Review

Antioxidant Activity of Foods: Development of Singlet Oxygen Absorption Capacity (SOAC) Assay Method

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Summary Recently a new assay method that can quantify the singlet oxygen absorption capacity (SOAC) of antioxidants (AOs) and food extracts was proposed. Singlet oxygen ($^1\text{O}_2$) quenching rates (k_Q) and the relative SOAC values were measured for many carotenoids and phenolic AOs in ethanol/chloroform/ D_2O (50 : 50 : 1, v/v/v) solution at 35°C using UV-vis spectrophotometry. It has been clarified that the SOAC method is useful to evaluate the $^1\text{O}_2$ -quenching activity of lipophilic and hydrophilic AOs having 5 orders of magnitude different rate constants (k_Q). Measurements of the k_Q and SOAC values were also performed for 39 kinds of food extracts. The results indicate that the SOAC method is useful to evaluate the $^1\text{O}_2$ -quenching activity of food extracts having two orders of magnitude different k_Q values. Further, the k_Q values for the reaction of $^1\text{O}_2$ with 8 carotenoids and 8 vitamin E homologues were measured in an aqueous Triton X-100 (5.0 wt %) micellar solution (pH 7.4). Results obtained demonstrate that the k_Q values of AOs in homogeneous and heterogeneous solutions vary notably depending on (i) polarity (dielectric constant (ϵ)) of the reaction field between AOs and $^1\text{O}_2$, (ii) local concentration of AOs, and (iii) mobility of AOs in solution. Measurements of k_Q and SOAC values in a micellar solution may be useful for evaluating the $^1\text{O}_2$ -quenching activity of AOs in biological systems. Furthermore, measurements of the SOAC values were performed for 32 kinds of food extracts using a microplate reader. The SOAC assay method was validated by inter-laboratory validation study due to 14 laboratories.

Key Words quenching rate, SOAC value, antioxidants, food extracts, carotenoids, vitamin E homologues, polyphenols, UV-vis spectrophotometry, microplate reader

Singlet oxygen ($^1\text{O}_2$) has attracted much attention as a biological oxidant (1, 2). In biological systems, $^1\text{O}_2$ is generated by type II photosensitization reaction, which is well known as the light-dependent $^1\text{O}_2$ generation mechanism (3, 4). $^1\text{O}_2$ is also generated by the light-independent mechanisms: that is, the reaction between two peroxy radicals ($\text{LOO}\cdot$) (i.e. Russell mechanism) (5, 6) and the reaction of hypochlorite ion (OCl^-) with hydrogen peroxide (H_2O_2) in macrophage (7, 8). Further, $^1\text{O}_2$ generation by a cytochrome *c* (cyt *c*)–cardiolipin (CL) complex in a model membrane containing CL was proposed (9).

$^1\text{O}_2$ reacts with many kinds of biological targets including lipids (10–12), proteins (3, 4), and DNA (13–15). Reactions with $^1\text{O}_2$ occur mainly by chemical reaction, inducing the degradation of biological systems. Many kinds of natural antioxidants (AOs) (such as carotenoids, vitamin E homologues, polyphenols, etc.) are widely present in foods, plants and biological systems (16–21) and may function as efficient $^1\text{O}_2$ quenchers (22–26).

Vitamin E homologues (i.e. D- α -, β -, γ -, δ -tocopherol and -tocotrienol (α -, β -, γ -, δ -Toc and -Toc-3)) can act as an efficient quencher of $^1\text{O}_2$ (27, 28). It was shown

that α -Toc quenches $^1\text{O}_2$ by a combination of physical quenching (k_q) and chemical reaction (chemical quenching, k_r). Because $k_q \gg k_r$, the quenching process is almost entirely “physical,” that is, α -tocopherol deactivates ~ 120 $^1\text{O}_2$ molecules before being destroyed by chemical reaction (Eq. (1)) (29).

As general phenolic AOs are easily soluble in ethanol, measurements of k_Q values were performed in ethanol, in previous works (27, 28). On the other hand, carotenoids (Cars) generally show low (or very low) solubility in ethanol, and, thus, ethanol/chloroform/ D_2O (50 : 50 : 1, v/v/v) solution (abbreviated as “mixed solvent”) was used for the measurements of the k_Q values for Cars (30).

Recently, singlet oxygen absorption capacity (SOAC) assay method was developed in our laboratory, in order to evaluate the $^1\text{O}_2$ -quenching activity of natural AOs and food and plant extracts (28, 31–36). The method is as follows: first, the second-order rate constants (k_Q) for the reaction of eight kinds of Cars and α -Toc (Table 1) with $^1\text{O}_2$ were measured in mixed solvent, using the competition reaction method, where endoperoxide (EP) was

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Table 1. Second-order rate constants ($k_Q^{AO}(S)$ and $k_Q^{AO}(t_{1/2})$) of eight carotenoids and α -tocopherol obtained from S_{blank}/S_{AO} vs $[AO]$ and $t_{1/2}^{AO}/t_{1/2}^{blank}$ vs $[AO]$ plots, respectively, relative rate constants ($k_Q^{AO}(S)/k_Q^{\alpha-Toc}(S)$), and relative SOAC values in ethanol/chloroform/D₂O solution.

Antioxidant (AO)	$k_Q^{AO}(S)$ /M ⁻¹ s ⁻¹	$k_Q^{AO}(t_{1/2})$ /M ⁻¹ s ⁻¹	$k_Q^{AO}(S)/k_Q^{\alpha-Toc}(S)$	Relative SOAC value
α -Tocopherol	av 1.31×10^{8a}	av 1.29×10^{8a}	1.00	1.00
Lycopene	av 1.40×10^{10a}	av 1.23×10^{10a}	av 107 ^b	av 123 ^b
Astaxanthin	av 1.17×10^{10a}	av 1.08×10^{10a}	av 89.3 ^b	av 109 ^b
β -Carotene	av 1.08×10^{10a}	av 1.02×10^{10a}	av 82.2 ^b	av 95.8 ^b
Capsanthin	1.21×10^{10}	1.07×10^{10}	107	99.3
Zeaxanthin	1.12×10^{10}	1.23×10^{10}	88.6	92.8
α -Carotene	av 1.00×10^{10a}	av 9.20×10^{9a}	av 75.8 ^b	av 92.4 ^b
Lutein	8.07×10^9	7.31×10^9	67.9	73.8
β -Cryptoxanthin	7.04×10^9	6.72×10^9	60.9	67.6

^a Average values of $k_Q^{AO}(S)$ and $k_Q^{AO}(t_{1/2})$ obtained (Ref. (32)).

^b Experimental errors in the rate constants and relative SOAC values (av) were estimated to be <10%.

used as a 1O_2 generator and 2,5-diphenyl-3,4-benzofuran (DPBF) as an UV-vis absorption probe (see Scheme 1) (31, 32). 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, where an UV-vis spectrophotometer, equipped with a six-channel cell-positioner, was used for measurement.

Second, the measurements were performed for various phenolic AOs, containing vitamin E homologues and polyphenols in mixed solvent (Table 2) (33). Examples for the measurement of $k_Q(S)$ and $k_Q(t_{1/2})$ values for γ -tocopherol and β -carotene were shown in Fig. 1 and Fig. 2, respectively. Third, the $k_Q(S)$ and $k_Q(t_{1/2})$ values were also measured for many food and plant extracts containing various AOs (28, 34–36).

From the results, a new assay method that can quantify the SOAC of AOs, including Cars, phenolic AOs and food extracts, was proposed (31–33). Relative SOAC value was defined in the following way.

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

where $[\alpha-Toc]$ and $[AO]$ are molar concentrations (M=mol/L) (or weight concentrations (g/L)) of α -Toc and AO, respectively. Equation (2) indicates that the AOs with fast 1O_2 -quenching rate constants (k_Q) show large

relative SOAC values (that is, high 1O_2 -quenching activity and high antioxidant activity). α -Tocopherol was used as a standard compound of SOAC assay. The reason was described in a previous study (31).

Further, measurements of the SOAC values for natural AOs (such as Cars, vitamin E homologues) and palm oil extracts were performed in an aqueous Triton X-100 (5.0 wt %) micellar solution (pH 7.4, 35°C), which was used as a simple model of biomembranes (37, 38).

The SOAC values were also measured for eight representative Cars, α -Toc, and various vegetable and fruit extracts using a versatile instrument, the microplate reader (39, 40). A validation test was also performed for the above AOs (41).

1. Materials

Abbreviations used for AOs are as follows (see Table 1 (32) and Table 2 (33)): (i) Tocopherol derivatives: D- α -, β -, γ -, δ -tocopherol and -tocotrienols (α -, β -, γ -, δ -Tocs and -Toc-3s), tocol, and trolox. (ii) Caffeic acids: isoeugenol (IE), caffeic acid (CA), and ferulic acid (FA). (iii) Catechins and related compounds: epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), 4-methylcatechol (4-MC), and 4-methyl gallate (4-MG). (iv) Carotenoids (Cars): astaxanthin (Ast), lutein (Lut), β -cryptoxanthin (β -Cry), zeaxanthin (Zea), capsanthin (Cap), α - and β -carotene (α - and β -Car), and lycopene (Lyc). (v) The others: ubiquinol-10 (UQ₁₀H₂) and vitamin C (Vit C).

DPBF was obtained from Tokyo Kasei Organic Chemicals, Japan. 3-(1,4-Epidioxy-4-methyl-1,4-dihydro-1-naphthyl)propionic acid (endoperoxide, EP) (Scheme 1) was obtained from Wakenyaku Co. Ltd., Japan. The result of the measurement of the UV spectrum of EP indicates that the powder sample of EP includes 95% EP and 5% EP-precursor unreacted (31, 32).

2. Measurements of Rate Constants (k_Q) and Molar Extinction Coefficient (ϵ_{max})

Measurements of rate constants (k_Q) were performed

Fig. 1. (A) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1\text{O}_2$ in the absence and presence of sample (α - or γ -tocopherol) in ethanol/chloroform/D₂O solution at 35 °C. [DPBF]_{*t*=0} = 7.84×10^{-5} M and [EP]_{*t*=0} = 7.40×10^{-4} M. The values of [α -Toc]_{*t*=0} and [γ -Toc]_{*t*=0} are shown in A. (B) Plot of ln (Absorbance) vs time. (C) Plot of $S_{\text{blank}}/S_{\gamma\text{-Toc}}$ vs [γ -Toc]. (D) Plot of $t_{1/2}^{\gamma\text{-Toc}}/t_{1/2}^{\text{blank}}$ vs [γ -Toc].

in (i) ethanol/chloroform/D₂O (50 : 50 : 1, v/v/v), (ii) ethanol, and (iii) an aqueous Triton X-100 micellar (5.0 wt %) solution (pH=7.4) by using a Shimadzu UV-vis spectrophotometer (UV-1800) (Kyoto, Japan), equipped with a six-channel cell-positioner and an electron-temperature control unit (CPS-240A). A micro-plate reader (SH-9000Lab; Corona Electric, Ibaraki, Japan) was also used for the measurement of k_Q value (39, 40). All the measurements were performed at $35.0 \pm 0.5^\circ\text{C}$. Detailed experimental conditions used for measurements were described in previous works (31, 32, 39). The values of molar extinction coefficient (ϵ_{max}) of Cars were determined, using Lambert-Beer's equation (Absorbance = ϵ_{max} [Carotenoid]).

3. Analyses of the Second-Order Rate Constants ($k_Q^{\text{AO}}(S)$ and $k_Q^{\text{AO}}(t_{1/2})$)

The rate constant $k_Q^{\text{AO}}(S)$ for the reaction of $^1\text{O}_2$ with AO was determined by Eq. (3), as reported in previous studies (31, 32, 42, 43).

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

where S_{blank} and S_{AO} are slopes of the first-order plots

(that is, ln (Absorbance) vs time plots) of disappearance of DPBF in the absence and presence of AO, respectively (Fig. 1B). k_d is the rate of natural deactivation of $^1\text{O}_2$ in the ethanol/chloroform/D₂O ($k_d = 3.03 \times 10^4$) (26) and ethanol ($k_d = 8.3 \times 10^4 \text{ s}^{-1}$) (44). Equation (3) indicates that the $k_Q^{\text{AO}}(S)$ value can be obtained from $S_{\text{blank}}/S_{\text{AO}}$ versus [AO] plot (Fig. 1C).

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

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

where $t_{1/2}^{\text{blank}}$ and $t_{1/2}^{\text{AO}}$ are the half-lives of DPBF in the absence and presence of AO, respectively (Fig. 1A). Equation (4) indicates that the $k_Q^{\text{AO}}(t_{1/2})$ value can be obtained from $t_{1/2}^{\text{AO}}/t_{1/2}^{\text{blank}}$ vs [AO] plot (Fig. 1D). If AO is α -Toc, we can obtain similar Eq. (5) for α -Toc.

$$t_{1/2}^{\alpha\text{-Toc}}/t_{1/2}^{\text{blank}} = 1 + \{k_Q^{\alpha\text{-Toc}}(t_{1/2}) [\alpha\text{-Toc}]\}/k_d \quad (5)$$

4. Development of Singlet Oxygen Absorption Capacity (SOAC) Assay Method

By eliminating k_d from Eqs. (4) and (5), we can obtain the following relation.

Table 2. The $k_Q^{AO}(S)$ and $k_Q^{AO}(t_{1/2})$ values for many kinds of antioxidants (AO) in ethanol/chloroform/D₂O solution at 35.0°C, relative rate constants ($k_Q^{AO}(S)/k_Q^{\alpha-Toc}(S)$), and relative SOAC values.

Antioxidant (AO)	$k_Q^{AO}(S)^a$ /M ⁻¹ s ⁻¹	$k_Q^{AO}(t_{1/2})^a$ /M ⁻¹ s ⁻¹	$k_Q^{AO}(S)/k_Q^{\alpha-Toc}(S)$	Relative SOAC value ^a
α -Toc 1	1.31×10^8	1.29×10^8	1.00	1.00
β -Toc 2	9.30×10^7	8.34×10^7	0.710	0.806–0.922 (av 0.866)
γ -Toc 3	8.44×10^7	7.09×10^7	0.644	0.666–0.764 (av 0.706)
δ -Toc 4	4.11×10^7	3.86×10^7	0.314	0.369–0.394 (av 0.380)
tocol 5	1.84×10^7	1.61×10^7	0.140	0.149–0.174 (av 0.162)
trolox 6	4.20×10^7	3.66×10^7	0.321	0.327–0.369 (av 0.349)
UQ ₁₀ H ₂ 7	6.22×10^7	5.86×10^7	0.475	0.468–0.594 (av 0.521)
IE 8	2.98×10^6	2.53×10^6	0.0227	0.0242–0.0278 (av 0.0258)
CA 9	6.85×10^5	5.85×10^5	0.00523	0.00637–0.00679 (av 0.00659)
FA 10	2.71×10^5	2.08×10^5	0.00207	0.00217–0.00257 (av 0.00228)
EC 11	8.31×10^6	7.85×10^6	0.0634	0.0724–0.0814 (av 0.0752)
EGC 12	1.31×10^7	1.30×10^7	0.100	0.117–0.129 (av 0.122)
ECG 13	4.94×10^6	3.61×10^6	0.0377	0.0367, 0.0383 (av 0.0375)
EGCG 14	5.05×10^6	4.27×10^6	0.0385	0.0442, 0.0405 (av 0.0424)
4-MC 15	4.96×10^6	4.12×10^6	0.0379	0.0421–0.0495 (av 0.0474)
4-MG 16	1.34×10^5	1.05×10^5	0.00102	0.00114–0.00126 (av 0.00119)
Vit C 17	1.92×10^6	1.88×10^6	0.0147	0.016, 0.016 (av 0.016)

^a The experimental errors in the rate constants ($k_Q^{AO}(S)$ and $k_Q^{AO}(t_{1/2})$) and relative SOAC values were estimated to be <10% (see Refs. (31) and (32)).

$$\begin{aligned} & (t_{1/2}^{AO} - t_{1/2}^{\text{blank}}) / (t_{1/2}^{\alpha-Toc} - t_{1/2}^{\text{blank}}) \\ & = k_Q^{AO} [AO] / k_Q^{\alpha-Toc} [\alpha-Toc] \end{aligned} \quad (6)$$

Equation (6) indicates that the half-life ($t_{1/2}^{AO}$) of the AO increases linearly with increasing the concentration of the AO and depends on the product of k_Q^{AO} and [AO], if the concentration of α -Toc is constant. From Eq. (6), we can derive Eq. (2) and define the relative SOAC value. Equation (2) indicates that the SOAC value corresponds to the ratio ($k_Q^{AO}/k_Q^{\alpha-Toc}$) of the quenching rate (k_Q^{AO}) of ¹O₂ by AO to that ($k_Q^{\alpha-Toc}$) by α -Toc. According to Eq. (2), the SOAC value was determined by the measurement of the half-life ($t_{1/2}$) of DPBF.

5. ¹O₂-Quenching Rates ($k_Q(S)$ and $k_Q(t_{1/2})$) and SOAC Values for 16 Phenolic Antioxidants

Measurements of $k_Q(S)$, $k_Q(t_{1/2})$, and SOAC values were performed for 16 kinds of phenolic AOs, including tocopherol derivatives (α -, β -, γ -, δ -Toc, tocol, trolox), ubiquinol-10, caffeic acids (IE, CA, FA), and Vit C (Table 2) (33). Figure 1A shows an example of measurement of the reaction between DPBF and EP in the absence ((a) blank) and presence of AOs ((b) [α -Toc] = 5.00×10^{-4} M, (c) [γ -Toc] = 2.22×10^{-4} M, (d) [γ -Toc] = 4.44×10^{-4} M, (e) [γ -Toc] = 7.77×10^{-4} M, (f) [γ -Toc] = 1.11×10^{-3} M) in mixed solvent at 35°C. The disappearance of DPBF at λ_{max} = 413 nm due to the chemical reaction with ¹O₂ was observed. The values of first-order decay rate constant (S_{blank} , $S_{\alpha-Toc}$, $S_{\gamma-Toc}$) (Table 3) were estimated by analyzing the decay curve of DPBF, as shown in Fig. 1B. The analysis of the decay curve was performed at $\sim 5 < t < \sim 60$ min. This is an important condition to obtain the correct rate constant (k_Q) for AOs (31–33). The values of half-life ($t_{1/2}^{\alpha-Toc}$, $t_{1/2}^{\gamma-Toc}$, $t_{1/2}^{\text{blank}}$) were

calculated carefully according to the method described in a previous work (31). The values obtained are summarized in Table 3.

Plots of $S_{\text{blank}}/S_{\gamma-Toc}$ and $t_{1/2}^{\gamma-Toc}/t_{1/2}^{\text{blank}}$ vs [γ -Toc] are shown in Fig. 1C and D, respectively. The $k_Q^{\gamma-Toc}(S)$ and $k_Q^{\gamma-Toc}(t_{1/2})$ values obtained by using Eqs. (3) and (4) are 8.44×10^7 and 7.09×10^7 M⁻¹s⁻¹, respectively. As the measurements were performed for one concentration of α -Toc and four concentrations of γ -Toc (AO-1–AO-4), we can determine four sets of relative SOAC values, using Eq. (2) (Table 3). The relative SOAC values (0.666–0.764, av = 0.706) obtained for γ -Toc are similar to each other and agree well with the ratio of the quenching rate constant of γ -Toc to that of α -Toc ($k_Q^{\gamma-Toc}(S)/k_Q^{\alpha-Toc}(S)$ = 0.644), as expected from Eq. (2). Similar measurements were performed for β - and δ -Toc, tocol, trolox, and ubiquinol-10. The rate constants ($k_Q^{AO}(S)$ and $k_Q^{AO}(t_{1/2})$) and the relative SOAC values obtained are listed in Table 2.

On the other hand, in the case of FA, the rate constant ($k_Q(S)$) is two–three orders of magnitude smaller than that of γ -Toc (Table 2). Consequently, we had to use two–three orders of magnitude higher concentrations to obtain reliable $k_Q(S)$ and SOAC values, as anticipated from Eqs. (3) and (6), respectively (see Table 3). We could not obtain the reliable SOAC value, when the difference between the half-lives of AO and blank ($t_{1/2}^{AO} - t_{1/2}^{\text{blank}}$) (that is, the value of a numerator in Eq. (6)) was smaller than ~ 5 min (data are not shown) (33). The rate constants and the SOAC values obtained for caffeic acids (IE, CA, and FA) are summarized in Tables 2 and 3.

Similar measurements were performed for EC, EGC, ECG, EGCG, 4-MC, and 4-MG, by varying concentrations

Table 3. Employed concentrations, first-order decay rates (S), and half-lives ($t_{1/2}$) of blank (DPBF only), α -tocopherol, and antioxidants (AOs) ((a) γ -tocopherol, (b) ferulic acid, (c) epicatechin, and (d) epicatechin gallate) and relative SOAC values in ethanol/chloroform/D₂O solution.

(a) γ -Tocopherol

	Blank	α -Toc	AO-1	AO-2	AO-3	AO-4
Concn (M)	0	5.19×10^{-4}	2.22×10^{-4}	4.44×10^{-4}	7.77×10^{-4}	1.11×10^{-3}
S_{AO} (s ⁻¹)	0.0255	0.0088	0.0148	0.0112	0.0083	0.0061
$t_{1/2}$ (min)	26.6	71.0	39.6	51.9	77.4	94.0
Relative SOAC value	—	—	0.685	0.666	0.764	0.710 (av. 0.706)

(b) Ferulic acid

	Blank	α -Toc	AO-1	AO-2	AO-3	AO-4
Concn (M)	0	5.04×10^{-4}	2.42×10^{-2}	4.85×10^{-2}	9.69×10^{-2}	1.21×10^{-1}
S_{AO} (s ⁻¹)	0.0360	0.0124	0.0282	0.0243	0.0191	0.0170
$t_{1/2}$ (min)	26.4	60.5	30.6	33.6	40.6	44.5
Relative SOAC value	—	—	0.00257	0.00219	0.00217	0.00221 (av. 0.00228)

(c) Epicatechin

	Blank	α -Toc	AO-1	AO-2	AO-3	AO-4
Concn (M)	0	5.28×10^{-4}	4.63×10^{-3}	6.94×10^{-3}	9.25×10^{-3}	1.16×10^{-2}
S_{AO} (s ⁻¹)	0.0315	0.0113	0.0140	0.0116	0.0094	0.0074
$t_{1/2}$ (min)	23.0	62.8	48.7	61.4	73.5	94.2
Relative SOAC value	—	—	0.0736	0.0734	0.0724	0.0814 (av. 0.0752)

(d) Epicatechin gallate

	Blank	α -Toc	AO-1	AO-2	AO-3	AO-4
Concn (M)	0	4.99×10^{-4}	9.11×10^{-4}	1.37×10^{-3}	2.73×10^{-3}	4.55×10^{-3}
S_{AO} (s ⁻¹)	0.0509	0.0169	0.0431	0.0404	0.0348	0.0290
$t_{1/2}$ (min)	14.7	38.6	17.0	17.8	19.5	23.1
Relative SOAC value	—	—	0.0516	0.0472	0.0367	0.0383 (av. 0.0375)

of the AOs. For instance, in the cases of EGC, 4-MC, and 4-MG, similar SOAC values were obtained for four concentrations of the AOs, being independent on the concentrations of the AOs. The rate constants ($k_Q^{AO}(S)$ and $k_Q^{AO}(t_{1/2})$) and the minimum and maximum SOAC values (and average SOAC values) obtained were listed in Tables 2 and 3 (33).

In the case of ECG, notable decrease of the SOAC values (0.0516, 0.0472, 0.0367, and 0.0383) (see Table 3(d)) was observed with increasing concentrations of ECG (9.11×10^{-4} , 1.37×10^{-3} , 2.73×10^{-3} , and 4.55×10^{-3} M, respectively), because, if the concentrations of ECG are low ($[ECG] = 9.11 \times 10^{-4}$ and 1.37×10^{-3} M), the differences between the half-lives for ECG and blank ($t_{1/2}^{ECG} - t_{1/2}^{blank}$) are smaller than ~ 5 min and, thus, we cannot obtain reliable SOAC values. At higher concentrations of ECG ($[ECG] = 2.73 \times 10^{-3}$

and 4.55×10^{-3} M), similar SOAC values (0.0367 and 0.0383, av 0.0375) were obtained. The SOAC values obtained for $t_{1/2}^{ECG} - t_{1/2}^{blank} > \sim 5$ min agree well with the relative rate constant ($k_Q^{AO}(S)/k_Q^{\alpha-Toc}(S) = 0.0377$), as expected from Eq. (2) (see Table 3(d)). Similar results were obtained for EGCG. The SOAC values of EGCG obtained for $t_{1/2}^{EGCG} - t_{1/2}^{blank} > \sim 5$ min are listed in Table 2.

As described above, measurements of the $k_Q(S)$ values were performed for 16 phenolic and polyphenolic AOs in mixed solvent (Table 2). As reported in a previous study (33), the values of $\log k_Q(S)$ were plotted against oxidation potential (E_P) of AOs. $\log k_Q(S)$ of AOs correlates well with E_P with a slope of $-(5.7 \pm 0.3) \text{ V}^{-1}$ (correlation coefficient = -0.98). The AOs that have smaller E_P values show higher reactivities (that is, higher $^1\text{O}_2$ -quenching activities). The chroman ring of trolox

Table 4. 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/D₂O (50 : 50 : 1, v/v/v) solution.

Molecules	$\lambda_{\max}^1/\text{nm}$ ($\varepsilon_1/\text{M}^{-1}\text{cm}^{-1}$)	$\lambda_{\max}^2/\text{nm}$ ($\varepsilon_2/\text{M}^{-1}\text{cm}^{-1}$)	$\lambda_{\max}^3$ and $\lambda_{\max}^4/\text{nm}$ (ε_3 and $\varepsilon_4/\text{M}^{-1}\text{cm}^{-1}$)	λ^5/nm ($\varepsilon_5/\text{M}^{-1}\text{cm}^{-1}$)
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 (Crx 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.

is substituted by an electron withdrawing COOH group, and thus trolox shows higher oxidation potential (E_P) (i.e. slower $^1\text{O}_2$ -quenching reaction rate, $k_Q(S)$).

6. $^1\text{O}_2$ -Quenching Rates ($k_Q(S)$ and $k_Q(t_{1/2})$) and SOAC Values for 8 Carotenoids

6-1. UV-vis absorption spectra of 8 representative carotenoids

Measurements of UV-vis absorption spectra have been performed for many Cars in organic solvents (30, 45). Although the values of the wavelengths of absorption maxima ($\lambda_{\max}$) were reported for many Cars, the Cars whose molar extinction coefficients ($\varepsilon_{\max}$) were determined are very limited, because solubility of Cars is generally low (or very low) in organic solvents and varies remarkably depending on the kinds of solvent. Furthermore, commercially-available Cars are very expensive.

Therefore, measurements of UV-vis absorption spectra of 8 Cars were performed in mixed solvent, and the correct $\lambda_{\max}$ and $\varepsilon_{\max}$ values were determined by repeating the measurements three to five times as reported (31). The average values reported are listed in Table 4. UV-vis absorption spectrum for β -Car is shown in Fig. 2A. Further, $\lambda_{\max}$ and $\varepsilon_{\max}$ values of α -Toc, EP, EP-precursor, and DPBF used for the measurement were also

listed in Table 4, together with those for Cars. As listed in Table 4, UV-vis absorptions of 8 Cars overlap to that of DPBF at 413 nm.

6.2. Measurement of k_Q and SOAC values of 8 representative carotenoids

Measurements of $k_Q(S)$, $k_Q(t_{1/2})$, and SOAC values were performed for 8 Cars (Table 1) (31, 32). Figure 2 shows an example of the reaction between β -Car and $^1\text{O}_2$ in mixed solvent. Decay curves of the absorbance of DPBF at 413 nm produced by the reaction with $^1\text{O}_2$ for β -Car are shown in Fig. 2B. As β -Car shows an absorption at 413 nm (Fig. 2A and Table 4), baseline corrections were performed by using an absorbance at 413 nm of UV-vis absorption spectrum in Fig. 2A. The decay curves corrected are shown in Fig. 2C. $\ln [\text{Absorbance}]$ vs time plots are shown in Fig. 2D, indicating that the decay of DPBF for β -Car follows first-order kinetics at $\sim 10 < t < \sim 50$ min (31). Plots of $S_{\text{blank}}/S_{\beta\text{-Car}}$ and $t_{1/2}^{\beta\text{-Toc}}/t_{1/2}^{\text{blank}}$ vs $[\beta\text{-Car}]$ is shown in Fig. 2E and 2F, respectively. The $k_Q^{\beta\text{-Toc}}(S)$ and $k_Q^{\beta\text{-Toc}}(t_{1/2})$ values obtained by using Eqs. (3) and (4) are 1.04×10^{10} and $1.01 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$, respectively, showing good agreement with each other. Similar measurements were performed for 8 Cars. The values obtained for 8 Cars are listed in Table 1, together with that for α -Toc. The k_Q^{Car} values obtained for 8 Cars

Fig. 2. (A) Absorption spectrum of β -carotene in ethanol/chloroform/D₂O. (B) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1\text{O}_2$ in the absence and presence of sample (α -tocopherol and β -carotene) in ethanol : chloroform : D₂O at 35 °C. $[\text{DPBF}]_{t=0} = 5.77 \times 10^{-5} \text{ M}$ and $[\text{EP}]_{t=0} = 4.36 \times 10^{-4} \text{ M}$. The values of $[\alpha\text{-Toc}]_{t=0}$ and $[\beta\text{-Car}]_{t=0}$ are shown in panel C. (C) Change in absorbance of DPBF, where the correction of baseline due to β -carotene was performed. (D) Plot of $\ln(\text{Absorbance})$ vs time. (E) Plot of $S_{\text{blank}}/S_{\beta\text{-Car}}$ vs $[\beta\text{-Car}]$. (F) Plot of $t_{1/2}^{\beta\text{-Car}}/t_{1/2}^{\text{blank}}$ vs $[\beta\text{-Car}]$.

Fig. 3. Comparison of the relative SOAC values for (A) tocopherol derivatives, ubiquinol-10, caffeic acids, catechins, and vitamin C and for (B) carotenoids, tocopherol derivatives, and ubiquinol-10 in ethanol/chloroform/D₂O solution.

are about two orders of magnitude larger than that for α-Toc.

As described above, ¹O₂-quenching rates ($k_Q^{AO}(S)$ and $k_Q^{AO}(t_{1/2})$) and the relative SOAC values were measured for 8 Cars and many phenolic AOs in mixed solvent at 35°C using UV-vis spectrophotometry (31–33). As listed in Tables 1 and 2, a fair agreement between the relative rate constant ($k_Q^{AO}(S)/k_Q^{\alpha-Toc}(S)$) and the average SOAC value was obtained for many phenolic AOs and 8 Cars. The result indicates that the method of the analysis used for estimating the SOAC value is reasonable. From the results, it has been clarified that the SOAC method is useful to evaluate the ¹O₂-quenching activity of lipophilic and hydrophilic antioxidants having five orders of magnitude different rate constants from $1.40 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ for Lyc to 2.71×10^5 for FA (see Tables 1 and 2). The relative SOAC values obtained for 8 Cars and many phenolic AOs in mixed solvent are shown as a bar graph in Fig. 3 (33).

7. Application of SOAC Assay Method to 23 Kinds of Food Extracts

7.1. Measurements of the k_Q and SOAC values for tomato extracts

Application of SOAC method to 23 kinds of vegetable and fruit extracts was performed (31, 32, 35). All vegetable and fruit samples were purchased at a local market. Basically, edible portions of foods were used for the extraction. In the cases of onion, satsuma mandarin, orange melon, persimmon, banana, and green melon, the part of peel was took off before freeze-drying, and the remaining part was used for extraction. The measurement of relative SOAC value was performed in the

following way; for example, the tomato extract prepared from 1.00 g of freeze-dried powder was dissolved in 25 mL of ethanol/chloroform/D₂O (50 : 50 : 1, v/v/v) solution. From this solution, four concentrations of tomato-1 were prepared (see Supplemental Online Material, Fig. S1B).

The tomato extract shows UV-vis absorption spectrum at 400–550 nm, suggesting that high concentrations of carotenoids are included in tomato (Supplemental Online Material, Fig. S1A) (18, 32, 35). Therefore, analysis of the $k_Q^{\text{Tomato}}(S)$ and $k_Q^{\text{Tomato}}(t_{1/2})$ values for tomato extract was performed, following the method similar to that of β-Car (see Fig. 2), as described below. Decay curves of the absorbance of DPBF due to the reaction with ¹O₂ for tomato extract are shown in Fig. S1B. Base line corrections were performed by using an absorbance at 413 nm of UV-vis absorption spectrum in Fig. S1A, and the decay curves corrected are shown in Fig. S1C. In [Absorbance] vs time plots are shown in Fig. S1D, indicating that the decay of DPBF for tomato extract also follows first-order kinetics at $\sim 5 < t < \sim 60$ min.

$S^{\text{blank}}/S^{\text{Tomato}}$ and $t_{1/2}^{\text{Tomato}}/t_{1/2}^{\text{blank}}$ vs [Tomato] (by g/L unit) plots are shown in Figs. S1E and S1F, respectively. Both the $S^{\text{blank}}/S^{\text{Tomato}}$ and $t_{1/2}^{\text{Tomato}}/t_{1/2}^{\text{blank}}$ values increase linearly with increasing the concentration of tomato extract ([Tomato]), and the plots show similar slopes, that is, similar rate constants ($k_Q^{\text{Tomato}}(S)$ and $k_Q^{\text{Tomato}}(t_{1/2})$), where the unit of k_Q^{Tomato} is $\text{L g}^{-1} \text{ s}^{-1}$. The $k_Q^{\text{Tomato}}(S)$ and $k_Q^{\text{Tomato}}(t_{1/2})$ values obtained are 1.69×10^4 and $1.53 \times 10^4 \text{ L g}^{-1} \text{ s}^{-1}$, respectively (32). The linear dependence of $S^{\text{blank}}/S^{\text{Tomato}}$ and $t_{1/2}^{\text{Tomato}}/t_{1/2}^{\text{blank}}$ values on [Tomato] suggests that the effects of the interactions between Cars included in tomato (mainly Lyc and β-Car (18, 31, 32, 35)) and among the Cars and many compounds included in solution are negligible.

7.2. Measurements of the k_Q and SOAC values for 23 kinds of food extracts

Similar measurements of the ¹O₂-quenching rates ($k_Q(S)$ and $k_Q(t_{1/2})$) were performed for 23 kinds of food extracts (see Table 5) (35). For example, tomato-1 and -2 extracts were prepared by repeating extraction twice from freeze-dried powder sample. The $k_Q^{\text{food}}(S)$ and $k_Q^{\text{food}}(t_{1/2})$ values obtained for food-1 and food-2 are similar to each other, respectively, as listed in Table 5. The $k_Q^{\text{food}}(S)$ values of food extracts varied notably depending on the kinds of food ones, as expected. The $k_Q^{\text{food}}(S)$ value ($3.18 \times 10^4 \text{ L g}^{-1} \text{ s}^{-1}$) of tomato-1 was 205 times larger than that ($1.55 \times 10^2 \text{ L g}^{-1} \text{ s}^{-1}$) of green melon-1.

As listed in Table 5A and 5B, a fair agreement between the $k_Q^{\text{food}}(S)$ and $k_Q^{\text{food}}(t_{1/2})$ values was observed for 14 kinds of vegetable extracts among 16 vegetable ones and all 7 kinds of fruits. Agreement was not sufficient only in the cases of eggplant-1, daikon radish-1 and -2, having slow ¹O₂-quenching rates (that is, low ¹O₂-quenching activity). However, the result of the present investigation indicates that measurements of the rate constants ($k_Q^{\text{food}}(S)$ and $k_Q^{\text{food}}(t_{1/2})$) based on the analyses of the first-order rate constant (S) and the half-life ($t_{1/2}$) of the decay curve of DPBF, respectively,

Table 5. $k_Q^{\text{food}}(S)$ and $k_Q^{\text{food}}(t_{1/2})$ values observed for (A) 16 vegetable and (B) 7 fruit extracts in ethanol/chloroform/D₂O solution at 35.0°C, relative rate constants ($k_Q^{\text{food}}(S)/k_Q^{\alpha\text{-Toc}}(S)$), relative SOAC values, $k_Q^{\text{food}}(S)$ values calculated, and ratio (B)/(A)).

(A) 16 Kinds of vegetable extracts

Vegetable extract	$k_Q^{\text{food}}(S)$ (Obsd.) (A) /L g ⁻¹ s ⁻¹	$k_Q^{\text{food}}(t_{1/2})$ /L g ⁻¹ s ⁻¹	$k_Q^{\text{food}}(S)/$ $k_Q^{\alpha\text{-Toc}}(S)$	Relative SOAC value	$k_Q^{\text{food}}(S)$ (Calcd.) (B) /L g ⁻¹ s ⁻¹	Ratio (B)/(A)
α -Tocopherol ^a	3.04×10^5	3.00×10^5	1.00	1.00	—	—
Tomato-1	3.18×10^4	3.05×10^4	0.1045	av 0.115 ^b	2.53×10^4	0.796
Tomato-2	3.16×10^4	2.88×10^4	0.1041	av 0.132	2.79×10^4	0.883
Tomato (reported) ^a	av 1.85×10^4	av 1.70×10^4	av 0.0608	av 0.0727	1.41×10^4	0.762
Garland chrysanthemum-1	2.97×10^4	2.82×10^4	0.0977	av 0.105	1.80×10^4	0.606
Garland chrysanthemum-2	2.79×10^4	2.61×10^4	0.0919	av 0.103	1.78×10^4	0.638
Spinach-1	2.73×10^4	2.70×10^4	0.0899	av 0.157	3.55×10^4	1.30
Spinach-2	2.94×10^4	2.81×10^4	0.0968	av 0.131	3.51×10^4	1.19
Red paprika-1	2.37×10^4	2.28×10^4	0.0778	av 0.114	3.54×10^4	1.49
Red paprika-2	2.42×10^4	2.26×10^4	0.0796	av 0.113	3.08×10^4	1.27
Red paprika (reported) ^a	av 3.09×10^4	av 2.86×10^4	av 0.102	av 0.117	7.88×10^3	0.255
Carrot-1	1.60×10^4	1.46×10^4	0.0528	av 0.0614	1.67×10^4	1.04
Carrot-2	1.63×10^4	1.51×10^4	0.0537	av 0.0687	1.66×10^4	1.02
Carrot (reported) ^a	av 1.48×10^4	av 1.25×10^4	av 0.0486	av 0.0530	1.42×10^4	0.959
Chinese leek-1	1.38×10^4	1.21×10^4	0.0454	av 0.0500	1.25×10^4	0.906
Chinese leek-2	1.47×10^4	1.29×10^4	0.0483	av 0.0530	1.26×10^4	0.857
Pumpkin-1	1.39×10^4	1.36×10^4	0.0458	av 0.0516	5.51×10^3	0.396
Pumpkin-2	1.16×10^4	1.11×10^4	0.0380	av 0.0436	5.01×10^3	0.433
Cucumber-1	5.23×10^3	5.14×10^3	0.0172	av 0.0146	3.03×10^3	0.579
Cucumber-2	5.74×10^3	5.45×10^3	0.0189	av 0.0143	3.02×10^3	0.526
Broccoli-1	5.17×10^3	5.13×10^3	0.0170	av 0.0172	2.52×10^3	0.487
Broccoli-2	4.68×10^3	4.39×10^3	0.0154	av 0.0183	3.65×10^3	0.780
Green pepper-1	4.72×10^3	4.58×10^3	0.0155	av 0.0151	3.48×10^3	0.737
Green pepper-2	3.41×10^3	3.62×10^3	0.0112	av 0.0108	3.90×10^3	1.14
Okra-1	3.09×10^3	2.94×10^3	0.0102	av 0.0122	9.66×10^2	0.313
Okra-2	4.07×10^3	3.94×10^3	0.0134	av 0.0173	9.45×10^2	0.232
Eggplant-1	8.09×10^2	1.29×10^3	0.0027	av 0.0058	4.47×10^2	0.553
Eggplant-2	7.61×10^2	6.83×10^2	0.0025	av 0.0030	4.77×10^2	0.627
Cabbage-1	5.93×10^2	5.50×10^2	0.0020	av 0.0029	1.28×10^2	0.216
Cabbage-2	5.48×10^2	5.89×10^2	0.0018	av 0.0032	1.30×10^2	0.237
Onion-1	3.09×10^2	3.79×10^2	0.0010	tenta 0.0021 ^c	0	0
Onion-2	5.88×10^2	5.10×10^2	0.0019	av 0.0036	0	0
Sweet potato-1	4.65×10^2	4.40×10^2	0.0015	av 0.0020	5.21×10^1	0.112
Sweet potato-2	3.83×10^2	3.89×10^2	0.0013	tenta 0.0020	4.85×10^1	0.127
Daikon radish-1	2.08×10^2	2.74×10^2	0.0007	tenta 0.0013	3.02×10^1	0.145
Daikon radish-2	2.81×10^2	3.46×10^2	0.0009	tenta 0.0017	8.05	0.0286

(B) 7 Kinds of fruit extracts

Fruit extract	$k_Q^{\text{food}}(S)$ (Obsd.) (A) /L g ⁻¹ s ⁻¹	$k_Q^{\text{food}}(t_{1/2})$ /L g ⁻¹ s ⁻¹	$k_Q^{\text{food}}(S)/$ $k_Q^{\alpha\text{-Toc}}(S)$	Relative SOAC value	$k_Q^{\text{food}}(S)$ (Calcd.) (B) /L g ⁻¹ s ⁻¹	Ratio (B)/(A)
Satsuma mandarin-1	4.15×10^3	4.01×10^3	0.0136	av 0.0197 ^b	8.16×10^2	0.197
Satsuma mandarin-2	4.43×10^3	4.43×10^3	0.0146	av 0.0192	8.42×10^2	0.190
Orange melon-1	3.49×10^3	3.44×10^3	0.0115	av 0.0163	3.25×10^3	0.931
Orange melon-2	3.92×10^3	3.69×10^3	0.0129	av 0.0190	3.65×10^3	0.931
Persimmon-1	1.67×10^3	1.45×10^3	0.0055	av 0.0079	5.10×10^2	0.305
Persimmon-2	1.83×10^3	1.65×10^3	0.0060	av 0.0095	7.85×10^2	0.429
Banana-1	3.99×10^2	4.73×10^2	0.0013	tenta 0.0020 ^c	1.20×10^2	0.301
Banana-2	4.74×10^2	4.74×10^2	0.0016	av 0.0020	1.46×10^2	0.308
Strawberry-1	4.26×10^2	4.32×10^2	0.0014	tenta 0.0020	7.69×10^1	0.181
Strawberry-2	4.26×10^2	4.11×10^2	0.0014	tenta 0.0022	6.60×10^1	0.155
Apple-1	3.76×10^2	4.08×10^2	0.0012	tenta 0.0022	5.23×10^1	0.139
Apple-2	4.04×10^2	3.99×10^2	0.0013	tenta 0.0021	4.63×10^1	0.115
Green melon-1	1.55×10^2	1.70×10^2	0.0005	tenta 0.0009	4.29×10^1	0.277
Green melon-2	2.23×10^2	2.41×10^2	0.0007	tenta 0.0012	4.45×10^1	0.200

^a See Table 6 in Ref. (32).

^b av: average SOAC value (see Ref. (35)).

^c tenta: tentatively determined SOAC value (see Ref. (35)).

Fig. 4. Comparison of (A) the relative rate constants ($k_Q^{\text{food}}(S)/k_Q^{\alpha\text{-Toc}}(S)$) and (B) the relative SOAC values for 16 vegetable and 7 fruit extracts in ethanol/chloroform/ D_2O solution.

are applicable to general food extracts having more than two orders of magnitude different 1O_2 -quenching rates. As expected from Eq. (2), a fair agreement between the relative rate constant ($k_Q^{\text{food}}(S)/k_Q^{\alpha\text{-Toc}}(S)$) and the average SOAC value was obtained for many food extracts (Table 5). Average values of the relative rate constants ($k_Q^{\text{food}}(S)/k_Q^{\alpha\text{-Toc}}(S)$) obtained for 16 vegetable and 7 fruit extracts in mixed solvent are shown as a bar graph in Fig. 4A (35). The relative SOAC values for 16 vegetable and 7 fruit extracts are also shown in Fig. 4B.

7.3. Comparison between $k_Q^{\text{food}}(S)$ (Obsd.) and $k_Q^{\text{food}}(S)$ (Calcd.) values for 23 kinds of food extracts

It is well known that various AOs coexist in foods and plants (16–21). As described above, the $k_Q^{\text{food}}(S)$ ($L\ g^{-1}s^{-1}$) values for 23 food extracts were measured by the $S_{\text{blank}}/S_{\text{food}}$ vs [food] (g/L) plot, using Eq. (7) (35).

$$S_{\text{blank}}/S_{\text{food}} = 1 + \{k_Q^{\text{food}}(S) (\text{Obsd.}) [\text{food}]\} / k_d \quad (7)$$

As reported in previous works (22–27, 30–34), $k_Q^{\text{Car}}(S)$ values of Cars show 2 to 5 orders of magnitude larger than those ($k_Q^{\text{phenolic AO}}(S)$) of general phenolic AOs, including tocopherol homologues, ubiquinol-10, catechins, and caffeic acids. The result suggests that the contribution of Cars to the 1O_2 -quenching activity of food extracts is considered to be very important. Therefore, measurements of the concentrations of Cars included in 16 vegetable and 7 fruit extracts were

performed using a HPLC technique, according to the method reported in a previous work (18). Analyses of the concentrations were performed for 7 Cars (α -Car, β -Car, Lut, Lyc, Cap, Zea, and β -Cry). The concentrations of 7 Cars included are summarized in Table S1 (Supplemental Online Material).

As listed in Table S1, 7 Cars (Car- i ($i=1-7$)) are included in 22 food extracts except for onion among 23 food extracts. Consequently, the $k_Q^{\text{food}}(S)$ values for 23 food extracts were calculated, using Eq. (8), where the $k_Q^{\text{Car-}i}(S)$ ($L\ g^{-1}s^{-1}$) values are the rate constants for 7 Cars in mixed solvent (31, 32). [Car- i] (unit: mg/100 g) (Table S1) is the concentration of 7 Cars included in food extracts. The $k_Q^{\text{food}}(S)$ (Calcd.) values calculated for 23 food extracts are listed in Table 5.

$$k_Q^{\text{food}}(S) (\text{Calcd.}) = \sum k_Q^{\text{Car-}i}(S) [\text{Car-}i] / 10^5 \quad (i=1-7) \quad (8)$$

We may expect that the $k_Q^{\text{food}}(S)$ (Obsd.) (A) values are larger than the corresponding $k_Q^{\text{food}}(S)$ (Calcd.) (B) values, that is, the values of ratio (B)/(A) in Table 5 are ≤ 1 , because not only 7 Cars, but also the other Cars are included in food extracts (16–18). Many phenolic AOs included in food extracts will also contribute to 1O_2 -quenching. Furthermore, chlorophyll a ($\lambda_{\text{max}}=430\text{ nm}$) is included in green vegetables (35). Chlorophyll a shows higher 1O_2 -quenching rate ($k_Q(S)=7.3 \times 10^8\text{ M}^{-1}s^{-1}$ in benzene) than α -Toc

($1.31 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ in mixed solvent), suggesting the contribution of chlorophyll *a* to the $k_Q(S)$ (Obsd.) values of green vegetable extracts (46).

As listed in Table 5, good agreement between observed $k_Q^{\text{carrot-1 and -2}}(S)$ values ($=1.60 \times 10^4$ and $1.63 \times 10^4 \text{ L g}^{-1}\text{s}^{-1}$) and calculated $\{\sum k_Q^{\text{Car-}i}(S) [\text{Car-}i]/10^5\}$ values ($=1.67 \times 10^4$ and $1.66 \times 10^4 \text{ L g}^{-1}\text{s}^{-1}$) was obtained for carrot-1 and -2 extracts, respectively (35). The result indicates that the total $^1\text{O}_2$ -quenching activity of carrot-1 and -2 extracts may be well explained by only considering the contribution of 3 kinds of Cars (α -Car, β -Car, and Lut) included in carrot extracts. The contribution of the other AOs is small and negligible. Similarly, fair agreement between $k_Q^{\text{food}}(S)$ (Obsd.) (A) and $k_Q^{\text{food}}(S)$ (Calcd.) (B) values was obtained for 4 food extracts (tomato, Chinese leek, green pepper, and orange melon), which include comparatively high concentrations of Cars (Table S1).

On the other hand, the $k_Q^{\text{food}}(S)$ (Obsd.) values observed for the other 16 food extracts are larger than the corresponding $k_Q^{\text{food}}(S)$ (Calcd.) values, as listed in Table 5, indicating that the other AOs included also contribute to the $k_Q^{\text{food}}(S)$ (Obsd.) values. In fact, the values of ratio (B)/(A) observed for 5 brightly colored vegetable extracts (garland chrysanthemum, pumpkin, cucumber, broccoli, and eggplant) are $0.70 > (B)/(A) > 0.50$. Generally, the values of ratio (B)/(A) observed for 5 vegetable extracts (okra, cabbage, onion, sweet potato, and dailikon radish extracts), which include low concentrations of carotenoids and show comparatively small $k_Q^{\text{food}}(S)$ (obsd.) values, are $0.313 > (B)/(A) \geq 0.000$. Similarly, 6 fruit extracts except for orange melon also show small values of ratio ($0.429 > (B)/(A) > 0.115$).

For example, 7 Cars are not included in onion-1 and -2 extracts. However, onion-1 and -2 extracts show $^1\text{O}_2$ -quenching activities ($k_Q^{\text{food}}(S)$ (Obsd.) $= 3.09 \times 10^2$ and $5.88 \times 10^2 \text{ L g}^{-1}\text{s}^{-1}$), respectively. Onion includes considerable amounts of quercetin which is well known as a representative flavone derivative (47). The $k_Q^{\text{quercetin}}(S)$ (Obsd.) value of quercetin reported is $4.57 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ ($= 1.51 \times 10^6 \text{ L g}^{-1}\text{s}^{-1}$ in ethanol) (48). Quercetin included in onion will contribute to the above $^1\text{O}_2$ -quenching rate.

On the other hand, in the cases of spinach-1 and -2 and red paprika-1 and -2, the values of the ratio (B)/(A) are > 1.00 (see Table 5). As listed in Table S1, the total Car concentrations included in spinach-1 and -2 (197.41 and 195.18 mg/100 g) and red paprika-1 and -2 (193.68 and 168.30 mg/100 g) are the highest ones among 23 kinds of food extracts studied. The result suggests that the interactions between 7 Cars and among 7 Cars and many molecules included in spinach and red paprika extracts may hinder the determination of correct $k_Q(S)$ (Obsd.) values. In fact, the total Car concentration included in red paprika (40.88 mg/100 g) reported in a previous work (32) is much lower than the above values (193.68 and 168.30 mg/100 g), and the value of the ratio (B)/(A) reported is 0.255, as listed in Table 5. However, the reason is not clear at present. Detailed study will be necessary to clarify the reason why the val-

ues of the ratio (B)/(A) obtained for spinach-1 and -2 and red paprika-1 and -2 are > 1.00 .

8. Application of SOAC Assay Method to 7 Rice Bran Extracts, 8 Vegetable Oils, and 5 Palm Oils

Further, measurements of the $^1\text{O}_2$ -quenching rates ($k_Q(S)$) and the relative SOAC values were performed for 7 rice bran extracts, which contained different concentrations of AOs (such as α -, β -, γ -, and δ -Tocs and -Toc-3s, 3 Cars (Lut, β -Car, and Zea), and γ -oryzanol), in ethanol at 35°C using UV-vis spectrophotometry (28). The concentrations of 4 Tocs and 4 Toc-3s, 3 Cars, and γ -oryzanol contained in the extracts were determined using HPLC-MS/MS, UV-HPLC, and UV-vis absorption spectroscopy, respectively. Similarly, comparisons of $k_Q(S)$ (Obsd.) values with $k_Q(S)$ (Calcd.) ($= \sum k_Q^{\text{AO-}i}(S) [\text{AO-}i]/10^5$) were performed. The ratios ($k_Q(\text{Calcd.})/k_Q(\text{Obsd.})$) for rice bran extracts 1–7 varied from 0.562 to 0.971. Similar measurements and analyses were performed for (i) 8 vegetable oils (36) and (ii) 5 palm oil and 1 soybean extracts (34), which contained different concentrations of AOs (such as 4 Tocs, 4 Toc-3s, and Cars). It has been ascertained that the SOAC method developed is applicable to general food extracts to evaluate the $^1\text{O}_2$ -quenching activity, as described above.

9. Measurements of the k_Q and SOAC Values for 8 Vitamin E Homologues and 8 Carotenoids in a Micellar Solution

Liposome is often used as a model of biomembrane. However, the measurement of k_Q^{AO} and SOAC values in liposome solution was difficult, because of low solubility of AOs in liposome solution. Solubility of AOs in an aqueous Triton X-100 micellar solution is higher than those in sodium dodecyl sulfate (SDS) and cetyl trimethyl ammonium bromide (CTAB) micellar solutions. Many kinds AOs (such as carotenoids, vitamin E homologues, and polyphenols) are soluble in a Triton X-100 micellar solution. Therefore, using a Triton X-100 (5.0 wt %) micellar solution (pH 7.4), the measurements of $k_Q^{\text{AO}}(S)$ and $k_Q^{\text{AO}}(t_{1/2})$ and SOAC values were performed for (i) vitamin E homologues (α -, β -, γ -, δ -Toc and -Toc-3) and (ii) 8 Cars (37, 38).

As reported in a previous work (38), $^1\text{O}_2$ -quenching rate constants ($k_Q^{\text{AO}}(S)$) of α -, β -, γ -, δ -Toc and -Toc-3 decreases in the order of Eq. (9) in mixed solvent and ethanol (Table 6).

$$\alpha\text{-Toc} \sim \alpha\text{-Toc-3} > \beta\text{-Toc} \sim \beta\text{-Toc-3} > \gamma\text{-Toc} \sim \gamma\text{-Toc-3} > \delta\text{-Toc} \sim \delta\text{-Toc-3} \quad (9)$$

The k_Q^{AO} values of α -, β -, γ -, and δ -Toc-3 show good agreement with those of the corresponding Toc in ethanol and mixed solvent, indicating that the difference in the alkyl side-chains between Toc and Toc-3 does not give a significant effect on the reaction rates. Similar results were reported by Gruska et al. (49).

On the other hand, the $^1\text{O}_2$ -quenching activity (i.e. $k_Q^{\text{AO}}(S)$ values) of α -, β -, γ -, δ -Toc and -Toc-3 decreases in the order of Eq. (10) in a Triton X-100 micellar solution (Table 6) (38).

$$\alpha\text{-Toc-3} > \alpha\text{-Toc} > \gamma\text{-Toc-3} > \beta\text{-Toc-3} > \beta\text{-Toc} >$$

Table 6. The k_Q^{AO} values for vitamin E homologues (AOs) in a Triton X-100 micellar solution, ethanol, and mixed solvent at 35.0°C, and ratio of the rate constants (k_Q (micelle)/ k_Q (EtOH) and k_Q^{AO} (micelle)/ k_Q^{AO} (mixed solvent)).

Antioxidant (AO)	k_Q^{AO} a (pH 7.4) / $M^{-1}s^{-1}$ micelle	k_Q^{AO} / $M^{-1}s^{-1}$ EtOH ^b	k_Q^{AO} (micelle)/ k_Q^{AO} (EtOH)	k_Q^{AO} / $M^{-1}s^{-1}$ mixed solvent ^b	k_Q^{AO} (micelle)/ k_Q^{AO} (mixed solvent)
Toc and Toc-3					
α -Toc	3.16×10^8	2.05×10^8	1.54	1.31×10^8	2.41
α -Toc-3	3.75×10^8	2.24×10^8	1.67	1.22×10^8	3.07
β -Toc	2.52×10^8	1.86×10^8	1.35	9.45×10^7	2.67
β -Toc-3	2.88×10^8	1.87×10^8	1.54	1.05×10^8	2.74
γ -Toc	2.28×10^8	1.58×10^8	1.44	8.15×10^7	2.80
γ -Toc-3	3.07×10^8	1.54×10^8	1.99	8.14×10^7	3.77
δ -Toc	1.09×10^8	6.63×10^7	1.64	4.79×10^7	2.28
δ -Toc-3	1.48×10^8	6.93×10^7	2.13	4.97×10^7	2.98
			av 1.66		av 2.84

^a $k_Q^{AO}(S)$ is abbreviated as k_Q^{AO} for the sake of simplicity.

^b The k_Q^{AO} (EtOH) and k_Q^{AO} (mixed solvent) values reported in Ref. (34).

$$\gamma\text{-Toc} > \delta\text{-Toc-3} > \delta\text{-Toc} \quad (10)$$

The values of the ratios ($k_Q^{\text{Toc-3}}(S)/k_Q^{\text{Toc}}(S)$) for α -, β -, γ -, and δ -Toc and -Toc-3 are 1.19, 1.14, 1.35, and 1.36, respectively. The result clearly indicates that the 1O_2 -quenching rates of Toc-3 are larger than those of the corresponding Toc in a micellar solution.

Toc-3 differs from Toc in the chemical nature of the side chain or tail. Toc has a saturated phytyl side chain, whereas Toc-3 has an unsaturated isoprenoid (or farne-syl) side chain (Fig. S2). A phytyl side chain will have a straight molecular structure. On the other hand, an unsaturated isoprenoid side chain possessing three double bonds will have a bent structure and shorter chain length than that of a phytyl side chain. This will give a change in the mobility of Toc and Toc-3 in a micellar solution. That is, the mobility of Toc-3 will be higher than that of Toc.

As listed in Table 6, the $k_Q^{\text{Toc and Toc-3}}$ values decreased in the following order:

$$k_Q^{\text{Toc and Toc-3}} (\text{micelle}) > k_Q^{\text{Toc and Toc-3}} (\text{EtOH}) > k_Q^{\text{Toc and Toc-3}} (\text{mixed solvent}) \quad (11)$$

The ratios of $k_Q^{\text{Toc and Toc-3}} (\text{micelle})$ to $k_Q^{\text{Toc and Toc-3}} (\text{mixed solvent})$ and $k_Q^{\text{Toc and Toc-3}} (\text{EtOH})$ for Toc and Toc-3 are 2.84 and 1.66, respectively (Eqs. (12) and (13)).

$$k_Q^{\text{Toc and Toc-3}} (\text{micelle}) = 2.84 k_Q^{\text{Toc and Toc-3}} (\text{mixed solvent}) \quad (12)$$

$$k_Q^{\text{Toc and Toc-3}} (\text{micelle}) = 1.66 k_Q^{\text{Toc and Toc-3}} (\text{EtOH}) \quad (13)$$

As 4 Toc and 4 Toc-3 are lipophilic, they are expected to localize within the micelle. Consequently, the local concentrations of these AO in a Triton X-100 micellar solution (5.0 wt %) should become ~ 20 times larger than in a homogeneous ethanol solution (or mixed solvent), if one assumes that the volume that Triton X-100 molecules (5.0 wt %) occupy in a micellar solution is 5.0% of the total volume. As the 1O_2 molecule is also lipid soluble (50), it is also expected to react with these AOs inside of the micelle. Therefore, if the polarity of the reaction field between 1O_2 and AO in a micellar solution

is similar to that of ethanol, the k_Q values of the AOs observed in micelles should become ~ 20 times larger than those in ethanol. To our surprise, however, the $k_Q^{\text{Toc and Toc-3}} (\text{micelle})/k_Q^{\text{Toc and Toc-3}} (\text{EtOH})$ ratio observed was only 1.66, as determined from Eq. (13).

As shown in Fig. S2, the 4 Toc and 4 Toc-3 investigated contain different numbers of CH_3 groups at an aromatic phenol ring and contain different side chains. Therefore, one might expect that the Toc and Toc-3 molecules are located at the positions having different polarities (i.e., different ϵ values) in a micellar solution. However, the measured λ_{max} values of the Toc and Toc-3 suggest that the part of polar head phenolic ring in 4 Toc and 4 Toc-3 molecules is localized to an environment with a polarity similar to that of chloroform (see Table S2) (38).

Gruszka et al. (49) reported that the 1O_2 -quenching rate constants (k_Q) of α -, β -, γ -, and δ -Toc increase linearly with an increasing solvent dielectric constant (ϵ) (i.e., polarity). The values of ratios ($k_Q (\text{EtOH}, \epsilon=24.58)/k_Q (\text{CHCl}_3, \epsilon=4.806)$) estimated from the k_Q versus ϵ plot (see Fig. 2 in Ref. (49)) are ca. 1.8, 2.7, 3.3, and 3.0 for α -, β -, γ -, and δ -Toc, respectively. Even after taking into account the decrease in $k_Q^{\text{Toc and Toc-3}}$ in chloroform described above, the small values of the observed ratios (i.e., 1.66), which are much smaller than the expected ratio of ~ 20 , cannot be explained. The significant decrease in the $k_Q^{\text{Toc and Toc-3}}$ values of Toc and Toc-3 may be due to suppression of the mobility of Toc and Toc-3 molecules in the heterogeneous micellar solution (37, 38, 51–53).

Toc-3 has received much attention in recent years (54, 55). Some biological functions such as cholesterol lowering, anti-cancer, anti-inflammatory, anti-angiogenic, and neuroprotection effects have been reported for Toc-3. These findings suggest that Toc-3 has a wide variety of health benefits. The difference in biological activity of Toc and Toc-3 may relate to the difference of

Table 7. The k_Q^{AO} values for antioxidants (AOs) in a Triton X-100 (5.0 wt %) micellar solution (pH 7.4), ethanol, and mixed solvent, and their ratios (k_Q (micelle)/ k_Q (EtOH) and k_Q^{AO} (micelle)/ k_Q^{AO} (mixed solvent)).

Antioxidant (AO)	k_Q^{AOa} (pH 7.4) /M ⁻¹ s ⁻¹ micelle	k_Q^{AO} /M ⁻¹ s ⁻¹ EtOH ^b	k_Q^{AO} (micelle)/ k_Q^{AO} (EtOH)	k_Q^{AO} /M ⁻¹ s ⁻¹ mixed solvent ^b	k_Q^{AO} (micelle)/ k_Q^{AO} (mixed solvent)
α -Tocopherol	3.16×10^8	2.06×10^8		1.31×10^8	
Lycopene	low solubility	low solubility		1.38×10^{10}	
Astaxanthin	7.11×10^9	low solubility		1.18×10^{10}	0.602
β -Carotene	4.23×10^9	1.71×10^{10}	0.247	1.08×10^{10}	0.393
Capsanthin	4.54×10^9	1.79×10^{10}	0.254	1.06×10^{10}	0.425
Zeaxanthin	5.83×10^9	1.82×10^{10}	0.320	1.05×10^{10}	0.555
α -Carotene	5.70×10^9	1.92×10^{10}	0.297	9.76×10^9	0.584
Lutein	5.53×10^9	1.76×10^{10}	0.314	9.24×10^9	0.598
β -Cryptoxanthin	4.40×10^9	1.54×10^{10}	0.286	7.31×10^9	0.602
			av 0.286 $\pm$ 0.012		av 0.530 $\pm$ 0.034

^a $k_Q^{AO}(S)$ is abbreviated as k_Q^{AO} for the sake of simplicity.

^b k_Q^{AO} (EtOH) and k_Q^{AO} (mixed solvent) values reported in Ref. (33).

the mobility of Toc and Toc-3, although the details are not clear at present. Measurements of k_Q and SOAC values in a micellar solution may be useful for evaluating the 1O_2 -quenching activity of AOs in biological systems.

Further, similar measurements of k_Q^{AO} (micelle) values were performed for 7 Cars in a micellar solution (37). The ratios of k_Q^{Car} (micelle) to k_Q^{Car} (mixed solvent) and k_Q^{Car} (EtOH) for Cars were calculated to be 0.530 and 0.286, respectively (see Table 7).

$$k_Q^{Car}(\text{micelle}) = 0.530 k_Q^{Car}(\text{mixed solvent}) \quad (14)$$

$$k_Q^{Car}(\text{micelle}) = 0.286 k_Q^{Car}(\text{EtOH}) \quad (15)$$

As Eqs. (14) and (12) indicate, the values of the ratios (k_Q^{Car} (micelle)/ k_Q^{Car} (EtOH)=0.286) for Cars are smaller than those for Toc and Toc-3 ($k_Q^{\text{Toc and Toc-3}}$ (micelle)/ $k_Q^{\text{Toc and Toc-3}}$ (EtOH)=1.66). The value of the latter is about 5.8 times larger than the former, and suggests that the mobility of the 7 Cars, which exhibit rigid molecular structures, should be more suppressed than those of Toc and Toc-3 in a micellar solution, resulting in greater decreases in k_Q^{Car} than $k_Q^{\text{Toc and Toc-3}}$. Furthermore, differences in the ratios (0.286 and 1.66) between Cars and Toc and Toc-3 may also be due to differences in the rigidity of the regions at which Cars and Toc and Toc-3 molecules are localized. However, details are not clear at present.

Fukuzawa et al. (52, 53) indicated that notable variations of the k_Q values of AOs in homogeneous ethanol and heterogeneous liposome solutions may be explained by (i) polarity (dielectric constant (ϵ)) of reaction field between AOs and 1O_2 , (ii) local concentration of AOs, and (iii) mobility of AOs in solutions. The results obtained in the present study also support the explanation given above, although large differences were observed for the k_Q values of AOs between micellar and liposome solutions.

As described above, the method to evaluate the 1O_2 -quenching activity of natural AOs and food and plant extracts in organic solvents and a Triton X-100

	1	2	3	4	5	6
A	Blank	α -Toc 500 μ M	Sample 1 $\times 0.2$	Sample 1 $\times 0.3$	Sample 1 $\times 0.6$	Sample 1 $\times 1.0$
B	Sample 2 $\times 1.0$	Sample 2 $\times 0.6$	Sample 2 $\times 0.3$	Sample 2 $\times 0.2$	α -Toc 500 μ M	Blank
C	Blank	α -Toc 500 μ M	Sample 3 $\times 0.2$	Sample 3 $\times 0.3$	Sample 3 $\times 0.6$	Sample 3 $\times 1.0$
D	Blank	Sample 3 $\times 1.0$	Sample 2 $\times 1.0$	Sample 1 $\times 1.0$	α -Toc 500 μ M	Blank

Fig. 5. Layout of the 24-well microplate. Gray wells (line D) are baseline wells in which DPBF and EP are not contained. The number of sample wells represents the sample dilution factor. The concentration of α -Toc (500 μ M) is final concentration.

micellar solution was established. It is well-known that representative AOs (such as α -Toc, UQ₁₀H₂, Cars, and Vit C) are contained in human plasma (56). Catechins are also found in plasma following oral ingestion (57). As endoperoxide (EP), DPBF, and plasma are soluble in a Triton X-100 micellar solution (5.0 wt %, pH 7.4), we have recently succeeded in measuring the $k_Q(S)$ and SOAC values of plasma (to be published). α -Toc and UQ₁₀H₂ are contained in mice tissues (heart, liver, kidney, and brain (homogenate and mitochondria)) and their concentrations were reported (56). Measurements of the SOAC value of mice tissues will be interesting to clarify the 1O_2 -quenching activity of biological systems.

10. Measurements of the k_Q and SOAC Values of Antioxidants and Food Extracts Using a Microplate Reader

As described above, the k_Q and SOAC values were measured in organic solvents and a micellar solution at 35°C, using an UV-vis spectrophotometer equipped with

Table 8. Second-order rate constants ($k_Q(S)$ and $k_Q(t_{1/2})$) for α -tocopherol, eight carotenoids, and three vegetable extracts measured using a microplate reader and an UV-vis spectrophotometer equipped with a six-channel cell positioner.

Carotenoid (Car)	$k_Q(S)/M^{-1}s^{-1}$		$k_Q(t_{1/2})/M^{-1}s^{-1}$	
	Microplate reader ^a	UV-vis spectro- photometer	Microplate reader ^a	UV-vis spectro- photometer
α -Toc	$(1.10 \pm 0.07) \times 10^8$	1.06×10^8	$(1.12 \pm 0.06) \times 10^8$	1.03×10^8
Lycopene	$(1.46 \pm 0.13) \times 10^{10}$	1.48×10^{10}	$(1.44 \pm 0.11) \times 10^{10}$	1.38×10^{10}
Astaxanthin	$(1.22 \pm 0.07) \times 10^{10}$	1.07×10^{10}	$(1.20 \pm 0.06) \times 10^{10}$	1.05×10^{10}
Capsanthin	$(1.10 \pm 0.09) \times 10^{10}$	1.15×10^{10}	$(1.05 \pm 0.07) \times 10^{10}$	1.10×10^{10}
Zeaxanthin	$(1.03 \pm 0.04) \times 10^{10}$	9.90×10^9	$(1.03 \pm 0.04) \times 10^{10}$	9.59×10^9
β -Carotene	$(1.02 \pm 0.06) \times 10^{10}$	1.02×10^{10}	$(9.74 \pm 0.64) \times 10^9$	9.94×10^9
α -Carotene	$(9.17 \pm 0.35) \times 10^9$	9.88×10^9	$(8.88 \pm 0.68) \times 10^9$	9.76×10^9
Lutein	$(7.42 \pm 0.36) \times 10^9$	8.81×10^9	$(7.58 \pm 0.39) \times 10^9$	8.12×10^9
β -Cryptoxanthin	$(7.54 \pm 0.70) \times 10^9$	7.26×10^9	$(8.24 \pm 0.27) \times 10^9$	6.84×10^9

Vegetable extract	$k_Q(S)/L\ g^{-1}s^{-1}$		$k_Q(t_{1/2})/L\ g^{-1}s^{-1}$	
	Microplate reader ^a	UV-vis spectro-photometer	Microplate reader ^a	UV-vis spectro-photometer
Tomato	$(1.56 \pm 0.04) \times 10^4$	1.52×10^4	$(1.56 \pm 0.07) \times 10^4$	1.38×10^4
Carrot	$(1.63 \pm 0.08) \times 10^4$	1.55×10^4	$(1.52 \pm 0.06) \times 10^4$	1.49×10^4
Red paprika	$(1.63 \pm 0.10) \times 10^4$	1.62×10^4	$(1.48 \pm 0.13) \times 10^4$	1.54×10^4

^a Data are represented as means $\pm$ SD ($n=3$).

a six-channel cell positioner and an electron-temperature control unit. Recently, Takahashi et al. (39) have succeeded in measuring the k_Q and SOAC values using a versatile instrument, microplate reader. A 24-well glass microplate (Fig. 5) was used for measurements, because a plastic microplate, which is generally used in laboratory, dissolves in the above mixed solvent. Measurements of the k_Q and SOAC values were performed for 8 representative carotenoids and 3 vegetable extracts (tomato, carrot, and red paprika). The $k_Q(S)$ and $k_Q(t_{1/2})$ and SOAC values of eight Cars and three vegetable extracts measured using a microplate reader were in good agreement with the corresponding those measured using an UV-vis spectrophotometer, as listed in Table 8 and Table S3, respectively. The result suggests that the microplate reader is applicable to measure reliable SOAC values for general AOs and food extracts in solution.

Using a microplate reader, it is possible to measure the SOAC value for three kinds of AOs simultaneously. It is a merit to use a microplate reader for the measurement of SOAC value. However, it is necessary to analyze pseudo-first-order rate constants (S_{blank} and S_{AO}) and half-lives ($t_{1/2}^{\text{blank}}$ and $t_{1/2}^{\text{AO}}$) (see Eqs. (3) and (4)) carefully, because the time range ($40 < t < 70$ min) that $\ln$ (Absorbance) vs [AO] (or [Blank]) plot shows a straight line is narrow in the case of a microplate reader, differing from that ($5 < t < 60$ min) in the case of an UV-vis spectrophotometer (Fig. 6). It took about 40 min before the solution temperature in 24-well glass microplate reaches 35°C (i.e. a steady-state), and, further, the formation rate of $^1\text{O}_2$ decreased gradually due to the con-

sumption of EP after 70 min.

Recently, $^1\text{O}_2$ -quenching activities of 32 kinds of fruits and vegetables typically consumed in Japan were evaluated using the SOAC method (40).

11. Validation Study

The precision of the SOAC assay method was evaluated in an inter-laboratory validation study (41). SOAC values were measured for 3 AO solutions (i.e. capsanthin, zeaxanthin, and astaxanthin solutions) and 3 food extracts (i.e. tomato, red paprika, and carrot extracts), using a 24-well glass microplate. This study involved 14 participating laboratories, with data sets from 8 successfully participating laboratories subjected to statistical analysis. In accordance with the harmonized protocol, this study showed that the intermediate precision relative standard deviation (RSDint) and the reproducibility relative standard deviation (RSDR) ranged from 3.4 to 9.9%, and from 6.2 to 14.0%, respectively, and the HorRat values ranged from 0.87 to 1.97. Therefore, the SOAC assay method was validated by this inter-laboratory study in accordance with the internationally harmonized protocol.

12. Conclusions

Lipid peroxy radical ($\text{LOO}\cdot$) and singlet oxygen ($^1\text{O}_2$) are well known as two representative reactive oxygen species (ROS) generated in biological systems. The method to assess the total oxygen radical absorption capacity (ORAC) of foods and plants has been established by several investigators (58–60). On the other

Fig. 6. (A) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1\text{O}_2$ in the absence and presence of sample ($\alpha\text{-Toc}$ and Lyc) in ethanol/chloroform/ D_2O at 35°C . $[\text{DPBF}] = 0.0614 \text{ mM}$ and $[\text{EP}] = 0.546 \text{ mM}$. The values of $[\alpha\text{-Toc}]$ and $[\text{Lyc}]$ are shown in A. (B) Change in absorbance of DPBF, where the correction of baseline due to Lyc was performed (see text). (C) Plot of $\ln(\text{Absorbance})$ vs time. (D) Plot of $S_{\text{Blank}}/S_{\text{Lyc}}$ vs $[\text{Lyc}]$. (E) Plot of $t_{1/2}^{\text{Lyc}}/t_{1/2}^{\text{Blank}}$ vs $[\text{Lyc}]$.

hand, a SOAC assay method to assess the total quenching activity of $^1\text{O}_2$ by foods and plants has not been established. Consequently, the development of the SOAC assay method is very important. In our laboratory, first, the quenching rates (k_Q) of $^1\text{O}_2$ by 8 representative Cars, $\alpha\text{-Toc}$, and three kinds of vegetable extracts containing high concentrations of Cars (i.e. tomato, carrot, and red paprika) were measured in ethanol/chloroform/ D_2O solution at 35°C , by using the competition reaction method (Scheme 1) (31, 32). From the results, the method to assess the total SOAC value of the AOs included in foods and plants has been proposed.

Many kinds of natural phenolic AOs (Table 2) are included in foods, plants, and animals, and may function as $^1\text{O}_2$ -quencher in biological systems. Therefore, in order to ascertain that the SOAC assay method proposed for Cars is applicable to general phenolic AOs, measure-

ments of k_Q and relative SOAC values were performed for the above phenolic AOs in mixed solvent. From the results, it has been clarified that the SOAC assay method is applicable to a variety of natural AOs having five orders of magnitude different $^1\text{O}_2$ -quenching rates (k_Q) (33). Measurements of the k_Q and SOAC values were also performed for 39 kinds of food extracts, indicating that the SOAC method is useful to evaluate the $^1\text{O}_2$ -quenching activity of food extracts having two orders of magnitude different rate constants (k_Q) (28, 34–36).

Further, the k_Q and SOAC values for the reaction of $^1\text{O}_2$ with 8 Cars and 8 vitamin E homologues were measured in an aqueous Triton X-100 (5.0 wt %) micellar solution (pH 7.4), which was used as a simple model of biomembranes (37, 38). Results obtained demonstrate that the k_Q values of AOs in homogeneous and heterogeneous solutions vary notably depending on (i) polar-

ity (dielectric constant (ϵ)) of the reaction field between AOs and $^1\text{O}_2$, (ii) local concentration of AOs, and (iii) mobility of AOs in solution. Measurements of k_Q and SOAC values in a micellar solution may be useful for evaluating the $^1\text{O}_2$ -quenching activity of AOs in biological systems.

Furthermore, measurements of the SOAC values were performed for 32 kinds of vegetable and fruit extracts using a versatile instrument, microplate reader (39, 40). The SOAC values measured using a microplate reader were in good agreement with the corresponding those measured using an UV-vis spectrophotometer. The SOAC assay method was validated by inter-laboratory validation study due to 14 laboratories (41).

As described in Introduction, the high reactivity of $^1\text{O}_2$ causes oxidative damage to biomolecules such as lipids (10–12), proteins (3, 4), and DNA (13–15). For example, lipids, particularly polyunsaturated fatty acids (PUFAs), are frequent targets of in vivo $^1\text{O}_2$ oxidation, producing lipid hydroperoxides. Generation of lipid hydroperoxides is associated with disease onset such as diabetes (61). The relationship between in vivo ROS production (including $^1\text{O}_2$) and the onset of diseases such as Alzheimer's disease, Parkinson's disease, and diabetes was described in the reviews of Terao (1) and Umeno et al. (61).

Disclosure of State of COI

No conflicts of interest to be declared.

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Supporting Information

Supplemental Online Material is available on J-STAGE.

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