JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Development of Singlet Oxygen Absorption Capacity (SOAC) Assay Method. 2. Measurements of the SOAC Values for Carotenoids and Food Extracts

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ABSTRACT: Recently a new assay method that can quantify the singlet oxygen absorption capacity (SOAC) of antioxidants was proposed. In the present work, kinetic study of the reaction of singlet oxygen ($^1O_2$) with carotenoids and vegetable extracts has been performed in ethanol/chloroform/D_2O (50:50:1, v/v/v) solution at 35 °C. Measurements of the second-order rate constants ($k_Q(S)$) and the SOAC values were performed for eight kinds of carotenoids and three kinds of vegetable extracts (red paprika, carrot, and tomato). Furthermore, measurements of the concentrations of the carotenoids included in vegetable extracts were performed, using a HPLC technique. From the results, it has been clarified that the total $^1O_2$-quenching activity (that is, the SOAC value) for vegetable extracts may be explained as the sum of the product $\{\Sigma\, k_Q^{\text{Car-}i}(S)\,[\text{Car-}i]_i\}$ of the rate constant ($k_Q^{\text{Car-}i}(S)$) and the concentration ([Car (i)]) of carotenoids included in vegetable extracts.

KEYWORDS: singlet oxygen, quenching rate, endoperoxide, carotenoids, β-carotene, lycopene, tomato, vegetable extracts, SOAC value, kinetic study

INTRODUCTION

Singlet oxygen ($^1O_2$) and lipid peroxyl radical (LOO•) are two well-known 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,[1,2] as well as the peroxyl radical does. Reactions with $^1O_2$ occur mainly by chemical reaction, inducing the degradation of biological systems. Carotenoids are widely present in vegetables and fruits in high concentrations[3−5] and may function as efficient $^1O_2$ quenchers in biological systems.[6−9]

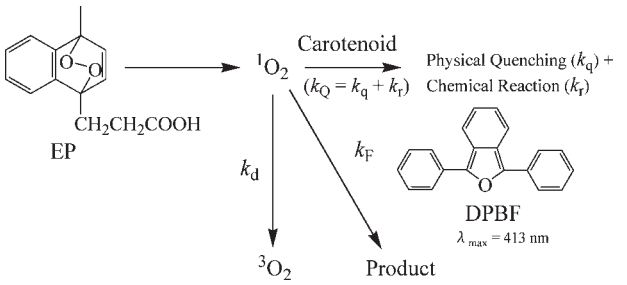
In recent years, the method to assess the total oxygen radical absorpton capacity (ORAC) of foods and plants has been developed, where oxygen radical indicates LOO•.[10−13] On the other hand, a singlet oxygen absorption capacity (SOAC) assay method to assess the total quenching activity of singlet oxygen by carotenoids and phenolic antioxidants included in foods and plants has not been developed.

In a previous work, a kinetic study of the quenching reaction of $^1O_2$ 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.[14] 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 and 2,5-diphenyl-3,4-benzofuran (DPBF) as an UV−vis absorption probe (see Scheme 1).

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

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, respectively, showing good accordance with each other. By reacting carotenoids with $^1O_2$ for 2−4 h at 35 °C, no changes of UV−vis spectra of carotenoids were observed. Consequently, the k_Q values obtained for carotenoids are thought to be due to physical quenching (k_q), that is, $k_Q \approx k_q$. Preliminary measurements of the $k_Q(S)$ and $k_Q(t_{1/2})$ values were performed for tomato and carrot extracts containing high concentrations of carotenoids. From the results, a new assay method that can quantify the SOAC of antioxidants, including carotenoids, α-tocopherol, and vegetable extracts, was proposed.[14] The relative SOAC value

Scheme 1



Received: December 27, 2010
Revised: March 8, 2011
Accepted: March 11, 2011
Published: March 11, 2011

dx.doi.org/10.1021/jf104955a | J. Agric. Food Chem. 2011, 59, 3717–3729

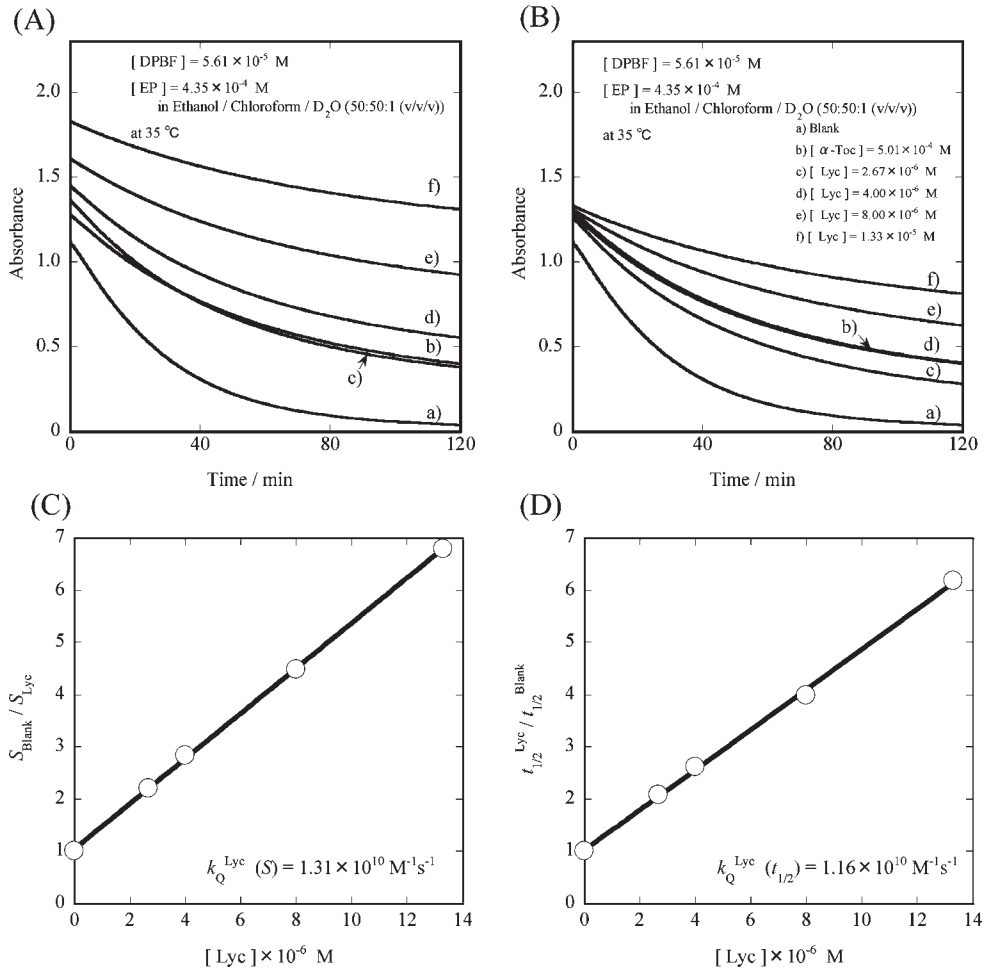


Figure 1. (A) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1O_2$ in the absence and presence of sample (α-tocopherol or lycopene) in ethanol/chloroform/D_2O at 35 °C. $[DPBF]_{t=0} = 5.61 \times 10^{-5}$ M and $[EP]_{t=0} = 4.35 \times 10^{-4}$ M. The values of $[\alpha\text{-Toc}]_{t=0}$ and $[Lyc]_{t=0}$ are shown in panel B. (B) Change in absorbance of DPBF, where the correction of baseline due to lycopene was performed (see text). (C) Plot of S_{blank}/S_{Lyc} versus [Lyc]. (D) Plot of $t_{1/2}^{Lyc}/t_{1/2}^{blank}$ versus [Lyc].

was defined in the following way

$$\text{relative SOAC value} = \{(t_{1/2}^{\text{sample}} - t_{1/2}^{\text{blank}})/(t_{1/2}^{\alpha\text{-Toc}} - t_{1/2}^{\text{blank}})\} \times \{[\alpha\text{-Toc}]/[\text{sample}]\} = k_Q^{\text{sample}}/k_Q^{\alpha\text{-Toc}} \quad (2)$$

where [α-Toc] and [sample] are molar concentrations of α-tocopherol and sample, respectively. α-Tocopherol was used as a standard compound.

In the present work, first, the measurements of the relative SOAC values were performed for eight carotenoids (lycopene (Lyc), astaxanthin (Ast), β-carotene (β-Car), capsanthin (Cap), zeaxanthin (Zea), α-carotene (α-Car), lutein (Lut), and β-cryptoxanthin (β-Cry)) in an ethanol/chloroform/D_2O (50:50:1, v/v/v) solution at 35 °C, because the SOAC values were determined for only Ast and β-Car in a previous work.[14] Second, measurements of the $k_Q(S)$ value were performed for the mixtures including two kinds antioxidants ((i) β-Car and Lyc and (ii) β-Car and α-Toc), to investigate the effect of the interaction between antioxidants on the quenching rate. Third, measurements of $k_Q(S)$, $k_Q(t_{1/2})$, and SOAC values for the reaction of $^1O_2$ with three vegetable extracts (red paprika, carrot, and tomato) were performed. Fourth, measurements of the concentrations of carotenoids included in the vegetable extracts were performed using a HPLC technique. Furthermore, comparison of the $k_Q(S)^{total}$ values observed for the above vegetable extracts with the sum of the product $\{\Sigma k_Q^{Car\text{-}i}(S)\,[Car\text{-}i]\}$ of the $k_Q^{Car\text{-}i}(S)$ values obtained for each carotenoid and the concentrations of caronenoids ([Car-*i*]) included in vegetable extracts was performed to ascertain the validity of the SOAC assay method developed.

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 and DPBF were obtained from Eisai Food Chemicals Co. Ltd., Japan, and Tokyo Kasei Chemicals, Japan, respectively. Sea sand was obtained from Wako Chemicals, Japan.

3-(1,4-Epidioxy-4-methyl-1,4-dihydro-1-naphthyl)propionic acid (endoperoxide, EP) was prepared according to a published procedure.[15−18] 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.[14]

Table 1. Employed Concentrations, First-Order Decay Rates (S), and Half-Lives ($t_{1/2}$) of Blank (DPBF Only), α-Tocopherol, and Sample ((a) Lycopene and (b) α-Carotene) and Relative SOAC Values in Ethanol/Chloroform/D_2O Solution

	blank	α-Toc	sample 1	sample 2	sample 3	sample 4
			(a) Lycopene-1			
concn (M)	0	5.01×10^{-4}	2.67×10^{-6}	4.00×10^{-6}	8.00×10^{-6}	1.33×10^{-5}
S_{sample} (s^{-1})	3.31×10^{-2}	1.17×10^{-2}	1.50×10^{-2}	1.17×10^{-2}	7.40×10^{-3}	4.90×10^{-3}
S_{blank}/S_{sample}	1	2.83	2.21	2.84	4.49	6.79
$t_{1/2}$ (min)	21.2	55.5	44.0	55.6	84.6	131
$t_{1/2}^{sample}/t_{1/2}^{blank}$	1	2.62	2.08	2.62	3.99	6.18
$t_{1/2}^{sample} \times S_{sample}/\ln 2$	1.013	0.937	0.952	0.936	0.900	0.922
relative SOAC value			125	126	116	120 (av 122)[a]
			(b) α-Carotene-1			
concn (M)	0	1.67×10^{-4}	2.60×10^{-6}	3.89×10^{-6}	7.79×10^{-6}	1.30×10^{-5}
S_{sample} (s^{-1})	4.39×10^{-2}	2.53×10^{-2}	2.16×10^{-2}	1.72×10^{-2}	1.10×10^{-2}	7.61×10^{-3}
S_{blank}/S_{sample}	1	1.73	2.03	2.55	3.99	5.76
$t_{1/2}$ (min)	16.0	25.9	30.0	38.0	59.4	84.4
$t_{1/2}^{sample}/t_{1/2}^{blank}$	1	1.62	1.88	2.38	3.71	5.29
$t_{1/2}^{sample} \times S_{sample}/\ln 2$	1.012	0.945	0.933	0.942	0.945	0.930
SOAC value			90.8	95.1	93.9	89.1 (av 92.2)[a]

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

Vegetable extracts were prepared in the following way;[19] 1.00 g of freeze-dried powder sample from vegetable (red paprika, carrot, and tomato) was mixed with 5 g of sea sand. Sample and sand were transferred to an 11 mL extraction cell, and extraction was performed 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 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). All of the 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, vegetable extracts, α-tocopherol, and DPBF. All of the measurements were done in a sealed system using a cuvette with a sealing cap 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 over 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. It 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.

Analyses of the Second-Order Rate Constants ($k_Q^{Car}(S)$ and $k_Q^{Car}(t_{1/2})$) and SOAC Values. The rate constant $k_Q^{Car}(S)$ for the reaction of $^1O_2$ with carotenoid (Car) (or sample) was determined by eq 3[14,20,21]

$$S_{blank}/S_{Car} = 1 + \{k_Q^{Car}(S)[Car]\}/k_d \quad (3)$$

where S_{blank} and S_{Car} are slopes of the first-order plots (that is, ln(absorbance) vs t plots) of disappearance of DPBF in the absence and presence of carotenoid, respectively (see Figure 1B). k_d (= 3.03×10^4 s^{-1}) is the rate of natural deactivation of $^1O_2$ in the solvent.[7,9] Equation 3 indicates that the $k_Q^{Car}(S)$ value can be obtained from the S_{blank}/S_{Car} versus [Car] plot (see Figure 1C).

We can easily obtain eq 4 by substituting the relation for the first-order reaction ($t_{1/2}^{Car} = \ln 2/S_{Car}$) into eq 3

$$t_{1/2}^{Car}/t_{1/2}^{blank} = 1 + \{k_Q^{Car}(t_{1/2})[Car]\}/k_d \quad (4)$$

where $t_{1/2}^{blank}$ and $t_{1/2}^{Car}$ are the half-lives of DPBF in the absence and presence of carotenoid, respectively. Equation 4 indicates that the $k_Q^{Car}(t_{1/2})$ value can be obtained from $t_{1/2}^{Car}/t_{1/2}^{blank}$ versus [Car] plot.[14] In fact, $t_{1/2}^{Car}$ increases linearly with increasing concentration of carotenoid, as shown in Figure 1D.

As proposed in a previous work,[14] the relative SOAC value for carotenoid (or sample) was defined as follows:

$$\begin{aligned}&\text{relative SOAC value (based on molar concentration unit (M = mol/L))}\\ &= \{(t_{1/2}^{sample} - t_{1/2}^{blank})/(t_{1/2}^{\alpha\text{-Toc}} - t_{1/2}^{blank})\} \times \{[\alpha\text{-Toc}]\ (M)/[sample]\ (M)\}\\ &= k_Q^{sample}(M^{-1}s^{-1})/k_Q^{\alpha\text{-Toc}}\ (M^{-1}s^{-1}) \quad (5)\end{aligned}$$

Equation 5 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. Equation 5 indicates that the SOAC value may be easily determined by the measurement of the half-life of DPBF.

Furthermore, the relative SOAC value for vegetable extracts was defined in the following way[14]

$$\begin{aligned}&\text{relative SOAC value (given on a weight basis (g/L))}\\ &= \{(t_{1/2}^{sample} - t_{1/2}^{blank})/(t_{1/2}^{\alpha\text{-Toc}} - t_{1/2}^{blank})\} \times \{[\alpha\text{-Toc}]\ (g/L)/[sample]\ (g/L)\}\\ &= k_Q^{sample}\ (L\ g^{-1}\ s^{-1})/k_Q^{\alpha\text{-Toc}}\ (L\ g^{-1}\ s^{-1}) \quad (6)\end{aligned}$$

where the unit of the concentration of sample ([sample]) and α-tocopherol ([α-Toc]) is g/L, and the unit of k_Q is not $M^{-1}\ s^{-1}$, but L

Table 2. 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, Relative Rate Constants ($k_Q^{sample}(S)/k_Q^{\alpha\text{-Toc}}(S)$), and Relative SOAC Values in Ethanol/Chloroform/D_2O Solution

sample	k_Q^{sample} (S) (M^{-1} s^{-1})	k_Q^{sample} ($t_{1/2}$) (M^{-1} s^{-1})	$k_Q^{sample}(S)/k_Q^{\alpha\text{-Toc}}(S)$	relative SOAC value
α-Toc (reported)	av 1.31×10^{8} [a]	av 1.29×10^{8} [a]	1.00	1.00
Lyc-1	1.31×10^{10}	1.16×10^{10}	100	122
Lyc-2	1.41×10^{10}	1.29×10^{10}	108	127
Lyc-3	1.48×10^{10}	1.23×10^{10}	113	120
	av 1.40×10^{10} [b]	av 1.23×10^{10} [b]	av 107 [c]	av 123 [c]
Lyc (reported)	av 1.38×10^{10} [a]	av 1.26×10^{10} [a]	av 105	
Ast-1	1.14×10^{10}	1.03×10^{10}	87.0	110
Ast-2	1.20×10^{10}	1.12×10^{10}	91.6	109
	av 1.17×10^{10} [b]	av 1.08×10^{10} [b]	av 89.3 [c]	av 109 [c]
Ast (reported)	av 1.18×10^{10} [a]	av 1.10×10^{10} [a]	av 90.1	av 82.2
β-Car-1	1.04×10^{10}	1.01×10^{10}	79.4	94.1
β-Car-2	1.05×10^{10}	9.94×10^{9}	80.2	92.8
β-Car-3	1.14×10^{10}	1.06×10^{10}	87.0	100.6
	av 1.08×10^{10} [b]	av 1.02×10^{10} [b]	av 82.2 [c]	av 95.8 [c]
β-Car (reported)	av 1.08×10^{10} [a]	av 1.06×10^{10} [a]	av 82.4	av 90.3
Cap	1.21×10^{10}	1.07×10^{10}	107	99.3
Cap (reported)	av 1.06×10^{10} [a]	av 1.07×10^{10} [a]	80.9	
Zea	1.12×10^{10}	1.23×10^{10}	88.6	92.8
Zea (reported)	av 1.05×10^{10} [a]	av 1.01×10^{10} [a]	av 80.2	
α-Car-1	1.10×10^{10}	1.01×10^{10}	82.6	92.2
α-Car-2	9.75×10^{9}	9.12×10^{9}	74.4	86.1
α-Car-3	9.24×10^{9}	8.36×10^{9}	70.5	98.8
α-Car (reported)	av 1.00×10^{10} [b]	av 9.20×10^{9} [b]	av 75.8 [c]	av 92.4 [c]
	av 9.76×10^{9} [a]	av 8.96×10^{9} [a]	av 74.5	
Lut	8.07×10^{9}	7.31×10^{9}	67.9	73.8
Lut (reported)	av 9.24×10^{9} [a]	av 9.05×10^{9} [a]	70.5	
β-Cry	7.04×10^{9}	6.72×10^{9}	60.9	67.6
β-Cry (reported)	av 7.31×10^{9} [a]	av 6.94×10^{9} [a]	55.8	

[a] Average values of $k_Q^{sample}(S)$ (reported) and $k_Q^{sample}(t_{1/2})$ (reported).[14] [b] Average values of $k_Q^{sample}(S)$ and $k_Q^{sample}(t_{1/2})$ obtained; experimental errors in the rate constants were estimated to be <10%. [c] Experimental errors in the relative rate constants ($k_Q^{sample}(S)/k_Q^{\alpha\text{-Toc}}(S)$) (av); relative SOAC values (av) were estimated to be <10%.

g^{-1} s^{-1}. Consequently, the relative SOAC value in eq 6 is not equivalent to the ratio of the second-order rate constants ($k_Q^{sample}/k_Q^{\alpha\text{-Toc}}$) given in M^{-1} s^{-1}. The relative SOAC values (given on a weight basis (g/L)) for vegetable extracts were calculated using eq 6.

In the SOAC assay method, the lipophilic α-tocopherol was used as a standard compound, instead of hydrophilic Trolox in the ORAC method. The reasons are as follows: (i) α-tocopherol and carotenoids are lipophilic; (ii) α-tocopherol is a representative phenolic antioxidant and shows the same order of k_Q value as those for the other phenolic antioxidants (such as tocopherol homologues, catechins, flavone derivatives, and ubiquinol-10);[22−26] (iii) α-tocopherol and carotenoids often coexist in general vegetables and fruits and function as singlet oxygen quenchers at similar lipophilic reaction field;[27−29] and (iv) Trolox is anα-tocopherol derivative.

In previous works,[22−26] measurements of rate constants (k_Q) for phenolic antioxidants were performed in ethanol. However, for example, lycopene and astaxanthin are insoluble in ethanol, and it was not easy to find a solvent in which many carotenoids are soluble. Consequently, the measurements were performed in ethanol/chloroform/D_2O (50:50:1, v/v/v) solution. This solution was used by Di Mascio et al.[7,9] to measure the k_Q values of many carotenoids.

RESULTS

Measurements of the $^1O_2$-Quenching Rates ($k_Q(S)$ and $k_Q(t_{1/2})$) and SOAC Values for Eight Kinds of Carotenoids. Masurements of $k_Q(S)$, $k_Q(t_{1/2})$, and SOAC values were performed for eight carotenoids (see Table 2). To ascertain the

validity of the SOAC method proposed for carotenoids (and the other antioxidants),[14] similar measurements were repeated several times for lycopene (lycopene-1, -2, -3), astaxanthin (astaxanthin-1, -2), β-carotene (β-carotene-1, -2, -3), and α-carotene (α-carotene-1, -2, -3) by varying the concentrations of α-tocopherol and carotenoids.

Figure 1A shows an example of the reaction between DPBF and EP in the absence ((a) blank) and presence of antioxidants ((b) [α-Toc] = 5.01×10^{-4} M, (c) [Lyc] = 2.67×10^{-6} M, (d) [Lyc] = 4.00×10^{-6} M, (e) [Lyc] = 8.00×10^{-6} M, (f) [Lyc] = 1.33×10^{-5} M) in ethanol/chloroform/D_2O solution at 35 °C. The disappearance of DPBF at λ_{max} = 413 nm due to the chemical reaction with $^1O_2$ was observed. The correction of the baseline in c−f was performed by using the value of ε (= 40900 M^{-1} cm^{-1}) at 413 nm of lycopene [14] (see Figure 1B). The values of first-order decay rate constant (S_{blank}, $S_{\alpha\text{-Toc}}$, S_{Lyc}) were calculated by analyzing the decay curve of DPBF, as listed in Table 1a. The analysis of the decay curve was performed at ∼5 < t < ∼60 min.[14] This is an important condition to obtain the correct rate constant (k_Q) for antioxidants. The values of half-life ($t_{1/2}^{\alpha\text{-Toc}}$, $t_{1/2}^{Lyc}$, $t_{1/2}^{blank}$) were calculated carefully according to the method described in a previous work.[14] The values obtained are summarized in Table 1a. As described under Materials and Methods, we can expect that the values of ($t_{1/2}^{sample} \times S_{sample}$)/ln 2 are ∼1, if the method of the analysis is right. In fact, the values of ($t_{1/2}^{sample} \times S_{sample}$)/ln 2 obtained were found to be ∼1 for lycopene-1 and α-carotene-1 (see Table 1).

Plots of S_{blank}/S_{Lyc} and $t_{1/2}^{Lyc}/t_{1/2}^{blank}$ versus [Lyc] are shown in Figure 1, panels C and D, respectively. The $k_Q^{Lyc}(S)$ and $k_Q^{Lyc}(t_{1/2})$ values obtained by using eqs 3 and 4 are 1.31×10^{10} and 1.16×10^{10} M^{-1} s^{-1}, respectively, showing fair agreement with each other.

As the measurements were performed for one concentration of α-tocopherol and four concentrations of lycopene, we can determine four sets of relative SOAC values, using eq 5 (see Table 1a). The relative SOAC values (116−126, av = 122) obtained for lycopene-1 are similar to each other and showed considerable agreement with the ratio ($k_Q^{Lyc}(S)/k_Q^{\alpha\text{-Toc}}(S)$ = 100, Table 2) of the quenching rate constant of lycopene to that of α-tocopherol, as expected from eq 5. Similar measurements were performed for lycopene three times, by varying the concentration of α-tocopherol and lycopene. The $k_Q^{Lyc}(S)$, $k_Q^{Lyc}(t_{1/2})$, and relative SOAC values obtained for lycopene-1, -2, and -3 showed good agreement with each other (see Table 2), if the concentrations of α-tocopherol and lycopene used were (2−7) × 10^{-4} and (2−7) × 10^{-6} M, respectively. We could not obtain the correct SOAC value if the differences between the half-lives of α-tocopherol and blank (that is, the value of a denominator in eq 5) and between those of lycopene and blank (that is, the value of a numerator in eq 5) were smaller than 10 min (deta are not shown).

A similar measurement was performed for α-carotene-1. The four sets of relative SOAC values obtained are listed in Table 1b. The relative SOAC values (89.1−95.1, av = 92.2) obtained are similar to each other and show a fair agreement with the ratio ($k_Q^{\alpha\text{-Car}}(S)/k_Q^{\alpha\text{-Toc}}(S)$ = 82.6, Table 2) of the quenching rate constant of α-carotene to that of α-tocopherol. The results obtained for α-carotene-2 and -3 are also listed in Table 2. Furthermore, the $k_Q(S)$, $k_Q(t_{1/2})$, and relative SOAC values obtained for astaxanthin-1 and -2, β-carotene-1, -2, and -3, capsanthin, zeaxanthin, lutein, and β-cryptoxanthin are also summarized in Table 2. The result indicates that the definition of eq 5 is useful for the estimation of the SOAC value of the carotenoids (and the other antioxidants).

Table 3. Observed and Calculated $\{(S_{blank}/S_{sample}) - 1\}$ Values for Mixtures of Two Kinds of Antioxidants

	sample 1	sample 2	sample 3	sample 4	sample 5
(a) β-Carotene and Lycopene ([Lyc] = Constant)					
[β-Car] (10^{-6} M)	0	0.897	1.79	3.59	5.38
[Lyc] (10^{-6} M)	3.35	3.35	3.35	3.35	3.35
(S_{blank}/S_{sample}) − 1 (obsd)	1.68	2.00	2.34	2.98	3.79
(S_{blank}/S_{sample}) − 1 (calcd)	1.53	1.85	2.16	2.80	3.44
(b) Lycopene and β-Carotene ([β-Car] = Constant)					
[Lyc] (10^{-6} M)	0	1.10	2.21	3.31	4.40
[β-Car] (10^{-6} M)	3.62	3.62	3.62	3.62	3.62
(S_{blank}/S_{sample}) − 1 (obsd)	1.33	1.94	2.47	2.86	3.57
(S_{blank}/S_{sample}) − 1 (calcd)	1.29	1.79	2.30	2.80	3.29
(c) β-Carotene and α-Tocopherol ([α-Toc] = Constant)					
[β-Car] (10^{-6} M)	0	0.596	1.19	2.38	3.58
[α-Toc] (10^{-6} M)	223	223	223	223	223
(S_{blank}/S_{sample}) − 1 (obsd)	0.930	1.08	1.28	1.75	2.24
(S_{blank}/S_{sample}) − 1 (calcd)	0.964	1.18	1.39	1.81	2.24
(d) α-Tocopherol and β-Carotene ([β-Car] = Constant)					
[α-Toc] (10^{-6} M)	0	57.7	115	173	289
[β-Car] (10^{-6} M)	1.57	1.57	1.57	1.57	1.57
(S_{blank}/S_{sample}) − 1 (obsd)	0.65	0.97	1.22	1.36	1.91
(S_{blank}/S_{sample}) − 1 (calcd)	0.560	0.809	1.06	1.31	1.81

Measurements of the $^1O_2$-Quenching Rates ($k_Q(S)$) for Mixtures of Two Kinds of Antioxidants. It is well-known that various antioxidants coexist not only in vegetables and fruits[27−29] but also in human tissues.[30−32] Consequently, measurement of the $k_Q(S)$ value has been performed for the solutions including two kinds of antioxidants to investigate the effect of the interaction between antioxidants on the quenching rate.

The measurement was performed for the solution including β-carotene and lycopene. In such a case, we can expect that the S_{blank}/S_{sample} value changes depending on eq 7, if the interaction between carotenoids is negligible.

$$S_{blank}/S_{sample} = 1 + \{k_Q^{Lyc}(S)[Lyc] + k_Q^{\beta\text{-Car}}(S)[\beta\text{-Car}]\}/k_d \quad (7)$$

$k_Q^{Lyc}(S)$ and $k_Q^{\beta\text{-Car}}(S)$ are the quenching rate constants for β-carotene and lycopene, respectively.

First, the measurement of $k_Q^{\beta\text{-Car}}(S)$ was performed by keeping lycopene ([Lyc]) at a constant concentration (3.35 × 10^{-6} M) and by varying the concentration of β-carotene ([β-Car]) (see Table 3a). The disappearance of DPBF was measured at 413 nm, as shown in Figure 2A. However, as the absorptions of β-carotene and lycopene overlap with that of DPBF at 413 nm, the correction of the baseline is necessary for each decay curve. The correction of the baseline was performed by using the values of molar extinction coefficient (ε) for β-carotene and lycopene reported in a previous work.[14] The decay curves corrected are shown in Figure 2B. Figure 2C shows the S_{blank}/S_{sample} versus [β-Car] plot (see Table 3a). From the gradient, we obtained the value of $k_Q^{\beta\text{-Car}}(S)$ (= 1.18×10^{10} M^{-1} s^{-1}). The $k_Q^{\beta\text{-Car}}(S)$ value (1.18×10^{10} M^{-1} s^{-1}) obtained showed good accordance

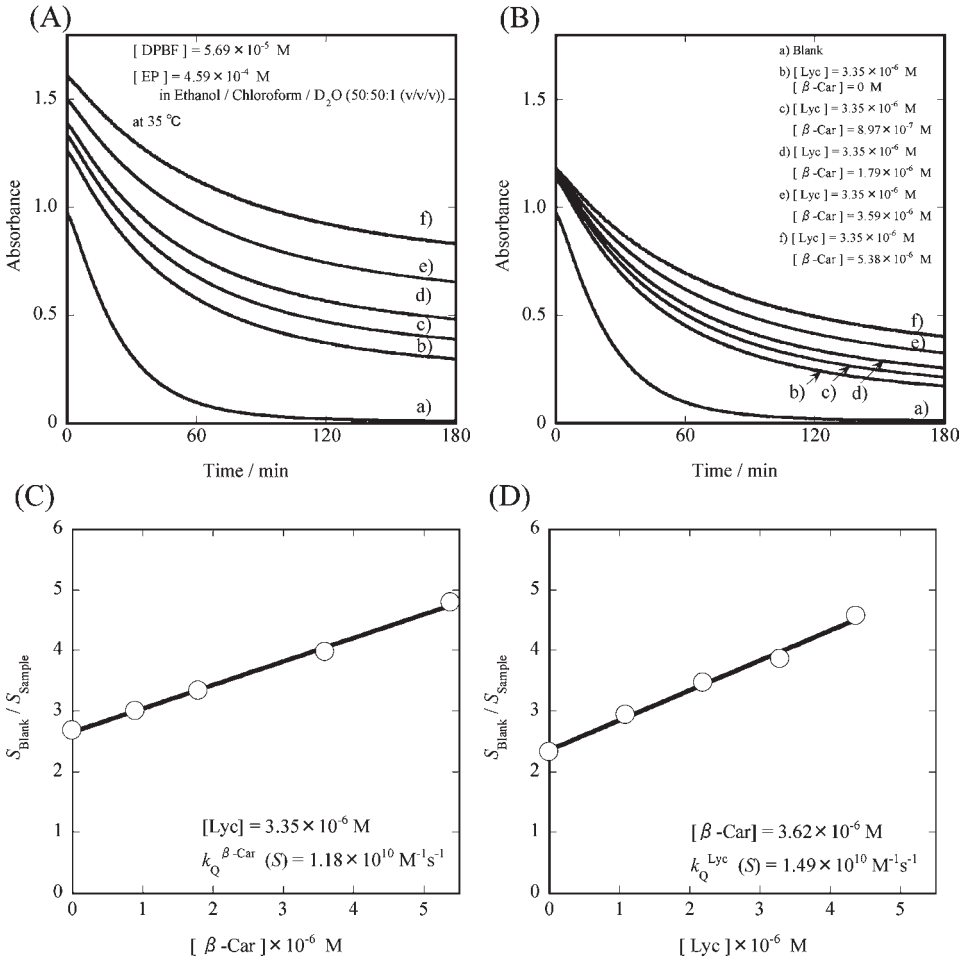


Figure 2. (A) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1O_2$ in the absence and presence of sample (β-carotene and lycopene) in ethanol/chloroform/D_2O at 35 °C. $[DPBF]_{t=0} = 5.69 \times 10^{-5}$ M and $[EP]_{t=0} = 4.59 \times 10^{-4}$ M. The values of $[\beta\text{-Car}]_{t=0}$ and $[Lyc]_{t=0}$ are shown in panel B. (B) Change in absorbance of DPBF, where the correction of baseline due to β-carotene and lycopene was performed (see text). (C) Plot of S_{blank}/S_{sample} versus [β-Car]. [Lyc] = 3.35×10^{-6} M. (D) Plot of S_{blank}/S_{sample} versus [Lyc]. [β-Car] = 3.62×10^{-6} M.

Table 4. Second-Order Rate Constants ($k_Q(S)$) Obtained for Mixtures of Two Kinds of Antioxidants

two component system		$k_Q(S)$ (M^{-1} s^{-1})[a] from a gradient	$k_Q(S)$ (M^{-1} s^{-1}) from an intercept at the *y*-axis	$k_Q(S)$ (M^{-1} s^{-1}),[b] the value reported for each antioxidant
β-Car and Lyc	β-Car	1.18×10^{10}	1.13×10^{10}	1.08×10^{10}
	Lyc	1.49×10^{10}	1.49×10^{10}	1.38×10^{10}
β-Car and α-Toc	β-Car	1.13×10^{10}	1.33×10^{10}	1.08×10^{10}
	α-Toc	1.28×10^{8}	1.20×10^{8}	1.31×10^{8}

[a] Experimental errors in the $k_Q(S)$ values obtained from a gradient for S_{blank}/S_{sample} vs [antioxidant] plot are <10%. [b] $k_Q(S)$ values are reported.[14]

with that (1.08×10^{10} M^{-1} s^{-1}) reported for β-carotene (Table 2).[14] Furthermore, we can determine the $k_Q^{Lyc}(S)$ value from the intercept at the *y*-axis (that is, $1 + \{k_Q^{Lyc}(S)[Lyc]\}/k_d$) in Figure 2C, using eq 7. The $k_Q^{\beta\text{-Car}}(S)$ and $k_Q^{Lyc}(S)$ values obtained showed good accordance corresponding those reported for β-carotene and lycopene, as listed in Table 4. Similar measurements were performed for the solution including β-carotene and lycopene, by keeping β-carotene ([β-Car]) at a constant concentration (3.62×10^{-6} M) and by varying the concentration of lycopene ([Lyc]) (see Figure 2D and Table 3b). Similarly, the $k_Q^{Lyc}(S)$ and $k_Q^{\beta\text{-Car}}(S)$ values were determined for lycopene and β-carotene, respectively. The $k_Q(S)$ values obtained are listed in Table 4, showing good agreement with those reported. The interaction between β-carotene and lycopene is considered to be negligibly small in solution. The result suggests that the total $^1O_2$-quenching ability may be evaluated by using eq 7.

Second, similar measurements were performed for the solutions including β-carotene and α-tocopherol, as shown in Figure 3. As the $k_Q^{\beta\text{-Car}}(S)$ value of β-carotene is 82 times as

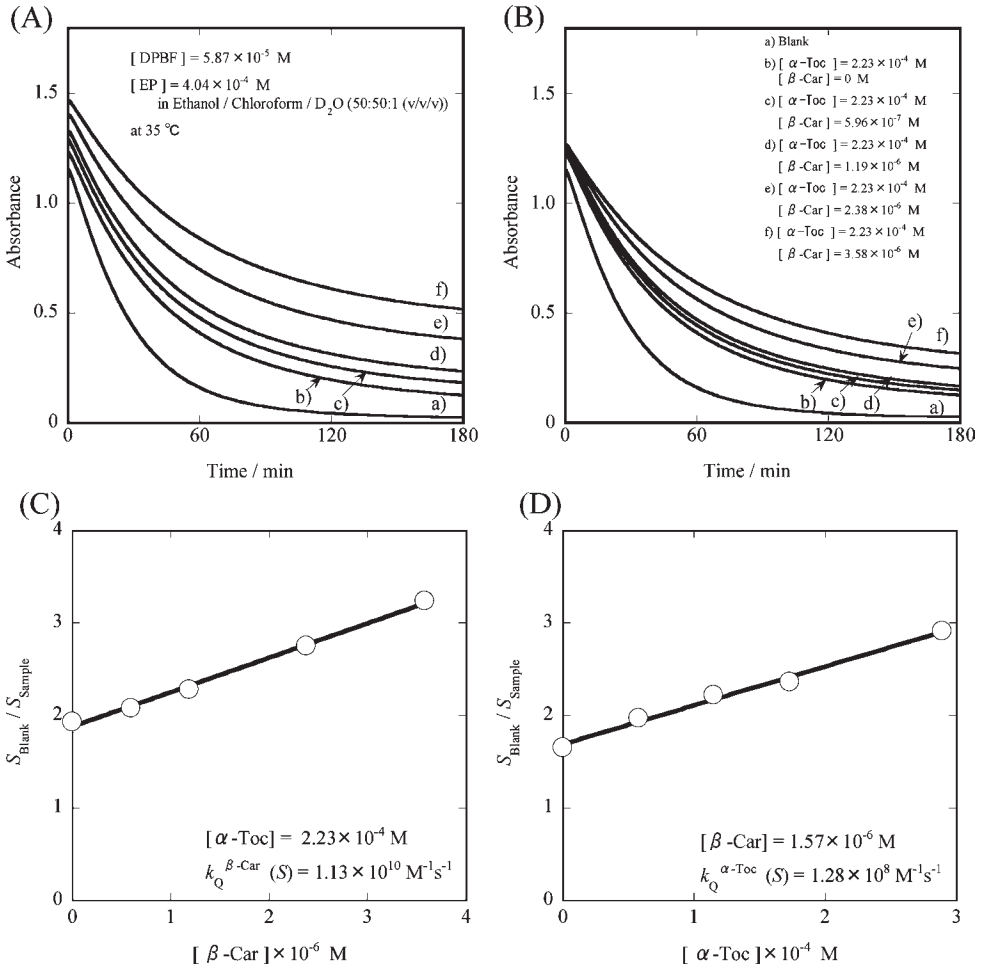


Figure 3. (A) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1O_2$ in the absence and presence of sample (β-carotene and α-tocopherol) in ethanol/chloroform/D_2O at 35 °C. $[DPBF]_{t=0} = 5.87 \times 10^{-5}$ M. and $[EP]_{t=0} = 4.04 \times 10^{-4}$ M. The values of $[\beta\text{-Car}]_{t=0}$ and $[\alpha\text{-Toc}]_{t=0}$ are shown in panel B. (B) Change in absorbance of DPBF, where the correction of baseline due to β-carotene was performed (see text). (C) Plot of S_{blank}/S_{sample} versus [β-Car]. [α-Toc] = 2.23×10^{-4} M. (D) Plot of S_{blank}/S_{sample} versus [α-Toc]. [β-Car] = 1.57×10^{-6} M.

large as $k_Q^{\alpha\text{-Toc}}(S)$ of α-tocopherol (see Table 4),[14] 2 orders of magnitude higher concentrations of α-tocopherol than those of β-carotene were used for the measurement (see Table 3c,d), so that the value of the product $\{k_Q^{\alpha\text{-Toc}}(S)\ [\alpha\text{-Toc}]\}/k_d$ in eq 7 is comparable to that of $\{k_Q^{\beta\text{-Car}}(S)\ [\beta\text{-Car}]\}/k_d$. If the value of the product is very small, the $k_Q(S)$ value of antioxidant estimated from the intercept at the y-axis will accompany a large experimental error. The $k_Q^{\beta\text{-Car}}(S)$ and $k_Q^{\alpha\text{-Toc}}(S)$ values obtained show good accordance with those reported for the solution including one component of antioxidant, respectively, as listed in Table 4.

Measurements of the $^1O_2$-Quenching Rates ($k_Q(S)$ and $k_Q(t_{1/2})$) and SOAC Values for Three Kinds of Vegetable Extract. The method of the preparation of red paprika, carrot, and tomato extracts was described under Material and Methods.[19] In the case of red paprika, three samples (red paprika-1, -2, -3) were prepared by repeating the extraction. Similarly, carrot and tomato extracts (carrot-1, -2, -3 and tomato-1, -2, -3) were prepared.

The measurement of relative SOAC value was performed in the following way: For example, the red paprika-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 red paprika-1 (see Table 5a) were prepared, where the concentration of red paprika-1 was defined as grams per liter (g/L) because we cannot use the mole concentration (M = mol/L) for red paprika-1. 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 5.01×10^{-4} M, that is, 2.16×10^{-1} g/L (see Table 5a).

The red paprika-1 extract shows an UV−vis absorption at 400−600 nm, suggesting that high concentrations of carotenoids are included in red paprika-1 (see Figure 4A).[33] Decay curves of the absorbance of DPBF due to the reaction with $^1O_2$ for red paprika-1 are shown in Figure 4B. Baseline corrections were performed by using an absorbance at 413 nm of UV−vis absorption spectrum in Figure 4A, and the decay curves corrected are shown in Figure 4C. ln[absorbance] versus t plots are shown in Figure 4D, indicating that the decay of DPBF for red paprika-1 also follows first-order kinetics at $\sim 10 < t < \sim 60$ min. The values of $S_{red\ paprika}$, $S_{\alpha\text{-Toc}}$, and S_{blank} and for $t_{1/2}^{red\ paprika}$, $t_{1/2}^{\alpha\text{-Toc}}$, and $t_{1/2}^{blank}$ obtained are listed in Table 5a. The values of $(t_{1/2}^{red\ paprika} \times S_{red\ paprika})/\ln 2$ obtained were found to be ∼1 (the values are not listed in Table 5a), indicating that the analysis of the rate constant ($k_Q(t_{1/2})$) using half-life ($t_{1/2}$) is appropriate.

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Table 5. Employed Concentrations, First-Order Decay Rates (S), and Half-Lives ($t_{1/2}$) of Blank (DPBF Only), α-Tocopherol, and Sample ((a) Red Paprika-1, (b) Carrot-1, and (c) Tomato-1), Relative SOAC Values, and Observed and Calculated $\{(S_{blank}/S_{sample}) - 1\}$ Values in Ethanol/Chloroform/D_2O

	blank	α-Toc	sample 1	sample 2	sample 3	sample 4
			(a) Red Paprika-1			
concn (g/L)	0	0.216	0.667	1.00	2.00	3.33
S_{sample} (s^{-1})	0.0440	0.0149	0.0239	0.0210	0.0139	0.0093
$t_{1/2}$ (min)	16.4	44.0	27.1	30.8	48.2	72.6
SOAC value			0.125	0.113	0.124	0.132 (av 0.124)
$(S_{blank}/S_{sample}) - 1$ (obsd)	0	1.94	0.84	1.10	2.17	3.73
$(S_{blank}/S_{sample}) - 1$ (calcd)			0.17	0.25	0.51	0.84
			(b) Carrot-1			
concn (g/L)	0	0.216	1.07	1.60	3.20	5.33
S_{sample} (s^{-1})	0.0342	0.0123	0.0225	0.0196	0.0140	0.0098
$t_{1/2}$ (min)	20.6	55.6	29.3	33.6	48.0	63.6
SOAC value			0.0503	0.0501	0.0528	0.0497 (av 0.0507)
$(S_{blank}/S_{sample}) - 1$ (obsd)	0	1.78	0.52	0.75	1.45	2.48
$(S_{blank}/S_{sample}) - 1$ (calcd)			0.49	0.74	1.47	2.46
			(c) Tomato-1[a]			
concn (g/L)	0	0.217	0.533	0.800	1.60	2.67
S_{sample} (s^{-1})	0.0384	0.0132	0.0288	0.0265	0.0200	0.0154
$t_{1/2}$ (min)	18.2	50.8	23.3	25.1	32.6	42.8
SOAC value			0.0635	0.0573	0.0598	0.0613 (av 0.0605)
$(S_{blank}/S_{sample}) - 1$ (obsd)	0	1.91	0.33	0.45	0.92	1.49
$(S_{blank}/S_{sample}) - 1$ (calcd)			0.22	0.33	0.67	1.12

[a] The data of tomato-1A are listed under (c) tomato-1.

$S_{blank}/S_{red\ paprika}$ and $t_{1/2}^{red\ paprika}/t_{1/2}^{blank}$ versus [red paprika] (by g/L unit) plots are shown in Figure 4, panels E and F, respectively. Both the $S_{blank}/S_{red\ paprika}$ and $t_{1/2}^{red\ paprika}/t_{1/2}^{blank}$ values increase linearly with increasing concentration of red paprika-1 ([red paprika]), and the plots show similar slopes, that is, similar rate constants ($k_Q^{red\ paprika}(S)$ and $k_Q^{red\ paprika}(t_{1/2})$), where the unit of $k_Q^{red\ paprika}$ is L g^{-1} s^{-1}. The $k_Q^{red\ paprika}(S)$ and $k_Q^{red\ paprika}(t_{1/2})$ values obtained are 3.35×10^4 and 3.12×10^4 L g^{-1} s^{-1}, respectively (see Table 6). The linear dependence of $S_{blank}/S_{red\ paprika}$ and $t_{1/2}^{red\ paprika}/t_{1/2}^{blank}$ values on [red paprika] suggests that the effects of the interactions between carotenoids included in red paprika and among the carotenoids and many compounds included in solution are negligible. Furthermore, the relative SOAC values (av 0.124) (given on a weight basis (g/L)) were determined, using eq 6, and are listed in Table 5a.

Similar measurements were performed for the carrot-1 and tomato-1 extracts. The results of analyses of the decay curves of DPBF are summarized in Table 5b,c. As observed for red paprika-1, both the S_{blank}/S_{sample} and $t_{1/2}^{sample}/t_{1/2}^{blank}$ values increase linearly with increasing concentration of carrot-1 and tomato-1, and the plots show similar slopes, that is, similar rate constants ($k_Q^{sample}(S)$ and $k_Q^{sample}(t_{1/2})$) for carrot-1 and tomato-1, respectively (see Table 6). The SOAC values were also determined, using eq 6 (see Table 5). Furthermore, in the case of tomato-1 extract, the measurements were repeated twice for the same extract (tomato-1A and -1B), by varying the concentrations of α-tocopherol and the extracts to ascertain the validity of the measurements. As listed in Table 6, the $k_Q^{sample}(S)$, $k_Q^{sample}(t_{1/2})$, and relative SOAC values obtained for tomato-1A and -1B are similar to each other.

Measurements were similarly performed for red paprika-2 and -3, carrot-2 and -3, and tomato-2 and -3 extracts. The observed rate constants ($k_Q^{sample}(S)$ and $k_Q^{sample}(t_{1/2})$) and relative SOAC values are listed in Table 6.

Concentrations of Carotenoids Included in Vegetable Extracts. Measurements of the concentrations of carotenoids included in vegetable extracts (red paprika, carrot, and tomato) were performed using a HPLC technique, according to the method reported in a previous work.[5] Analyses of the concentrations were performed for seven carotenoids (α-carotene, β-carotene, lutein, lycopene, capsanthin, zeaxanthin, and β-cryptoxanthin) included in vegetable extracts. However, zeaxanthin and β-cryptoxanthin were not detected, showing that the concentrations of these carotenoids are low in these extracts (< ~0.05 mg/100 g). The concentrations of five carotenoids included in vegetable extracts (red paprika-1, -2, -3, carrot-1, -2, -3, and tomato-1A, -1B, -2, and -3) are summarized in Table 7.

In the case of tomato-1 extract, the HPLC analyses were repeated twice for the same tomato-1 extracts (tomato-1A and -1B) to ascertain the reliability of the analysis. The concentrations of carotenoids obtained for tomato-1A and -1B are similar to each other, indicating that the method of analysis is reliable.

In the case of carrot extracts, the concentrations of α-carotene, β-carotene, and lutein included in carrot-1, -2, and -3 independently prepared were similar to one another, respectively. On the other hand, in the case of tomato extracts, the concentrations of four carotenoids included in tomato-2 and -3 were by 20−30% larger than the corresponding ones in tomato-1A and -1B (see Table 7). In the case of red paprika-1, -2, and -3, 20−30%

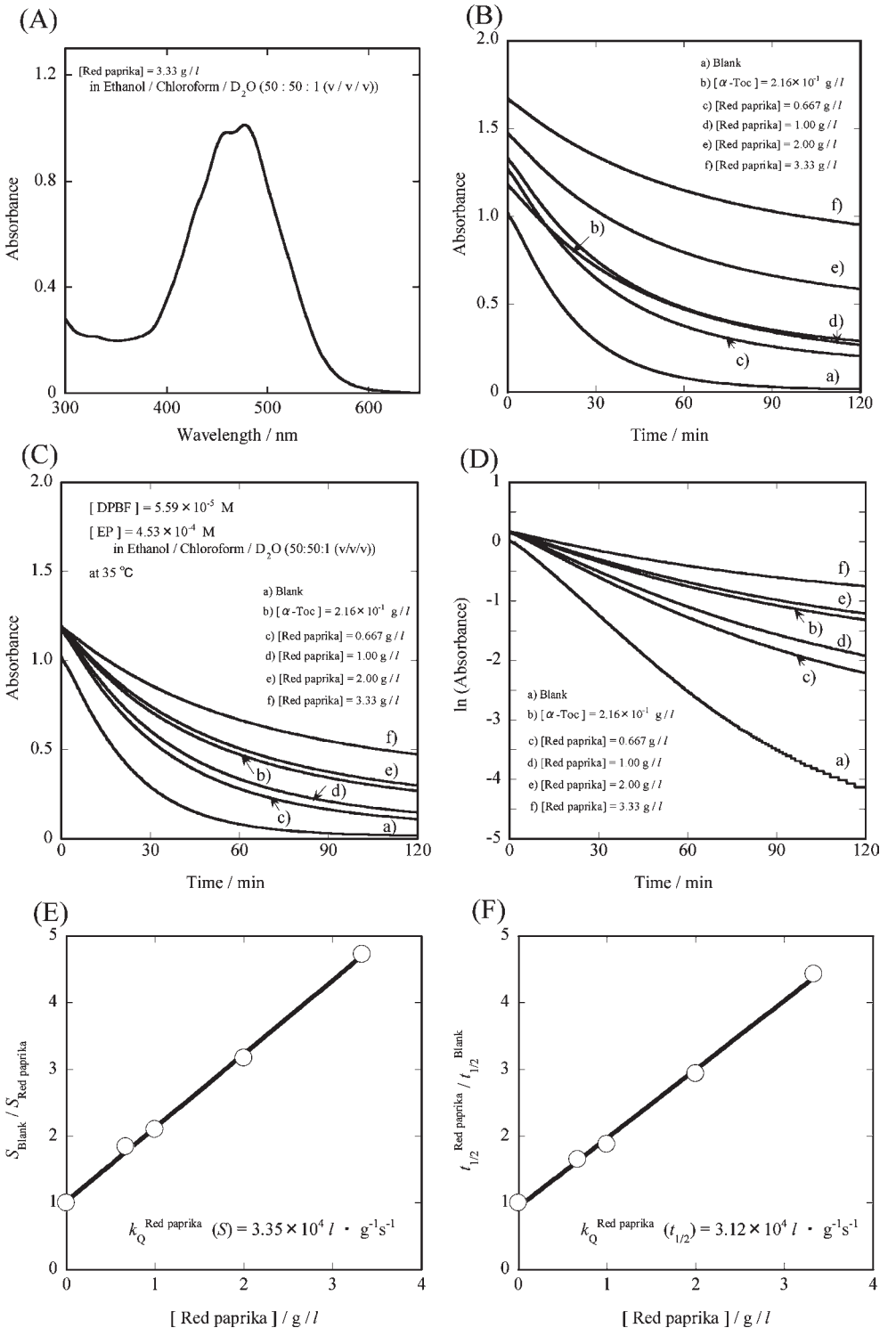

Figure 4. (A) Absorption spectrum of red paprika-1 extract in ethanol/chloroform/D_2O. The concentration of red paprika extract is 3.33 g/L. (B) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1O_2$ in the absence and presence of sample (α-tocopherol and red paprika extracts) in ethanol/chloroform/D_2O at 35 °C. $[DPBF]_{t=0} = 5.59 \times 10^{-5}$ M and $[EP]_{t=0} = 4.53 \times 10^{-4}$ M. The values of $[\alpha\text{-Toc}]_{t=0}$ and $[\text{red paprika}]_{t=0}$ are shown in panel C. (C) Change in absorbance of DPBF, where the correction of baseline due to red paprika extract was performed (see text). (D) Plot of ln(absorbance) versus *t*. (E) Plot of $S_{blank}/S_{red\ paprika}$ versus [red paprika]. (F) Plot of $t_{1/2}^{red\ paprika}/t_{1/2}^{blank}$ versus [red paprika].

differences in the concentration of carotenoids were observed, depending on the sample preparation. The differences will be due to the inhomogeneous distribution of carotenoids included in freeze-dried powder sample.

DISCUSSION

Relative SOAC Values for Eight Kinds of Carotenoids. In a previous work,[14] the SOAC assay method to assess the total

dx.doi.org/10.1021/jf104955a |J. Agric. Food Chem. 2011, 59, 3717–3729

Table 6. Rate Constants ($k_Q^{sample}(S)$ and $k_Q^{sample}(t_{1/2})$ (in L g^{-1} s^{-1})) Obtained from S_{blank}/S_{sample} versus [Sample] (in g/L) and $t_{1/2}^{sample}/t_{1/2}^{blank}$ versus [Sample] Plots, Respectively, the Ratios ($k_Q^{sample}(S)/k_Q^{\alpha\text{-Toc}}(S)$), 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})	$k_Q^{sample}(t_{1/2})$ (L g^{-1} s^{-1})	$k_Q^{sample}(S)/k_Q^{\alpha\text{-Toc}}(S)$	relative SOAC value
α-Toc	av 3.04×10^5	av 3.00×10^5	1.00	1.00
red paprika-1	3.35×10^4	3.12×10^4	0.110	0.124
red paprika-2	3.36×10^4	3.07×10^4	0.110	0.130
red paprika-3	2.57×10^4	2.38×10^4	0.0845	0.0977
	av 3.09×10^4[a]	av 2.86×10^4[a]	av 0.102	av 0.117
carrot-1	1.40×10^4	1.20×10^4	0.0460	0.0507
carrot-2	1.47×10^4	1.27×10^4	0.0483	0.0535
carrot-3	1.56×10^4	1.27×10^4	0.0513	0.0547
	av 1.48×10^4[a]	av 1.25×10^4[a]	av 0.0486	av 0.0530
tomato-1A	1.69×10^4	1.53×10^4	0.0556	0.0605
tomato-1B	1.62×10^4	1.48×10^4	0.0533	0.0596
tomato-2	2.04×10^4	1.86×10^4	0.0671	0.0842
tomato-3	2.05×10^4	1.91×10^4	0.0674	0.0865
	av 1.85×10^4[a]	av 1.70×10^4[a]	av 0.0608	av 0.0727

[a] Average values of $k_Q^{sample}(S)$ and $k_Q^{sample}(t_{1/2})$.

Table 7. Contents of Five Kinds of Carotenoids Included in Vegetable Extracts and Rate Constants ($k_Q^{sample}(S)$)

vegetable extract	α-carotene (mg/100 g)	β-carotene (mg/100 g)	lutein (mg/100 g)	lycopene (mg/100 g)	capsanthin (mg/100 g)	$k_Q^{sample}(S)$ (L g^{-1} s^{-1})
red paprika-1	7.93	21.30	nd[a]	nd	9.62	3.35×10^4
red paprika-2	9.30	24.48	nd	nd	11.24	3.36×10^4
red paprika-3	6.86	23.81	nd	nd	8.11	2.57×10^4
carrot-1	24.00	44.55	2.31	nd	nd	1.40×10^4
carrot-2	26.17	49.00	2.57	nd	nd	1.47×10^4
carrot-3	24.28	45.24	2.33	nd	nd	1.56×10^4
tomato-1A	0.57	5.19	0.23	43.33	nd	1.69×10^4
tomato-1B	0.52	5.44	0.20	44.41	nd	1.62×10^4
tomato-2	0.77	7.55	0.43	59.18	nd	2.04×10^4
tomato-3	0.64	6.24	0.31	50.17	nd	2.05×10^4

[a] nd, not detected because of low concentration.

quenching activity of singlet oxygen by carotenoids and phenolic antioxidants included in foods and plants was proposed. SOAC values were determined for only two carotenoids (β-carotene and astaxanthin). In the present work, measurements of the SOAC values were performed for eight carotenoids in ethanol/chloroform/D_2O solution. As described under Results, the measurements were performed by varying the concentrations of α-tocopherol and carotenoids to clarify the conditions that are necessary to obtain the correct SOAC value for carotenoids (and antioxidants).

As listed in Table 2, the SOAC values decrease in the order

$$\text{lycopene} > \text{astaxanthin} > \beta\text{-carotene} \sim \text{capsanthin} \sim \text{zeaxanthin} \sim \alpha\text{-carotene} > \text{lutein} > \beta\text{-cryptoxanthin} > > \alpha\text{-tocopherol} \quad (8)$$

However, the difference among the SOAC values of carotenoids is not remarkable. The value of lycopene is only 1.82 times larger than that of β-cryptoxanthin. On the other hand, the SOAC value of lycopene is 123 times larger than that of α-tocopherol. As reported in a previous work,[14] the $k_Q(S)$ and $k_Q(t_{1/2})$ values obtained for these carotenoids also decrease in the order of eq 8 (see Table 2), as expected from eq 5. The relative magnitudes of $k_Q(S)$, $k_Q(t_{1/2})$, and SOAC values, which are obtained with three different analytical methods (see eqs 3, 4, and 5, respectively), agree well with each other. The result indicates that these three methods are available to assess the singlet oxygen quenching activity of carotenoids (and antioxidants).

Relative SOAC Values for Three Kinds of Vegetable Extract. In a previous work,[14] application of the SOAC method to vegetable extracts (tomato and carrot) was performed, and the preliminary results obtained were reported. In the present work, relative SOAC values were measured for three kinds of vegetable extracts, and detailed analyses were performed to ascertain the validity of the application of the SOAC method to vegetable extracts.

As listed in Table 7, the concentrations of α-carotene, β-carotene, and lutein included in carrot-1, -2, and -3 are similar

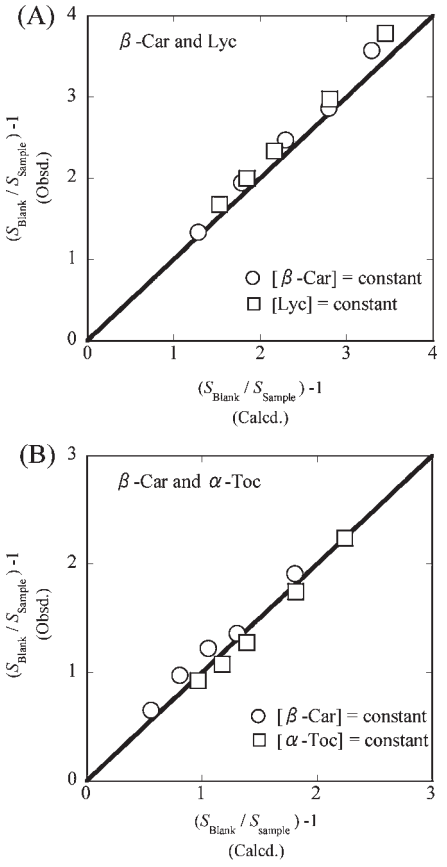

Figure 5. Plot of $\{(S_{blank}/S_{sample}) - 1\}_{obsd}$ versus $\{(S_{blank}/S_{sample}) - 1\}_{calcd}$: (A) β-carotene and lycopene; (B) β-carotene and α-tocopherol. Measurements of $\{(S_{blank}/S_{sample}) - 1\}_{obsd}$ values were performed for the solutions including two kinds of antioxidants in ethanol/chloroform/D_2O at 35 °C.

to each other. In fact, the $k_Q^{carrot}(S)$, $k_Q^{carrot}(t_{1/2})$, and relative SOAC values obtained for carrot-1, -2, and -3 are similar to each other, as listed in Table 6. The result indicates that both the methods of sample preparation and the measurement of rate constants used for carrot extracts are efficient and reliable.

On the other hand, in the case of tomato extracts, the concentrations of four carotenoids included in tomato-2 and -3 extracts are by 20−30% larger than the corresponding ones in tomato-1A (and -1B) extract (see Table 7). In fact, the $k_Q^{tomato}(S)$, $k_Q^{tomato}(t_{1/2})$, and SOAC values obtained for tomato-2 and -3 are by 20−30% larger than the corresponding those for tomato-1A (and -1B), as listed in Table 6. The difference in the rate constants (and SOAC values) seems to be due to that in the concentrations of carotenoids included in tomato-1, -2, and -3.

In the case of red paprika-1, -2, and -3, good correlations between the rate constants ($k_Q^{tomato}(S)$ and $k_Q^{tomato}(t_{1/2})$) (and SOAC values) and the concentrations of carotenoids included in red paprika extracts were not observed. As described in the following section, the concentrations of only about 30% of carotenids included in red paprika extracts were analyzed in the present work, using a HPLC technique.[33] It will be necessary to analyze the concentrations of the other carotenoids included in red paprika extracts in order to discuss the details of the relation between the rate constants and the carotenoid concentrations in red paprika.

The average values of $k_Q^{tomato}(S)$, $k_Q^{carrot}(t_{1/2})$, and SOAC are summarized in Table 6. As listed in Table 6, the relative SOAC values for vegetable extracts decrease in the order of eq 9.

$$\text{red paprika} > \text{tomato} > \text{carrot} \quad (9)$$

The SOAC value for red paprika is 2.2 times larger than that for carrot.

Comparison between Observed and Calculated $^1O_2$-Quenching Activities for Mixtures of Two Kinds of Antioxidants. As described under Results, measurements of the $k_Q(S)$ values were performed for the mixtures of two kinds of antioxidants. The S_{blank}/S_{sample} values for the solutions including β-carotene and lycopene (see Figure 2C,D) can be determined by analyzing the decay curve of DPBF in solution. On the other hand, the S_{blank}/S_{sample} values can also be calculated by using eq 7, the concentrations of β-carotene and lycopene used, and the $k_Q^{\beta\text{-Car}}(S)$ and $k_Q^{Lyc}(S)$ values in Table 2. The calculated $\{(S_{blank}/S_{sample}) - 1\}_{calcd}$ values are listed in Table 3a,b, together with the those observed. The observed $\{(S_{blank}/S_{sample}) - 1\}_{obsd}$ values were plotted against the calculated $\{(S_{blank}/S_{sample}) - 1\}_{calcd}$ ones. As shown in Figure 5A, the observed and calculated values showed good agreement with each other.

Similar calculations were performed for the mixtures including β-carotene and α-tocopherol. The calculated $\{(S_{blank}/S_{sample}) - 1\}_{calcd}$ values are listed in Table 3c,d. As shown in Figure 5B, good agreement between the observed $\{(S_{blank}/S_{sample}) - 1\}_{obsd}$ and calculated $\{(S_{blank}/S_{sample}) - 1\}_{calcd}$ values was obtained. The results indicate that the total $^1O_2$-quenching activity may be calculated as the sum of the products of the rate constants ($k_Q^{antioxidant}(S)$) and the concentrations ([antioxidant]) of the antioxidants included in the solution.

β-Carotene, lycopene, and α-tocopherol coexist in many biological systems, such as vegetables and fruits[27−29] and human tissue.[30−32] Our result suggests that we can estimate the total $^1O_2$-quenching activity (that is, the total SOAC value) for the solutions including various antixoidants. It will be necessary to estimate the total $^1O_2$-quenching activity for the vegetable extracts used in the present work.

Comparison between Observed and Calculated $^1O_2$-Quenching Activities for Three Kinds of Vegetable Extract. As performed for the mixtures of two kinds of carotenoids (or antioxidants), the S_{blank}/S_{sample} values for vegetable extracts may be calculated as the sum of the product $\{\Sigma k_Q^{Car\text{-}i}(S)\,[Car\text{-}i]\}/k_d$ of the rate constant ($k_Q^{Car\text{-}i}(S)$) and the concentration ([Car-i]) of carotenoids included.

$$S_{blank}/S_{sample} = 1 + \{\sum_i k_Q^{Car\text{-}i}(S)[Car\text{-}i]\}/k_d \quad (10)$$

Three carotenoids (α-carotene, β-carotene, and lutein) are included in carrot, as listed in Table 7. Consequently, the calculations of the S_{blank}/S_{sample} values were performed for carrot-1 extract, using eq 10. The $k_Q^{Car\text{-}i}(S)$ values used are listed in Table 2. As listed in Table 5b, good accordance between observed and calculated $\{(S_{blank}/S_{sample}) - 1\}$ values was obtained for carrot-1. Similar calculations were performed for carrot-2 and -3 (data not shown). As shown in Figure 6, the $\{(S_{blank}/S_{sample}) - 1\}_{obsd}$ values observed for carrot-1, -2, and -3 extracts were plotted against the $\{(S_{blank}/S_{sample}) - 1\}_{calcd}$ values calculated. Good agreement between the observed and calculated values was obtained, and the gradient for the $\{(S_{blank}/S_{sample}) - 1\}_{obsd}$ versus $\{(S_{blank}/S_{sample}) - 1\}_{calcd}$ plot was ~1. The result indicates that the total $^1O_2$-quenching activity of carrot may be

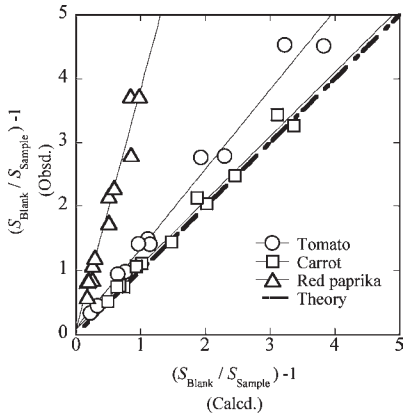

Figure 6. Plot of $\{(S_{blank}/S_{sample}) - 1\}_{obsd}$ versus $\{(S_{blank}/S_{sample}) - 1\}_{calcd}$: (○) tomato-1A, -2, and -3; (□) carrot-1, -2, and -3; (△) red paprika-1, -2, and -3. Measurements of $\{(S_{blank}/S_{sample}) - 1\}_{obsd}$ values were performed for the solutions including vegetable extracts (tomato, carrot, and red paprika) in ethanol/chloroform/D_2O at 35 °C.

explained by considering the contribution of only three carotenoids (α-carotene, β-carotene, and lutein) included in carrot. The contribution of the other antioxidants is negligible. Furthermore, the result suggests that the interactions between carotenoids and among carotenoids and many molecules included in carrot extracts are weak and negligible in solution.

As listed in Table 7, four carotenoids (α-carotene, β-carotene, lutein, and lycopene) are included in tomato extracts. Consequently, the calculations of the $\{(S_{blank}/S_{sample}) - 1\}_{calcd}$ values were performed for tomato-1A extracts, using eq 10. As listed in Table 5c, the observed $\{(S_{blank}/S_{sample}) - 1\}_{obsd}$ values are by about 20−30% larger than the calculated ones, suggesting contribution from other carotenoids included in tomato extracts. Similar calculations of the $\{(S_{blank}/S_{sample}) - 1\}_{calcd}$ values were performed for tomato extracts (tomato-2 and -3). As shown in Figure 6, the observed values for tomato-1A, -2, and -3 were plotted against the calculated ones. The gradient obtained for the plot is ∼1.3, suggesting ∼30% of contribution due to the other antioxidants. In fact, it has been reported that, in addition to the above four carotenoids, high concentrations (20−30%) of the other carotenoids (such as phytoene and phytofluene) are included in tomato extracts.[34−36]

In the case of red paprika, similar calculations were performed, using the concentrations of three carotenoids (α-carotene, β-carotene, and capsanthin) included (see Table 7). However, as listed in Table 5a and as shown in Figure 6, the observed $\{(S_{blank}/S_{sample}) - 1\}_{obsd}$ values could not be explained by the calculated $\{(S_{blank}/S_{sample}) - 1\}_{calcd}$ ones. As reported in a previous work,[33] many other carotenoids are included in red paprika extracts, and only about 30% of the contents of carotenoids are analyzed in the present work.

In the present work, measurements of the $^1O_2$-quenching rates ($k_Q(S)$ and $k_Q(t_{1/2})$) and the relative SOAC values were performed for eight carotenoids, mixtures of two kinds of antioxidants, and three kinds of vegetable extracts (red paprika, carrot, and tomato) in ethanol/chloroform/D_2O (50:50:1, v/v/v) solution, by using a competition reaction method. Furthermore, measurements of the concentrations of the carotenoids included in vegetable extracts were performed, using a HPLC technique. From the results, it has been clarified that the total $^1O_2$-quenching activity (that is, the relative SOAC value) for vegetable extracts may be explained as the sum of the product $\{\Sigma k_Q^{Car\text{-}i}(S)[Car\text{-}i]_i\}$ of the rate constant ($k_Q^{Car\text{-}i}(S)$) and the concentration ([Car (i)]) of carotenoids included in vegetable extracts. The result suggests that the interactions between carotenoids and among carotenoids and many molecules included in carrot extracts are weak and negligible in solution.

Measurements of the SOAC values for phenolic antioxidants, such as tocopherol homologues, caffeic acids, catechins, and flavone derivatives, and many vegetable and fruit extracts including mainly phenolic antioxidants are now in progress in our laboratory.

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Funding Sources

This work was partly supported by a grant from SUNATEC, Japan (to K.M. and S.N.).

ACKNOWLEDGMENT

We are very grateful to Dr. Akihiko Nagao, Head of the Lipid Laboratory of the National Food Research Institute, for his helpful discussions. We are also grateful to Takashi Hirose, General Manager of Kagome Co. Ltd., for his continuous encouragement throughout this work.

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