SHORT COMMUNICATION

Effects of Bleached and Unbleached Rosemary Oleoresin and Rosmariquinone on Light-Sensitized Oxidation of Soybean Oil

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Synthetic rosmariquinone (RQ), an antioxidant naturally present in rosemary (*Rosmarinus officinalis* L.), tertiary butylhydroquinone (TBHQ) and rosemary oleoresin (bleached and unbleached), was tested for antioxidant activity in stripped and nonstripped soybean oil in a light-induced oxidation system. In the stripped soybean oil system, RQ was significantly less ($P < 0.05$) effective than TBHQ at controlling oxidation of the oil. In light-induced oxidation of nonstripped soybean oil, RQ had significantly lower ($P < 0.05$) peroxide values (PV) than TBHQ after 36 h. RQ had PV that was significantly ($P < 0.05$) lower than those for both the bleached and nonbleached rosemary oleoresin throughout the 72-h study.

KEY WORDS: Bleached rosemary oleoresin, light-induced oxidation, nonstripped soybean oil, rosmariquinone (RQ), stripped soybean oil, tertiary butylhydroquinone (TBHQ).

A crude rosemary (*Rosmarinus officinalis* L.) oleoresin (RO) is currently sold as a flavoring with the added feature of exhibiting antioxidant activity. The use of crude RO is limited because the oleoresin is a mixture of mono- and diglycerides and vegetable oil, and it has dark brown pigmentation. Although RO has proven to be an effective antioxidant in autoxidized systems (1-3), only limited data have been reported regarding its effect on light-induced oxidation (photooxidation) (4).

Isolation and purification of antioxidants from rosemary have resulted in the identification of compounds with a range of antioxidant activity, including carnosol (5,6), rosmanol (7), rosmadial (8), rosmaridiphenol (9) and rosmariquinone (RQ) (10). Isolation of antioxidants from rosemary requires large quantities of solvent and plant material to obtain significant amounts of the pure antioxidant.

Synthetic chemistry can be an alternative for the production of pure antioxidants, such as RQ. Knapp and Sharma (11) completed the synthesis of RQ by means of a Diels-Alder cycloaddition between 3-isopropylcatechol and 6,6-dimethyl-1-vinylcyclohexene by converting 3-isopropylcatechol to 3-isopropyl-*o*-benzoquinone before the cycloaddition reaction. Lee *et al.* (12) reported the synthesis of miltirone (i.e., RQ) by a regioselective Diels-Alder's cycloaddition between 3-isopropyl catechol and 6,6-dimethyl-1-vinylcyclohexene with ultrasound.

This study was designed to compare the antioxidant effectiveness of synthetic RQ, bleached and unbleached ROs and tertiary butylhydroquinone (TBHQ) in light-induced oxidation of stripped and nonstripped soybean oil.

MATERIALS AND METHODS

Analysis of soybean oil components. Tocopherols (α, γ, δ), β-carotene and chlorophyll standards were obtained from Sigma Chemical Co. (St. Louis, MO). Tocopherols were determined by the high-performance liquid chromatography (HPLC) method of Carpenter (13). Chlorophyll and β-carotene were analyzed by modified Association of Official Analytical Chemists (AOAC) methods (14,15) with spectrophotometry (4).

Antioxidants. TBHQ was obtained from Eastman Chemical Products Inc. (Kingsport, TN). RO (Herbalox® Seasoning, type O) was obtained from Kalsec® Inc.(Kalazoo, MI). Product literature indicated that the RO contained a mixture of vegetable oil and mono- and diglycerides with chlorophyll substantially removed. RQ was synthesized in this laboratory by the method of Lee *et al.* (12). Quantitation of RQ in the commercial RO was completed by HPLC (13).

Stripping of soybean oil. Commercial soybean oil was purchased from a local supermarket and stored in the dark at −18°C until needed. The oil was stripped by the method of Kiritsakis and Dugan (16) as described by Hall and Cuppett (4).

Bleaching of RO. Commercially available RO used in this study was pigmented and could contain prooxidant compounds, such as chlorophyll and pheophytin. Therefore, it was decided to determine the effect of this pigmentation on the light-induced oxidation of both the stripped and nonstripped soybean oils by bleaching part of the RO by the procedure described previously for the stripping of soybean oil (4).

Oxidation of soybean oils. Prior to each study, hexane was evaporated from the stripped soybean oil under vacuum at 25°C and protected from light. Samples (100 g) of stripped or nonstripped soybean oil were weighed into 110-mL glass jars. Then, appropriate amounts of either bleached or unbleached RO (0.02%), RQ (0.02%) or TBHQ (0.02%) were added. Each sample was thoroughly mixed to ensure complete dispersion of the antioxidants. Hexane was mixed with nonstripped oil and evaporated for a control to determine the effect of hexane on oil oxidation. A total of ten treatments, five for the stripped and five for the nonstripped oil, not including the hexane treatment, were prepared. The study was replicated three times.

The jars were covered with clear plastic wrap and randomly placed under two 15-W cool fluorescent lamps at a level sufficient to generate 4200 lux of fluorescent radiation at 25 ± 1°C. Aluminum foil was placed in the open areas between the side of the jars and the bottom of the lamps to create uniform lighting. Peroxide values (PV) were determined every 12 h during the 72-h light exposure by the American Oil Chemists' Society (AOCS) Method Cd 8-35 (17).

For PV analysis, the oil samples were removed sequentially and were returned immediately to the same position under the light after sampling. The entire sampling and peroxide determination was completed in less than

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1.5 h. Oil samples were then illuminated for an additional 12 h before PVs were measured again.

Statistical analysis. Data were analyzed by analysis of variance in which the least significant means (18) were used to determine 95% confidence level ($P < 0.05$) between the mean values of the treatments in both the stripped and nonstripped soybean oil tests.

RESULTS AND DISCUSSION

β-Carotene, chlorophyll and tocopherol compositions of the stripped soybean oil, nonstripped soybean oil and bleached and nonbleached ROs are given in Table 1. Chlorophyll content of the nonstripped soybean oil was comparable to that of commercially refined soybean oil (19). Both β-carotene (0.12 ppm) and total tocopherol (560 ppm) content of the nonstripped soybean oil were lower than the reported 28–30 and 580–1530 ppm, respectively, found in commercially refined soybean oil (19,20).

ROs exhibited the highest antioxidant activity at the 0.02% level in a previous study (4), in which the same model system was used. Therefore, this level was also used for comparison in this study. The effect of bleaching reduced the RQ from 16 to 10 ppm (Table 1).

When stripped soybean oil was treated with 0.02% TBHQ; 0.02% RQ; 0.02% unbleached RO (URO); 0.02% bleached RO (BRO) or left untreated and exposed to fluorescent light, the following results were obtained. For the first 48 h, PV for RQ and TBHQ were not significantly ($P > 0.05$) different (Fig. 1). After 48 h, the RQ treatment showed increasing PV, and by 72 h, RQ had significantly ($P < 0.05$) higher PV than TBHQ. The untreated control, URO and BRO had PVs that were not significantly ($P > 0.05$) different from each other until 36 h, but were significantly ($P < 0.05$) greater than PV for the RQ and TBHQ treatments after 36 h (Fig. 1). URO had PVs that were significantly ($P < 0.05$) lower than the PV for the control after 48 h and significantly ($P < 0.05$) lower than the PV for the BRO after 60 h. The BRO had PV that were significantly ($P < 0.05$) lower than the PV of the control at 72 h (Fig. 1). Results from the stripped soybean oil exposed to fluorescent light indicated that the RO contained compounds, such as chlorophyll, pheophytin and mono- and diglycerides, that interfere with the antioxidant components, thus reducing the antioxidant activity. This was confirmed by the higher level of antioxidant activity exhibited by the RQ in comparison to the RO.

When nonstripped soybean oil was treated with 0.02% TBHQ, 0.02% RQ, 0.02% URO, 0.02% BRO or left untreated and exposed to fluorescent light, the following results were found. The RQ and TBHQ activity were not significantly ($P > 0.05$) different from each other at 24 h but were significantly different ($P < 0.05$) after 36 h, as indicated by the lower PV in the RQ sample (Fig. 2). At 36 h, the untreated control (URO) and BRO had PVs that were not significantly different ($P > 0.05$) from one another

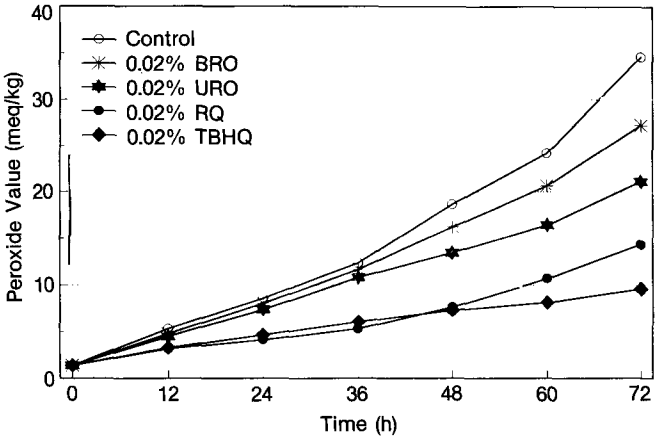

FIG. 1. The effects of rosmariquinone (RQ), tertiary butylhydroquinone (TBHQ), bleached rosemary oleoresin (BRO) and unbleached rosemary oleoresin (URO) on light-induced oxidation, as measured by peroxide values of stripped soybean oil.

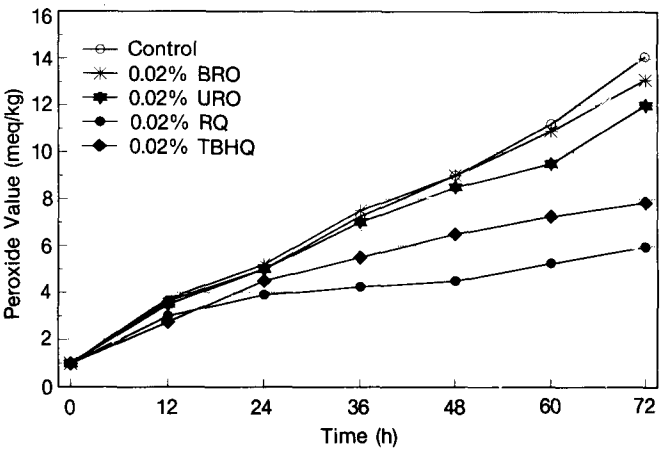

FIG. 2. The effects of RQ, TBHQ, BRO and URO on light-induced oxidation, as measured by peroxide value of nonstripped soybean oil. Abbreviations as in Figure 1.

TABLE 1

Composition of Stripped and Nonstripped Soybean Oils and Bleached and Nonbleached Rosemary Oleoresin

Sample	β-Carotene (ppm)	Chlorophyll (ppm)	Tocopherol[a] (ppm)	Rosmariquinone (ppm)
Nonstripped SBO[b]	0.12	0.10	560	0
Stripped SBO	0.03	0.01	>2	0
Nonbleached RO[c]	104	4.21	390	16
Bleached RO	0.20	0.67	79	10

[a]Tocopherol level based on α, δ, γ.
[b]Soybean oil.
[c]Rosemary oleoresin.

but were significantly ($P < 0.05$) greater than those of the RQ and TBHQ samples. After 60 h, the untreated control and BRO had PVs that were significantly ($P < 0.05$) greater than the PV for the URO, but not significantly ($P > 0.05$) different from each other throughout the duration of the study (Fig. 2).

The RQ had greater antioxidant activity than the ROs probably because RQ was a pure compound and, as a result, there was little interference from other components, as in the case with the oleoresins. Although the URO contained substantial β-carotene and tocopherol (Table 1), it also contained chlorophyll (Table 1), mono- and diglycrides, as well as other extraneous materials. The mono- and diglycerides are believed to reduce the surface tension between the oxygen and the lipid, allowing the oxidation to occur more readily (4). Because the bleaching of RO reduced the β-carotene and tocopherol levels, the overall effectiveness was also reduced in comparison to URO. The RQ level was also reduced from 16 to 10 ppm during the bleaching process (Table 1). RQ was found to have greater activity in nonstripped soybean oil vs. stripped soybean oil, indicating a possible synergism with the tocopherol or β-carotene inherent to the oil.

The antioxidant activity of RQ is equivalent to butylated hydroxytoluene and superior to butylated hydroxyanisole in an autoxidized lard system (10). Others (6,7, 9,21) have reported that carnosol, carnosic acid, rosmanol and rosmaridiphenol are most active in autoxidized systems, but nothing has been reported for light-sensitized systems. RQ is not among the most active in autoxidized systems and only accounts for 16 ppm of the RO (Table 1) used in this study. RQ in this study was found to inhibit light-sensitized oxidation of nonstripped soybean oil as effectively as TBHQ. Although RQ was not as effective as TBHQ in stripped soybean oil, it was more effective than the ROs. The ability of RQ to inhibit oxidation, and the present health concerns about synthetic antioxidants indicates the possibility that RQ could be an alternative antioxidant after all toxicological data have been determined.

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