

# Effects, Quenching Mechanisms, and Kinetics of Nickel Chelates in Singlet Oxygen Oxidation of Soybean Oil<sup>†</sup>

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Effects of  $0, 7 \times 10^{-8}$ ,  $14 \times 10^{-8}$ , and  $21 \times 10^{-8}$  M bis(di-*n*-butyldithiocarbamate)nickel chelate (BTC) and  $0, 9 \times 10^{-7}$ ,  $18 \times 10^{-7}$ , and  $27 \times 10^{-7}$  M [2,2'-thiobis(4-(1,1,3,3-tetramethylbutyl)phenolato)](*n*-butylamine)nickel chelate (TPB) on the singlet oxygen oxidation of soybean oil containing  $4.4 \times 10^{-9}$  M chlorophyll were determined by measuring peroxide value and depleted headspace oxygen of oil. The samples were stored in the 4000-lx light storage box for 24 h at 20 °C and evaluated for the oxidative stabilities every 4 h in duplicate. The reaction rate constant of singlet oxygen with soybean oil was  $1.38 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ . As the concentrations of BTC and TPB increased, peroxide values and depleted headspace oxygen decreased significantly at  $P < 0.05$ . BTC was significantly more effective than TPB in reducing the singlet oxygen oxidation of soybean oil at  $P < 0.05$ . Quenching mechanisms and kinetics of  $0, 0.75 \times 10^{-5}$ ,  $1.50 \times 10^{-5}$ , and  $3.0 \times 10^{-5}$  M BTC and  $0, 1.25 \times 10^{-4}$ ,  $2.50 \times 10^{-4}$ , and  $5.0 \times 10^{-4}$  M TPB in the singlet oxygen oxidation of soybean oil containing  $3.3 \times 10^{-9}$  M chlorophyll were studied by measuring the depleted headspace oxygen of sample bottles. BTC and TPB quenched singlet oxygen to minimize the chlorophyll photosensitized oxidation of soybean oil. The total singlet oxygen quenching rate constants of BTC and TPB were  $1.23 \times 10^9$  and  $3.70 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ , respectively.

## INTRODUCTION

Triplet oxygen free-radical lipid oxidation to improve the oxidative stability of lipid foods has been extensively studied for the past 50 years (Labuza, 1971; Frankel, 1980, 1985). However, it does not fully explain the initiation step of lipid oxidation (Richardson and Korycka-Dahl, 1983; Frankel, 1985). The role of singlet oxygen at the initiation step of lipid oxidation was reported, and the reaction rate of singlet oxygen with linoleic acid was at least 1450 times greater than that of triplet oxygen (Rawls and Van Santen, 1970).

Singlet oxygen is formed by photosensitizers such as chlorophylls in the presence of light and triplet oxygen and reacts with vegetable oils to form off-flavor compounds (Clements et al., 1973; Terao and Matsushita, 1977; Frankel et al., 1982; Usuki et al., 1984; Frankel, 1985; Lee and Min, 1988).

To reduce the undesirable singlet oxygen oxidation in lipid foods, the effects of naturally occurring tocopherols and carotenoids on singlet oxygen oxidation have been extensively studied (Matsushita and Terao, 1980; Fakourelis et al., 1987; Warner and Frankel, 1987; Min and Lee, 1988; Lee and Min, 1990). However, information on the application of synthetic compounds to minimize singlet oxygen oxidation in lipid foods is very limited. Nickel chelates have been reported as good quenchers to minimize the photosensitized singlet oxygen oxidation of olefin polymers and oils (Carlsson et al., 1972, 1976; Zweig and Henderson, 1975).

The objective of this research was to study the effects, quenching mechanisms, and kinetics of synthetic bis(di-*n*-butyldithiocarbamate)nickel chelate and [2,2'-thiobis(4-(1,1,3,3-tetramethylbutyl)phenolato)](*n*-butylamine)-

nickel chelate on the chlorophyll-photosensitized singlet oxygen oxidation of soybean oil.

## MATERIALS AND METHODS

**Materials.** Soybean oil was obtained from Capital City Products Co. (Columbus, OH). Chlorophyll was purchased from Sigma Chemical Co. (St. Louis, MO). Bis(di-*n*-butyldithiocarbamate)nickel chelate (BTC) and [2,2'-thiobis(4-(1,1,3,3-tetramethylbutyl)phenolato)](*n*-butylamine) nickel chelate (TPB) were purchased from P & B Research Chemicals (Waterbury, CT) and Aldrich Chemical Co. (Milwaukee, WI), respectively.

**Purification of Soybean Oil and Light Storage Conditions for Singlet Oxygen Oxidation of Soybean Oil.** The method used for the purification of soybean oil and the light storage conditions for singlet oxygen oxidation of soybean oil were essentially the same as described by Lee and Min (1988). Three hundred milliliters of soybean oil were passed through a chromatographic column (60 cm × 4 cm) packed with a series of 120 g of activated 100-mesh silicic acid (Mallinkrodt), 40 g of a 2:1 mixture of activated charcoal (J. T. Baker Chemical Co.) and Celite (Sargent Welch), 120 g of a 2:1 mixture of powdered sugar and Celite, and 120 g of activated silicic acid. The soybean oil that passed through the column was referred to as purified soybean oil.

**Chemical Analyses of Purified Soybean Oil.** Tocopherols were determined by high-pressure liquid chromatography (Carpenter, 1979), and carotenoids were analyzed by the spectrometric method of Proctor and Snyder (1987). Phospholipids and free fatty acids in the oil were determined according to AOCS (1980) methods.

**Sample Preparation, Storage, and Analyses for Effects of BTC and TPB on Singlet Oxygen Oxidation of Soybean Oil.** To study the effects of BTC and TPB on the singlet oxygen oxidation of soybean oil, the samples of soybean oil containing  $0, 7 \times 10^{-8}$ ,  $14 \times 10^{-8}$ , and  $21 \times 10^{-8}$  M BTC and  $0, 9 \times 10^{-7}$ ,  $18 \times 10^{-7}$ , and  $27 \times 10^{-7}$  M TPB were prepared in duplicate (Lee and Min, 1988).

Fifteen milliliters of oil sample was transferred into a 30-mL serum bottle. The bottles were airtightly sealed with Teflon septa and aluminum caps and then placed without shaking in the light storage box which was described in detail by Lee and Min (1988) for 24 h. The light storage box consisted of a rectangular glass chamber (60 cm × 30 cm × 50 cm) and a wooden box (70 cm × 50 cm × 60 cm) and was maintained at 20 °C. Samples in duplicate were placed on wire netting that was 10 cm

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<sup>†</sup> Salaries and research support provided by State and Federal funds appropriated to The Ohio Agricultural Research and Development Center, The Ohio State University; Journal Article No. 345-89.

above Sylvania cool-white fluorescent lamps (Danvers, MA), whose light intensity was 4000 lx. Samples were rearranged in the storage box at 20 °C every hour to have as much uniform light exposure as possible. The oxidative stability of soybean oil was determined by a combination of measuring the peroxide value and depleted headspace oxygen of sample bottles every 4 h for 24 h. The peroxide value was determined by the AOCS (1980) method. Depleted headspace oxygen of sample bottles was measured by using a Hewlett-Packard 5880A gas chromatograph equipped with a thermal conductivity detector and a Hewlett-Packard 3390 electronic integrator (Fakourelis et al., 1987; Lee and Min, 1988). A stainless steel column (1.83 m × 0.32 cm i.d.) packed with 80/100 mesh molecular sieve 13× (Altech Associates, Inc., Deerfield, IL) was used, and the flow rate of nitrogen gas was 30 mL/min. One milliliter of headspace gas from the sample bottle was injected into a gas chromatograph by using a 2-mL gastight syringe (Hamilton Co., Reno, NV). Electronic units of the gas chromatographic oxygen peak of 1 mL of headspace gas were converted to micromoles of O<sub>2</sub> per milliliter of headspace by using the formula of Min and Lee (1988), and the depleted headspace oxygen of sample bottles was expressed as micromoles of O<sub>2</sub> per milliliter of headspace gas.

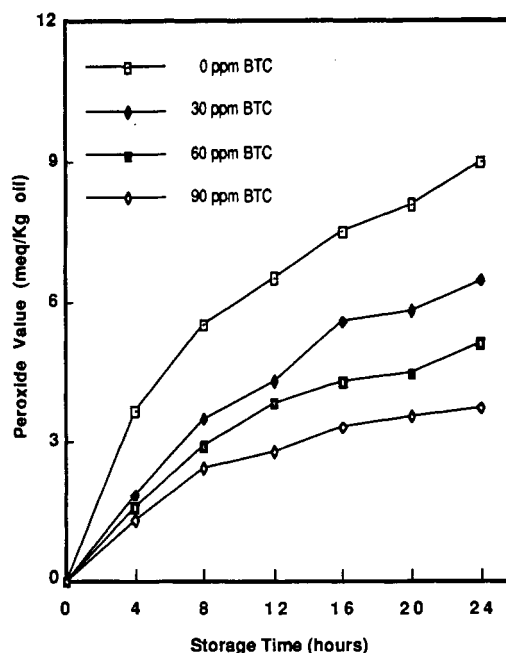
**Sample Preparation and Analyses for the Studies of Quenching Mechanisms and Kinetics of BTC and TPB in Singlet Oxygen Oxidation of Soybean Oil.** The quenching mechanisms and kinetics of BTC and TPB in the singlet oxygen oxidation of soybean oil were studied by using the steady-state kinetic equation (Foote and Denny, 1968; Zweig and Henderson, 1975; Foote, 1979). Samples of 0.025, 0.05, 0.067, 0.10, or 0.20 M soybean oil in methylene chloride containing  $3.3 \times 10^{-9}$  M chlorophyll and 0,  $0.75 \times 10^{-5}$ ,  $1.50 \times 10^{-5}$ , and  $3.0 \times 10^{-5}$  M BTC or 0,  $1.25 \times 10^{-4}$ ,  $2.50 \times 10^{-4}$ , and  $5.0 \times 10^{-4}$  M TPB were prepared in duplicate (Yamauchi and Matsushita, 1977; Lee and Min, 1988, 1990). Fifteen milliliters of sample was transferred into a 30-mL serum bottle. The bottles were airtightly sealed with Teflon septa and aluminum caps and then put in the light storage box for 2 h. The quenching mechanisms and kinetics of BTC and TPB in soybean oil oxidation were studied by measuring the depleted headspace oxygen of the sample bottle by gas chromatography (Fioriti, 1977; Fakourelis et al., 1987). The depleted headspace oxygen content was calculated according to the method of Lee and Min (1988) and expressed as micromoles of O<sub>2</sub> per milliliter of headspace gas.

**Statistical Analyses.** The peroxide values and depleted headspace oxygen contents reported in this paper are the mean value of duplicate samples. Tukey's range test (SAS, 1985) was used to determine the effects of different levels of BTC and TPB on the peroxide values and depleted headspace oxygen of soybean oil during storage.

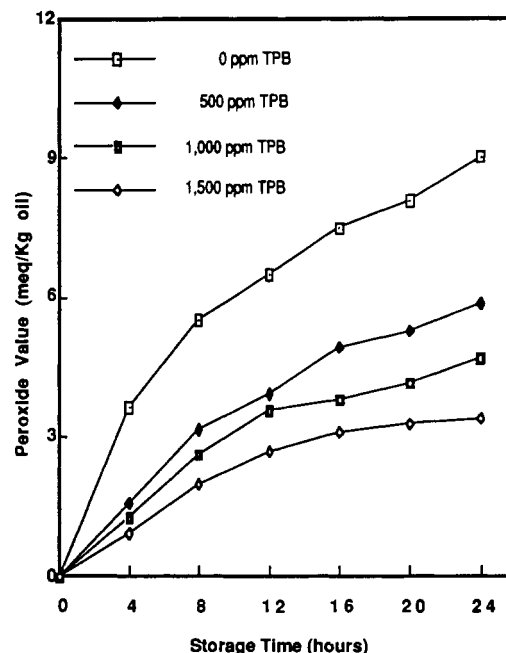
## RESULTS AND DISCUSSION

**Purification of Soybean Oil.** The purified soybean oil obtained by silicic acid column chromatography was colorless and did not contain chlorophylls, carotenoids, phospholipids, peroxides, and free fatty acids. These results agree with the report of Min and Lee (1988).

**Effects of BTC and TPB on the Singlet Oxygen Oxidation of Soybean Oil.** The quantitative effects of BTC and TPB on the peroxide value of soybean oil containing  $4.4 \times 10^{-9}$  M chlorophyll under light at 20 °C for 24 h are shown in Figures 1 and 2, respectively. The peroxide value increased as the storage time increased from 0 to 24 h. Preliminary study showed that the peroxide values of soybean oil containing no chlorophyll did not change as the storage time increased from 0 to 24 h at 20 °C under dark or light. Therefore, the increase of peroxide value of soybean oil containing chlorophyll under light storage must be due to the chlorophyll-photosensitized singlet oxygen oxidation. Chlorophyll has been reported as a photosensitizer to produce singlet oxygen in the presence of light and triplet oxygen (Matsushita and Terao, 1980; Usuki et al., 1984; Fakourelis et al., 1987; Warner and Frankel, 1987; Lee and Min, 1988). As the concen-



**Figure 1.** Effects of bis(di-*n*-butylthiocarbamato)nickel chelate (BTC) on the peroxide value of soybean oil containing  $4.4 \times 10^{-9}$  M chlorophyll under light storage at 20 °C.



**Figure 2.** Effects of [2,2'-thiobis(4-(1,1,3,3-tetramethylbutyl)-phenolato)](*n*-butylamine)nickel chelate (TPB) on the peroxide value of soybean oil containing  $4.4 \times 10^{-9}$  M chlorophyll under light at 20 °C.

trations of BTC and TPB increased, the peroxide values decreased as shown in Figures 1 and 2. Tukey's range test (data not presented) showed that the effects of different levels of BTC and TPB on the peroxide value were significantly different at  $P < 0.05$ .

The effects of different levels of BTC and TPB on the depleted headspace oxygen of soybean oil are shown in Table I. The coefficient of variation of headspace oxygen determination by gas chromatography was 3%. As the concentrations of BTC and TPB increased, the depleted headspace oxygen content decreased. The correlation coefficient ( $r$ ) between peroxide formation (Figures 1 and 2) and depleted headspace oxygen content (Table I) was  $-0.99$ .

Tukey's range test (Table I) shows that the effects of

**Table I. Tukey's Range Test for the Effect of Bis(di-*n*-butyldithiocarbamate)nickel Chelate (BTC) and [2,2'-Thiobis(4-(1,1,3,3-tetramethylbutyl)phenolato)]-(*n*-butylamine)nickel Chelate (TPB) on the Depleted Headspace Oxygen Soybean Oil Containing  $4.4 \times 10^{-9}$  M Chlorophyll under Light at 20 °C for 24 h**

Ni chelate in soybean oil, $\times 10^{-8}$ M	depleted headspace oxygen, $\mu\text{mol of O}_2/\text{mL of headspace}^a$							
	0 h	4 h	8 h	12 h	16 h	20 h	24 h	mean <sup>b</sup>
BTC (0)	0	1.28	1.48	1.87	2.40	2.53	2.82	1.77 <sup>a</sup>
BTC (7)	0	0.86	1.09	1.45	1.63	1.75	2.01	1.25 <sup>b</sup>
BTC (14)	0	0.79	0.97	1.29	1.39	1.52	1.70	1.09 <sup>c</sup>
BTC (21)	0	0.71	0.85	1.00	1.24	1.35	1.39	0.93 <sup>d</sup>
TPB (0)	0	1.28	1.48	1.87	2.40	2.53	2.82	1.77 <sup>a</sup>
TPB (90)	0	0.66	0.99	1.44	1.56	1.84	1.96	1.20 <sup>b</sup>
TPB (180)	0	0.56	0.88	1.14	1.34	1.44	1.59	0.99 <sup>c</sup>
TPB (270)	0	0.44	0.73	0.84	1.01	1.15	1.23	0.77 <sup>d</sup>

<sup>a</sup> Depleted headspace oxygen is the mean value of analyses of duplicate samples. <sup>b</sup> Mean of depleted headspace oxygen after 0, 4, 8, 12, 16, 20, and 24 h of storage; means in a column of same nickel chelate with different letters are significantly different at  $P < 0.05$ .

different levels of BTC and TPB on the depleted headspace oxygen of soybean oil were significantly different at  $P < 0.05$ . The combined results of peroxide values (Figures 1 and 2) and depleted headspace oxygen of soybean oil (Table I) indicate that BTC and TPB reduced the chlorophyll-photosensitized singlet oxygen oxidation of soybean oil.

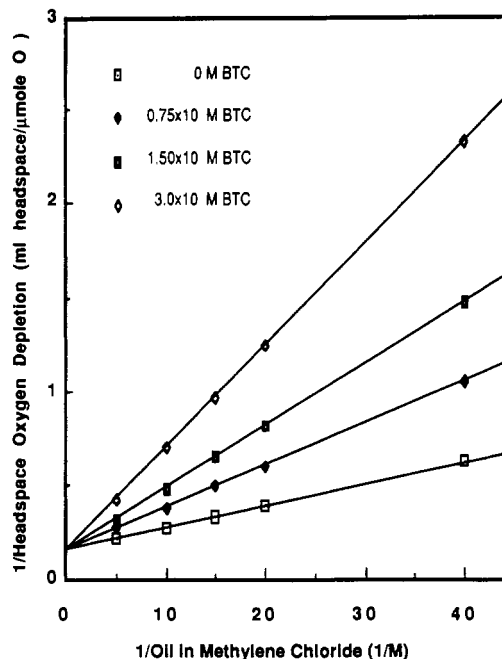
The BTC and TPB might reduce the chlorophyll-photosensitized singlet oxygen oxidation of soybean oil by one or a combination of the following: (1) free-radical scavenging mechanism, (2) light screening effects, (3) singlet oxygen quenching, and (4) excited triplet state chlorophyll quenching.

To study whether BTC and TPB acted as antioxidants by free-radical scavenging or not, the purified soybean oils containing no chlorophyll with and without 90 ppm BTC or 1500 ppm TPB were stored at 20 °C for 5 days under light, and the peroxide values were determined daily. The peroxide values of soybean oils with and without BTC or TPB were essentially the same. Therefore, BTC and TPB did not scavenge the free radicals to reduce soybean oil oxidation.

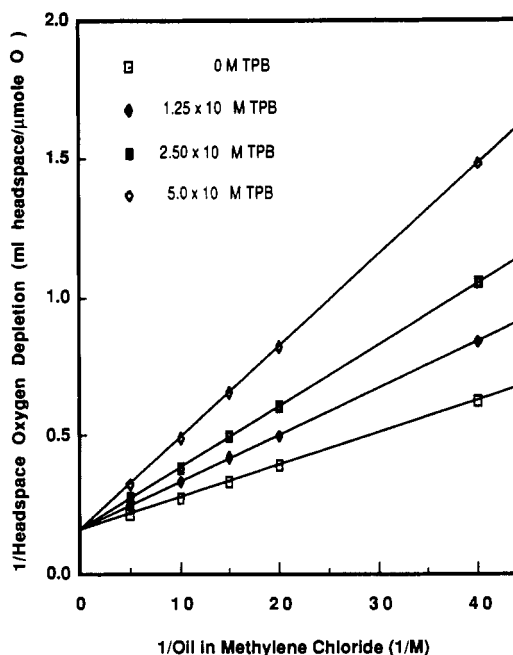
To study the possible light screening effects of BTC and TPB on the oxidative stability of soybean oil, the screening effects of 90 ppm BTC and 1500 ppm TPB on the purified soybean oils were determined by measuring headspace oxygen content according to the method of Fakourelis et al. (1987). The samples were stored under light at 20 °C for 24 h, and headspace oxygen was determined every 4 h. The screening effects of BTC and TPB on the headspace oxygen of sample bottles were not significantly different from the control at  $P > 0.05$ .

Whether BTC and TPB quenched singlet oxygen and/or excited triplet state chlorophyll to reduce the chlorophyll-photosensitized oxidation of soybean oil or not was studied, and the results will be discussed in detail in the following section.

**Quenching Mechanisms and Kinetics of BTC and TPB in Singlet Oxygen Oxidation of Soybean Oil.** The effects of 0,  $0.75 \times 10^{-5}$ ,  $1.50 \times 10^{-5}$ , and  $3.0 \times 10^{-5}$  M BTC and 0,  $1.25 \times 10^{-4}$ ,  $2.50 \times 10^{-4}$ , and  $5.0 \times 10^{-4}$  M TPB on the headspace oxygen depletion of soybean oil in methylene chloride containing  $3.3 \times 10^{-9}$  M chlorophyll during 2 h of light storage are shown in Figures 3 and 4, respectively. As the concentrations of BTC and TPB increased, the depleted headspace oxygen content of sample bottles decreased (Figures 3 and 4). The BTC



**Figure 3.** Effects of bis(di-*n*-butylthiocarbamate)nickel chelate (BTC) on the headspace oxygen depletion of soybean oil in methylene chloride containing  $3.3 \times 10^{-9}$  M chlorophyll under light storage at 20 °C for 2 h.



**Figure 4.** Effects of [2,2'-thiobis(4-(1,1,3,3-tetramethylbutyl)phenolato)](*n*-butylamine)nickel chelate (TPB) on the headspace oxygen depletion of soybean oil in methylene chloride containing  $3.3 \times 10^{-9}$  M chlorophyll under light storage at 20 °C for 2 h.

and TPB acted as antioxidants in the chlorophyll-photosensitized oxidation of soybean oil. If BTC or TPB reduced the chlorophyll-photosensitized singlet oxygen oxidation by singlet oxygen quenching, the steady-state kinetic equation

$$\{-d[\text{O}_2]/dt\}^{-1} = \{d[\text{ROOH}]/dt\}^{-1} = K^{-1}\{1 + (k_q[\text{Q}] + k_{\text{ox-Q}}[\text{Q}] + k_d)/k_r[\text{RH}]\}$$

is established (Foote and Denny, 1968; Foote, 1979), where ROOH is oxidized soybean oil,  $K$  is the rate of singlet oxygen formation,  $k_q$  is the reaction rate constant of physical singlet oxygen quenching by BTC or TPB,  $\text{Q}$  is quencher such as BTC or PTB,  $k_{\text{ox-Q}}$  is the reaction rate

constant of chemical singlet oxygen quenching by BTC or TPB,  $k_d$  is the decaying rate constant of singlet oxygen,  $k_r$  is the reaction rate constant of soybean oil with singlet oxygen, and RH is soybean oil.

The plots of  $(-d[O_2]/dt)^{-1}$  vs  $[RH]^{-1}$  at various  $[Q]$  have the constant intercepts of  $K^{-1}$  and the intercepts are  $[Q]$  independent if quencher (Q) quenched singlet oxygen only. That is, the constant intercepts of the plots of  $(-d[O_2]/dt)^{-1}$  vs  $[RH]^{-1}$  at various  $[Q]$  are diagnostic of singlet oxygen quenching (Foote, 1979). The slope of the plot of  $(-d[O_2]/dt)^{-1}$  vs  $[RH]^{-1}$  is  $K^{-1}(k_q[Q] + k_{ox-Q}[Q] + k_d)/k_r$ . The plot of  $S_Q/S_0$  (slopes in the presence and absence of quencher) vs  $[Q]$  is a straight line, and the slope of the straight line is  $(k_q + k_{ox-Q})/k_d$  (Foote et al., 1974; Yamachi and Matsushita, 1977; Foote, 1979).

If BTC or TPB reduced the chlorophyll-photosensitized singlet oxygen oxidation of soybean oil by quenching the excited triplet state chlorophyll sensitizer, the steady-state kinetic equation

$$\{-d[O_2]/dt\}^{-1} = \{d[ROOH]/dt\}^{-1} = K^{-1}\{1 + (k_q[Q]/k_0[{}^3O_2])\}\{1 + (k_d/k_r[RH])\}$$

is established (Foote, 1979), where ROOH is oxidized soybean oil,  $K$  is the rate of excited triplet sensitizer formation,  $k_q$  is the rate of excited triplet sensitizer physical quenching by BTC or TPB,  $Q$  is quencher such as BTC or TPB,  $k_0$  is the reaction rate between triplet oxygen and excited triplet sensitizer to form singlet oxygen,  $k_d$  is the decaying rate constant of singlet oxygen,  $k_r$  is the reaction rate constant of soybean oil with singlet oxygen, and RH is soybean oil.

The plots of  $(-d[O_2]/dt)^{-1}$  vs  $[RH]^{-1}$  at various  $[Q]$  give  $y$  intercepts equal to  $K^{-1}\{1 + (k_q[Q]/k_0[{}^3O_2])\}$  if quencher (Q) quenched the excited triplet chlorophyll sensitizer to reduce the photosensitized oxidation of soybean oil. The variable  $y$  intercepts of the plots of  $(-d[O_2]/dt)^{-1}$  vs  $[RH]^{-1}$  at various  $[Q]$  are diagnostic of excited triplet sensitizer quenching (Foote, 1979).

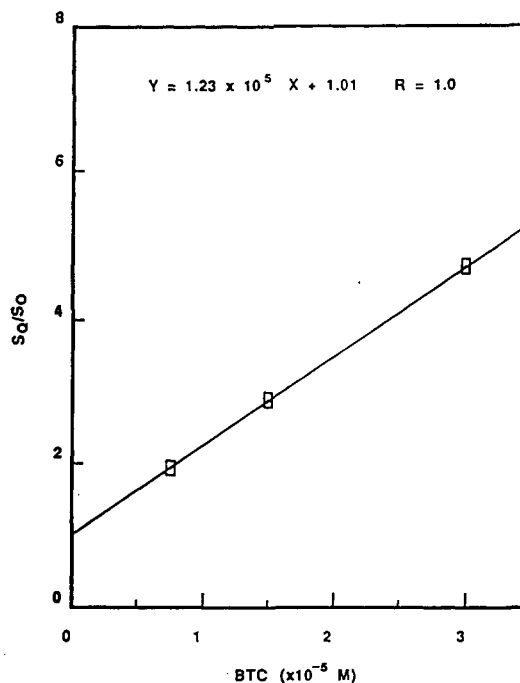
The results of the study of the quenching mechanisms of BTC in singlet oxygen oxidation of soybean oil are shown in Figure 3. The  $y$  intercepts of the plots  $(-d[O_2]/dt)^{-1}$  vs  $[soybean\ oil]^{-1}$  at different levels of BTC are the same, but the slopes of the plots are different (Figure 3). The steady-state kinetic equation (Zweig and Henderson, 1975; Foote, 1979) states that when the  $y$  intercepts of the plots  $(-d[O_2]/dt)^{-1}$  vs  $[soybean\ oil]^{-1}$  at different concentrations of quencher are the same and the slopes of the plots are different, the quencher minimizes singlet oxygen oxidation by quenching singlet oxygen only. Since the  $y$  intercepts of the plots having different levels of BTC are the same and the slopes are different (Figure 3), BTC quenched singlet oxygen only and did not quench the excited triplet state of chlorophyll to minimize the chlorophyll-photosensitized oxidation of soybean oil. Similarly, TPB quenched singlet oxygen only to reduce the singlet oxygen oxidation of soybean oil containing chlorophyll and did not quench the excited triplet state chlorophyll (Figure 4).

The slopes and the ratios of  $S_Q/S_0$  of the plots with different levels of BTC of Figure 3 are given in Table II. The slope and  $y$  intercept of the plot  $(-d[O_2]/dt)^{-1}$  vs  $[soybean\ oil]^{-1}$  of sample containing no BTC were 0.0116 mL of headspace gas/ $\mu$ mol of  $O_2$  and 0.1598 mL of headspace gas/ $\mu$ mol of  $O_2$ , respectively, as shown in Table II. The slope/ $y$  intercept of the plot  $(-d[O_2]/dt)^{-1}$  vs  $[soybean\ oil]^{-1}$  for samples containing no BTC is  $k_d/k_r$ , where  $k_d$  is the decaying rate of singlet oxygen in methylene chloride and  $k_r$  is the reaction rate constant of singlet oxygen with

**Table II. Parameters in the Plot of  $(-d[O_2]/dt)^{-1}$  vs  $[Soybean\ Oil]^{-1}$  of Figure 3 for the Effects of Bis(di-*n*-butyldithiocarbamate)nickel Chelate (BTC) on the Headspace Oxygen Depletion of Soybean Oil in Methylene Chloride Containing  $3.3 \times 10^{-9}$  M Chlorophyll under Light at 20 °C for 2 h**

BTC in methylene chloride, $\times 10^{-5}$ M	intercept (mL of headspace/ $\mu$ mol of $O_2$ )	slope (M-mL headspace/ $\mu$ mol of $O_2$ )	$S_Q/S_0^a$
0	0.1598	0.0116	
0.75	0.1609	0.0223	1.9924
1.50	0.1582	0.0331	2.8534
3.0	0.1602	0.0543	4.6810

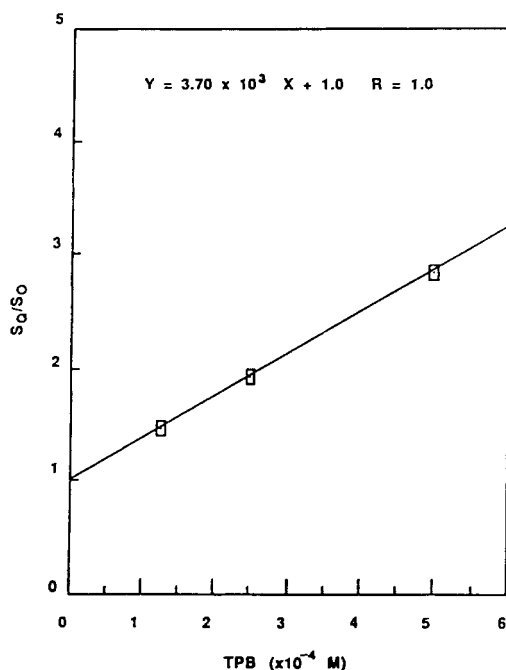
<sup>a</sup>  $S_Q$  and  $S_0$  are the slopes of the plot  $(-d[O_2]/dt)^{-1}$  vs  $[soybean\ oil]^{-1}$  in the presence and absence of bis(di-*n*-butyldithiocarbamate)nickel chelate (BTC), respectively.



**Figure 5. Regression line of  $S_Q/S_0$  vs concentrations of bis(di-*n*-butyldithiocarbamate)nickel chelate (BTC) in soybean oil [ $S_Q$  and  $S_0$  are the slopes of the plot  $(-d[O_2]/dt)^{-1}$  vs  $[soybean\ oil]^{-1}$  in the presence and absence of BTC, respectively].**

soybean oil (Foote, 1979). The  $k_d/k_r$  value was 0.0726 M as shown in Table II. The decaying rate ( $k_d$ ) of singlet oxygen in methylene chloride was  $1.0 \times 10^4$  s<sup>-1</sup> (Hurst et al., 1982). Therefore, the reaction rate constant ( $k_r$ ) of singlet oxygen with soybean oil was  $1.38 \times 10^5$  M<sup>-1</sup> s<sup>-1</sup> in methylene chloride. Doleiden et al. (1974) reported that the reaction rate constants of singlet oxygen with methyl oleate and methyl linoleate were  $0.7 \times 10^5$  and  $1.3 \times 10^5$  M<sup>-1</sup> s<sup>-1</sup> in pyridine, respectively. The reaction rate of soybean oil with singlet oxygen in methylene chloride determined by measuring headspace oxygen of sample bottles was very similar to that of methyl linoleate in pyridine determined by measuring the oxidized methyl linoleate (Doleiden et al., 1974). The fatty acid analyses indicated that the composition of purified soybean oil was 9.76% palmitic acid, 3.89% stearic acid, 23.49% oleic acid, 54.71% linoleic acid, and 8.15% linolenic acid.

To measure the total singlet oxygen quenching rate constant ( $k_q + k_{ox-Q}$ ) of BTC, the regression line of  $S_Q/S_0$  vs  $[BTC]$  was plotted by using the data of Table II and is shown in Figure 5.  $k_q$  and  $k_{ox-Q}$  are physical and chemical singlet oxygen quenching rate constants of the quencher, respectively (Foote, 1979).  $S_Q$  and  $S_0$  are the slopes of the plot  $(-d[O_2]/dt)^{-1}$  vs  $[soybean\ oil]^{-1}$  in the presence and



**Figure 6.** Regression line of  $S_Q/S_0$  vs concentrations of [2,2'-thiobis(4-(1,1,3,3-tetramethylbutyl)phenolato)](*n*-butylamine)-nickel chelate (TPB) in soybean oil [ $S_Q$  and  $S_0$  are the slopes of the plot  $(-d[O_2]/dt)^{-1}$  vs [soybean oil] $^{-1}$  in the presence and absence of TPB, respectively].

absence of quencher, respectively. The slope of the regression line of  $S_Q/S_0$  vs [quencher] is  $(k_q + k_{ox-Q})/k_d$  (Foote and Denny, 1968; Yamauchi and Matsushita, 1977; Foote, 1979). The slope of the regression line of  $S_Q/S_0$  vs [BTC] was  $1.23 \times 10^5 \text{ M}^{-1}$  (Figure 5). Since the decaying rate constant ( $k_d$ ) of singlet oxygen in methylene chloride was  $1.0 \times 10^4 \text{ s}^{-1}$  (Hurst et al., 1982), the total singlet oxygen quenching rate constant ( $k_q + k_{ox-Q}$ ) of BTC in methylene chloride was  $1.23 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ . Since the slope of the regression line of  $S_Q/S_0$  vs [TPB] was  $3.70 \times 10^3 \text{ M}^{-1}$  (Figure 6) and the  $k_d$  value in methylene chloride was  $1.0 \times 10^4 \text{ s}^{-1}$ , the total singlet oxygen quenching rate constant ( $k_q + k_{ox-Q}$ ) of TPB was  $3.70 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  in methylene chloride. Carlsson et al. (1976) reported that the singlet oxygen quenching rate constants of BTC and TPB were  $17 \times 10^9$  and  $2.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  in isooctane, respectively. The marked variations of singlet oxygen quenching rates have been explained in terms of significant solvent polarity effects (Foote et al., 1974). As an example, the singlet oxygen quenching rate of  $\alpha$ -tocopherol is  $9 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  in cyclohexane and  $53 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  in methanol (Foote, 1979).

The singlet oxygen quenching rate constant of BTC ( $1.23 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ) was about 30 times greater than that of TPB ( $3.70 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ ) in methylene chloride and was comparable to the quenching rate ( $5.72 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ) of lutein in methylene chloride. Lutein is one of the major carotenoids in soybean oil and the most effective naturally occurring singlet oxygen quenchers (Lee and Min, 1990).

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Received for review June 19, 1990. Revised manuscript received November 13, 1990. Accepted November 27, 1990.

**Registry No.** BTC, 13927-77-0; TPB, 14516-71-3;  $O_2$ , 7782-44-7.