JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 9967–9978 9967
DOI:10.1021/jf101947a

Kinetic Study of the Quenching Reaction of Singlet Oxygen by Carotenoids and Food Extracts in Solution. Development of a Singlet Oxygen Absorption Capacity (SOAC) Assay Method

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A kinetic study of the quenching reaction of singlet oxygen ($^1O_2$) with eight kinds of carotenoids and α-tocopherol was performed in ethanol/chloroform/D_2O (50:50:1, v/v/v) solution at 35 °C. The overall rate constants, k_Q (= k_q + k_r, physical quenching + chemical reaction), for the reaction of carotenoids with $^1O_2$ were measured, using the competition reaction method, where endoperoxide was used as a singlet oxygen generator, 2,5-diphenyl-3,4-benzofuran (DPBF) as an UV−vis absorption prove, and α-tocopherol as a standard compound. The rate constants, k_Q (S) and k_Q ($t_{1/2}$), were determined by analyzing the first-order rate constant (S) and the half-life ($t_{1/2}$) of the decay curve of DPBF with carotenoids, respectively, showing good accordance with each other. Similar measurements were performed for tomato and carrot extracts. From the results, a new assay method that can quantify the singlet oxygen absorption capacity (SOAC) of antioxidants, including carotenoids, α-tocopherol, and vegetable extracts, has been proposed.

KEYWORDS: Singlet oxygen; quenching rate; endoperoxide; carotenoids; β-carotene; lycopene; astaxanthin; foods; SOAC value; kinetic study

INTRODUCTION

Carotenoids have attracted much attention because of their high antioxidant capacity and their abundance in the human diet (*1*, *2*). Vegetables and fruits contain considerable amounts of carotenoids (*3*−*5*). Several epidemiolologic studies show an inverse association between serum/adipose β-carotene levels and coronary heart disease risk (*6*). Furthermore, there is now growing evidence that carotenoids may possess inhibitory effects against cancer, although the human epidemiology remains inconclusive (*7*−*11*). An inverse relationship between β-carotene ingestion and the incidence of certain types of cancer, such as lung and intestinal tract cancer, has been reported (*12*). Dietary consumption of the carotenoid lycopene (mostly from tomato products) has been associated with a lower risk of prostate cancer in men (*13*−*15*). The cancer preventive effects often have been attributed to antioxidant actions (*16*). Carotenoids have been recognized to be efficient singlet oxygen quenchers (*17*−*19*).

Measurement of the quenching rates k_Q (= k_q + k_r, physical quenching + chemical reaction) of singlet oxygen with several carotenoids in solution has been performed by Di Mascio et al. (*18*, *19*), using a near-IR $^1O_2$ luminescence method (reaction 1)

$$^1O_2 + \text{Carotenoid} \xrightarrow{k_Q} \text{physical quenching } (k_q) + \text{chemical reaction } (k_r) \quad (1)$$

where the former results in energy transfer and de-excitation of the singlet state but no chemical change in the energy acceptor. The latter results in oxidation of the target. Di Mascio et al. reported that carotenoids generally show very high activity for the quenching of singlet oxygen. For instance, the second-order rate constants (k_Q) (~10^{10} M^{-1} s^{-1}) for carotenoids (*18*, *19*) are about 2−3 orders of magnitude larger than those for α-tocopherol (*20*, *21*), ubiquinol-10 (*22*), catechins (*23*), and flavone derivatives (*24*). Measurement of the quenching rate of singlet oxygen by carotenoids in liposome has also been performed, indicating that carotenoids inhibit $^1O_2$-dependent lipid peroxidation in liposome (*25*). On the other hand, the activities of carotenoids to trap chain-carrying peroxyl radicals were much less than that of α-tocopherol (*26*).

In recent years, the method to assess the total oxygen radical absorpton capacity (ORAC) of foods and plants has been established (*27*−*30*). On the other hand, a singlet oxygen absorption capacity (SOAC) assay method to assess the total quenching activity of singlet oxygen by carotenoids and phenolic antioxidants included in foods and plants has not been established. Lipid peroxyl radical (LOO•) and singlet oxygen ($^1O_2$) are well-known as two representative reactive oxygen species (ROS) generated in biological systems. $^1O_2$ reacts with many kinds of biological targets including lipids, sterols, proteins, DNA, and RNA (*31*, *32*), as well as peroxyl radical. Reactions with $^1O_2$ occur mainly by chemical reaction, inducing the degradation of biological systems. Carotenoids are widely present in vegetables and

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Published on Web 08/20/2010

Table 1. UV−Vis Absorption Maxima ($\lambda_{max}{}^{i}$ (i = 1−5)) and Molar Extinction Coefficients (ε_i (i = 1−5)) of the Carotenoids **1**−**8** and Used Compounds in Ethanol/Chloroform/D_2O (50:50:1, v/v/v) Solution

molecule	$\lambda_{max}{}^{1}$/nm (ε_1/M^{-1} cm^{-1})	$\lambda_{max}{}^{2}$/nm (ε_2/M^{-1} cm^{-1})	$\lambda_{max}{}^{3}$ and $\lambda_{max}{}^{4}$/nm (ε_3 and ε_4/M^{-1} cm^{-1})	λ^{5}/nm (ε_5/M^{-1} cm^{-1})
lycopene (Lyc, **1**)	479 (160000)[a]	511 (140000)	452 (110000) 298 (40500)	413 (40900)[a]
astaxanthin (Ast, **2**)	486 (124000)[a]			413 (35600)[a]
β-carotene (β-Car, **3**)	459 (133000)[a]	485 (117000)	277 (21500)	413 (56800)[a]
capsanthin (Cap, **4**)	481 (106000)[a]		292 (18700)	413 (32900)[a]
zeaxanthin (Zea, **5**)	459 (129000)[a]	485 (114000)	278 (21900)	413 (55200)[a]
α-carotene (α-Car, **6**)	453 (138000)[a]	481 (123000)	270 (24000)	413 (64700)[a]
lutein (Lut, **7**)	452 (126000)[a]	480 (113000)	269 (26000)	413 (61100)[a]
β-cryptoxanthin (Crp, **8**)	459 (95100)[a]	485 (82400)	262 (22700)	413 (43500)[a]
α-tocopherol (α-Toc)	237 (1930)	293 (2980)[a]		
endoperoxide (EP)	236 (1630)			
EP-precursor	290 (7670)	281 (6350)	239 (9130)	
DPBF	413 (20900)[b]	315 (7950)	264 (25600)	413 (20900)[b]

[a] Average of values obtained by measurements repeated three to five times. The values of ε were not varied by the dilution of the solution, indicating that the carotenoids **1**−**8** are completely dissolved in the solution. Experimental errors in the values of ε were estimated to be 5%. [b] Average of values obtained by measurements repeated five times.

fruits in high concentrations (*3*−*5*) and may function as quenchers of $^1O_2$ in biological systems (*16*, *18*, *19*, *26*). Consequently, the development of a SOAC method is very important.

In previous works, to clarify the structure−activity relationship in the quenching reaction of $^1O_2$ by natural phenolic antioxidants, we measured the second-order rate constant (k_Q) for the reaction of $^1O_2$ with many phenolic antioxidants such as vitamin E homologues (*21*), biological hydroquinones (*22*), and polyphenols (*23*, *24*) in ethanol solution (reaction 1), using the competition reaction method, where endoperoxide (EP) was used as a generator of singlet oxygen and 2,5-diphenyl-3,4-benzofuran (DPBF) was used as an indicator of the singlet oxygen quenching capacity.

In the present work, first, the measurements of UV−vis absorption spectra were performed for eight kinds of carotenoids **1**−**8** (lycopene (Lyc, **1**), astaxanthin (Ast, **2**), β-carotene (β-Car, **3**), capsanthin (Cap, **4**), zeaxanthin (Zea, **5**), α-carotene (α-Car, **6**), lutein (Lut, **7**), β-cryptoxanthin (β-Cry, **8**)), to determine the values of wavelengths of absorption maxima (λ_{max}) and correct molar extinction coefficients (ε) in ethanol/chloroform/D_2O (50:50:1, v/v/v) solution. Second, the stability of carotenoids **1**−**8** in ethanol/chloroform/D_2O solution was ascertained by the measurement of UV−vis absorption spectra at 35 °C. Third, the quenching rates (k_Q) of $^1O_2$ by carotenoids **1**−**8**, α-tocopherol, and two kinds of food extracts (from tomato and carrot) were measured in ethanol/chloroform/D_2O at 35 °C, by using the above competition reaction method (*21*−*24*). α-Tocopherol was used as a standard compound. Furthermore, the chemical reaction of carotenoids **1**−**8** with $^1O_2$ in solution was studied spectrophotometrically, by reacting the carotenoids with $^1O_2$ at 35 °C. From the results, the method to assess the total SOAC of the antioxidants included in foods and plants has been proposed.

MATERIALS AND METHODS

Materials. Lutein, β-cryptoxanthin, zeaxanthin, and capsanthin were obtained from Extrasynthese (Genay, France). α- and β-carotene and lycopene were obtained from Wako Chemicals, Japan. Astaxanthin was obtained from Funakoshi Co. Ltd., Japan. D-α-Tocopherol (Eisai Food Chemicals Co. Ltd., Japan) and DPBF (Tokyo Kasei Chemicals, Japan) are commercially available. $Na_2MoO_4 \cdot 2H_2O$ (KANTO Chemical Co., Inc., Japan) is also commercially available. Sea sand was obtained from Wako Chemicals, Japan.

3-(4-Methyl-1-naphthyl)propionic acid (EP-precursor) was prepared according to a published procedure (*33*−*35*). 3-(1,4-Epidioxy-4-methyl-1,4-dihydro-1-naphthyl)propionic acid (endoperoxide, EP) was prepared through the reaction of EP-precursor with $^1O_2$ produced in H_2O_2 solution including Na_2MoO_4 (see reaction 2) (*36*).

$$\text{EP-Precursor} + {}^1O_2(Na_2MoO_4, H_2O_2) \rightarrow \text{EP} \xrightarrow{35°C} \text{EP-Precursor} + {}^1O_2 \quad (2)$$

Tomato and carrot extracts were prepared according to a procedure similar to that used by Wu et al. (*37*). The method is as follows: 1.00 g of freeze-dried powder sample from tomato (or carrot) was mixed with 5 g of sea sand. Sample and sand were transferred to an 11 mL extraction cell and were extracted with ethanol/chloroform/D_2O (50:50:1, v/v/v) three times, using an ASE-200 accelerated solvent extractor (Dionex Corp., Sunnyvale, CA). The extracts were combined, and the volume was adjusted to 25.0 mL with the same solvent in a volumetric flask. This solution was used to measure the SOAC value.

Measurements of UV−Vis Absorption Spectra and Molar Extinction Coefficients of Carotenoids 1−8 and DPBF. Carotenoids (3−4 mg) were dissolved into 200 mL of ethanol/chloroform/D_2O solution. Then, each of 1.00, 2.00, or 3.00 mL of the above solution was diluted to 10.0 mL, and the measurement of UV−vis spectrum was performed for each solution. The values of molar extinction coefficients (ε^1) at $\lambda_{max}{}^{1}$ for the three solutions showed good accordance with each other. The experimental errors in ε^1 values were $< 5\%$, indicating that carotenoids were completely dissolved in the solution. Similar measurements were repeated three times for each carotenoid, and the value of ε^1 was determined. Measurement of UV−vis absorption spectrum of DPBF was also performed five times in ethanol/chloroform/D_2O solution. The values of $\lambda_{max}{}^{i}$ and the average values of ε_i ($\varepsilon_{Av}{}^{i}$) obtained for carotenoids **1**−**8** and DPBF are summarized in **Table 1**.

Measurements of Rate Constants (k_Q). Measurements of rate constants (k_Q) were performed in ethanol/chloroform/D_2O (50:50:1, v/v/v) solution, by using a Shimadzu UV−vis spectrophotometer (UV-1800), equipped with a six-channel cell positioner and an electron-temperature control unit (CPS-240A). The rate constants, k_Q (S) and k_Q ($t_{1/2}$), were determined by analyzing the first-order rate constant (S) and the half-life ($t_{1/2}$) of the decay curve of DPBF with carotenoids, respectively, as described under Results and Discussion. All measurements were performed at 35.0 ± 0.5 °C.

Measurements of UV−vis absorption spectra were performed under nitrogen atmosphere, to avoid the degradation of carotenoids **1**−**8**, vegetable extracts, α-tocopherol, and DPBF. A closed system (that is, a cuvette with a sealing cap) was used to avoid loss of solvent, because the solvents show high vapor pressures at 35 °C.

The production of $^1O_2$ due to the thermal decomposition of EP occurs at 25 °C. Consequently, sample preparation was performed by adding 1.00 mL of EP solution to 2.00 mL of solution including DPBF and an antioxidant in a quartz cuvette at ∼20 °C to avoid the decomposition of EP, and measurements of the UV−vis absorption spectra were then started at 35 °C. We took about 5 min to prepare solutions of six cuvettes.

About 3 min was necessary before the solution temperature in the cuvette rose from ~20 to 35 °C.

RESULTS AND DISCUSSION

UV−Vis Absorption Spectra and Stability of Carotenoids 1−8 in Solution. The wavelengths of absorption maxima (λ_{max}) of UV−vis absorption spectra were reported for many carotenoids (*38*, *39*). However, carotenoids for which molar extinction coefficients (ε) have been reported are very limited (*38*). One reason for this is that carotenoids, except for β-carotene, are costly, and at least several milligrams of carotenoids is necessary to determine the ε value. Measurements of the quenching rate (k_Q) for phenolic antioxidants (such as vitamin E analogues, polyphenols, and biological hydroquinones) were performed in ethanol (*21*−*24*). However, the solubility of carotenoids is low in organic solvents, including ethanol, and depends on the molecular structure of carotenoids. Carotenoids are soluble in chloroform. However, the degradation of DPBF occurs in chloroform. Therefore, measurements of the k_Q values were performed in ethanol/chloroform/D_2O (50:50:1, v/v/v) solution. This solution was used by Di Mascio et al. (*18*, *19*) and Beutner et al. (*39*) to measure the k_Q values of carotenoids.

The measurements of UV−vis absorption spectra were carefully performed for carotenoids **1**−**8** in ethanol/chloroform/D_2O, and the values of λ_{max} and ε obtained are summarized in **Table 1**. The values of ε are important to determine the concentrations of carotenoids used for the reaction and to obtain the correct rate constant (k_Q). As listed in **Table 1**, absorption spectra of carotenoids **1**−**8** overlap with that of DPBF at λ_{max} = 413 nm. Therefore, the correction of baseline due to the absorption of carotenoids **1**−**8** at 413 nm is necessary (see **Figure 1**). The ε_{Av} values of carotenoids **1** −**8** at 413 nm are also listed in **Table 1**.

It is important to investigate the stability of carotenoids **1**−**8** at 35 °C in ethanol/chloroform/D_2O solution because the corrections of the baseline due to the absorption of carotenoids at 413 nm are necessary for the analysis of the rate constant (k_Q), as described below. Therefore, the measurements of the time dependence of the absorbance of carotenoids at 413 nm were performed at 35 °C in solution under nitrogen atmosphere. As shown in **Figure 1A**, line e, the change of the absorbance of β-carotene (1.41×10^{-5} M) was negligible at t = 0−260 min. Similar results were obtained for the other carotenoids, indicating that these carotenoids are stable in ethanol/chloroform/D_2O at 35 °C (data are not shown).

Overall Rate Constants (k_Q) for the Reaction of $^1O_2$ with α-Tocopherol and Carotenoids 1−8. Singlet oxygen was generated by the thermal decomposition of the endoperoxide (EP) (see eq 2) (*21*−*24*). The UV absorption spectra of EP-precursor and EP show absorption maxima at λ_{max} (ε) = 290 (7670), 281 (6350), and 239 nm (9130 M^{-1} cm^{-1}) and λ_{max} (ε) = 236 nm (1630 M^{-1} cm^{-1}), respectively, in ethanol/chloroform/D_2O (50:50:1, v/v/v) solution (see **Table 1**). The result of the measurement of the UV spectrum of EP indicates that the powder sample of EP includes 95.6% EP and 4.4% EP-precursor unreacted.

DPBF was used as the UV−vis absorption probe. **Figure 2A** shows an example of the reaction between DPBF (7.95×10^{-5} M) and EP (4.68×10^{-4} M) in the absence or presence of α-tocopherol ((0−9.46) $\times 10^{-4}$ M) in ethanol/chloroform/D_2O solution at 35 °C. By the reaction, the disappearance of DPBF at λ_{max} = 413 nm due to the chemical reaction between DPBF and $^1O_2$ produced was observed. The overall rate constant k_Q ($= k_q + k_r$) for the reaction of $^1O_2$ with α-tocopherol was determined by eq 3 (Stern−Volmer plot), as reported in previous works (*40*, *41*).

$$S_{Blank}/S_{\alpha\text{-Toc}} = 1 + (k_Q{}^{\alpha\text{-Toc}}/k_d)[\alpha\text{-Toc}] \quad (3)$$

In eq 3, S_{Blank} and $S_{\alpha\text{-Toc}}$ are slopes of the first-order plots (that is, ln(absorbance) versus t plots) of disappearance of DPBF in the absence or presence of α-tocopherol, respectively (see **Figure 2B**). k_d is the rate of natural deactivation of $^1O_2$ in the solvent.

As described above, eq 3 was derived from the steady-state approximation to $^1O_2$ (*40*, *41*), that is, by assuming that

$$-d[^1O_2]/dt = -k_f[EP] + k_{DPBF}[DPBF][^1O_2] + k_Q[\alpha\text{-Toc}][^1O_2] + k_d[^1O_2] = 0 \quad (4)$$

where k_f and k_{DPBF} are the formation rate constant of $^1O_2$ from EP at 35 °C and the second-order rate constant for the chemical reaction of DPBF with $^1O_2$, respectively. As shown in **Figure 2B**, the first-order plots indicate straight lines at ~5 < t < ~60 min. At t < ~5 min, the plots deviate from straight lines, because it takes about 5 min for the solution temperature in quartz cuvette to increase from 20 to 35 °C and for the steady-state approximation (eq 4) to be fulfilled. This approximation will be broken at t > ~60 min, because the formation rate of $^1O_2$ (k_f[EP]) in eq 4 decreases gradually at t > ~60 min due to the consumption of EP. Therefore, the analysis of the decay curve was performed at ~5 < t < ~60 min. This is an important condition to obtain a correct rate constant (k_Q) for antioxidants.

A plot of $S_{Blank}/S_{\alpha\text{-Toc}}$ versus the concentration of α-tocopherol ([α-Toc]) is shown in **Figure 2C**. The rate constant (k_Q) was calculated by using the value of k_d in ethanol/chloroform/D_2O (50:50:1, v/v/v) ($k_d = 3.03 \times 10^5$ s^{-1}) (*18*, *19*, *39*). The k_Q value obtained is 1.24×10^8 M^{-1} s^{-1}. Similar measurements were repeated three times, and the individual and average k_Q values are listed in **Table 2**.

Similarly, solutions containing EP (4.56×10^{-4} M), DPBF (5.65×10^{-5} M), and various amounts of β-carotene (0−1.41×10^{-5} M) in ethanol/chloroform/D_2O were reacted at 35 °C (see **Figure 1A**). The disappearance of DPBF was measured at 413 nm. However, as the absorption of β-carotene overlaps with that of DPBF at 413 nm (see **Figure 1A**), the correction of the baseline is necessary for each decay curve. As shown in **Figure 1A**, the measurement of the baseline was performed by observing the time dependence of the absorption of β-carotene (1.41×10^{-5} M) at 413 nm (see line e in **Figure 1A**), where 1.41×10^{-5} M is the highest concentration of β-carotene used for the measurement. The correction of the baseline for each decay curve of DPBF was performed by taking the absorbance of this baseline into account and using Lambert−Beer's equation (absorbance = ε[β-Car]). The decay curves corrected are shown in **Figure 1B**. The ln(absorbance) versus t plot is shown in **Figure 1C**, indicating that the first-order plots are straight lines at ~5 < t < ~60 min, as observed for α-tocopherol. A plot of $S_{Blank}/S_{\beta\text{-Car}}$ versus [β-Car] is shown in **Figure 1D**. The $k_Q{}^{\beta\text{-Car}}$ value obtained is 1.10×10^{10} M^{-1} s^{-1}. Similar measurements were performed for β-carotene three times. As listed in **Table 2**, the $k_Q{}^{\beta\text{-Car}}$ values obtained are nearly equal to each other.

Similar measurements were performed for carotenoids **1**, **2**, and **4**−**8** in ethanol/chloroform/D_2O solution. $S_{Blank}/S_{Carotenoid}$ versus [Carotenoid] plots are shown in **Figure 3A**. Measurements were repeated three times for each carotenoid, to obtain the correct k_Q values. The individual k_Q values obtained and the average values of k_Q (k_Q (av)) for carotenoids **1**−**8** are summarized in **Table 2**, together with those obtained for α-tocopherol. The experimental error in k_Q value for each carotenoid was ±5% at maximum.

The k_Q values of carotenoids **1**−**8** decrease in the order lycopene > astaxanthin > β-carotene ~ capsanthin ~ zeaxanthin > α-carotene > lutein > β-cryptoxanthin in ethanol/chloroform/D_2O.

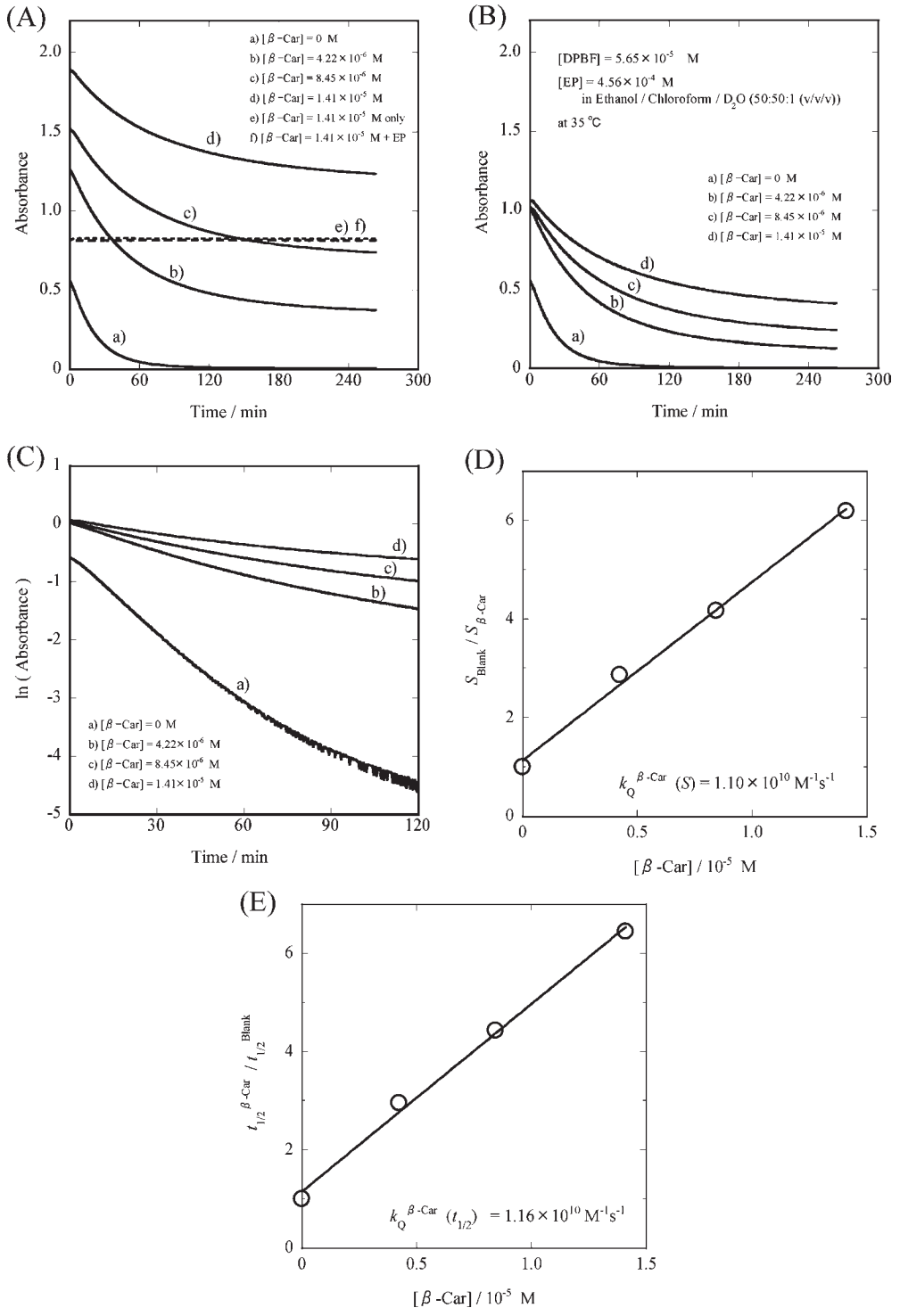

Figure 1. (**A**) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1O_2$ in the absence (line a) and presence (lines b−d) of β-carotene in ethanol/chloroform/D_2O at 35 °C. $[DPBF]_{t=0} = 5.65 \times 10^{-5}$ M and $[EP]_{t=0} = 4.56 \times 10^{-4}$ M. The values of $[\beta\text{-Car}]_{t=0}$ are shown in **A**. Lines e and f indicate the time dependence of absorbance of β-carotene (1.41×10^{-5} M) at 413 nm in the absence and presence of EP, respectively, where DPBF is not included. (**B**) Change in absorbance of DPBF, where the correction of baseline due to β-carotene was performed (see text). (**C**) Plot of ln(absorbance) versus *t*. (**D**) Plot of $S_{Blank}/S_{\beta\text{-Car}}$ versus [β-Car]. (**E**) Plot of $t_{1/2}{}^{\beta\text{-Car}}/t_{1/2}{}^{Blank}$ versus [β-Car].

However, the difference among the k_Q values is not remarkable. The value of lycopene is only 1.89 times larger than that of β-cryptoxanthin, although the value of lycopene is 105 times larger than that of α-tocopherol.

Measurements of the k_Q values have been reported for many carotenoids. However, for instance, the k_Q values reported for β-carotene in solutions vary from 5×10^9 to 30×10^9 M^{-1} s^{-1} (*18*). The variety may be due to the difference in the experimental methods used, solvent, temperature, etc. Di Mascio et al. (*18*, *19*) and Beutner et al. (*39*) reported the k_Q values of carotenoids **1**−**3** and **5**−**8** obtained by measuring the photoemission of $^1O_2$ at 1270 nm in ethanol/chloroform/D_2O (50:50:1, v/v/v). The values are listed in **Table 2**. The order of the k_Q values for carotenoids **1**−**3** and **5**−**8** is the same as that obtained in the present work. However, the value of lycopene is 5.2 times as large as that of β-cryptoxanthin, and the k_Q values of carotenoids **1**−**3** and **5**−**8** obtained in the

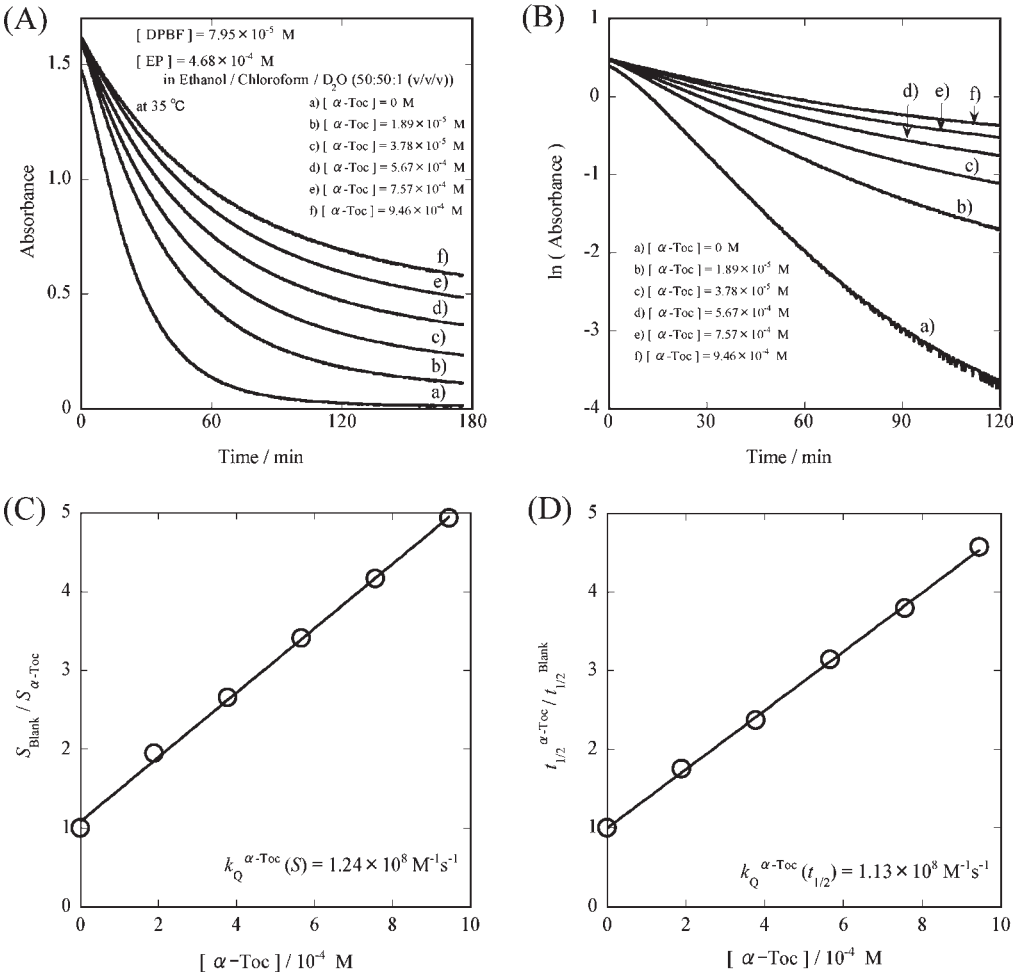

Figure 2. (**A**) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1O_2$ in the absence and presence of α-tocopherol in ethanol/chloroform/D_2O at 35 °C. $[DPBF]_{t=0} = 7.95 \times 10^{-5}$ M and $[EP]_{t=0} = 4.68 \times 10^{-4}$ M. The values of $[\alpha\text{-Toc}]_{t=0}$ are shown in **A**. (**B**) Plot of ln(absorbance) versus *t*. (**C**) Plot of $S_{Blank}/S_{\alpha\text{-Toc}}$ versus [α-Toc]. (**D**) Plot of $t_{1/2}^{\alpha\text{-Toc}}/t_{1/2}^{Blank}$ versus [α-Toc].

persent work are 1.5−4.1 times larger than those of the corresponding carotenoids reported by Di Mascio et al. The reason for such a difference is not clear at present.

Chemical Reaction of $^1O_2$ with Carotenoids 1−8. The measurement of the rate constant (k_r) for the chemical reaction of β-carotene with $^1O_2$ was performed by a few investigators (*42*−*44*), showing that the k_r values (3.66×10^7, 3.8×10^6, and 2.0×10^6 M^{-1} s^{-1}) are much smaller than the k_q values (2.99×10^{10}, 7.00×10^9, and 1.2×10^{10} M^{-1} s^{-1}) in *n*-hexane, CCl_4, and acetonitrile/benzene (4:1, v/v) solution, respectively. β-Carotene quenches $^1O_2$ through a very efficient physical reaction. On the other hand, cyclic endoperoxides of β-carotene were obtained as products of chemical quenching of $^1O_2$, if the generation of $^1O_2$ was performed by using a photosensitizer (*45*, *46*). However, measurements of the k_r values and the $^1O_2$-quenching products for the other carotenoids have not been performed, as far as we know.

In the present work, the chemical reaction of carotenoids **1**−**8** with $^1O_2$ in ethanol/chloroform/D_2O solution was studied spectrophotometrically, by reacting the carotenoids with $^1O_2$ generated by the thermal decomposition of the EP at 35 °C. As shown in **Figure 1A**, line f, the chemical reaction between $^1O_2$ and β-carotene is very slow, and a measurable change in the absorbance of β-carotene was not observed at 413 nm. Similarly, by reacting carotenoids **1**−**8** with $^1O_2$ for 2−4 h at 35 °C, no changes of UV−vis spectra of carotenoids were observed (data are not shown). Consequently, the k_Q values obtained for carotenoids are thought to be due to physical quenching (k_q), that is, $k_Q \approx k_q$.

Measurement of the Rate Constant (k_Q) Based on the Half-Life ($t_{1/2}$) of DPBF. It will be better to use $t_{1/2}^{\alpha\text{-Toc}}$ instead of $S_{\alpha\text{-Toc}}$ to assay the k_Q value (that is, the SOAC value) of antioxidants and vegetable extracts in solution, because the analysis of $t_{1/2}^{\alpha\text{-Toc}}$ is more direct and easier than that of $S_{\alpha\text{-Toc}}$. Furthermore, the use of the half-life ($t_{1/2}^{\alpha\text{-Toc}}$) is better than that of the first-order rate constant ($S_{\alpha\text{-Toc}}$) to define the SOAC value for vegetable extracts.

The decay rate ($S_{\alpha\text{-Toc}}$) of DPBF due to the chemical reaction with $^1O_2$ decreases with increasing the concentration of α-tocopherol, as shown in **Figure 2A**. The k_Q values were determined by using eq 3. As the decay of DPBF is a first-order reaction, there exists the following relationship between $S_{\alpha\text{-Toc}}$ and half-life ($t_{1/2}^{\alpha\text{-Toc}}$) at $\sim 5 < t < \sim 60$ min:

$$A_{1/2} = A_0 \exp(-S_{\alpha\text{-Toc}} \times t_{1/2}^{\alpha\text{-Toc}}) \quad (5)$$

A_0 is the absorbance of DPBF at $t_0 = 5$ min, and ($t_{1/2}^{\alpha\text{-Toc}} + t_0$) is the time at which $A_{1/2} = A_0/2$ (see **Figure 2A**). We can easily obtain the following relationship from eq 5:

$$t_{1/2}^{\alpha\text{-Toc}} = \ln 2/S_{\alpha\text{-Toc}} \quad (6)$$

By substituting eq 6 into eq 3, we can obtain eq 7a

$$t_{1/2}^{\alpha\text{-Toc}}/t_{1/2}^{Blank} = 1 + (k_Q^{\alpha\text{-Toc}}/k_d)[\alpha\text{-Toc}] \quad (7a)$$

where $t_{1/2}^{Blank}$ and $t_{1/2}^{\alpha\text{-Toc}}$ are the half-lives in the absence and presence of α-tocopherol, respectively. Equation 7a indicates that

Table 2. Second-Order Rate Constants (k_Q^{Sample} and k_Q^{Sample} (av)) and Relative Rate Constants (k_Q^{Sample} (Av) → k_Q^{Sample} (av)) for the Reaction of $^1O_2$ with Carotenoids **1**−**8** and α-Tocopherol in Ethanol/Chloroform/D_2O (50:50:1, v/v/v)

sample	k_Q^{Sample}/ M^{-1} s^{-1}	k_Q^{Sample} (av) [a]/ M^{-1} s^{-1}	k_Q^{Sample}/M^{-1} s^{-1} Di Mascio et al. [b] (Beutner et al.) [c]	k_Q^{Sample} (av)/ $k_Q^{α\text{-Toc}}$ (av)
α-tocopherol (α-Toc)	1.24×10^8 1.33×10^8 1.36×10^8	1.31×10^8	8.5×10^7	1.00
lycopene (Lyc, **1**)	1.43×10^{10} 1.31×10^{10} 1.41×10^{10}	1.38×10^{10}	9.4×10^9 (8.8×10^9)	105
astaxanthin (Ast, **2**)	1.16×10^{10} 1.23×10^{10} 1.14×10^{10}	1.18×10^{10}	7.3×10^9 (9.0×10^9)	90.1
β-carotene (β-Car, **3**)	1.10×10^{10} 1.04×10^{10} 1.11×10^{10}	1.08×10^{10}	4.2×10^9 (8.4×10^9)	82.4
capsanthin (Cap, **4**)	9.36×10^{10} 1.16×10^{10} 1.07×10^{10}	1.06×10^{10}		80.9
zeaxanthin (Zea, **5**)	1.04×10^{10} 1.02×10^9 1.11×10^{10}	1.05×10^{10}	3.0×10^9	80.2
α-carotene (α-Car, **6**)	1.08×10^{10} 8.82×10^9 9.66×10^9	9.76×10^9	5.7×10^9	74.5
lutein (Lut, **7**)	9.16×10^9 9.14×10^9 9.43×10^9	9.24×10^9	2.4×10^9	70.5
β-cryptoxanthin (Crp, **8**)	7.04×10^9 7.25×10^9 7.63×10^9	7.31×10^9	1.8×10^9	55.8

[a] Experimental errors in the rate constants (k_Q^{Sample} (av)) were estimated to be <5%. [b] Values reported by Di Mascio et al. (*18*, *19*). [c] Values reported by Beutner et al. (*39*).

the $k_Q^{α\text{-Toc}}$ value can be obtained from $t_{1/2}^{α\text{-Toc}}/t_{1/2}^{Blank}$ versus [α-Toc] plot. In fact, $t_{1/2}^{α\text{-Toc}}$ increases linearly with increasing concentration of α-tocopherol, as shown in **Figure 2D**.

If the concentration of α-tocopherol is low ([α-Toc] = 0, 1.89×10^{-5}, 3.78×10^{-5}, 5.67×10^{-4} M) and the value of $t_{1/2}^{α\text{-Toc}}$ is < 60 min (see **Figure 2A**), we can directly determine the half-life from the decay curve of DPBF. However, if the concentration of α-tocopherol is large (7.57×10^{-4} or 9.46×10^{-4} M), the time (t) at which $A_{1/2} = A_0/2$ (that is, the seeming half-life) is > 60 min. In such a case, we cannot directly determine the $t_{1/2}^{α\text{-Toc}}$ value from the decay curve of DPBF, because the decay does not follow the first-order kinetics, as described above. The half-life must be calculated in the following way.

There exists the following relationship between $A_{m/n}$ and $t_{m/n}^{α\text{-Toc}}$.

$$A_{m/n} = A_0 \exp(-S_{α\text{-Toc}} \times t_{m/n}^{α\text{-Toc}}) \quad (8)$$

where $A_{m/n} = (m/n)A_0$ and ($t_{m/n}^{α\text{-Toc}} + t_0$ ($t_0 = 5$ min)) is the time at which $A_{m/n} = (m/n)A_0$. By substituting eq 6 into eq 8, we can obtain eq 9, that is, we can estimate $t_{1/2}^{α\text{-Toc}}$ from $t_{m/n}^{α\text{-Toc}}$.

$$t_{1/2}^{α\text{-Toc}} = t_{m/n}^{α\text{-Toc}}(\ln(1/2)/\ln(m/n)) \quad (9)$$

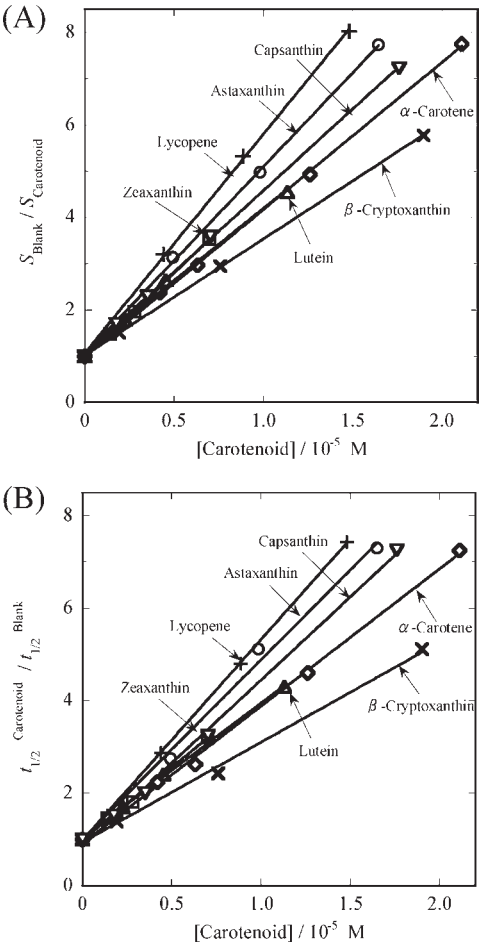

Figure 3. (**A**) Plot of $S_{Blank}/S_{Carotenoid}$ versus concentrations of carotenoids (lycopene, astaxanthin, capsanthin, zeaxanthin, α-carotene, lutein, and β-cryptoxanthin). (**B**) Plot of $t_{1/2}^{Carotenoid}/t_{1/2}^{Blank}$ versus concentrations of carotenoids.

For instance, the value of $t_{1/2}^{α\text{-Toc}}$ for [α-Toc] = 9.46×10^{-4} M is 81.4 min, when the correction is performed by using eq 9. On the other hand, if the correction is not performed, the value of $t_{1/2}^{α\text{-Toc}}$ obtained is 97.6 min, showing a larger value. The correction is very important to obtain a correct $t_{1/2}^{α\text{-Toc}}$ value. In conclusion, the k_Q value can be calculated from $t_{1/2}^{α\text{-Toc}}/t_{1/2}^{Blank}$ versus [α-Toc] plot, as shown in **Figure 2D**. The $k_Q^{α\text{-Toc}}$ ($t_{1/2}$) value (1.13×10^8 M^{-1} s^{-1}) obtained by using eq 7a is nearly equal to the $k_Q^{α\text{-Toc}}$ (S) value (1.24×10^8 M^{-1} s^{-1}) obtained by using eq 3 (see **Table 3**).

A similar analysis of the half-life ($t_{1/2}^{β\text{-Car}}$) was performed for β-carotene (see **Figure 1B**). The $k_Q^{β\text{-Car}}$ ($t_{1/2}$) value (1.16×10^{10} M^{-1} s^{-1}) obtained is nearly equal to the $k_Q^{β\text{-Car}}$ (S) value (1.10×10^{10} M^{-1} s^{-1}) (see **Figure 1** and **Table 3**).

Similarly, $t_{1/2}^{Carotenoid}$ and $t_{1/2}^{Blank}$ values were calculated for the carotenoids **1**, **2**, **4**−**8**. The $t_{1/2}^{Carotenoid}/t_{1/2}^{Blank}$ versus [Carotenoid] plots are shown in **Figure 3B**, indicating the linear correlation between $t_{1/2}^{Carotenoid}/t_{1/2}^{Blank}$ and [Carotenoid]. As summarized in **Table 3**, the k_Q^{Car} ($t_{1/2}$) values obtained are close to the corresponding k_Q^{Car} (S) values obtained by the analyses of S_{Car} values. The result indicates that the analysis based on half-life is reliable. The values of the ratio (k_Q^{Car} ($t_{1/2}$)/k_Q^{Car} (S)) are listed in the last column of **Table 3**.

Development of Singlet Oxygen Absorption Capacity (SOAC) Assay Method. There are the following relationships (eqs 10

Table 3. Second-Order Rate Constants (k_Q^{Sample} (S) and k_Q^{Sample} ($t_{1/2}$)) Obtained from S_{Blank}/S_{Sample} versus [Sample] and $t_{1/2}^{Sample}/t_{1/2}^{Blank}$ versus [Sample] Plots, Respectively, and the Ratios of the Rate Constants (k_Q^{Sample} ($t_{1/2}$)/k_Q^{Sample} (S))

sample	k_Q^{Sample} (S)/M^{-1} s^{-1} (S_{Blank}/S_{Sample} plot)	k_Q^{Sample} ($t_{1/2}$)/M^{-1} s^{-1}[a] ($t_{1/2}^{Sample}/t_{1/2}^{Blank}$ plot)	k_Q^{Sample} ($t_{1/2}$)/k_Q^{Sample} (S)
α-tocopherol	1.24×10^8	1.13×10^8	0.911
	1.33×10^8	1.22×10^8	0.917
	1.36×10^8	1.52×10^8	1.07
	av: 1.31×10^8	av: 1.29×10^8	av: 0.984
lycopene	1.43×10^{10}	1.32×10^{10}	0.923
	1.31×10^{10}	1.16×10^{10}	0.885
	1.41×10^{10}	1.29×10^{10}	0.914
	av: 1.38×10^{10}	av: 1.26×10^{10}	av: 0.913
astaxanthin	1.16×10^{10}	1.09×10^{10}	0.940
	1.23×10^{10}	1.18×10^{10}	0.959
	1.14×10^{10}	1.03×10^{10}	0.904
	av: 1.18×10^{10}	av: 1.10×10^{10}	av: 0.932
β-carotene	1.10×10^{10}	1.16×10^{10}	1.00
	1.04×10^{10}	1.01×10^{10}	0.971
	1.11×10^{10}	9.96×10^9	0.892
	av: 1.08×10^{10}	av: 1.06×10^{10}	av: 0.976
capsanthin	9.36×10^9	1.04×10^{10}	1.11
	1.16×10^{10}	1.16×10^{10}	1.00
	1.07×10^{10}	1.09×10^{10}	1.02
	av: 1.06×10^{10}	av: 1.07×10^{10}	av: 1.01
zeaxanthin	1.04×10^{10}	1.06×10^{10}	1.03
	1.02×10^{10}	1.00×10^{10}	0.985
	1.11×10^{10}	9.56×10^9	0.861
	av: 1.05×10^{10}	av: 1.01×10^{10}	av: 0.961
α-carotene	1.08×10^{10}	9.85×10^9	0.912
	8.82×10^9	8.03×10^9	0.910
	9.66×10^9	9.01×10^9	0.933
	av: 9.76×10^9	av: 8.96×10^9	av: 0.918
lutein	9.16×10^9	9.30×10^9	1.01
	9.14×10^9	9.01×10^9	0.985
	9.43×10^9	8.83×10^9	0.936
	av: 9.24×10^9	av: 9.05×10^9	av: 0.979
β-cryptoxanthin	7.04×10^9	7.55×10^9	1.07
	7.25×10^9	7.01×10^9	0.967
	7.63×10^9	6.27×10^9	0.822
	av: 7.31×10^9	av: 6.94×10^9	av: 0.949

[a] Experimental errors in the rate constants (k_Q^{Sample} ($t_{1/2}$) (av)) were estimated to be <10%.

and 7b) between the half-lives ($t_{1/2}^{Sample}$ and $t_{1/2}^{\alpha\text{-Toc}}$) and the concentrations of carotenoid sample ([Sample]) and α-tocopherol ([α-Toc]), respectively.

$$t_{1/2}^{Sample}/t_{1/2}^{Blank} = 1 + (k_Q^{Sample}/k_d)[\text{Sample}] \quad (10)$$

$$t_{1/2}^{\alpha\text{-Toc}}/t_{1/2}^{Blank} = 1 + (k_Q^{\alpha\text{-Toc}}/k_d)[\alpha\text{-Toc}] \quad (7b)$$

By eliminating k_d from eqs 7b and 10, we can obtain the following relationship:

$$(t_{1/2}^{Sample} - t_{1/2}^{Blank})/(t_{1/2}^{\alpha\text{-Toc}} - t_{1/2}^{Blank}) = k_Q^{Sample}[\text{Sample}]/k_Q^{\alpha\text{-Toc}}[\alpha\text{-Toc}] \quad (11)$$

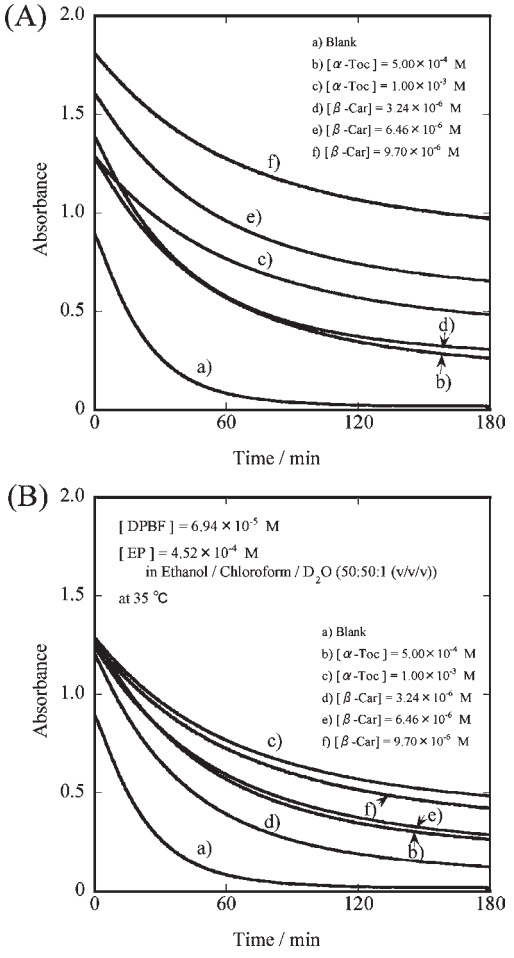

Figure 4. (**A**) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1O_2$ in the absence or presence of sample (α-tocopherol or β-carotene) in ethanol/chloroform/D_2O at 35 °C. $[DPBF]_{t=0} = 6.94 \times 10^{-5}$ M and $[EP]_{t=0} = 4.52 \times 10^{-4}$ M. The values of $[\alpha\text{-Toc}]_{t=0}$ and $[\beta\text{-Car}]_{t=0}$ are shown in Figure **A**. (**B**) Change in absorbance of DPBF, where the correction of baseline due to β-carotene was performed (see text).

Equation 11 indicates that the half-life ($t_{1/2}^{Sample}$) of the sample increases linearly with increasing concentration of the sample and depends on the product of k_Q^{Sample} and [Sample], if the concentration of α-tocopherol is constant. From eq 11, we can derive eq 12 and define the relative SOAC value as follows:

$$\text{relative SOAC value (given in molar units (M = mol/L))} = \{(t_{1/2}^{Sample} - t_{1/2}^{Blank})/(t_{1/2}^{\alpha\text{-Toc}} - t_{1/2}^{Blank})\} \times [\alpha\text{-Toc}]/[\text{Sample}] = k_Q^{Sample}/k_Q^{\alpha\text{-Toc}} \quad (12)$$

Equation 12 indicates that the SOAC value corresponds to the ratio ($k_Q^{Sample}/k_Q^{\alpha\text{-Toc}}$) of the quenching rate of singlet oxygen (k_Q^{Sample}) by sample to that ($k_Q^{\alpha\text{-Toc}}$) by α-tocopherol. α-Tocopherol is used as a standard compound of SOAC assay.

The measurement of the SOAC value was performed for β-carotene and astaxanthin. **Figure 4A** shows an example of the reaction between DPBF (6.94×10^{-5} M) and EP (4.52×10^{-4} M) in the absence ((a) Blank) and presence of antioxidants ((b) [α-Toc] = 5.00×10^{-4} M, (c) [α-Toc] = 1.00×10^{-3} M, (d) [β-Car] = 3.24×10^{-6} M, (e) [β-Car] = 6.46×10^{-6} M, (f) [β-Car] = 9.70×10^{-6} M) in ethanol/chloroform/D_2O solution at 35 °C. The disappearance of DPBF at $\lambda_{max} = 413$ nm due to the chemical reaction with $^1O_2$ was observed. The correction of the baseline

Table 4. Half-Lives ($t_{1/2}$) and Employed Concentrations of α-Tocopherol, Sample, and DPBF, Relative SOAC Values, and Relative Rate Constants ($k_Q^{Sample}(S)/k_Q^{\alpha\text{-Toc}}(S)$) in Ethanol/Chloroform/$D_2O$

sample	$t_{1/2}^{\alpha\text{-Toc}}$/min ([α-Toc]/M)	$t_{1/2}^{Sample}$/min ([Sample]/M)	$t_{1/2}^{Blank}$/min ([DPBF]/M)	relative SOAC value[a]	$k_Q^{Sample}(S)/k_Q^{\alpha\text{-Toc}}(S)$
β-carotene	50.5 (5.00×10^{-4})	35.0 (3.26×10^{-6})	16.6 (6.94×10^{-5})	83.8	82.4
		58.7 (6.46×10^{-6})		96.1	
		76.0 (9.70×10^{-6})		90.3	
	84.1 (10.0×10^{-4})	35.0 (3.26×10^{-6})		84.1	
		58.7 (6.46×10^{-6})		96.5	
		76.0 (9.70×10^{-6})		90.7	
				av = 90.3	
astaxanthin	33.5 (2.00×10^{-4})	33.5 (2.80×10^{-6})	15.8 (6.99×10^{-5})	71.4	90.1
		55.7 (5.59×10^{-6})		80.7	
		74.2 (8.39×10^{-6})		78.7	
	54.7 (5.00×10^{-4})	33.5 (2.80×10^{-6})		81.3	
		55.7 (5.59×10^{-6})		91.7	
		74.2 (8.39×10^{-6})		89.5	
				av = 82.2	

[a] Experimental errors in the relative SOAC values (av) were estimated to be <10%.

Table 5. Half-Lives ($t_{1/2}$) and Employed Concentrations of α-Tocopherol, Sample (Tomato and Carrot Extracts) and DPBF, and Relative SOAC Values in Ethanol/Chloroform/D_2O

vegetable extract	$t_{1/2}^{\alpha\text{-Toc}}$/min ([α-Toc]/(g/L))	$t_{1/2}^{Sample}$/min ([Sample]/(g/L))	$t_{1/2}^{Blank}$/min ([DPBF]/M)	relative SOAC value
tomato-1	50.8 (2.17×10^{-1})	23.3 (5.33×10^{-1})	18.2 (5.55×10^{-5})	0.0633
		25.1 (8.00×10^{-1})		0.0572
		32.6 (1.60)		0.0598
		42.8 (2.67)		0.0612
				av = 0.0604
tomato-2	25.3 (7.16×10^{-2})	37.0 (2.67)	21.8 (6.07×10^{-5})	0.0638
		47.7 (4.00)		0.0651
		76.2 (8.00)		0.0625
		118.5 (13.3)		0.0643
				av = 0.0639
carrot	55.6 (2.16×10^{-1})	29.3 (1.07)	18.2 (5.55×10^{-5})	0.0503
		33.6 (1.60)		0.0501
		48.0 (3.20)		0.0528
		63.6 (5.33)		0.0497
				av = 0.0507

was performed by using the value of ε at 413 nm of β-carotene (see **Table 1**). The values of half-life ($t_{1/2}^{\alpha\text{-Toc}}$, $t_{1/2}^{\beta\text{-Car}}$, $t_{1/2}^{Blank}$) were calculated according to the method described in a previous section (see **Table 4**). As the measurements were performed for two different concentrations of α-tocopherol and three concentrations of β-carotene, we can determine six sets of relative SOAC values, using eq 12, as listed in **Table 4**. The relative SOAC values (83.8−96.5, av = 90.3) obtained for β-carotene are similar to each other and are close to the ratio ($k_Q^{\beta\text{-Car}}/k_Q^{\alpha\text{-Toc}} = 82.4$, **Table 2**) of the quenching rate constant of β-carotene to that of α-tocopherol, as expected from eq 12.

A similar measurement was performed for astaxanthin. The six sets of relative SOAC values obtained are listed in **Table 4**. The relative SOAC values (71.4−91.7, av = 82.2) obtained for astaxanthin are similar to each other and are close to the ratio ($k_Q^{Ast}/k_Q^{\alpha\text{-Toc}} = 90.1$, **Table 2**) of the quenching rate constant of astaxanthin to that of α-tocopherol. The result indicates that the definition of eq 12 is useful for the estimation of the SOAC value of the carotenoids.

Application of SOAC Assay Method to Vegetable Extracts. Application of SOAC method to vegetable extracts (tomato and carrot) was performed. The preliminary results obtained are as follows. The preparation of tomato and carrot extracts was described under Material and Methods. For example, the tomato-1 extract prepared from 1.00 g of freeze-dried powder was dissolved in 25 mL of ethanol/chloroform/D_2O (50:50:1, v/v/v) solution. From this solution, four concentrations of tomato extract (tomato-1, see **Table 5**) were prepared, where the concentration of tomato-1 extract was defined as grams per liter because we cannot use the molar concentration (M = mol/L) for tomato extracts. Similarly, the concentration of α-tocopherol as a standard sample was expressed as grams per liter. The concentration of α-tocopherol used for the measurement was 5.03×10^{-4} M, that is, 2.17×10^{-1} g/L (see **Table 5**).

The tomato-1 extract shows an UV−vis absorption spectrum at 400−550 nm, suggesting that high concentrations of carotenoids are included in tomato-1 (see **Figure 5A**) (*5*). Decay curves of the absorbance of DPBF due to the reaction with $^1O_2$ for tomato-1 extract are shown in **Figure 5B**. Baseline corrections were performed by using an absorbance at 413 nm of UV−vis absorption spectrum in **Figure 5A**, and the decay curves corrected are shown in **Figure 5C**. ln [Absorbance] versus *t* plots are shown in **Figure 5D**, indicating that the decay of DPBF for tomato-1 extract also follows first-order kinetics at $\sim 5 < t < \sim 60$ min.

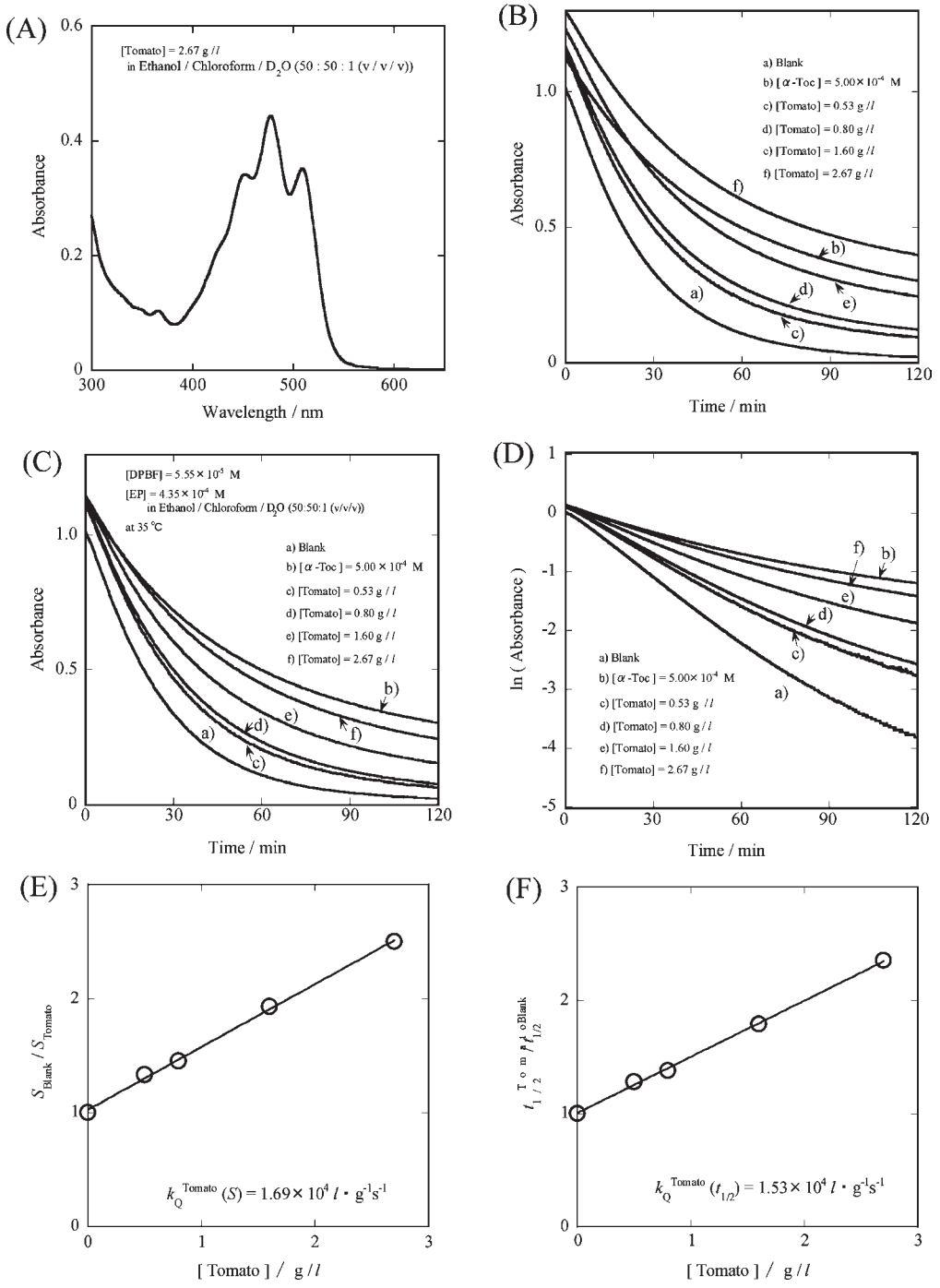

Figure 5. (**A**) Absorption spectrum of tomato-1 extract in ethanol/chloroform/D_2O. The concentration of tomato extract is 2.67 g/L. (**B**) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1O_2$ in the absence or presence of sample (α-tocopherol and tomato extracts) in ethanol/chloroform/D_2O at 35 °C. $[DPBF]_{t=0} = 5.55 \times 10^{-5}$ M and $[EP]_{t=0} = 4.35 \times 10^{-4}$ M. The values of $[\alpha\text{-Toc}]_{t=0}$ and $[Tomato]_{t=0}$ are shown in **B**. (**C**) Change in absorbance of DPBF, where the correction of baseline due to tomato extract was performed (see text). (**D**) Plot of ln(absorbance) versus *t*. (**E**) Plot of S_{Blank}/S_{Tomato} versus concentration of tomato extract. (**F**) Plot of $t_{1/2}^{Tomato}/t_{1/2}^{Blank}$ versus concentration of tomato extract.

Table 6. Rate Constants ($k_Q^{Sample}(S)$ and $k_Q^{Sample}(t_{1/2})$ (in L g^{-1} s^{-1} Units)) Obtained from S_{Blank}/S_{Sample} versus [Sample] (in g/L Units) and $t_{1/2}^{Sample}/t_{1/2}^{Blank}$ versus [Sample] Plots, Respectively, the Ratios ($k_Q^{Sample}(t_{1/2})/k_Q^{Sample}(S)$), and Relative SOAC Values for the Reaction of $^1O_2$ with Vegetable Extracts in Ethanol/Chloroform/D_2O

vegetable extract	$k_Q^{Sample}(S)$/(L g^{-1} s^{-1}) (S_{Blank}/S_{Sample} plot)	$k_Q^{Sample}(t_{1/2})$/(L g^{-1} s^{-1}) ($t_{1/2}^{Sample}/t_{1/2}^{Blank}$ plot)	$k_Q^{Sample}(t_{1/2})$ / $k_Q^{Sample}(S)$	relative SOAC value
tomato-1	1.69×10^4	1.53×10^4	0.905	av = 0.0604
tomato-2	1.56×10^4	1.37×10^4	0.878	av = 0.0639
carrot	1.41×10^4	1.20×10^4	0.851	av = 0.0507

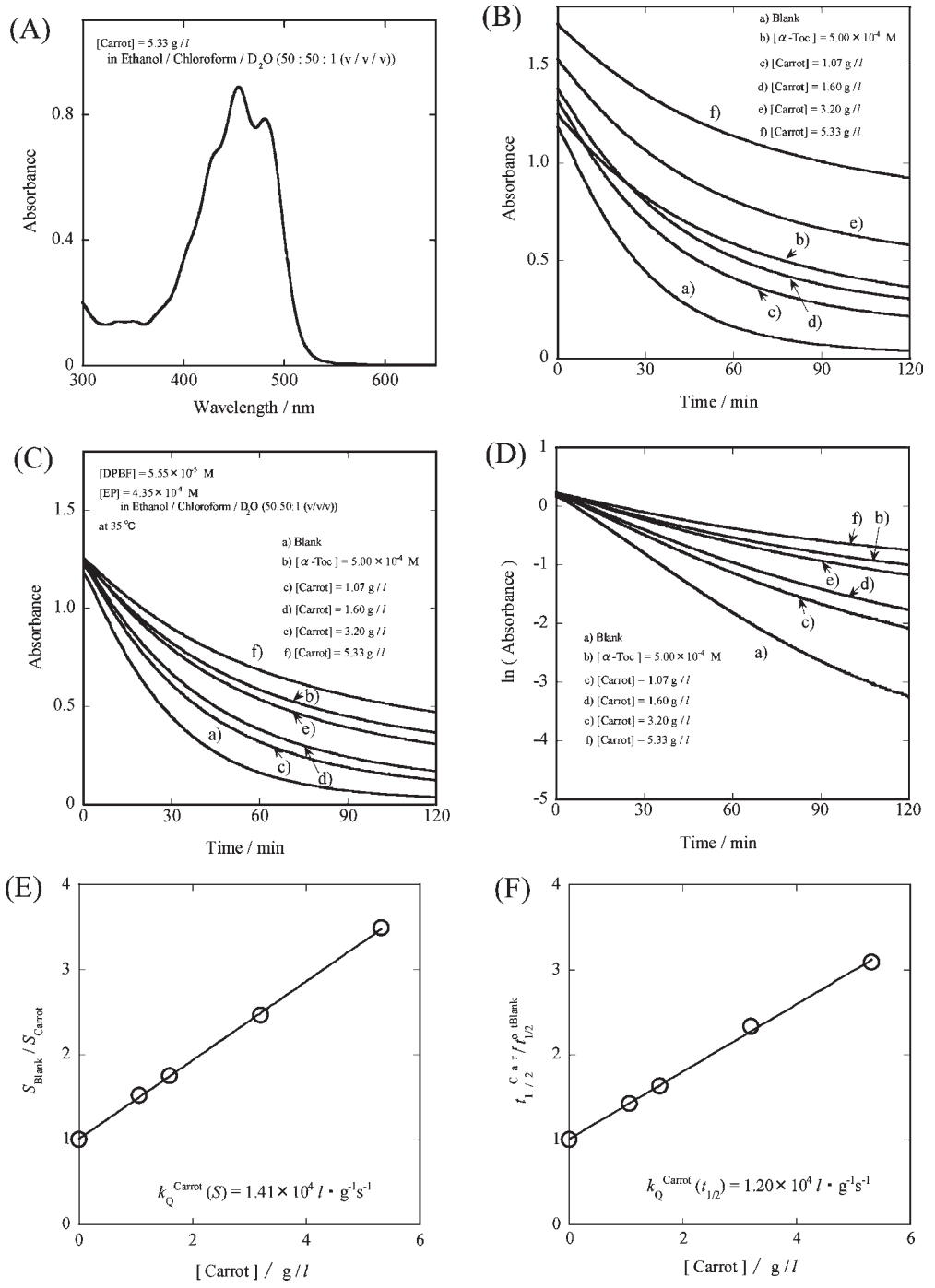

Figure 6. (**A**) Absorption spectrum of carrot extract in ethanol/chloroform/D_2O. The concentration of carrot extract is 5.33 g/L. (**B**) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1O_2$ in the absence or presence of sample (α-tocopherol and carrot extracts) in ethanol/chloroform/D_2O at 35 °C. $[DPBF]_{t=0} = 5.55 \times 10^{-5}$ M and $[EP]_{t=0} = 4.35 \times 10^{-4}$ M. The values of $[\alpha\text{-Toc}]_{t=0}$ and $[Carrot]_{t=0}$ are shown in **B**. (**C**) Change in absorbance of DPBF, where the correction of baseline due to carrot extract was performed (see text). (**D**) Plot of ln(absorbance) versus *t*. (**E**) Plot of S_{Blank}/S_{Carrot} versus concentration of carrot extract. (**F**) Plot of $t_{1/2}^{Carrot}/t_{1/2}^{Blank}$ versus concentration of carrot extract.

The values of $t_{1/2}^{Tomato}$, $t_{1/2}^{\alpha\text{-Toc}}$, and $t_{1/2}^{Blank}$ obtained are listed in **Table 5**.

S^{Blank}/S^{Tomato} and $t_{1/2}^{Tomato}/t_{1/2}^{Blank}$ versus [Tomato] (by g/L unit) plots are shown in **Figure 5**, panels **E** and **F**, respectively. Both the S^{Blank}/S^{Sample} and $t_{1/2}^{Sample}/t_{1/2}^{Blank}$ values increase linearly with increasing concentration of tomato-1 extract ([Tomato]), and the plots show similar slopes, that is, similar rate constants ($k_Q^{Tomato}(S)$ and $k_Q^{Tomato}(t_{1/2})$), where the unit of k_Q^{Tomato} is L g^{-1} s^{-1}. The $k_Q^{Tomato}(S)$ and $k_Q^{Tomato}(t_{1/2})$ values obtained are 1.69×10^4 and 1.53×10^4 L g^{-1} s^{-1}, respectively (see **Table 6**). Furthermore, the linear dependence of S^{Blank}/S^{Sample} and $t_{1/2}^{Sample}/t_{1/2}^{Blank}$ values on [Tomato] suggests that the effects of the interactions between carotenoids included in tomato (mainly lycopene and β-carotene (*5*)) and among the carotenoids and many compounds included in solution are negligible.

A similar measurement was repeated again by varying the concentrations of tomato extract ([Tomato]) and α-tocopherol ([α-Toc]). Sample of tomato-2 extract was prepared similarly, and the measurements of the rate constants ($k_Q^{Tomato}(S)$ and $k_Q^{Tomato}(t_{1/2})$) were performed again (data are not shown).

The two rate constants obtained for tomato-2 were similar to each other, as listed in **Table 6**. Furthermore, the rate constants obtained for tomato-2 were similar to those for tomato-1, as listed in **Table 6**, indicating that both the methods of sample preparation and measurement of the rate constants are reliable for the assay of singlet oxygen quenching activity of vegetable extracts.

A similar measurement was performed for the carrot extract, as shown in **Figure 6**. The results of analyses of the decay curves of DPBF are summarized in **Tables 5** and **6**. As observed for tomato extracts, both the $S^{\text{Blank}}/S^{\text{Sample}}$ and ${t_{1/2}}^{\text{Sample}}/{t_{1/2}}^{\text{Blank}}$ values increase linearly with increasing the concentration of carrot extract [Carrot], and the plots show similar slopes, that is, similar rate constants (${k_Q}^{\text{Carrot}}$ (S) and ${k_Q}^{\text{Carrot}}$ ($t_{1/2}$)) (see **Figure 6E**,**F**).

In the case of tomato and carrot extracts, the relative SOAC assay value will be defined as

$$\begin{aligned}&\text{relative SOAC value (given on a weight basis (g/L))}\\&= \{({t_{1/2}}^{\text{Sample}} - {t_{1/2}}^{\text{Blank}})/({t_{1/2}}^{\alpha\text{-Toc}} - {t_{1/2}}^{\text{Blank}})\}\\&\quad \times [\alpha\text{-Toc}]\ (\text{g/L})/[\text{Sample}]\ (\text{g/L})\\&= {k_Q}^{\text{Sample}}\ (\text{L g}^{-1}\ \text{s}^{-1})/{k_Q}^{\alpha\text{-Toc}}\ (\text{L g}^{-1}\ \text{s}^{-1}) \qquad (15)\end{aligned}$$

where the unit of the concentration of sample ([Sample]) and α-tocopherol ([α-Toc]) in eq 15 is g/L and the unit of k_Q is not $M^{-1}\ s^{-1}$, but $L\ g^{-1}\ s^{-1}$. Consequently, the relative SOAC value in eq 15 is not equivalent to the ratio of the second-order rate constants (${k_Q}^{\text{Sample}}/{k_Q}^{\alpha\text{-Toc}}$) given in $M^{-1}\ s^{-1}$ unit. The relative SOAC values (given on a weight basis (g/L)) were calculated, using eq 15, and are listed in **Tables 5** and **6**.

The relative SOAC values obtained for four concentrations of tomato-1 extracts are similar to each other. Similar results were obtained for tomato-2 extract. The average SOAC value (av = 0.0639) for tomato-2 is close to the value (av = 0.0604) for tomato-1 (see **Table 6**). The relative SOAC values obtained for four concentrations of carrot extracts are also similar to each other, and the average SOAC value is 0.0507. The SOAC value obtained for tomato extract is 1.2 times larger than that for carrot extract. Furthermore, the result indicates that the intake of 1.00 g of tomato and carrot extracts has the singlet oxygen quenching activity equal to that of 0.0622 and 0.0507 g of pure α-tocopherol, respectively.

In the present work, the measurements of the SOAC values were performed for tomato and carrot extracts. The measurements of the SOAC values for many extracts of foods and plants are now in progress in our laboratory.

Lipid peroxyl radical ($LOO^{\bullet}$) and singlet oxygen ($^1O_2$) are well-known as two representative ROS generated in biological systems. As described in the Introduction, the method to assess the total ORAC of foods and plants has been established by several investigators (*27*−*30*). On the other hand, a SOAC assay method to assess the total quenching activity of $^1O_2$ by carotenoids and phenolic antioxidants included in foods and plants has not been established. Consequently, the development of the SOAC method is very important. In the present work, the quenching rates (k_Q) of $^1O_2$ by carotenoids **1**−**8**, α-tocopherol, and two kinds of food extracts (from tomato and carrot) were measured in ethanol/chloroform/D_2O solution at 35 °C, by using the competition reaction method. From the results, the method to assess the total SOAC value of the antioxidants included in foods and plants has been proposed.

ACKNOWLEDGMENT

We are very grateful to Prof. Kunihiko Tajima of Kyoto Institute of Technology, Prof. Tateaki Ogata of Yamagata University, and Honorary Prof. Nagao Azuma of Ehime University for their helpful discussions. We are also grateful to Takashi Origuchi and Miyabi Fujii of Ehime University for their kind help in the preparation of endoperoxide (EP).

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Received for review May 21, 2010. Revised manuscript received July 27, 2010. Accepted August 6, 2010. This work was partly supported by a grant from SUNATEC, Japan (to K.M. and S.N.).