Rosmariquinone Interactions in Autoxidation and Light-Sensitized Oxidation of Stripped Soybean Oil

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ABSTRACT: Two studies were conducted, using a stripped soybean oil model system, to evaluate the antioxidant interaction(s) between rosmariquinone (RQ) and oil components, including chlorophyll (CHL), β-carotene (CAR), and tocopherol(s) (TOCO), under both autoxidation and light-sensitized oxidation. In autoxidation, CHL alone had no effect on the level of oxidation, whereas CAR alone showed prooxidant ($P < 0.05$) activity. RQ and RQ + CHL or RQ + CAR were significantly ($P < 0.05$) better in controlling autoxidation. RQ appeared to be responsible for the activity in the test combinations. However, among the treatments containing TOCO, RQ, and RQ + TOCO, RQ + TOCO gave a level ($P < 0.05$) of inhibition that indicated a possible synergistic relationship. When tested in a light-sensitized oxidative system, CHL and CAR had no effect on oxidation relative to the control and, again, RQ alone or in combination with CHL or CAR was responsible for the greatest ($P < 0.05$) inhibition of oxidation. RQ + TOCO had better ($P < 0.05$) inhibitory activity than the individual compounds. In the second study, the interaction between RQ and TOCO was studied. TOCO, added at levels found in the nonstripped soybean oil, and RQ, added at two levels (100 and 200 ppm), were tested alone or in combination in both autoxidation and light-sensitized oxidation. In autoxidation, the combination of RQ at 200 ppm and TOCO was synergistic; however, in light-sensitized oxidation, this combination was not synergistic. Monitoring the loss of both RQ and TOCO suggested that during autoxidation RQ acted as a secondary antioxidant relative to TOCO.

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KEY WORDS: Autoxidation, light-sensitized oxidation, rosmariquinone, tocopherol.

The chemical system controlled by an antioxidant (AO) must be defined to ensure the maximum activity of the compound. Two chemical pathways responsible for oxidative rancidity in foods are autocatalytic/autoxidation and photooxidation. Although both of these pathways involve the classic stages of lipid oxidation (i.e., initiation, propagation, and termination of free radicals), they differ in their initiation process. In autoxidation, an abstraction of hydrogen from the lipid (LH) or decomposition of the lipid hydroperoxide (LOOH) initiates oxidation. Photooxidation is initiated *via* a light reaction in the presence of a sensitizer.

In edible oils, chlorophyll (CHL), β-carotene (CAR), and tocopherols (TOCO) can impact the development of lipid oxidation. CHL is a common sensitizer that acts as a promoter of photooxidation in oil systems. Usuki *et al.* (1) found that the combined CHL and pheophytin levels in commercial soybean oils ranged from 57 to 166 ppb. In the presence of CHL and light, methyl oleate and methyl linoleate were readily oxidized (2). Unlike CHL, CAR has been shown to inhibit photooxidation *via* a singlet oxygen-quenching mechanism (3,4). A hydrogen donation (5,6) or free radical-scavenging (7,8) mechanism accounts for the majority of the antioxidant activity of TOCO. However, $^1O_2$ quenching of TOCO correlated well with their biological activity, i.e., α-tocopherol was most reactive toward $^1O_2$ and had the highest vitamin E activity (9,10).

Rosmariquinone (RQ) or miltirone is a diterpene found in a variety of plant materials. Hayashi *et al.* (11) first isolated miltirone from Chinese sage (*Salvia miltiorrhia* Bunge) and Houlihan *et al*. (12) isolated RQ from rosemary (*Rosmarinus officinalis* L.). Hall *et al*. (13) found RQ was an effective inhibitor of light-sensitized oxidation of soybean oil. RQ was less active than tertiary butylhydroquinone (TBHQ) in a stripped soybean oil (SBO) system, but in a nonstripped SBO it exhibited significantly better AO activity than TBHQ. This observation indicated that synergists were present in the nonstripped SBO. Using a stripped SBO model system, the objectives of this study were (i) to compare the antioxidant activity of RQ in the presence of TOCO, CAR, and CHL and (ii) to determine the effect of RQ level, in combination with TOCO, on controlling both autoxidation and light-sensitized oxidation.

EXPERIMENTAL PROCEDURES

Project overview. Two studies were conducted in stripped SBO (SSBO) model systems. In a preliminary study (study 1), RQ was tested alone and in combination with CAR or CHL added at levels found in the original oil or mixed TOCO (α and δ). γ-TOCO was not available at the time of the preliminary study so higher concentrations of α- and δ-TOCO than normally

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found in soybean oil were used to replace the TOCO removed during bleaching. Because the antioxidant activity of δ-TOCO more closely resembles γ-TOCO than α-TOCO (5,7), higher concentrations (654 ppm) of δ-TOCO were used to replace γ-TOCO. The positive results from study 1 prompted further investigation. To account for the role of γ-TOCO, a second study was completed in which the individual tocopherols (α, γ, and δ) were added to SSBO as a mixture that represented the level of each as found in the original oil. The mixture was tested with and against two levels (100 and 200 ppm) of RQ.

Analysis of soybean oil components. TOCO (α, δ), CAR, and CHL standards were obtained from Sigma Chemical Co. (St. Louis, MO). γ-Tocopherol was obtained from Eastman Chemical Products Inc. (Kingsport, TN). TOCO were determined by the high-performance liquid chromatography (HPLC) method of Carpenter (14), whereas CHL and CAR, respectively, were spectrophotometrically analyzed by modified Association of Official Analytical Chemists (AOAC) methods (15,16).

Antioxidants. TBHQ was obtained from Eastman Chemical Products Inc. Rosmariquinone was synthesized in the laboratory using the method of Lee *et al.* (17) as modified by Hall (18). TOCO and RQ loss was simultaneously determined using a modified HPLC procedure of Carpenter (14). Modifications included detection at 280 nm and a flow rate of 0.5 mL/min.

Stripping of soybean oil. Commercial SBO was purchased from a local supermarket and stored in the dark at –18°C until it was stripped using the batch process method (18). TOCO bleaching/removal process was complete after no TOCO was detected by HPLC (2 to 4 h). Bleaching material was filtered out under a stream of nitrogen and the solvent was removed *in vacuo* at 30°C from the SSBO to give a TOCO-free SBO. To remove CAR, the TOCO-free SBO was resuspended in hexane using a 2:1 ratio of solvent to SBO. The SBO/solvent mixture was passed through a column (18) and purification was monitored by spectrophotometry at 436 nm (β-carotene) and 452 nm (lutein). The SBO was repurified if pigments were observed. The SSBO was stored at –18°C and the solvent removed *in vacuo* at 30°C just prior to use.

Oxidation of soybean oils. Samples (100 g) of SSBO were weighed into 100-mL glass jars. Treatments for study 1 included appropriate amounts of CHL, CAR, α- and β-TOCO, RQ, or combinations of these compounds (Table 1). Appropriate amounts of α-, β-, δ-TOCO, RQ, or combinations of these compounds (Table 2) were used in study 2. Each sample was thoroughly mixed to ensure complete dispersion of the antioxidants. TBHQ acted as a positive control in the second study. To better represent the statistical soybean oil population, the addition of treatments to the SSBO varied based on the original content of the materials in the nonstripped soybean oil. No significant differences were observed in CHL, CAR, or TOCO between the replications.

The jars were covered with clear plastic wrap prior to storage. For the photooxidation study, jars were randomly placed under two 15-W cool fluorescent lamps at a level sufficient to illuminate 4,200 lux of fluorescent radiation at 25 ± 1°C. To create uniform lighting, aluminum foil was used to cover the open areas on the sides and bottom of the test area. For the autoxidation study, jars were randomly placed in an oven at 60°C. During testing, oil samples were removed sequentially from under the light source or from the oven and were returned immediately to the same position after sampling. The entire sampling was completed in less than 1.5 h. Oil samples were either heated or illuminated for an additional 12 h before sampling again.

In the first study, peroxide values (PV) were determined every 12 h for a total of 72 h. In the second study, all treatments were evaluated every 12 h for a total of 96 h and those not at a PV of 20 meq/kg were monitored until they reached a PV of 20 meq/kg. PV were determined using the American Oil Chemists' Society (AOCS) Official Method Cd-8-53 (19).

TABLE 1
Individual and Combinations of Antioxidants Added to Stripped Soybean Oil (SSBO) for Study 1

	Additive level[a] (ppm)		
Treatment[b]	Replication 1	Replication 2	Replication 3
CHL	0.201	0.201	0.112
CAR	0.109	0.249	0.107
TOCO[c]	828.6	988.4	866.4
RQ	200.0	200.0	200.0
RQ + CHL	200.0 + 0.201	200.0 + 0.201	200.0 + 0.112
RQ + CAR	200.0 + 0.109	200.0 + 0.249	200.0 + 0.107
RQ + TOCO	200.0 + 828.6	200.0 + 988.4	200.0 + 866.4

[a]Additive level refers to the concentration of a particular compound added back to the SSBO at a level that was found in the original non-SSBO.
[b]Treatment abbreviations are as follows: CAR = carotene, CHL = chlorophyll, TOCO = tocopherol, RQ = rosmariquinone, RQ + CAR = rosmariquinone and carotene, RQ + CHL = rosmariquinone and chlorophyll; and RQ + TOCO = rosmariquinone and tocopherol. The control was an untreated SSBO.
[c]The averaged TOCO concentration (α = 240 ppm and δ = 654 ppm) added to the SSBO.

TABLE 2
Individual and Combinations of Antioxidants Added to Stripped Soybean Oil (SSBO) for Study 2

	Additive level[a] (ppm)		
Treatment[b]	Replication 1	Replication 2	Replication 3
TOCO (total)	824.9	819.5	840.8
α[c]	74.7	68.4	69.7
γ	564.5	545.7	560.8
δ	185.6	205.4	210.3
RQ1	100.0	100.0	100.0
RQ2	200.0	200.0	200.0
RQ1 + TOCO[c]	100.0 + 824.9	100.0 + 819.5	100.0 + 840.8
RQ2 + TOCO[c]	200.0 + 824.9	200.0 + 819.5	200.0 + 840.8
TBHQ	200.0	200.0	200.0

[a]Additive level refers to the concentration of a particular compound added back to the SSBO at a level that was found in the original non-SSBO.
[b]Treatment abbreviations are as follows: TBHQ = tertiary butylhydroquinone; RQ + TOCO = rosmariquinone and tocopherol; for other abbreviations see Table 1. Control was an untreated SSBO.
[c]TOCO reported are the total concentration present in the non-SSBO and are added back to the SSBO in the proper concentrations of α-, γ- and δ-TOCO. Individual TOCO did not act as individual treatments.

Statistical analysis. Data were analyzed using analysis of variance (ANOVA) in which the least significant means (20) were used to determine 95% confidence level ($P < 0.05$) between the mean values of the treatments. Repeated measures analysis (21) was completed using Statistical Analysis System (SAS) software (22). Comparison of contrasts was used in the repeated measures statement where antioxidant contrasts were determined every 12 h until the end of the studies. Each treatment was completed three times, to represent three replications.

RESULTS AND DISCUSSION

The first study evaluated the role of each of the soybean oil components, i.e., CHL, CAR, and TOCO, alone or in combination with RQ, on autoxidation in SSBO. Oil treated with CHL, RQ, and RQ + CHL showed differences between the treatments within the first 12 h of heating (Table 3). The oxidation of the SSBO and the CHL-treated oil samples was significantly ($P < 0.05$) higher than the RQ-treated samples. As the study continued, the treatments stayed grouped such that by the end of the study (72 h) the control and the CHL-treated oils were significantly (P<0.05) more oxidized than the RQ-treated oil. In this study, CHL, at the levels used, did not contribute to prooxidant or antioxidant activity. The antioxidant activity of the RQ + CHL treatment was due solely to RQ.

In the SSBO treated with CAR, RQ, and RQ + CAR and subjected to autoxidative conditions, the observed oxidation patterns were similar to those found with the CHL treatments. By the end of the first 12 h, the control and the CAR samples were oxidizing at a greater ($P < 0.05$) extent than those treated with RQ and RQ + CAR (Table 3). At the end of 72 h, the RQ samples were significantly ($P < 0.05$) less oxidized than the control and the CAR-treated oils. However, by 72 h the CAR-treated oil was significantly ($P < 0.05$) more oxidized (Table 3) than the control, indicating prooxidant activity at this level of addition. The observed prooxidant activity of CAR may be due to a complete loss of CAR during the oxidation process; thus, CAR would not contribute an antioxidant effect at 60 h. Alternatively, β-carotene oxidation products may have reached a level that could catalyze the oxidation of SSBO, similar to that proposed by Frankel *et al.* (23). RQ was capable of counteracting the prooxidant effect as seen by the low level of oxidation occurring in the (RQ + CAR)-treated oil ,which was not different from the RQ-treated oil.

The autoxidized SSBO had a PV that was significantly ($P < 0.05$) greater than oils treated with RQ, TOCO, and RQ + TOCO starting with the 12-h measurement (Table 3). The PV of the TOCO- and RQ-treated oils were not significantly different from each other but were significantly ($P < 0.05$) greater than the PV of the (RQ + TOCO)-treated sample throughout the study. The low level of oxidation in the RQ + TOCO samples indicated a synergism between the two antioxidants during autoxidation. Synergism was not noted in the RQ + CAR and RQ + CHL because the PV were not significantly lower than the PV of the RQ-treated oil. Under autoxidation conditions, the combination of RQ and TOCO proved to be a valuable method to control oxidation of SSBO.

When CHL, RQ, and RQ + CHL were added to SSBO (Table 4) and exposed to light, the results were similar to those observed in autoxidation. Again, the control and the CHL treatment were significantly ($P < 0.05$) more oxidized than the RQ and RQ + CHL counterparts. The RQ and RQ + CHL oxidation levels were not significantly different over the 72 h study, but the RQ + CHL had a slightly lower ($P > 0.05$) PV than the RQ at the end of the test period (Table 4). At the level used, CHL was not prooxidant or antioxidant during the light-sensitized oxidation of the SSBO.

The control and the CAR-treated SSBO were not significantly different throughout the 72 h of light exposure (Table 4). However, these treatments were more oxidized ($P < 0.05$) than the RQ and RQ + CAR treatments as early as 12 h. The oxidation of the RQ and RQ + CAR was not significantly different throughout the 72 h study, suggesting that no advantage was gained by adding β-CAR at the level tested.

The TOCO and RQ treatments had oxidation patterns that were not significantly different at the end of the 72 h light exposure. After 36 h and for the duration of the study, RQ + TOCO had an antioxidant activity that was significantly ($P < 0.05$) better than either the individual RQ or TOCO, indicating a possi-

TABLE 3
Autoxidation of SSBO Treated with CHL, CAR, or TOCO Alone or in Combination with RQ as Measured by Peroxide Values (meq/kg)[a]

	Hours[b]						
Treatment	0	12	24	36	48	60	72
CAR	2.5	27.3^{d}	42.3^{d}	54.6^{c}	80.9^{c}	108.7^{d}	236.9^{d}
CHL	2.5	25.6^{d}	39.7^{d}	48.9^{c}	74.6^{c}	95.3^{c}	170.3^{c}
SSBO (control)	2.5	25.8^{d}	38.5^{d}	46.8^{c}	78.0^{c}	96.6^{c}	162.1^{c}
TOCO	2.5	10.4^{b}	18.7^{b}	22.1^{b}	32.0^{b}	37.6^{b}	73.8^{b}
RQ	2.5	12.6^{b}	19.0^{b}	21.1^{b}	32.9^{b}	40.6^{b}	66.8^{b}
RQ + CAR	2.5	15.8^{c}	21.3^{b}	24.5^{b}	30.9^{b}	40.9^{b}	64.1^{b}
RQ + CHL	2.5	16.5^{c}	28.7^{c}	31.7^{b}	34.4^{b}	44.6^{b}	75.2^{b}
RQ + TOCO	2.5	5.4^{a}	5.8^{a}	6.0^{a}	6.9^{a}	8.7^{a}	11.8^{a}

[a]See Table 1 for abbreviations and concentrations of the antioxidants.
[b]Roman letters within a column indicate significant ($P < 0.05$) differences between treatments at the specified analysis time.

TABLE 4
Light-Sensitized Oxidation of SSBO Treated with CHL, CAR, or TOCO Alone or in Combination with RQ as Measured by Peroxide Values (meq/kg)

	Hours[b]						
Treatment	0	12	24	30	48	60	72
CAR	2.5	12.8^{b}	18.6^{c}	23.1^{e}	27.3^{d}	34.9^{d}	40.6^{d}
CHL	2.5	13.0^{b}	9.2^{c}	23.7^{e}	27.7^{d}	33.8^{d}	40.6^{d}
SSBO (control)	2.5	12.2^{b}	18.3^{c}	20.7^{d}	26.5^{d}	32.4^{d}	39.1^{d}
TOCO	2.5	5.9^{a}	8.7^{b}	11.3^{c}	14.4^{c}	17.7^{c}	20.3^{c}
RQ	2.5	5.9^{a}	6.9^{a,b}	7.6^{b}	10.2^{b}	13.7^{b}	18.3^{b,c}
RQ + CAR	2.5	5.5^{a}	7.0^{b}	8.4^{b}	10.1^{b}	13.9^{b}	17.1^{b,c}
RQ + CHL	2.5	6.0^{a}	7.2^{b}	8.0^{b}	9.8^{b}	12.2^{b}	16.3^{b}
RQ + TOCO	2.5	4.3^{a}	4.7^{a}	5.1^{a}	5.8^{a}	6.1^{a}	7.0^{a}

[a]See Table 1 for abbreviations and concentrations of the antioxidants.
[b]Roman letters within a column indicate significant ($P < 0.05$) differences between treatments at the specified analysis time.

ble synergism between the TOCO and RQ. Again, CAR or CHL and RQ combinations did not exhibit synergism because these combinations were not significantly different from RQ treatments (Table 4).

Data from study 1 indicated that, at the levels tested, CAR and CHL did not affect the activity of RQ. However, TOCO in combination with RQ enhanced antioxidant activity when compared to the individual compounds. Based on this, a second study was conducted to determine if synergism exists between tocopherol(s) and RQ.

In study 2, the antioxidant activity of TOCO in combination with two levels of RQ (RQ1 = 100 ppm and RQ2 = 200 ppm) was evaluated in SSBO until each treatment reached a PV of 20 meq/kg. In addition, the loss of TOCO and RQ was monitored at time 0, 72 h (midpoint), and until a PV of 20 was reached (final). In the autoxidation system, the control oxidized more quickly ($P < 0.05$), as would be expected (Fig. 1A). The three treatments (RQ1, RQ2, and TOCO) oxidized more slowly ($P < 0.05$) than the control but more quickly ($P < 0.05$) than the combinations of RQ and TOCO and the positive control TBHQ. Within the two combinations of RQ and TOCO, the sample containing the greater concentration of RQ had a significantly ($P < 0.05$) lower level of oxidation; however, the least ($P < 0.05$) oxidized sample was the TBHQ-treated SSBO (Fig. 1A).

Synergism between RQ and TOCO can be determined by plotting the number of hours each treatment and treatment combination inhibits the SSBO from reaching a PV of 20 meq/kg. In autoxidation, a clear synergism occurred in the RQ2TOCO sample (Fig. 1A). TOCO and RQ2 alone prevented the SSBO PV from reaching 20 meq/kg for 51 and 73 h, respectively. The combination (RQ2TOCO) delayed the oxidation to 20 meq/kg for 225 h, which is in excess of the 124 h needed to be more than an additive effect, i.e., synergism.

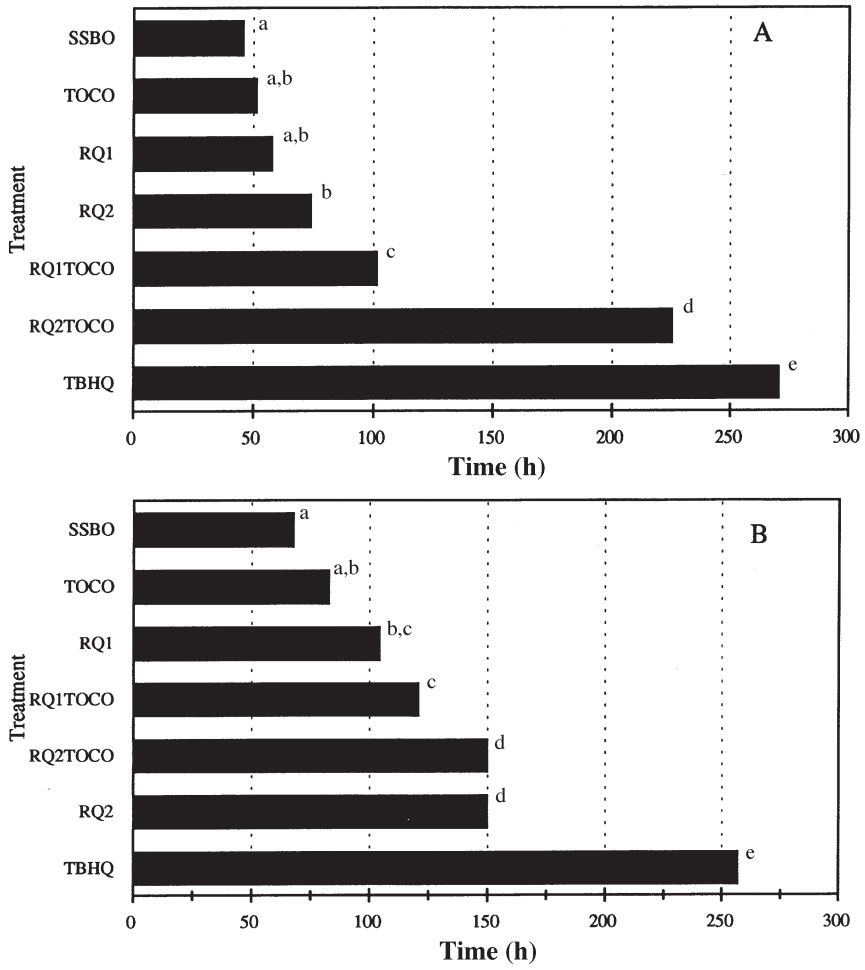


FIG. 1. The oxidation of stripped soybean oil (SSBO) treated with tocopherols (TOCO) alone or in combination with rosmariquinone (RQ) as measured by the time at which the treated SSBO reached a peroxide value of 20 meq/kg in autoxidation (A) and light-sensitized oxidation (B). TOCO were added at the levels originally found in the nonstripped oil and RQ was added at two levels, i.e., 100 ppm (RQ1) or 200 ppm (RQ2). Roman superscript letters indicate significant ($P < 0.05$) differences between treatments once a peroxide value of 20 meq/kg had been reached.

TOCO and RQ1 alone prevented the oil from reaching a PV of 20 meq/kg for 51 and 57 h, respectively. The RQ1TOCO treatment prevented the SSBO from reaching a PV of 20 meq/kg for 101 h. An additive, rather than synergistic, effect was observed because 108 h (51 + 57 h) would be required to delay the oxidation to 20 meq/kg. Neither of the RQTOCO combinations was superior to TBHQ in inhibiting the autoxidation of SSBO.

To determine the role antioxidants play in synergism, one can measure the loss of the individual components for an indication whether the compound is acting as a primary or secondary antioxidant. The antioxidant that has the fastest rate of loss is considered to be the secondary compound, since it acts to spare the primary antioxidant. The losses of RQ and TOCO (Table 5) were determined so as to evaluate each compound's role in the observed synergism during autoxidation. By the midpoint of the study, the sample containing 100 ppm RQ alone had the greatest ($P < 0.05$) loss of RQ. Treatments RQ1TOCO, RQ2, and RQ2TOCO were not significantly different at the midpoint (Table 5). At the conclusion of the study, the sample with the lowest ($P < 0.05$) RQ loss was the RQ2TOCO-treated sample. The sample treated with RQ2 alone tended to have less residual RQ than the sample treated with RQ1TOCO, but the results were not significantly different. The RQ1 sample had the lowest ($P < 0.05$) level of residual RQ by the end of the study (Table 5). Overall, the loss of RQ was between 60 and 100% (0–40% remaining).

The loss of TOCO in the RQ- and TOCO-treated samples during autoxidation showed that the sample treated with TOCO alone had the lowest ($P < 0.05$) residual level of TOCO by the end of the study (Table 5). The RQ1TOCO and RQ2TOCO lost TOCO essentially at the same rate during the study. However, since there was less TOCO lost (83–90% remaining), it can be determined that TOCO was acting as the primary antioxidant in this system.

In the light-sensitized (study 2) experiment, the PV data indicated that the RQ2 and RQ2TOCO were significantly ($P < 0.05$) better antioxidants for inhibiting SSBO oxidation relative to the other RQ/TOCO treatments (Fig. 1B). However, they were not as effective ($P < 0.05$) as the TBHQ. The RQ1TOCO and RQ1 tended to be more effective than the TOCO treatments in preventing oxidation relative to the control (Fig. 1B), but only RQ1TOCO was a significantly ($P < 0.05$) better antioxidant.

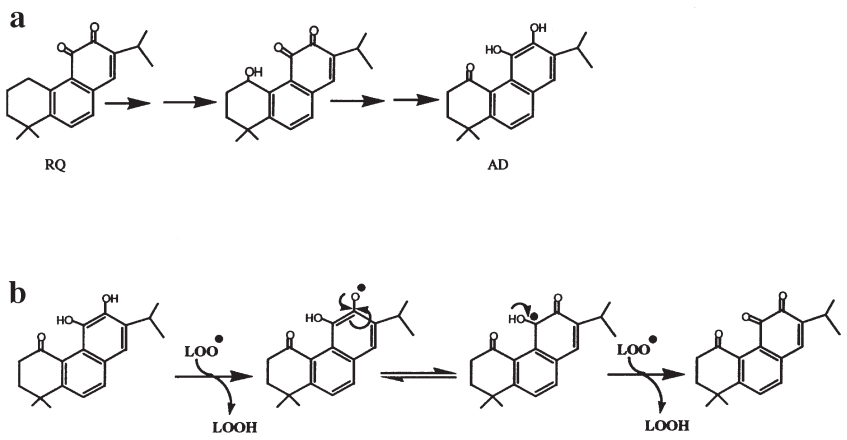
Unlike during autoxidation, no synergistic activity existed in the light-sensitized oxidation system (Fig. 1B). RQ2 and RQ2TOCO both inhibited the oxidation for 149 h while RQ1 and RQ1TOCO delayed the oxidation until 104 and 120 h, respectively. The discrepancy between the observed synergism in the light-sensitized oxidation in study 1 and the lack of synergism during study 2 may be due to the higher concentration of α-TOCO present in the TOCO treatments. The α-TOCO content of 240 ppm (study 1) was significantly higher than the average in study 2 (71 ppm). In study 1, α- and β-TOCO were used to make up the total TOCO, whereas in study 2, the total TOCO was a mixture of α-, γ-, and δ-TOCO, thus, accounting for the variation in α-tocopherol. α-TOCO has the highest $^1O_2$ quenching activity (9,10); the higher concentrations of α-TOCO in study 1 may contribute to the synergism.

During study 2, RQ and TOCO remaining in the oil were monitored at time 0, 72 h (midpoint), and until a PV of 20 meq/kg was reached (final). The reduction patterns observed in the light-sensitized oxidation indicated that between 16 and 38% of the RQ and 84 and 90% of the TOCO remained at the end of the study (Table 5). The RQ2TOCO treatment tended to have the highest level of RQ remaining but was not significantly ($P > 0.05$) different from the RQ2 and RQ1TOCO treatments. However, the TOCO level of the RQ2TOCO was significantly higher than the amounts left in the TOCO and RQ1TOCO treatments. In both RQ and TOCO measurements, the within-treatment losses of RQ and TOCO were significantly ($P < 0.05$) different from the preceding time period (data not shown). Although no true synergism was found between RQ and TOCO during photooxidation, based on data

TABLE 5
Percentage of Tocopherol (TOCO) and Rosmariquinone (RQ) Remaining During the Oxidation of Stripped Soybean Oil [a]

	Autoxidation			Light-sensitized oxidation[b]		
	Initial	Midpoint	Final	Initial	Midpoint	Final
TOCO remaining (%)						
TOCO	100	86.9[a]	82.9[a]	100	88.4[a]	84.4[a]
RQ1TOCO	100	94.4[b]	89.0[b]	100	92.7[b]	84.9[a]
RQ2TOCO	100	94.9[b]	90.2[b]	100	92.6[b]	89.5[b]
RQ remaining (%)						
RQ1	100	12.8[a]	0.0[a]	100	20.6[a]	16.3[a]
RQ2	100	48.7[b]	19.6[b]	100	38.5[a,b]	20.0[a,b]
RQ1TOCO	100	37.3[b]	27.4[b]	100	45.3[b,c]	23.9[a,b]
RQ2TOCO	100	54.8[b]	38.9[c]	100	65.2[c]	37.5[b]

[a]Treatments include TOCO added at levels originally found in the nonstripped oil RQ1 (100 ppm), and RQ2 (200 ppm). Each antioxidant was tested individually and in combination.
[b]Roman letters within a column indicate significant ($P < 0.05$) differences between treatments at various intervals.



SCHEME 1

that is not significant, 200 ppm RQ did tend to "spare" the loss of TOCO during the oxidation process.

The synergistic activity in autoxidation observed between RQ and TOCO can be explained using the proposed AO mechanism for RQ (24). RQ is hypothesized to undergo several oxidation-reduction rearrangements to form the active intermediate arucadiol (AD, Scheme 1). In a previous report (24), AD was found in both photo- and autoxidation model systems as early as 30 min suggesting that AD may contribute to antioxidant activity of RQ. AD's donation of hydrogen to the lipid peroxy radical is one mechanism hypothesized for the observed antioxidant activity of RQ (Scheme 1). The regeneration of TOCO by AD (Scheme 2), through hydrogen donation may explain the higher concentrations of TOCO remaining at the end of this study and contribute to the observed synergistic activity between TOCO and RQ in autoxidation.

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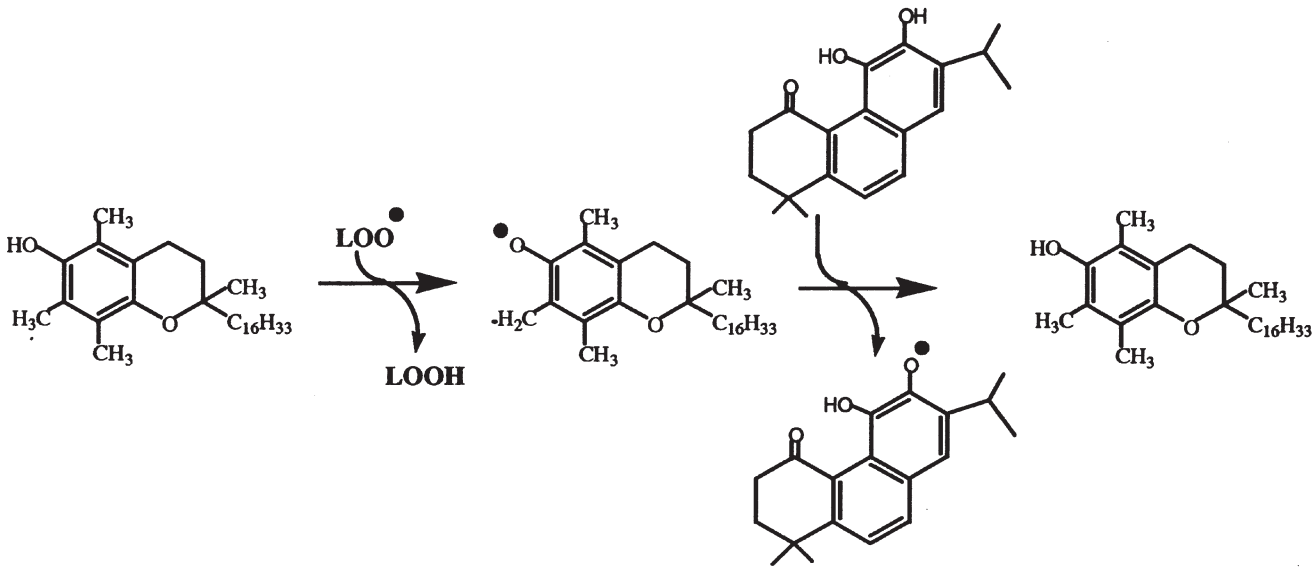

SCHEME 2

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