxidant index: ratio of treatment OSI induction time/induction time of control. [c] Mean absorbance value measured at 470 nm over 105 min at 15 min. intervals. [d] ND, β-carotene assay not performed on 150 ppm of SE.

(Table 1). However, at 250 ppm, SE significantly delayed the oxidation of β-carotene at a level greater than that at 200 ppm of BHT. Because SE extracts appeared to be more effective than FC extracts, as demonstrated by both the β-carotene assay and the OSI assay, subsequent studies (Models II and III) compared the antioxidant activity of only the SE extract to that of BHT.

Model System II. The SE extract was added to a safflower oil system at concentrations ranging from 200 ppm up to 8800 ppm. On the basis of hexanal formation (Figure 1), SE treatments at 3520 and 8800 ppm were significantly ($p < 0.05$) more protective than the control. At 8800 ppm, SE was more effective than BHT at 200 ppm and less effective than the combined BHT and SE at 100 and 3520 ppm, respectively. However, at equal concentrations of 200 ppm each, BHT possessed more antioxidant activity than SE.

The lack of effectiveness of the SE extracts compared to that of BHT can be logically explained on the basis of "purity of extracts." SE extracts were added to model systems with no additional efforts made to separate the compounds most likely responsible for the antioxidant activity from those having either a pro-oxidant activity or no activity. However, some studies also show that in many cases natural antioxidants are more effective as mixtures rather than as pure compounds (*4*, *6*). Differences between treatments containing BHT and SE appeared to be concentration dependent as noted by decreases in hexanal concentration with increases in SE concentration.

Synergism between SE and BHT was noted by lower concentrations of hexanal produced in the treatment containing SE at 3520 ppm and BHT addition at 100 ppm. Reductions of SE and BHT from 8800 ppm and 200 ppm to 3520 ppm and 100 ppm represent a 40−50% decrease in the addition of the two treatments. However, the two antioxidants appeared to act in synergism as noted by a major decrease of more than 94% in hexanal production over treatments containing SE or BHT at the same concentration or higher.

The measurement of conjugated dienes (Figure 2) for bulk oil showed a pattern similar to that observed for hexanal measurement in that a concentration-dependent reduction in conjugated dienes occurred with increased addition of SE. The addition of SE at 500 ppm produced conjugated diene level of 19.4 ug/mL that was not significantly different ($P < 0.05$) from that of the control at 18.9 μg/mL. However, the addition of SE at higher concentrations of 3520 and 8800 ppm produced conjugated dienes with mean values of 6.5 ug/mL and 4.8 μg/mL, respectively. Thus, the addition of SE at higher concentrations of 3520 and 8800 resulted in conjugated diene levels that were equal to and lower than that of BHT addition which was 6.3 μg/mL. Again, synergism between BHT and SE appears to be evident as noted by further reductions in conjugated dienes from

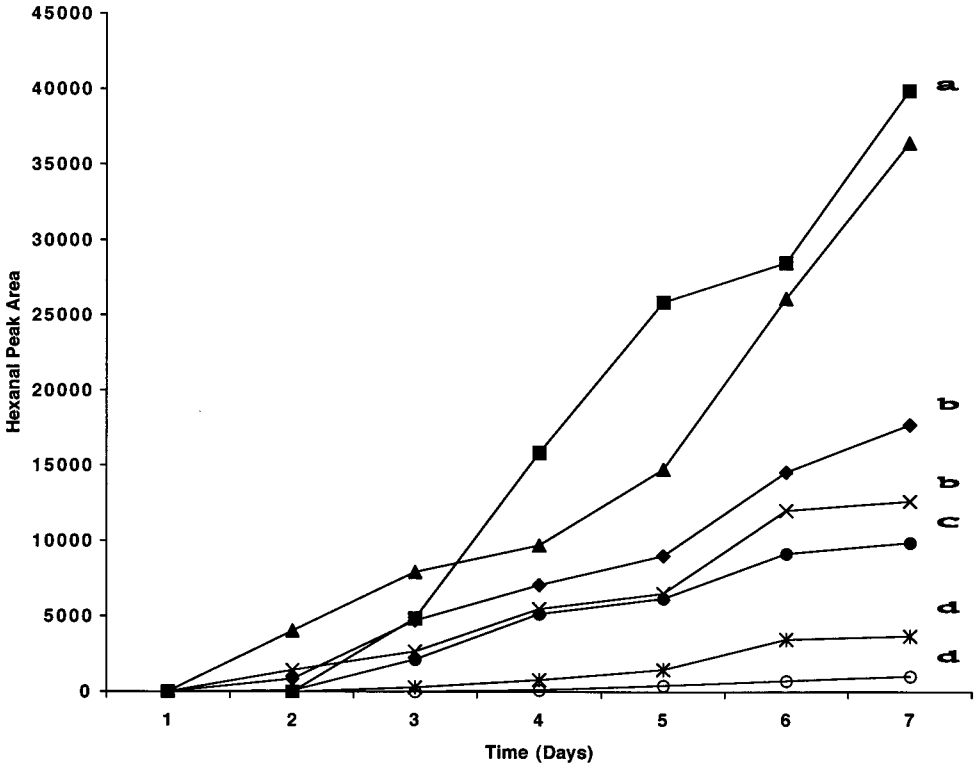

Figure 1. Change in hexanal peak area of an oil model system containing no seed extract (control), BHT, and seed extract (SE) at various concentrations. Treatments followed by different letters (a, b, and c) were significantly different ($p < 0.05$). ■ Control, ● BHT, ▲ SE (200 ppm), ♦ SE (500), × SE (3520), * SE (8800), ○ SE and BHT (3520 and 100, respectively).

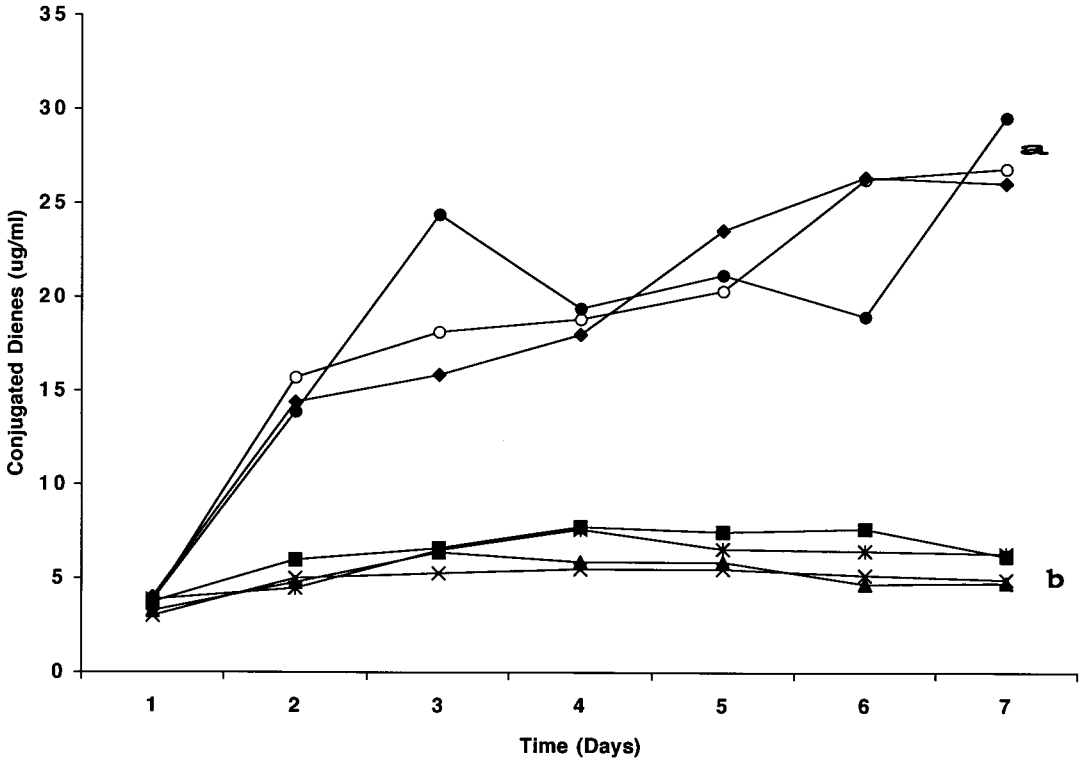

Figure 2. Change in conjugated dienes concentration of an oil model system containing no seed extract (control), BHT, and seed extracts (SE) at various concentrations. Treatments followed by different letters (a, b, and c) were significantly different ($p < 0.05$). ♦ Control, * BHT, ● SE (200 ppm), ○ SE (500), ■ SE (3520), ▲ SE (8800), × SE and BHT (3520 and 100, respectively).

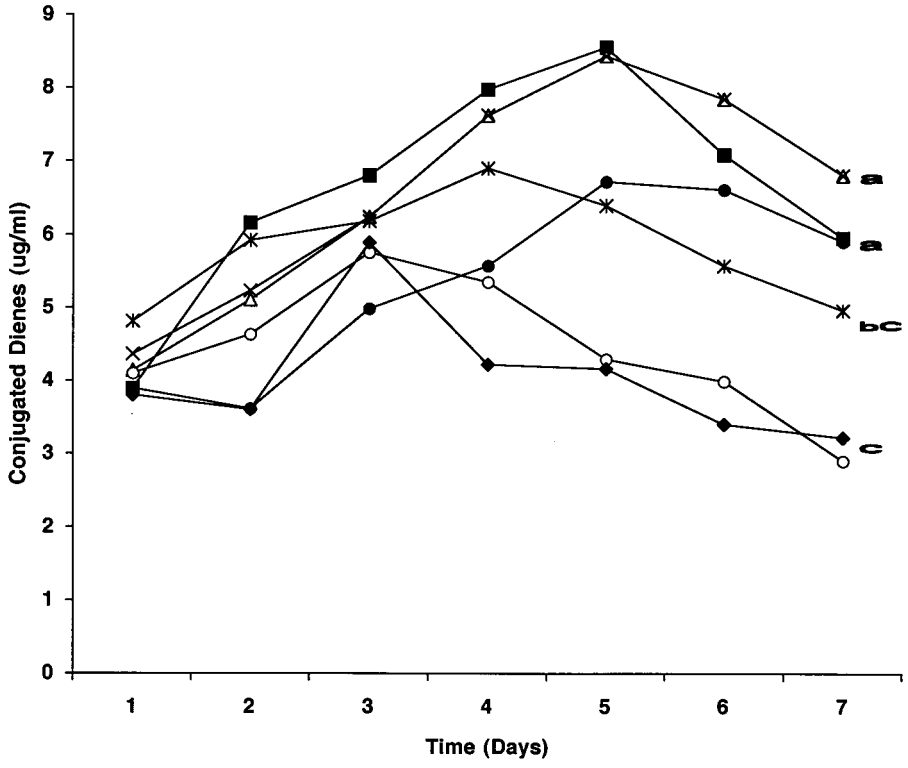

Figure 3. Change in conjugated dienes of oil-in-water emulsion system containing no seed extract (control), BHT, and seed extract (SE) at various concentrations. Treatments followed by different letters (a, b, and c) were significantly different ($p < 0.05$). ■ Control, △ BHT, × SE (200 ppm), * SE (500 ppm), ○ SE (3520), ● SE (8800), ● SE and BHT (3520 and 100, respectively).

an average of 6.2 ug/mL to 4.9 μg/mL following the combined addition of SE and BHT.

Model System III. A similar trend in antioxidant activity was observed for SE extracts added to emulsion systems (Figure 3). However, BHT was less effective in preventing oxidation as noted by conjugated dienes and hexanal values that were not significantly different from those of the control and treatments containing SE at 200 and 500 ppm. The addition of SE at 3520, SE at 8800 ppm, and the combined SE and BHT treatments showed the best antioxidative activity. Activity of SE at 500 ppm was equivalent to that of BHT at 200 ppm

Table 2. Hexanal Peak Areas for Safflower Oil−Water Emulsions Containing BHT and Evening Primrose Extracts at Various Concentrations[a]

time (days)	treatment						
	control	BHT	SE (200)	SE (500)	SE (3520)	SE (8800)	SE + BHT[b]
0	0a	0a	0a	0a	0a	0a	0a
1	3099c	0b	0b	0b	0b	0b	0b
2	4166f	0a	1219e	0d	0d	0d	0d
3	5877h	0g	3593h	0g	0g	0g	0g
4	7273j	276i	5992j	855i	472i	236i	0i
5	5169l	556k	3546l	1279kl	988k	367k	331k
6	3563n	760m	1301m	1795m	1416m	633m	663m
average	4163a	227d	2236b	561c	411c	176d	142d

[a] Numbers in a treatment row followed by different letters were significantly different at $p < 0.05$. [b] SE (3520 ppm) and BHT (100 ppm).

throughout the entire 7-day experiment. After day 3, the higher concentrations of SE proved to be more effective at preventing the formation of hydroperoxides.

The combined addition of SE and BHT produced a numerical reduction in conjugated dienes even though mean differences were not statistically different from those of treatments containing 3520 and 8800 ppm of SE. For example, treatments containing 200 ppm of SE and 3550 ppm of SE produced conjugated dienes that averaged 6.1 ug/mL and 4.34 ug/mL, respectively. The combined addition of BHT at 100 and SE at 3520 ppm averaged 3.73 ug/mL, representing in a 28.6% drop in the production of conjugated dienes.

Hexanal peak areas for the emulsion followed a pattern similar to that observed for conjugated dienes in that increasing addition of SE resulted in decreased hexanal headspace area counts. The control and the treatment containing 200 ppm of the SE were not significantly different from each other, whereas treatments containing BHT and higher levels of SE showed similar patterns (Table 2). The combination of BHT and SE resulted in a significant reduction of approximately 55% in the amount of hexanal compared to the average of that of the two treatments added separately. Thus, the pattern for both hexanal and conjugated dienes in the oil-in-water emulsion were similar to that observed in the bulk oil system with BHT being less effective in the emulsion system. As there were major differences in temperature between model systems I and II (100 °C) and model system III (60 °C), the amount of conjugated dienes and total headspace reflected the differences in temperature as well as the effectiveness of the two types of antioxidant preparations in combating oxidation.

Comparison of the antioxidant properties of BHT, SE, and FC over the course of the three models revealed several trends. In model I, the SE extract appeared to be more effective than equal concentrations of FC extract. As the FC was obtained from a commercial evening primrose grower and had been pressed and held at room temperature for more than six months, some deterioration of antioxidant properties had probably began. By contrast, the SE extract was obtained from freshly harvested seeds and tested in the model system immediately after extraction. However, both extracts improved the stability of the oil as noted by increased induction times on the OSI and increased resistance of β-carotene to oxidation. Even though the β-carotene assay has been criticized because it uses linoleic acid rather than a typical triacylglycerol generally found in oils and mixed food systems, in this particular model, the β-carotene assay generally supported the results of the OSI measurements showing that evening primrose extracts contained antioxidant activity.

Models systems II and III tested the activity of SE extracts against the activity of BHT in a bulk oil system and an oil−water emulsion system. As expected from previous research (*4*, *6*, *23*) on synthetic antioxidants and their effectiveness, BHT was a more potent antioxidant at equal concentrations. However, with increasing concentration of crude SE extracts, it too, was shown to have the capacity to decrease oxidation under stressful conditions of heat and oxygen. Thus, the lack of functionality at low concentrations of 500 ppm or less may be more a function of lack of purity than lack of antioxidant activity.

Results from the safflower oil model system II and the oil-in-water model system III may also be explained on the basis of interfacial phenomena having a significant impact on the effectiveness of an antioxidant to stabilize lipids in any given system. The mechanism behind the interfacial phenomena is based on the affinities of the antioxidants toward the air−oil interfaces in bulk oil and toward the water−oil interfaces in emulsions. Uri (*23*) also made the observation that low solubility with a good antioxidant is no disadvantage if the rate of diffusion or dissolution is not a determining factor. At high temperatures, as used in model system I, this can potentially be a secondary factor resulting in ineffectiveness of the antioxidant.

In the oil-in-water emulsion system, lipophilic antioxidants are surface active and are oriented in the oil−water interface to provide better protection against oxidation (*15*, *18*). Even though BHT is a lipophilic antioxidant and should have been more effective in the oil-in-water emulsion system, it appeared to be more effective in the bulk oil system. The SE extract provided a degree of protection against oxidation in the bulk oil system and in the oil-in-water emulsion but was more effective in the emulsion. This activity would imply that the evening primrose seed extract works well at both interfaces and has both lipophilic and hydrophilic properties. However, the results indicate that the SE extract would work best at the oil−water interface (lipophilic) by providing surface protection to lipids but also at the lipid−air interface (hydrophilic) of bulk oils to provide protection, though less effective. The theoretical basis for these results is supported by others (*19*, *24*, *25*) showing that natural plant materials contain significant antioxidants, and that the activities can be explained on the basis of interfacial phenomena associated with the nature of the individual antioxidants, but also with the nature of the lipid substrate involved. These results are slightly different from that of Frankel's

(*15*, *18*) findings using rosemary extracts. Hydrophilic rosemary antioxidants provided more protection in the bulk fish oil system by being oriented in the air−oil interface and less protection in the oil-in-water emulsion system. The difference in their actions (SE extracts vs rosemary extracts) may be indicative of differences in the natures of the individual antioxidant components present. Identification of the individual antioxidants in evening primrose seeds responsible for this activity is ongoing.

LITERATURE CITED

(1) Wanasundara, U. N.; Shahidi, F. Stabilization of Marine Oils with Flavonoids. *J. Food Lipids* **1998**, *5*, 183−196.

(2) Barlow, S. M. Toxicological Aspects of Antioxidants used as Food Additives. In *Food Additives*; Hudson, B. J. F., Ed. Elsevier: Amsterdam, 1990; pp. 253−307.

(3) Barnen, A. L. Toxicology and Biochemistry of Butylated Hydroxyanisole and Butylated hydroxytoluene. *J. Am. Chem. Soc.* **1975**, *52*, 59−63.

(4) Porter, W. L. Paradoxical Behavior of Antioxidants in Food and Biological Systems. In *Antioxidants: Chemical, Physiological, Nutritional and Toxicological Aspects*; Williams, G. M., Ed. Princeton Scientific: Princeton, NJ, 1993; pp 93−122.

(5) Cassady, J. M.; Baird, W. M.; Chang, C. J. Natural Products as Source of Potential Cancer Chemotherapeutic and Chemopreventive Agents. *J. Nat. Prod.* **1990**, *53*, 23−41.

(6) Pokorný, J. Natural Antioixdants for Food Use. *Trends Food Sci. Technol.* **1991**, *9*, 223−227.

(7) Shahidi, F.; Wanasundara, P. K. J. P. D.; Hong, C. Antioxidant Activity of Phenolic Compounds in Meat Model Systems. In *Phenolic Compounds in Food and Their Effects on Health;* Ho, C.-T., Lee, C. Y., Haung, M.-T. Eds.; ACS Symp. Ser. 507; American Chemical Society: Washington, DC, 1992; vol. I, Chapter 17.

(8) Wu, J. W.; Lee, M. H.; Ho, C. T.; Chang, S. S. Elucidation of Chemical structures of Natural Antioxidants Isolated from Rosemary. *J. Am. Chem. Soc.* **1982**, *59*, 339−345.

(9) Aruoma, O. I.; Halliwell, B.; Aeschbach, R.; Loligers, J. Antioxidant and Pro-oxidant Properties of Active Rosemary Constituents: Carnosol and Carnosic Acid. *Xenobiotica* **1992**, *22*, 257−268.

(10) Pratt, D. E.; Hudson, B. J. F. Natural Antioxidants not Exploited Commercially. In *Food Antioxidants*; Hudson, B. J. F., Ed.; Elsevier: Amsterdam, 1990; pp 171−192.

(11) Wang, H.; Murphy, P. A. Isoflavone Composition of American and Japanese Soybeans in Iowa: Effects of Variety, Crop Year, and Location. *J. Agric. Food Chem.* **1994**, *42*, 1674−1677.

(12) Kato, R.; Nakadate, T.; Yammamoto, S.; Sugimura, T. Inhibition of 12-*O*-tetradecacanoylphobol-13-acetate-Induced Tumor Promotion and Ornithine Decarboxylase Activity by Quercetin: Possible Involvement of Lipoxygenase Inhibition. *Carcinogenesis* **1983**, *4*, 1301−1305.

(13) Matsushita, S.; Ibuki, F. Antioxidative and Prooxidative Abilities of Some Biological Substances and Physiologically Active Substances on the Oxidation of Unsaturated Fatty Acids. *Agric. Biol. Chem.* **1965**, *29* (9), 792−795.

(14) Resurreccion, A. V. A.; Reynolds, A. E. Evaluation of Natural Antioxidants in Frankfurters Containing Chicken and Pork. *J Food Sci.* **1990**, *55*, 629−631.

(15) Frankel, E. N.; Haung, S. Evaluation of Antioxidant Activity of Rosemary Extracts, Carnosol and Carnosic Acid in Bulk Vegetable Oils and Fish Oil and Their Emulsions. *J. Sci. Food Agric.* **1996**, *72*, 201−208.

(16) Hudson, B. J. F. Evening Primrose (*Oenothera* Spp.) Oil and Seed. *J. Am. Oil Chem. Soc.* **1984**, *61* (3), 540−543.

(17) Hammerschmidt, P. A.; Pratt, D. E. Phenolic Antioxidants of Dried Soybeans. *J. Food Sci.* **1978**, *443*, 556−559.

(18) Frankel, E. N.; Haung, S.; Kanner, J.; German, J. B. Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oils vs Emulsions. *J. Agric. Food Chem.* **1994**, *42*, 1054−1059.

(19) Frankel, E. N.; Haung, S.; Aeschbach, R.; Prior, E. Antioxidant Activity of a Rosemary Extract and Its Constituents, Carnosic Acid, Carnosol, and Rosmarinic Acid, in Bulk Oil and Oil-in-Water Emulsion. *J. Agric. Food Chem.* **1996**, *44*, 132−135.

(20) Yang, X.; Peppard, T. Solid-Phase Microextraction for Flavor Analysis. *J. Agric. Food Chem.* **1994**, *42* (9), 1925−1930.

(21) Boyd, L. C.; King, M. F.; Sheldon, B. W. A Rapid Method for Determining the Oxidation of *n*-3 Fatty Acids. *J. Am. Oil Chem. Soc.* **1992**, *69*, 325−328.

(22) SAS Institute, Inc. SAS/STAT User's Guide, Version 6; SAS Institute, Inc.: Cary, NC, 1990.

(23) Uri, N. *Mechanism of Antioxidant in Autoxidation and Antioxidants*. Lundbert, W. O., Ed.; Interscience Publishers: New York, 1961.

Received for review April 26, 2001. Revised manuscript received July 26, 2001. Accepted July 26, 2001. Paper number FSR 01-13 of the journal series of the Department of Food Science, North Carolina State University, Raleigh, NC. The North Carolina Agricultural Research Service of the College of Agricultural and Life Sciences funded research reported in this publication.

JF010542F