Finding of Remarkable Synergistic Effect on the Aroxyl-Radical-Scavenging Rates \( (k_s) \) under the Coexistence of Vitamin E Homologues (or Vegetable Oils) and Ubiquinol-10: Proposal of A New Mechanism to Explain An Increase of \( k_s \) Value

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Abstract: Measurements of aroxyl \( (\text{ArO}^\cdot) \)-radical-scavenging rate constants \( (k_{\text{AOH}}) \) of antioxidants \( (\text{AOHs}) \) (i.e., \( \alpha-, \beta-, \gamma-, \delta- \)tocopherol (TocH) and ubiquinol-10 \( (\text{UQ}_{10}\text{H}_2) \) ) were performed in ethanol/chloroform/\( \text{H}_2\text{O} \) \( (50/50/1, \text{v/v}) \) solution, using stopped-flow spectrophotometry. \( k_{\text{AOH}} \) values were measured not only for each \( \text{AOH} \), but also for the mixtures of two \( \text{AOHs} \) (i.e., TocH and \( \text{UQ}_{10}\text{H}_2 \) ). \( k_{\text{AOH}} \) values for \( \alpha-, \beta-, \gamma-, \delta- \)tocopherol increased \( 1.21, 1.28, 1.55, \) and \( 1.19 \) times, respectively, under the coexistence of constant concentrations of \( \text{UQ}_{10}\text{H}_2 \). Similar measurements were performed for eight vegetable oils \( 1-8 \) containing different concentrations of \( \alpha-, \beta-, \gamma-, \delta- \)tocopherol (TocH) and \( \alpha-, \beta-, \gamma-, \delta- \)tocotrienol (Toc-3H). \( k_{\text{AOH}} \) values of all eight vegetable oils \( 1-8 \) also increased \( 1.24-1.54 \) times under the coexistence of constant concentrations of \( \text{UQ}_{10}\text{H}_2 \). A new mechanism to explain the notable increase of \( k_{\text{AOH}} \) values under the coexistence of two kinds of phenolic \( \text{AOHs} \) was proposed. UV-vis absorption of \( \alpha-, \beta-, \gamma- \)tocopherol radicals, produced by reaction of \( \alpha-, \beta-, \gamma- \)tocopherols (or vegetable oils \( 1-8 \) ) with \( \text{ArO}^\cdot \), disappeared under the coexistence of \( \alpha-, \beta-, \gamma- \)tocopherol and ubiquinol-10. This suggests that the prooxidant reaction resulting from the presence of \( \alpha-, \beta-, \gamma- \)tocopherol radicals is suppressed in the presence of ubiquinol-10.

Key words: vegetable oils, tocopherol and tocotrienol, ubiquinol-10, aroxyl-radical-scavenging rates, proposal of new mechanism for synergistic effect, stopped-flow spectrophotometry

1 Introduction

Vitamin E homologues, including \( \alpha-, \beta-, \gamma-, \delta- \)tocopherols \((\text{TocHs})\) and tocotrienols \((\text{Toc-3Hs})\), are well known as the most important lipophilic antioxidants \((\text{AOHs})\). \( \alpha-, \beta-, \gamma-, \delta- \)tocopherol function as efficient inhibitors of lipid peroxidation in both foods and biological systems \((1-5)\). The antioxidant action of \( \text{TocHs} \) and \( \text{Toc-3Hs} \) has been ascribed to their ability to scavenge lipid peroxyl \((\text{LOO}^\cdot)\) radicals, producing the tocopheroxyl \((\text{TOO}^\cdot)\) and tocotrienoxyl \((\text{TTOO}^\cdot)\) radicals, respectively \((\text{reaction 1})\) \((1,2,6)\). However, if \( \text{TocHs} \) and \( \text{Toc-3Hs} \) exist in edible oils and bio-membranes, \( \alpha-, \beta-, \gamma-, \delta- \)tocopherol and tocotrienol radicals may react with unsaturated lipids \((\text{LHs})\) \((\text{reaction 2})\), causing a prooxidant reaction which induces the degradation of these unsaturated lipids \((5,7-11)\).

\[
k_{\text{inh}} \quad \text{LOO}^\cdot + \text{TocH} \rightarrow \text{LOOH} + \text{TOO}^\cdot \quad (1)
\]

\[
k_{\text{p}} \quad \text{TOO}^\cdot + \text{LH} \rightarrow \text{Toc}^\cdot + \text{L} \quad (2)
\]

\[
k_{\text{inh}} \quad \text{LOO}^\cdot + \text{UQ}_{10}\text{H}_2 \rightarrow \text{LOOH} + \text{UQ}_{10}\text{H}_2^\cdot \quad (3)
\]

\[
k_{\text{inh}} \quad \alpha-\text{Toc}^\cdot + \text{UQ}_{10}\text{H}_2 \rightarrow \alpha-\text{Toc}^\cdot + \text{UQ}_{10}\text{H}_2^\cdot \quad (4)
\]

where \( \text{UQ}_{10}\text{H}_2^\cdot \) denotes a ubisemiquinone radical. Kinetic studies were performed for reactions 3 and 4 in organic solvents, indicating that both reactions are important for the antioxidant actions of \( \text{UQ}_{10}\text{H}_2^\cdot \) \((17-20)\).

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Vitamin C (Vit C)\textsuperscript{21–24} and tea catechins (CatHs) (i.e., EC, ECG, EGC, and EGCG)\textsuperscript{25–34} are well known water soluble AOHs, which may also function as AOHs (i) by scavenging LOO\textsuperscript{•} (reaction 3) and (ii) by reducing \(\alpha\)-Toc\textsuperscript{•} to \(\alpha\)-TocH (reaction 4).

Second-order rate constants \((k_s)\) for the reaction of many phenolic AOHs (including \(\alpha\), \(\beta\), \(\gamma\), and \(\delta\)-TocHs\textsuperscript{35–38} and catechins\textsuperscript{39}) with 2,6-di-t-buty1-4-(4-methoxyphenyl)...

Fig. 1 (A) Change in electronic absorption spectra of ArO\textsuperscript{•} and \(\alpha\)-Toc\textsuperscript{•} radicals during reaction of ArO\textsuperscript{•} with \(\alpha\)-TocH in mixed solvent at 25.0°C. Initial concentrations are [ArO\textsuperscript{•}] = 7.29 \times 10^{-5}\ M and [\(\alpha\)-TocH] = 5.68 \times 10^{-4}\ M. Spectra were recorded at 50 ms intervals. Arrow indicates a decrease (ArO\textsuperscript{•}) and an increase (\(\alpha\)-Toc\textsuperscript{•}) in absorbance with time. (B) Time dependences of the absorbance of ArO\textsuperscript{•} radical at 376 nm in solutions including six different concentrations of \(\alpha\)-TocH at 25.0°C. (C) Plots of \(k_{obsd}\) versus [\(\alpha\)-TocH] for reactions of ArO\textsuperscript{•} radical with (i) \(\alpha\)-TocH alone (○) and (ii) mixture of \(\alpha\)-TocH and UQ\textsubscript{10}H\textsubscript{2} (●). (D) Similar plots for \(\beta\)-TocH. (E) Similar plots for \(\gamma\)-TocH. (F) Similar plots for \(\delta\)-TocH.
Kinetic Study of Synergistic Effect between Tocopherol and Ubiquinol

The ArO• radical was used as a model for active oxygen radicals (LOO• and others) and the rate constants ($k_i$) were measured using stopped-flow spectrophotometry.

$$\text{ArO}_2^- + \text{AOH} \rightarrow \text{ArOH} + \text{AO}^-$$

It is well known that mixtures of AOHs (i.e., (i) $\alpha$-TocH and UQ$_{10}$H$_2$, (ii) $\alpha$-TocH and Vit C, and (iii) $\alpha$-TocH and CatHs) may function synergistically as AOHs in foods and biological systems. In a previous study, the second-order rate constants were measured for these AOHs, kinetic study has not been performed for the mixtures of these AOHs. In a previous study, $k_{\text{AOH}}$ values were measured for each AOH and for combinations of these AOHs (i.e., $\alpha$-TocH and UQ$_{10}$H$_2$ and (ii) $\alpha$-TocH and Vit C (i.e., sodium ascorbate)) in a 2-PrOH/H$_2$O solution. A notable synergistic effect (that is, an increase of $k_{\text{AOH}}$ value) was observed for mixtures of two AOHs. In addition, measurement was performed for the mixture of $\alpha$-TocH and CatHs (EC, EGC, and EGC-G) in ethanol solution, and a similar synergistic effect was observed. However, examples are very limited, and mechanism of synergistic effect has not been clarified.

In the present study, first, measurements of the second-order rate constants ($k_{\text{AOH}}$) were performed for reactions of $\text{ArO}_2^•$ with one of four TocHs (i.e., $\alpha$, $\beta$, $\gamma$, $\delta$-TocH) and UQ$_{10}$H$_2$ in mixed solvent, using stopped-flow spectrophotometry. Second, the $k_{\text{AOH}}$ values were measured for solution including two AOHs (i.e., TocH and UQ$_{10}$H$_2$) to investigate the synergistic effect of AOHs on the ArO•-scavenging rate constant ($k_{\text{AOH}}$). The result indicated that the rate constant ($k_{\text{AOH}}$) increases notably under the coexistence of two AOHs in the solution. Given this data we propose a novel mechanism to explain the increase in $k_{\text{AOH}}$ values for mixed AOH solutions.

Edible vegetable oils are used for cooking in ordinary homes every day. Vegetable oils 1–8 (see Table 2) generally contain high concentrations of unsaturated lipids, and they are exposed to danger of lipid peroxidation. Vegetable oils 1–8 contain different concentrations of vitamin E homologues (see supplementary Table S1), and the degradation of oils 1–8 is prevented by the action of these vitamin E homologues.

Recently, a detailed kinetic study was performed for the reactions of ArO• radical with oils 1–8. In the present study, measurements of $k_{\text{AOH}}$ values were performed for the mixtures of oils 1–8 and UQ$_{10}$H$_2$. Vegetable oils 1–8 contain high concentrations of fatty acids (75.7–85.5 g/100 g vegetable oil), which means that it is interesting to understand whether a similar synergistic effect (that is, an increase in the $k_{\text{AOH}}$ value) is observed for oils 1–8 containing UQ$_{10}$H$_2$.

### 2 Materials and Methods

#### 2.1 Materials

$\alpha$, $\beta$, $\gamma$, and $\delta$-tocopherol (-TocH) were supplied by the Eisai Co. Ltd. (Tokyo, Japan). Ubiquinone-10 (UQ$_{10}$H$_2$) was kindly supplied by Kaneka Co. Ltd. (Osaka, Japan). Ubiquinol-10 (UQ$_{10}$H$_2$) was prepared by reducing UQ$_{10}$ with sodium phenoxyl (aroxyl (ArO•)) radical (Fig. 1B) were measured in organic solvents and micellar solutions (reaction 5), in order to clarify the free radical-scavenging activity of these AOHs. The ArO• radical was used as a model for active oxygen radicals (LOO• and others) and the rate constants ($k_i$) were measured using stopped-flow spectrophotometry.
Table 2  Second-order rate constants ($k_s^{\alpha}(\text{alone})$ and $k_s^{\alpha}(+\text{UQ}_{10}H_2_2)$) and ratio ($k_s^{\alpha}(+\text{UQ}_{10}H_2_2)/k_s^{\alpha}(\text{alone})$) for eight vegetable oils 1 - 8 in mixed solvent at 25.0°C, and concentrations of (i) α-TocH+α-Toc-3H and (ii)γ-TocH+γ-Toc-3H contained in oils 1 - 8.

| Tocopherol   | $k_s^{\alpha}(\text{alone})$ | $[\text{UQ}_{10}H_2]$ (constant) | $k_s^{\alpha}(+\text{UQ}_{10}H_2_2)$ | $k_s^{\alpha}(+\text{UQ}_{10}H_2_2)/k_s^{\alpha}(\text{alone})$ | α-TocH + α-Toc-3H | γ-TocH + γ-Toc-3H |
|--------------|-------------------------------|----------------------------------|------------------------------------|-------------------------------------------------|-----------------|-----------------|
| Rice bran oil 1 | $18.5 \pm 0.1 \times 10^{-3}$ | $2.24 \times 10^{-4}$              | $(26.4 \pm 0.2) \times 10^{-3}$   | 1.43                                             | 62.9            | 31.6            |
| Perilla oil 2   | $(9.97 \pm 0.1) \times 10^{-3}$ | $2.33 \times 10^{-4}$              | $(15.4 \pm 0.3) \times 10^{-3}$   | 1.54                                             | 1.0             | 75.9            |
| Rape seed oil 3 | $8.73 \pm 0.28 \times 10^{-3}$ | $2.30 \times 10^{-4}$              | $(12.6 \pm 0.6) \times 10^{-3}$   | 1.44                                             | 16.6            | 46.3            |
| Safflower oil 4 | $8.63 \pm 0.30 \times 10^{-3}$ | $2.47 \times 10^{-4}$              | $(10.8 \pm 0.2) \times 10^{-3}$   | 1.25                                             | 46.6            | 2.8             |
| Grape seed oil 5 | $8.06 \pm 0.19 \times 10^{-3}$ | $2.36 \times 10^{-4}$              | $(11.8 \pm 0.2) \times 10^{-3}$   | 1.46                                             | 19.8            | 25.6            |
| Sesame oil 6     | $8.11 \pm 0.17 \times 10^{-3}$ | $2.36 \times 10^{-4}$              | $(11.6 \pm 0.2) \times 10^{-3}$   | 1.43                                             | 5.8             | 49.3            |
| Extra virgin olive oil 7 | $6.11 \pm 0.09 \times 10^{-3}$ | $2.33 \times 10^{-4}$              | $(7.57 \pm 0.29) \times 10^{-3}$  | 1.24                                             | 24.7            | 1.9             |
| Olive oil 8      | $3.29 \pm 0.20 \times 10^{-3}$ | $2.38 \times 10^{-4}$              | $(4.61 \pm 0.30) \times 10^{-3}$  | 1.40                                             | 16.2            | 1.7             |

Values are expressed as mean ± SD (standard deviation).

3 Results

3.1 Measurements of the aroyl-radical-scavenging rates ($k_s^{\alpha AOH}(\text{alone})$) for α-, β-, γ-, and δ-tocopherol and ubiquinol-10 in mixed solvent

By reacting TocH (α-, β-, γ-, and δ-TocH) with ArO• radical, the absorbances at 376 and 580 nm of the ArO• decrease, and the absorbances of the Toc• (α-, β-, and γ-Toc•) radicals increase. The absorption of the δ-Toc• radical was not observed, but this is to be expected, as δ-Toc• radical is unstable. An example for α-TocH is shown in Fig. 1(A). The scavenging rate ($k_{\text{obsd}}$) of ArO• was measured by following the decrease in absorbance at 376 or 580 nm of the ArO• radical (Fig. 1(B)). The $k_{\text{obsd}}$ value was linearly dependent on the concentration of TocH [TocH], and thus, the rate equation can be expressed as

$$-d[\text{ArO•}]/dt = k_{\text{obsd}}[\text{ArO•}] = k_s^{\alpha \text{TocH}}[\text{TocH}][\text{ArO•}]$$  (6)

where $k_s^{\alpha \text{TocH}}$ is the second-order rate constant for reaction of ArO• radical with TocH. The $k_s^{\alpha \text{TocH}}$ value was obtained by plotting $k_{\text{obsd}}$ against [TocH] (Fig. 1(C), (D), (E), and (F) (open circle)). The $k_s^{\alpha \text{TocH}}(\text{alone})$ value obtained for α-, β-, γ-, and δ-TocH are listed in Table 1, where $k_s^{\alpha \text{TocH}}(\text{alone})$ represents the ArO•-radical-scavenging rate constants obtained for solutions containing only one component of AOH.

Similar measurements were performed for reaction of UQ$_{10}$H$_2$ with ArO•.

$$k_s^{\text{UQ}_{10}H_2}$$  (7)

ArO• + UQ$_{10}$H$_2$ → ArOH + UQ$_{10}$H.

For example, by reacting UQ$_{10}$H$_2$ with ArO• radical, the absorbance at 376 nm of ArO• decreases rapidly (data not shown). We could not observe the absorption spectrum for the UQ$_{10}$H• radical because of its instability. The $k_s^{\text{UQ}_{10}H_2}(\text{alone})$ value obtained for UQ$_{10}$H$_2$ is $(4.64 \pm 0.04) \times 10^{-3}$ M$^{-1}$ s$^{-1}$ (Table 1).

As listed in Table 1, the $k_s^{\alpha AOH}(\text{alone})$ value increases in

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the order of
\[ \delta-\text{TocH} < \UQ_{10}H_{2} < \gamma-\text{TocH} < \beta-\text{TocH} < \alpha-\text{TocH} \] (8)

3.2 Measurement of the aroxyl-radical-scavenging rates 
\( k_{s}^{\text{TocH}} (+ \UQ_{10}H_{2}) \) for mixtures of tocopherol homologues and ubiquinol-10 in mixed solvents

First, measurement of the \( k_{s}^{\alpha-\text{TocH}} (+ \UQ_{10}H_{2}) \) value was performed for a solution including \( \alpha-\text{TocH} \) and \( \UQ_{10}H_{2} \). \( k_{s}^{\alpha-\text{TocH}} (+ \UQ_{10}H_{2}) \) indicates the rate constant obtained for solutions including two components of AOHs. \( k_{s}^{\alpha-\text{TocH}} (+ \UQ_{10}H_{2}) \) value was measured by keeping the \([\UQ_{10}H_{2}]\) constant (\([\UQ_{10}H_{2}] = 3.15 \times 10^{-4} \) M) (Table 1) and varying the \([\alpha-\text{TocH}]\) (\((0 - 5.68) \times 10^{-4} \) M). By analyzing the decay curves of \( \text{ArO}^\cdot \) at 376 nm, \( k_{\text{obsd}} \) values were determined.

Figure 1 (C) (closed circle) shows the \( k_{\text{obsd}} \) versus \([\alpha-\text{TocH}]\) plot (34, 43).

Similar measurements were performed for solutions including (i) \( \beta-\text{TocH} \) and \( \UQ_{10}H_{2} \), (ii) \( \gamma-\text{TocH} \) and \( \UQ_{10}H_{2} \), and (iii) \( \delta-\text{TocH} \) and \( \UQ_{10}H_{2} \). \( k_{\text{obsd}} \) versus \([\text{TocH}]\) (\( \beta^{-}, \gamma^{-}, \delta^{-}\text{TocH} \)) plots (closed circle) are shown in Fig. 1 (D), (E), and (F), respectively.

If \( \text{TocH} \) and \( \UQ_{10}H_{2} \) coexist in a solution, competitive reactions 9 and 10 will occur, because the \( k_{s}^{\text{TocH}}(\text{alone}) \) values for \( \alpha^{-}, \beta^{-}, \gamma^{-}, \delta^{-}\text{TocH} \) show the same order of rate constant as \( k_{s}^{\UQ_{10}H_{2}}(\text{alone}) \) value of \( \UQ_{10}H_{2} \) (Table 1).

\[ \text{ArO}^\cdot + \text{TocH} + \UQ_{10}H_{2} \rightarrow \text{ArOH} + \text{Toc}^\cdot + \UQ_{10}H_{2} \] (9)

\[ \text{ArO}^\cdot + \text{TocH} + \UQ_{10}H_{2} \rightarrow \text{ArOH} + \text{Toc} + \UQ_{10}H_{2} \] (10)

In such a case, we can expect that the \( k_{\text{obsd}} \) value depends on Eq. (11), if the interaction between \( \text{TocH} \) and \( \UQ_{10}H_{2} \) is negligible (34, 43).

\[ k_{\text{obsd}} = k_{s}^{\text{TocH}}(\text{alone}) \times [\text{TocH}] + k_{s}^{\UQ_{10}H_{2}}(\text{alone}) \times [\UQ_{10}H_{2}] \] (11)

Next we will assume that a synergistic effect is present. By substituting \( k_{s}^{\text{TocH}}(\text{alone}) \) and \( k_{s}^{\UQ_{10}H_{2}}(\text{alone}) \) values and the \([\UQ_{10}H_{2}]\) value (\( = 3.19 \times 10^{-4} \) M for \( \alpha^{-}\text{TocH} \), \( 2.39 \times 10^{-4} \) M for \( \beta^{-}\text{TocH} \), \( 2.37 \times 10^{-4} \) M for \( \gamma^{-}\text{TocH} \), and \( 2.32 \times 10^{-4} \) M for \( \delta^{-}\text{TocH} \)) used for measurements (Table 1) into Eq. (11), \( k_{\text{obsd}} \) was plotted against \([\text{TocH}] \) (see dotted line in Fig. 1 (C), (D), (E), and (F), respectively).

As described above, all measurements were performed, keeping the \( \UQ_{10}H_{2} \) at a constant concentration and varying the \([\text{TocH}] \) (\( \alpha^{-}, \beta^{-}, \gamma^{-}, \delta^{-}\text{TocH} \)). Consequently, the \( k_{s}^{\text{TocH}}(\text{alone}) \) value (Table 1) was determined from the gradient of the \( k_{\text{obsd}} \) versus \([\text{TocH}] \) plot (closed circle) in Fig. 1 (C), (D), (E), and (F), respectively, using Eq. (12).

\[ k_{s}^{\text{TocH}}(\text{alone}) = \frac{1}{k_{s}^{\text{TocH}}\times[\text{TocH}]} \] (12)

As expected from the gradient in Fig. 1 (C), (D), (E), and (F), the \( k_{s}^{\text{TocH}}(\text{alone}) \) values of \( \alpha^{-}, \beta^{-}, \gamma^{-}, \delta^{-}\text{TocH} \) are 1.21, 1.28, 1.55, and 1.19 times, respectively, larger than the corresponding \( k_{s}^{\text{TocH}}(\text{alone}) \) values (Table 1). A notable synergistic effect due to the coexistence of \( \text{TocH} \) and \( \UQ_{10}H_{2} \) in solution was observed for the rate constant (\( k_{s}^{\text{TocH}}(\text{alone}) \)). The \( k_{s}^{\text{TocH}}(\text{alone}) \) and \( k_{s}^{\text{TocH}}(\text{alone}) \) values obtained for \( \alpha^{-}, \beta^{-}, \gamma^{-}, \delta^{-}\text{TocH} \) are shown as a bar graph in Fig. 2 (A).

3.3 Measurements of the aroxyl radical-scavenging rates 
\( k_{s}^{\text{ArO}}(+ \UQ_{10}H_{2}) \) for mixtures of vegetable oils 1 - 8 and ubiquinol-10 in mixed solvent

The rate constants \( k_{s}^{\text{ArO}}(\text{alone}) \) for the reaction of the \( \text{ArO}^\cdot \) radical with vegetable oils 1 - 8 were measured in mixed solvent, as performed for \( \alpha^{-}, \beta^{-}, \gamma^{-}, \delta^{-}\text{TocH} \).

\[ \text{ArO}^\cdot + \text{Vegetable oil} \rightarrow \text{ArOH} + \text{Vegetable oil}^\cdot \] (13)

For example, by reacting grape seed oil 5 with the \( \text{ArO}^\cdot \) radical, the absorbance at 376 and 580 nm of \( \text{ArO}^\cdot \) decreases, and the absorbance at \( \approx 429 \text{ nm} \) of the \( \text{Toc}^\cdot \) radicals, which will be due to \( \alpha^{-}\text{Toc}^\cdot, \alpha^{-}\text{Toc}^\cdot-3^\cdot, \gamma^{-}\text{Toc}^\cdot, \text{and} \gamma^{-}\text{Toc}^\cdot-3^\cdot \), increases, as shown in Fig. 3 (A), because \( \alpha^{-}\text{TocH}, \alpha^{-}\text{Toc}-3\text{H}, \gamma^{-}\text{TocH}, \text{and} \gamma^{-}\text{Toc}-3\text{H} \) are contained in grape seed oil 5 (Table 2 or Table S1) (46, 49).

Decay rates \( (k_{\text{obsd}}) \) for the \( \text{ArO}^\cdot \) radical were measured by

![Fig. 2](image-url)
following the decrease in absorbance at 376 nm (Fig. 3 (B)). The pseudo-first-order rate constant \( k_{\text{obsd}} \) was obtained by varying the concentration of grape seed oil 5.

The value of \( k_{\text{obsd}} \) (alone) \( = (8.06 \pm 0.19) \times 10^{-3} \text{ Lg}^{-1} \text{s}^{-1} \) was obtained by plotting \( k_{\text{obsd}} \) against [grape seed oil] (g/L), as shown in Fig. 3 (D) (open circle). Similar measurements

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**Fig. 3** (A) Change in electronic absorption spectra of ArO· and α-Toc· (and γ-Toc·) radicals during reaction of ArO· with grape seed oil 5 in mixed solvent at 25.0°C. Initial concentrations are [ArO·] = 6.59 \times 10^{-5} \text{ M} and [grape seed oil] = 90.6 \text{ gL}^{-1}. Spectra were recorded at 250 ms intervals. Arrow indicates a decrease (ArO·) and an increase (α-Toc· and γ-Toc·) in absorbance with time. (B) Time dependences of the absorbance of ArO· radical at 376 nm in solutions including six different concentrations of oil 5 at 25.0°C. (C) Plots of \( k_{\text{obsd}} \) versus [Pelilla oil] for reactions of ArO· radical with (i) perilla oil 2 alone (○) and (ii) mixture of oil 2 and UQ10H2 (●). Similar plots for (D) grape seed oil 5, (E) sesame oil 6, and (F) extra virgin olive oil 7.
were performed for the other vegetable oils 1 - 4 and 6 - 8 in mixed solvents. Units of g/L were used for the concentrations of vegetable oils 1 - 8.

$k_{\text{diss}}$ against [vegetable oil (g/L)] plots for oils 2, 6, and 7 are also shown in Fig. 3 (C), (E), and (F), respectively, showing a good linear relationship between $k_{\text{diss}}$ and [vegetable oil]. The $k_{\text{diss}}$ (alone) values obtained for oils 1 - 8 are listed in Table 2.

Further, measurements of the $k_{10}^{\text{obs}}$ (+ UQ$_{10}$H$_2$) values were performed for the solutions including vegetable oils and UQ$_{10}$H$_2$. $k_{10}^{\text{obs}}$ (+ UQ$_{10}$H$_2$) indicates the rate constant obtained for solutions containing two components of AOHs. Measurement of the $k_{10}^{\text{obs}}$ (+ UQ$_{10}$H$_2$) for grape seed oil 5 was performed by keeping the[UQ$_{10}$H$_2$] at constant (2.36 x $10^{-4}$ M) and varying the [grape seed oil] (0 - 90.4 g/L). By mixing the solution of ArO$^\cdot$ radical with the solutions including oil 5 and UQ$_{10}$H$_2$, absorption of the ArO$^\cdot$ at 376 and 580 nm decreases rapidly (data are not shown). By analyzing the decay curves for ArO$^\cdot$ at 376 nm, $k_{\text{diss}}$ values were determined. Figure 3 (D) (closed circle) shows the $k_{\text{diss}}$ versus [grape seed oil] plot. The $k_{10}^{\text{obs}}$ (+ UQ$_{10}$H$_2$) value obtained for oil 5 is (11.8 ± 0.2) x $10^{-1}$ L mol$^{-1}$ s$^{-1}$.

Similar measurements were performed for oils 1 - 8, and $k_{\text{diss}}$ versus [vegetable oil] plot (closed circle) for oils 2, 6, and 7 are shown in Fig. 3 (C), (E), and (F), respectively. The $k_{10}^{\text{obs}}$ (+ UQ$_{10}$H$_2$) values obtained for oils 1 - 8 are listed in Table 2.

If vegetable oil and UQ$_{10}$H$_2$ coexist in solution, reactions 14 and 15 will occur competitively in solution, as observed for the solutions containing TocH (i.e., $\alpha$, $\beta$, $\gamma$, and $\delta$-TocH) and UQ$_{10}$H$_2$, because vegetable oils 1 - 8 contain different concentrations of $\alpha$, $\beta$, $\gamma$, and $\delta$-TocH and -Toc-3H (Table 2 or Table S1).

\[
k_{10}^{\text{obs}} = k_{\alpha}^{\text{obs}}(\text{Oil}) + k_{\alpha}^{\text{UQ}_{10}H_2}(\text{Oil}) + k_{\beta}^{\text{UQ}_{10}H_2}(\text{Oil}) + k_{\gamma}^{\text{UQ}_{10}H_2}(\text{Oil}) + k_{\delta}^{\text{UQ}_{10}H_2}(\text{Oil})
\]

As expected from the gradient in Fig. 3 (C), (D), (E), and (F), the $k_{10}^{\text{obs}}$ (+ UQ$_{10}$H$_2$) values are larger than $k_{10}^{\text{obs}}$ (alone) obtained for the solution including only Oil. All the $k_{10}^{\text{obs}}$ (+ UQ$_{10}$H$_2$) values and ratios of $k_{10}^{\text{obs}}$ (+ UQ$_{10}$H$_2$) to $k_{10}^{\text{obs}}$ (alone) obtained for Oils 1 - 8 are listed in Table 2. The values in Table 2 demonstrate a notable synergistic effect between vegetable oils 1 - 8 and UQ$_{10}$H$_2$. The value of the ratios of $k_{10}^{\text{obs}}$ (+ UQ$_{10}$H$_2$) to $k_{10}^{\text{obs}}$ (alone) varied from 1.24 for extra virgin olive oil 7 (containing mainly $\alpha$-TocH) to 1.54 for perilla oil 2 (containing mainly $\gamma$-TocH) (Table 2). The $k_{10}^{\text{obs}}$ (alone) and $k_{10}^{\text{obs}}$ (+ UQ$_{10}$H$_2$) values obtained for oils 1 - 8 are shown as a bar graph in Fig. 2 (B).

### 3.4 UV-Vis absorption of the tocopheroxyl radicals disappears under the coexistence of tocopherols (or vegetable oils) and ubiquinol-10: Suppression of proxidant effect of tocopherols

As described in Section 3.1, by reacting ArO$^\cdot$ radical with $\alpha$, $\beta$, and $\gamma$-TocH, absorptions of $\alpha$, $\beta$, and $\gamma$-Toc radicals appear at $\lambda_{\text{max}}$ = 429, 432, and 433 nm, respectively (see Fig. 4 (A), (C), and (E)). As $\delta$-Toc is unstable, absorption of $\delta$-Toc$^\cdot$ was not observed\cite{11}. On the other hand, if TocH and UQ$_{10}$H$_2$ coexist in solution, reactions 9 and 10 and/or Toc$^\cdot$ + UQ$_{10}$H$_2$ will occur competitively in solution, because $\alpha$, $\beta$, $\gamma$, and $\delta$-TocH and UQ$_{10}$H$_2$ show the same order of reaction rate constant ($k_r$) (Table 1). UQ$_{10}$H$_2$ - radical is very unstable, and disappears rapidly\cite{30}. Further, $\alpha$, $\beta$, $\gamma$, $\delta$-Toc$^\cdot$ produced by the reaction of $\alpha$, $\beta$, $\gamma$-TocH with ArO$^\cdot$, respectively, also disappear rapidly, if UQ$_{10}$H$_2$ coexists in solution, as shown in Fig. 4 (B), (D), and (F). The result suggests that $\alpha$, $\beta$, $\gamma$-Toc$^\cdot$ produced are reduced to $\alpha$, $\beta$, $\gamma$-TocH by fast regeneration reaction between $\alpha$, $\beta$, $\gamma$-TocH and UQ$_{10}$H$_2$, respectively\cite{18}. In fact, the rate constants ($k_r$) of regeneration reaction (reaction 4) of $\alpha$- and $\beta$-Toc radicals to $\alpha$- and $\beta$-TocH by UQ$_{10}$H$_2$ reported are very fast ($k_r = 2.15 \times 10^5$ and $2.95 \times 10^5$ M$^{-1}$ s$^{-1}$, respectively) at 25.0°C in ethanol\cite{18,20}.

Similar measurements were performed for the solutions containing vegetable oils 1 - 8 and UQ$_{10}$H$_2$. Vegetable oils 1 - 8 contain different concentrations of $\alpha$, $\beta$, $\gamma$, $\delta$-TocH and -Toc-3H (Table 2 or Table S1)\cite{19}. For example, by reacting ArO$^\cdot$ radical with vegetable oils (perilla oil 2, grape seed oil 5, and olive oil 8), the absorptions of Toc$^\cdot$ and Toc-3$^\cdot$ radicals appear rapidly, as shown in Fig. 5 (A), (C), and (E). On the other hand, if vegetable oils 2, 5, and 8 and UQ$_{10}$H$_2$ coexist in solution, absorptions of Toc$^\cdot$ and Toc-3$^\cdot$ radicals disappear rapidly, as shown in Fig. 5 (B), (D), and (F), respectively. These results indicate that fast regeneration reactions between Toc$^\cdot$ and/or Toc-3$^\cdot$ radicals (contained in vegetable oils 2, 5, and 8) and UQ$_{10}$H$_2$ proceed in solution. Similar results were obtained for the other vegetable oils 1, 3, 4, 6, and 7 used in the present study.

\[
k_{\text{diss}} = k_{\alpha}^{\text{obs}}(\text{Oil}) + k_{\alpha}^{\text{UQ}_{10}H_2}(\text{Oil})
\]
Toc-3· radicals react with LH, and show a pro-oxidant effect (reaction 2). High concentrations of fatty acids (i.e., 75.7−85.5 g/100 g oil) are contained in the vegetable oils 1-8. The result obtained in the present study suggests that the prooxidant reaction due to Toc-3· and Toc-3· radicals may be suppressed by the coexistence of UQ₁₀H₂. Addition of UQ₁₀H₂ to vegetable oils will be effective to protect the degradation of vegetable oils 7-11. Any examples for such a direct observation of the disappearance of α-, β-, and γ-Toc· radicals produced by the reactions with ArO· under the coexistence of vegetable oils and UQ₁₀H₂ have not been reported, as far as we know.

Fig. 4 (A) and (B) Change in electronic absorption spectra of ArO· and α-Toc· radicals during reaction of ArO· with (A) α-TocH alone and (B) a mixture of α-TocH and UQ₁₀H₂ in mixed solvent at 25.0°C. Initial concentrations of [ArO·], [α-TocH], and [UQ₁₀H₂] used for reaction are shown in Fig. 4(A) and (B). (C) and (D) Similar plots for β-TocH. (E) and (F) Similar plots for γ-TocH.
Discussion

4.1 Aroxyl-radical-scavenging rate ($k_{s, AOH}$) remarkably increases under the coexistence of $\alpha$-, $\beta$-, $\gamma$-, and $\delta$-tocopherol (or vegetable oils) and ubiquinol-10

It is well known that various AOHs coexist in many foods, plants, and biological systems. These AOHs function not only individually but also synergistically with other AOHs to scavenge free radicals. The interaction between $\alpha$-TocH and Vit C is the most well-known one. Hydrophilic Vit C present in the aqueous phase of biological systems efficiently reduces $\alpha$-Toc radical located within the membranes and lipoproteins to regenerate $\alpha$-TocH and inhibits initiation of a chain reaction induced by $\alpha$-Toc (that is, the prooxidant effect of $\alpha$-TocH) (reaction 2). In

![Fig. 5](A) and (B) Change in electronic absorption spectra of ArO• and $\alpha$-Toc• ($\gamma$-Toc•) radicals during reaction of ArO• with (A) perilla oil 2 alone and (B) a mixture of perilla oil 2 and UQ$_{10}$H$_2$ in mixed solvent at 25.0°C. Initial concentrations of [ArO•], [Perilla oil], and [UQ$_{10}$H$_2$] used for reaction are shown in Fig. 5(A) and (B). (C) and (D) Similar plots for grape seed oil. (E) and (F) Similar plots for olive oil.

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fact, values of inhibition times ($t_{inh}$) of $\alpha$-TocH increase by adding Vit C to an $\alpha$-TocH solution. Similarly, lipophilic UQ$_{10}$H$_2$ regenerates $\alpha$-TocH during lipid peroxidation in homogeneous solution, liposomal membranes, low density lipoprotein, and mitochondrial membranes$^{23,40}$.

Measurements of the inhibition rate constant ($k_{inh}$) for the scavenging of LOO$^\cdot$ radical were performed for $\alpha$-TocH, Vit C, and mixtures of two AOHs (i.e., $\alpha$-TocH and Vit C) in tert-butyl alcohol/methanol (3/1, v/v) solution at 37°C$^{21}$. The $k_{inh}$ value obtained for the mixture of $\alpha$-TocH and Vit C was similar to that for $\alpha$-TocH alone, and increase of the $k_{inh}$ value was not observed under the coexistence of two AOHs. However, the details are not clear, because the $k_{inh}$ values reported include large experimental errors.

The synergistic antioxidant effect of polyphenols extracted from green tea (CatHs: EC, EGC, EGC, and EGCG) with $\alpha$-TocH against the peroxidation of linoleic acid has been studied in homogeneous solutions$^{27}$, in homogeneous micelles and liposomes$^{28,29,32}$, and in human LDL$^{60}$. Mixtures of green tea polyphenols and $\alpha$-TocH act synergistically to protect lipid peroxidation. In fact, $t_{inh}$ values of $\alpha$-TocH increased by adding CatHs to a $\alpha$-TocH solution$^{27}$. However, the synergistic effect was almost negligible in oil/water emulsions$^{53}$ and a phosphatidylcholine-based liposome system$^{44}$. While the effect was varied in heterogeneous solutions. Further, inhibition rate constants ($k_{inh}$) were measured for $\alpha$-TocH, CatHs, and mixtures of these two AOHs in homogeneous solutions and micelles. However, increase of $k_{inh}$ values was not observed under the coexistence of two AOHs. The $k_{inh}$ values rather decreased under the coexistence of $\alpha$-TocH and CatH in homogeneous and micellar solutions$^{27}$.

In a previous study, measurements of the ArO$^\cdot$-scavenging rate constants ($k_{s, AOH}$) of AOHs ($\alpha$-TocH, UQ$_{10}$H$_2$ and Vit C (sodium ascorbate, Na"AsH") were performed in 2-propanol/water, using stopped-flow spectrophotometry$^{49}$. The $k_{s, AOH}$ values were measured not only for each AOH, but also for the mixtures of two AOHs ([i] $\alpha$-TocH and UQ$_{10}$H$_2$, and [ii] $\alpha$-TocH and Na"AsH") For example, the measurement of the $k_{s, \alpha$-TocH$}^\cdot$+(+UQ$_{10}$H$_2$) value was performed by keeping [UQ$_{10}$H$_2$] constant and varying [$\alpha$-TocH]. The value of $k_{s, \alpha$-TocH$}^\cdot$+(+UQ$_{10}$H$_2$) obtained was 1.63 times larger than that of $k_{s, \alpha$-TocH$}^\cdot$( alone). Similarly, the value of $k_{s, \alpha$-TocH$}^\cdot$+(Na"AsH") was 1.42 times larger than that of $k_{s, \alpha$-TocH$}^\cdot$( alone). ArO$^\cdot$-scavenging rates increased notably under the coexistence of two AOHs in solution$^{49}$.

Recently, rate constants ($k_{s, AOH}$) for AOHs ($\alpha$-TocH, EC, EGC, and EGCG) in ethanol solutions were evaluated for both single AOHs and combinations of these AOHs ([i] $\alpha$-TocH and EC, [ii] $\alpha$-TocH and EGC, and [iii] $\alpha$-TocH and EGCG)$^{34}$. First, measurements of the $k_{s, \alpha$-TocH$}^\cdot$+(CatH) values were performed by keeping the [CatH] constant and varying the [$\alpha$-TocH]. $k_{s, \alpha$-TocH$}^\cdot$+(CatH) values of $\alpha$-TocH were 1.29, 1.84, and 1.65 times larger than the correspond-
very high and vary from 75.707 g/100 g of rice bran 1 to 85.520 g/100 g of extra virgin olive oil 6−8. However, the result of the present kinetic study indicates that effect of fatty acids contained in oils 1 − 8 on \( k_{\text{act}} \) values of vegetable oils 1 − 8 seems to be small and negligible.

4.2 Mechanism for the increase of the aroxyl-radical-scavenging rate \( k_{\text{AORH}} \) under the coexistence of vitamin E homologues (or vegetable oils 1 − 8) and ubiquinol−10

In previous studies, measurements of the scavenging rate \( k_{\text{ar}} \) of ArO− by many phenolic AOHs including \( \alpha, \beta, \gamma, \) and \( \delta \)-TocH and UQ_{10}H were performed in ethanol solution (reaction 5) 35−38. The logarithms of the rate constants \( \log k_{\text{ar}} \) were found to correlate well with their peak oxidation potentials \( E_{p} \); the AOHs which have smaller \( E_{p} \) values show higher scavenging rates 35−37. From detailed analysis of the temperature dependence of \( k_{\text{ar}} \) values, the activation energy \( (E_{a}) \) for the reaction was determined 39. The \( E_{a} \) values obtained were also found to correlate well with the \( E_{p} \) values. A similar result was obtained for biological hydroquinones including UQ_{10}H 18, 39. These facts suggest that the transition states in the ArO− scavenging reactions by TocH (i.e., \( \alpha, \beta, \gamma, \) and \( \delta \)-TocH) and UQ_{10}H have the property of electron-transfer intermediates (i.e., (i) transition state A \( ([\text{ArO}: \cdots \leftarrow \text{α\text{-TocH}:}^{\cdot}\text{−}] \rightarrow \text{ArOH} + \text{α\text{-TocH}}^{\cdot} \) (18)); (ii) transition state B \( ([\text{ArO}: \cdots \leftarrow \text{UQ}_{10}\text{H}^{\cdot}]) \)) respectively (see Scheme 1 (Eqs. (18) and (19)) 35−38), which has also been described for interaction between LOO· and vitamin E homologues 35, 56. An example for \( \alpha \)-TocH is shown in Fig. 6. In the case of \( \alpha \)-TocH, \( k_{\text{a\text{-TocH}}} \) (alone) > \( k_{\text{a\text{-UQ}_{10}\text{H}}} \) (alone) (Table 1), and thus, \( E_{\text{act}} > E_{\text{act}} \).

Scheme 1: One component system ---

(i) Transition State A --- Reaction with \( \alpha \)-TocH
\[
k_{\text{a\text{-TocH}}} \quad \text{ArO}^{\cdot} + \text{α\text{-TocH}} \rightarrow [\text{ArO}: \cdots \leftarrow \text{α\text{-TocH}:}^{\cdot}\text{−}] \rightarrow \text{ArOH} + \text{α\text{-TocH}}^{\cdot} \tag{18}
\]

(ii) Transition State B --- Reaction with UQ_{10}H
\[
k_{\text{a\text{-UQ}_{10}\text{H}}} \quad \text{ArO}^{\cdot} + \text{UQ}_{10}\text{H}^{\cdot} \rightarrow [\text{ArO}: \cdots \leftarrow \text{UQ}_{10}\text{H}^{\cdot}] \rightarrow \text{ArOH} + \text{UQ}_{10}\text{H}^{\cdot} \tag{19}
\]

As described above, ArO·-scavenging rate constants \( k_{\text{a\text{-TocH}}} \) (alone) increased significantly when a second antioxidant \( \text{UQ}_{10}\text{H} \) was added to the solution. In such cases, Scheme 2 (Eqs. (20), (21), and (22)) has been proposed to explain the mechanism of the reactions.

If \( \alpha \)-TocH and \( \text{UQ}_{10}\text{H} \) coexist in the reaction mixtures and there is no interaction between \( \text{α\text{-TocH}} \) and \( \text{UQ}_{10}\text{H} \), reactions 20 and 21 proceed competitively. This would mean that, during the transition state, electron transfer intermediates (transition state A \( ([\text{ArO}: \cdots \leftarrow \text{α\text{-TocH}:}^{\cdot}\text{−}] \rightarrow \text{ArOH} + \text{α\text{-TocH}}^{\cdot} \) + \( \text{UQ}_{10}\text{H}^{\cdot} \)) and transition state B \( ([\text{ArO}: \cdots \leftarrow \text{UQ}_{10}\text{H}^{\cdot}] + \text{α\text{-TocH}}^{\cdot} \)) would coexist in solution. On the other hand, as suggested by Eq. (22), an equilibrium between transition states A and B (i.e., a quasi-resonance stabilization) should occur easily due to fast electron transfer. As a result, activation energy decreases from \( E_{\text{act}} \) to \( E_{\text{act}} \). Aspects of these changes to the potential curves under the coexistence of \( \alpha \)-TocH and \( \text{UQ}_{10}\text{H} \) is shown in Scheme 2 of Fig. 6. This would be the reason why the ArO·-scavenging rate constant \( (k_{\text{a\text{-TocH}}} + \text{UQ}_{10}\text{H}) \) increases under the coexistence of \( \alpha \)-TocH and \( \text{UQ}_{10}\text{H} \) in solution.

Scheme 2: Two component systems --- Coexistence of \( \alpha \)-TocH and \( \text{UQ}_{10}\text{H} \)

(i) Transition State A --- Reaction with \( \alpha \)-TocH under the coexistence of \( \text{UQ}_{10}\text{H} \)
\[
k_{\text{a\text{-TocH}}} \quad \text{ArO}^{\cdot} + \text{α\text{-TocH}} + \text{UQ}_{10}\text{H} \rightarrow [\text{ArO}: \cdots \leftarrow \text{α\text{-TocH}:}^{\cdot}\text{−}] + \text{UQ}_{10}\text{H}^{\cdot} \rightarrow \text{ArOH} + \text{α\text{-TocH}}^{\cdot} + \text{UQ}_{10}\text{H}^{\cdot} \tag{20}
\]

(ii) Transition State B --- Reaction with \( \text{UQ}_{10}\text{H} \) under the coexistence of \( \alpha \)-TocH
\[
k_{\text{a\text{-UQ}_{10}\text{H}}} \quad \text{ArO}^{\cdot} + \text{UQ}_{10}\text{H} + \text{α\text{-TocH}} \rightarrow [\text{ArO}: \cdots \leftarrow \text{UQ}_{10}\text{H}^{\cdot}] + \text{α\text{-TocH}} \rightarrow \text{ArOH} + \text{UQ}_{10}\text{H}^{\cdot} + \text{α\text{-TocH}} \tag{21}
\]

(iii) Transition State C --- Activation energy \( (E_{\text{act}}) \) decreases as a result of the fast electron transfer (i.e., a quasi-resonance) between transition states A and B.
\[
\text{electron} \quad \text{ArO}^{\cdot} + \text{UQ}_{10}\text{H}^{\cdot} + \text{α\text{-TocH}} \rightarrow [\text{ArO}: \cdots \leftarrow \text{UQ}_{10}\text{H}^{\cdot}] + \text{α\text{-TocH}} \tag{22}
\]

(iv) Reaction Products A and B --- An equilibrium proceeds between reaction products A and B due to hydrogen atom transfer \( (\text{H}^{\cdot}) \), if \( \alpha \)-TocH and \( \text{UQ}_{10}\text{H}^{\cdot} \) radicals are stable.
\[
\text{Product A} \quad \text{H}^{\cdot} \quad \text{Product B} \quad \text{ArOH} + \text{α\text{-TocH}}^{\cdot} + \text{UQ}_{10}\text{H}^{\cdot} \rightarrow \text{ArOH} + \text{α\text{-TocH}}^{\cdot} + \text{UQ}_{10}\text{H}^{\cdot} \tag{23}
\]

Our experimental results demonstrate a notable increase in \( k_{\text{a\text{-TocH}}} + \text{UQ}_{10}\text{H} \) values for not only \( \alpha \)-TocH, but also for \( β, γ, \) and \( δ \)-TocH when they were evaluated in the presence of \( \text{UQ}_{10}\text{H} \) (Table 1). This increase in \( k_{\text{a\text{-TocH}}} + \text{UQ}_{10}\text{H} \) values for \( β, γ, \) and \( δ \)-TocH can also be explained by the scheme described in Fig. 6. As listed in Table 1, the value of ratio \( (k_{\text{a\text{-TocH}}} + \text{UQ}_{10}\text{H})/k_{\text{a\text{-TocH}}} \) for \( γ \)-TocH is 1.55,
and is larger than those (1.21, 1.28, and 1.19) for $\alpha$, $\beta$, and $\delta$-TocH, respectively. However, the reason why the value for $\gamma$-TocH shows the highest one is not clear at present. More detailed experimental and theoretical studies will be necessary to explain the reason.

As described in Section 3.3, an increase in $k_{\text{Oil-UQ10H2}}$ values for vegetable oils 1–8 was observed under the coexistence of oils 1–8 and UQ$_{10}$H$_2$ (Table 2). This increase can also be explained by the scheme described above as $\alpha$, $\beta$, $\gamma$, and $\delta$-TocH and -Toc-3H are all present in vegetable oils 1–8 (Table S1).

If $\alpha$-TocH and UQ$_{10}$H$_2$ are contained in a reaction mixture, reactions 20 and 21 proceed competitively. Further, if $\alpha$-Toc$^.$ and UQ$_{10}$H$^.$ radicals are stable in solution, hydrogen atom transfer reaction (i.e., reaction 23) may occur between reaction product A [ArOH + $\alpha$-Toc$^.$ + (UQ$_{10}$H$_2$)] and reaction product B [ArOH + UQ$_{10}$H$^.$ + ($\alpha$-TocH)]. That is, an equilibrium may take place between reaction products A and B due to the hydrogen atom transfer. In such a case, the total energy of reaction products A and B may decrease. This may induce a decrease of activation energy ($E_{\text{act}}$) as expected from the Evans-Polanyi theory. However, $\alpha$-Toc$^.$ and UQ$_{10}$H$^.$ radicals in reaction products A and B are unstable in solution, and rates of the hydrogen atom transfer are slower than those of the electron transfer. Further, as the stability of $\beta$, $\gamma$, and $\delta$-Toc$^.$ radicals is lower than that of the $\alpha$-Toc$^.$ radical, the effects of the hydrogen atom transfer on the increase of the reaction rate constants (i.e., (i) $k_{\text{Oil-UQ10H2}}$ (UQ$_{10}$H$_2$) and (ii) $k_{\text{Oil-UQ10H2}}$) would be small and negligible. However, details are not clear at present.
5 Conclusions

Aroxyl (ArO•) radical scavenging rate constants \(k_{s,100}(+)\) for AOHs (\(\alpha\)-, \(\beta\)-, \(\gamma\)-, TocH and UQ\(_{100}H_2\)) were measured in ethanol/chloroform/H\(_2\)O (50/50/1, v/v) solution, using stopped-flow spectrophotometry. \(k_{s,100}\) values were measured not only for each AOH, but also for the mixtures of two AOHs (i.e., TocH and UQ\(_{100}H_2\)), \(k_{s,100}(+)\) values for \(\alpha\)-, \(\beta\)-, \(\gamma\)-, \(\delta\)-TocH increased 1.21, 1.28, 1.55, and 1.19 fold, respectively, under the coexistence of a constant concentration of UQ\(_{100}H_2\). Similarly, \(k_{s,100}(+)\) values for eight vegetable oils 1 - 8, containing different concentrations of \(\alpha\)-, \(\beta\)-, \(\gamma\)-, \(\delta\)-TocH and Toc-3H, also increased 1.24 – 1.54 fold when UQ\(_{100}H_2\) was added. A new mechanism to explain a notable increase of \(k_{s,100}\) values under the coexistence of two kinds of phenolic AOHs was proposed.

UV-Vis absorption spectra for \(\alpha\)-, \(\beta\)-, \(\gamma\)-Toc• radicals, produced by the interactions between \(\alpha\)-, \(\beta\)-, \(\gamma\)-TocHs (or vegetable oils 1 - 8) and ArO•, disappeared when both TocHs (or oils 1 - 8) and UQ\(_{100}H_2\) were added to the solution, suggesting that the prooxidant reaction due to Toc• radicals is suppressed by the presence of UQ\(_{100}H_2\).

Many phenolic AOHs, including polyphenols, are found in foods, plants, and biological systems. Consequently, we may expect similar synergistic effects on the free-radical-scavenging rates for any system containing two or more kinds of AOH. Here we describe the results of a kinetic study performed in homogeneous solutions but future work should focus on investigating whether a similar synergistic effect on \(k_s\) is observed in heterogeneous systems such as micelle solutions, liposome and plasma.

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Conflict of Interest

The authors declare no conflicts of interest.

Supporting Information

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