Quenching Mechanisms and Kinetics of Quercetin in Inhibition of Photosensitized Oxidation of Palm Oil and Linoleic Acid

Posman Silalu1, Suparmo1, Umar Santoso2, Zuheid Noor1, Mary Astuti1 and Sri Raharjo1

1Dept. of Agricultural Product of Technology, St Thomas Catholic University, Medan, Indonesia. 
2Dept. of Food & Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia.

ABSTRACT

Effect of 0, 200, 400, 600, 800 and 1000 ppm (w/vol) quercetin on the erythrosine sensitized photosoxidations of palm oil and linoleic acid in methylene chloride containing 100 ppm erythrosine, were studied during storage under 4000 lux fluorescent light for 3 h by measuring peroxide value. Steady-state kinetic approximation was used to determine a quenching mechanism and quenching rate constant of quercetin in the erythrosine-sensitized photosoxidation of palm oil and linoleic acid in methylene chloride model system. Erythrosine greatly increased the photosoxidation of palm oil and linoleic acid, as was expected. Quercetin was extremely effective in minimizing erythrosine-sensitized photosoxidation of palm oil and linoleic acid. As the concentration of quercetin increased from 0 to 200, 400, 600, 800 and 1000 ppm, the peroxide values of palm oil and linoleic acid decreased significantly (P < 0.05). The steady-state kinetic studies indicated that quercetin quenched singlet oxygen only to minimize the erythrosine-sensitized photosoxidation of palm oil and linoleic acid. The calculated total quenching rate of quercetin on erythrosine photosensitized oxidation of palm oil in methylene chloride was 4.3 x 10³ M⁻¹ s⁻¹ and total quenching rate of quercetin on erythrosine photosensitized oxidation of linoleic acid in methylene chloride was 3.2 x 10⁷ M⁻¹ s⁻¹.

Keywords: Quercetin, photosensitized oxidation, singlet oxygen, palm oil and linoleic acid.

INTRODUCTION

Lipid oxidation in foods is a serious problem, difficult to overcome often, and leads to loss of shelf life, palatability, functionality, and nutritional quality. Loss of palatability is due to the generation of off-flavors that arise primarily from the breakdown of unsaturated fatty acids during autoxidation. The high reactivity of the carbon double bonds in unsaturated fatty acids makes these substances as primary targets for free radical reactions (Resche, et al., 2002; Zhuang, et al., 2002).

Oxidation reactions can be formed by either diindical triplet oxygen or nonradical singlet oxygen. Nonradical singlet oxygen oxidation of foods has been only studied during the last 30 years, but triplet oxygen free radical lipid oxidation to improve the oxidative stability of lipid foods has been extensively studied during 70 years and is well understood through the extensive and concerted efforts of university, industry, and government scientist (Labaza, 1971; Min and Boff, 2002a). However, it
(Labuza, 1971; Min and Boff, 2002a). However, it does not fully explain the initiation step of lipid oxidation. The role of singlet oxygen at the initiation step of lipid oxidation was reported, and the reaction rate of singlet oxygen with linoleic acid and methyl linolate was at least 1450 and 1500 time faster than normal triplet oxygen (Rawls and Van Santen, 1970; Frankel, 1996). Singlet oxygen rapidly increases the oxidation rate of food even at very low temperature. Singlet oxygen oxidation can produce new compounds, which are not found in ordinary triplet oxygen oxidation in foods (Min and Boff, 2002b; Kolakowska, 2002).

Singlet oxygen is produced by photosensitizers in the presence of light and triplet oxygen. Photosensitizers such as chlorophyll, phaeophytin, riboflavin, myoglobin, and synthetic colorants in foods can absorb energy from light and transfer it to triplet oxygen to form singlet oxygen (Huang, et al., 2004; Lledias and Hansberg, 2000). The photosensitizer absorbs the ultraviolet or visible radiation energy rapidly and becomes an unstable, excited, singlet state molecule (‘sen’). The excited singlet photosensitizer loses its energy by internal conversion, emission of light, or intersystem crossing.

Synthetic food colorants, like erythrosine, which have been used to improve the appearance of foods, may act as photosensitizers due to the highly conjugated double bonds. Photosensitizing synthetic colorants affect the lipid oxidation and the safety of foods. Erythrosine or FD&C Red No.3 has been reported to be a photosensitizer leading to the oxidation of pork product, methyl linolate, and cholesterol (Chung, et al., 1997; Yang, et al., 2002).

To reduce the undesirable singlet oxygen oxidation in lipid foods, the effects of naturally occurring tocopherols and tocotrienols, ascorbic acid, and carotenoids on singlet oxygen oxidation have been extensively studied (Lee and Min, 1991; Jung and Min, 1991; Lee et al., 1997; Lee, et al., 2004). However, information on the application of flavonoid compounds to minimize singlet oxygen oxidation in lipid foods is very limited. Meanwhile, the quenching mechanisms and kinetics of quercetin on the photosensitized oxidations of palm oils and linoleic acid has not been studied.

The objectives of this work were to study (1) the effects of quercetin on the erythrosine sensitized photooxidation of palm oil or linoleic acid and (2) determine the quenching mechanism and quenching rate constant of quercetin in erythroseine-sensitized photooxidation of palm oil or linoleic acid.

MATERIALS AND METHODS

Materials

Refined, bleached and deodorized palm oils was obtained from PT Astra Agro Lestari, Medan, North Sumatera. Silicic acid, cellie, activated charcoal, quercetin, a-tocopherol and b-carotene was purchased from Aldrich Chemical Co. Methylene chloride was purchased from J.T. Baker Chemical Co. Linoleic acid was purchased from Sigma Chemical Co. (St. Louis, MO). Erythrosine was obtained from Insti, Yogyakarta.

Purification of Palm Oil

To prepare purified palm oil, it was passed through a chromatographic column (60 cm x 4 cm) packed with a series of activated silicic acid, 2:1 mixture of activated charcoal and cellite, 2:1 mixture of powder sugar and cellite, and activated silicic acid as described by Lee and Min (1988). The oil passed through the column was purified palm oil. It was colorless and contained peroxide value 0.73 meq/ kg oil, free fatty acids 0.08%, tocopherol 7.67 ppm or carotenoids 4.21 ppm, and did not contain detectable concentrations of conjugated dienes.

Chemical Analysis of Purified Palm Oil

Tocopherols were determined by the high pressure liquid chromatography of Carpenter (1979), and carotenoids were determined by the spectrometric method of Proctor and Snyder (1987). Peroxide value, and free fatty acids were determined by A.O.C.S (1980) methods (Shahidi and Wanasundara, 2002).
Effects of Quercetin on Erythrose Sensitized Photoxidation of Palm Oil and Linoleic Acid

To study the effects of quercetin on the photosensitized oxidation of palm oil, samples of 0, 200, 400, 600, 800, and 1000 ppm (wt/vol) quercetin in 10.0% (wt/vol) purified palm oil in methylene chloride containing 100 ppm (wt/vol) erythrose were prepared according to the methods of Lee, et al (1997). Samples containing 400 ppm (wt/vol) α-tocopherol were used as a positive control in the system. Fifteen mL of the prepared oil samples was transferred into 25 mL serum bottles in duplicate. The bottles were sealed airtight with rubber septa and aluminium caps and place in a light storage box described in detail by Lee and Min (1988). The light sources, four Sylvania 15 watt cool white fluorescent lamps, were placed on the bottom of wooden box. The light intensity at the sample level was 4,000 lux.

To study the effects of quercetin on the photosensitized oxidation of linoleic acid, samples of 0, 200, 400, 600, 800, and 1000 ppm (wt/vol) quercetin in 1.0% (wt/vol) linoleic acid in methylene chloride containing 100 ppm (wt/vol) erythrose were prepared according to the methods of Lee, et al (1997). Samples containing 400 ppm (wt/vol) α-tocopherol were used as a positive control in the system. Fifteen mL of the prepared oil samples was transferred into 25 mL serum bottles in duplicate. The bottles were sealed airtight with rubber septa and aluminium caps and place in a light storage box described in detail by Lee and Min (1988). The light sources, four Sylvania 15 watt cool white fluorescent lamps, were placed on the bottom of wooden box. The light intensity at the sample level was 4,000 lux. The degree of oxidation palm oil and linoleic acid was determined by measuring peroxide value every hour for 5 h by using the AOCs method (Shahidi and Vanasundara, 2002).

Determination of Quenching Mechanism and Rate Constant.

The quenching mechanism and kinetics of quercetin in erythrose-sensitized photoxidation of palm oil and linoleic acid were studied by the steady-state kinetic method of Foote (Min and Boff, 2002a). To study the quenching mechanism and singlet oxygen quenching rates of quercetin, samples of 0.01, 0.02, 0.03, and 0.04 M palm oil or linoleic acid in a solvent methylene chloride containing 100 ppm (wt/vol) erythrose were prepared according to the methods of Lee, et al (1997). The fatty acid composition of purified palm oil determined by the gas chromatographic method of Jung and Min (1991) was 0.21% lauric acid, 1.03% myristic acid, 43.33% palmitic acid, 4.41% stearic acid, 39.10% oleic acid, and 10.83% linoleic acid. The average molecular weight of the palm oil was estimated from palmitic acid, the most dominant fatty acid. The 0.01, 0.02, 0.03, and 0.04 molal concentration of palm oil in methylene chloride was obtained from the average molecular weight of palm oil triglycerides.

Fifteen mL of the prepared oil samples was transferred into 25 mL serum bottles in triplicate. The bottles were sealed airtight with rubber septa and aluminium caps and place in a light storage box described in detail by Lee and Min (1988). The light sources, four Sylvania 15 watt cool white fluorescent lamps, were placed on the bottom of wooden box. The light intensity at the sample level was 4,000 lux. The degree of oxidation palm oil and linoleic acid was determined by measuring peroxide value every hour for 5 h by using the AOCs method (Shahidi and Vanasundara, 2002).

RESULT AND DISCUSSION

Effect of Quercetin on the Photosensitized Oxidation of Palm Oil

Effect of 0, 200, 400, 600, 800, and 1000 ppm (wt/vol) quercetin on erythrose-sensitized photoxidation of palm oil in methylene chloride during 5-h storage under 4,000 lux fluorescent light are shown in Figure 1. Erythrose greatly increased the photoxidation of palm oil in methylene chloride, as was expected. Preliminary studies showed that the peroxide values of purified palm oil in methylene chloride containing no erythrose did not change during 5 hr of storage under light and the peroxide values of the oils with and without erythrose after 5 hr of storage in the dark were not detectable.

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The peroxide value of palm oil in the presence of 100 ppm ethyelene in 5-h storage under light illumination was 20.39 meq/kg oil. Addition of either quercetin or α-tocopherol greatly decreased the ethyelene-sensitized photoxidation of quercetin. As the concentration of quercetin increased, the reduction of peroxide formation in palm oil increased. The peroxide value of palm oil in the presence of 0, 200, 400, 600, 800, and 1000 ppm quercetin after 5-h storage under light were 20.39, 14.21, 10.20, 9.15, 7.14, 5.42 meq/kg oil. The peroxide values of purified palm oil in methylene chloride containing no ethyelene was 0.90 meq/kg oil. Duncan’s multiple range tests showed that the peroxide value of samples treated with quercetin were significantly lower than the control (no quercetin added) after 5-h storage under fluorescent light (P < 0.05). Quercetin was much more effective than α-tocopherol (Figure 1).

![Figure 1. Effect of 0, 200, 400, 600, 800, 1000 ppm (w/v) quercetin, and 400 ppm (w/v) α-tocopherol on ethyelene-sensitized photoxidation of palm oil in methylene chloride during storage under fluorescent light.](image)

The peroxide value of palm oil in the presence of 400 ppm α-tocopherol was 15.31 meq/kg oil after 5-h storage under fluorescent light, showing the significantly lower activity of 400 ppm α-tocopherol than even 200 ppm quercetin (P < 0.05).

**Effect of Quercetin on the Photosensitized Oxidation of Linoleic Acid**

Quercetin was extremely effective at minimizing ethyelene-sensitized photoxidation of linoleic acid (Figure 2). As quercetin was increased from 200 to 1000 ppm, its effectiveness increased significantly (P < 0.05). The peroxide values of ethyelene-sensitized photoxidation of linoleic acid with 0, 200, 400, 600, 800, and 1000 ppm quercetin after 5-h storage under fluorescent light were 52.14, 34.46, 30.32, 23.02, 19.98, 14.24 meq/kg oil, respectively.

The peroxide value of linoleic acid in the presence of 400 ppm α-tocopherol was 40.78 meq/kg oil after 5-h storage under fluorescent light, showing the significantly lower activity of 400 ppm α-tocopherol than even 200 ppm quercetin (P < 0.05).

![Figure 2. Effect of 0, 200, 400, 600, 800, 1000 ppm (w/v) quercetin, and 400 ppm (w/v) α-tocopherol on ethyelene-sensitized photoxidation of linoleic acid in methylene chloride during storage under fluorescent light.](image)

Alpha-Tocopherol and β-carotene are natural compounds that are frequently used to prevent the oxidation of food. Alpha-Tocopherol was reported to quench singlet oxygen in riboflavin photosensitized milk (King and Min, 1998) and in chlorophyll photosensitized soybean oil (Jeng et al., 1991). Beta-Carotene is an effective singlet oxygen quencher on the chlorophyll photosensitized soybean and riboflavin photosensitized vitamin D3, due to the highly conjugated diene structure, which can dissipate the high energy of singlet oxygen as heat (Edge et al., 1997; Hpsia and Heinonen, 1999).
Flavonoids may act as antioxidants by scavenging radicals that include superoxide anions, lipid peroxide radicals and hydroxyl radicals. Other mechanisms of action of selected flavonoids include singlet oxygen quenching (Nakagawa, et al. 2000, Tahahama, 1984; Cuppett, 1992). The relative activities of flavonoids in quenching water-soluble radical cation ABTS⁺ (ABTS = 2,2'-azinobis (3-ethyl benzothiazoline-6-sulfonic acid)) decreased in the order quercetin > Myricetin > rutin > α-tocopherol (Rice-Evaas, et al 1995; Penman and Gordon, 1998). In lipid systems flavonol aglycones are generally reported to be more active than their glycosides; quercetin was more active than quercitin and rutin in feroxos-induced oxidation of rat brain mitochondria suspension (Ratty and Das, 1988; Kandzwar and Middleton, 1997; Szymusiak and Zielinski, 2003).

Quenching Mechanism and Rate Constant of Quercetin

Because quercetin decreased photosensitized oxidation of oil, the authors decided to study the mechanism and kinetics of quercetin for the reduction of photosensitized oxidation of oils by using a steady-state kinetic approach. The schematic diagram for the formation of oxidized products (AO₂) via singlet-oxygen oxidation is as follow (Foote, 1979):

![Figure 3. Formation of singlet oxygen and its reaction with substrate A to produce the oxidized product AO₂. The formation of AO₂ can be prevented by the reaction of 'Sen' or 'O₂' with a quenching agent. (Min and Boff, 2002a)](image)

Quenching agents, such as quercetin, may be involved to minimize the development or activity of singlet oxygen at several stages in the oxidation of foods. Figure 3 shows the development of singlet oxygen and its subsequent reaction with compound (A) to form the oxidized product (AO₂). At every stage in this reaction, there is at least 2 alternate routes, which, if taken, would minimize the oxidation of the compound (A). The 1st step represents when a sensitizer (Sen) such as erythorine, in oil absorbs light energy, it becomes an excited singlet sensitizer ('Sen'). The return of the excited singlet sensitizer ('Sen') to ground state ('Sen') without interval crossing (isc) to form the excited triplet sensitizer ('Sen'). The 2nd step represents reaction with a quenching agent (Q) at a rate represented as kₚ, returning the excited triplet sensitizer ('Sen') to ground state ('Sen') prior to reaction with triplet oxygen (O₂) to form singlet oxygen (O). The excited triplet sensitizer ('Sen') may react with triplet oxygen (O₂) to form singlet oxygen (O)

Following its creation, there are 3 fates for singlet oxygen in foods: (1) it may naturally decay to the ground state at a rate represented as kₚ; (2) it may react with a singlet-state compound (L) at a rate represented as kₘ forming the oxidized product AO₂; and (3) it may be destroyed by a quenching agent by either combining with the quencher, at a rate represented as kₚ, to form the product QO₂, or by passing its energy to the quenching agent and reuming to free triplet oxygen, at a rate represented as kₚ (Min and Boff, 2002a).

The formation of oxidized product (AO₂) could be reduced by the quenching of the singlet oxygen and/or the excited triplet sensitizer. If quercetin reduces photosensitized oxidation of oils by singlet oxygen quenching, the following steady-state kinetic equation is established:

\[ d[AO_2]/dt = k' [1 + (k_{q}[Q]) + k_{O_2}[Q]/(k_A + k_{O_2})] \]

Where k denotes the rate of singlet oxygen formation; AO₂, oxidized palm oil or linoleic acid; kₚ, reaction rate constant of palm oil or linoleic acid with singlet oxygen; A, palm oil or linoleic acid; kₚ,
reaction rate constant of physical singlet oxygen quenching by quercetin; \( k_{q,op} \) reaction rate constant of chemical singlet oxygen quenching by quercetin; \( Q \), quercetin; and \( k_q \), decaying rate of singlet oxygen.

The intercept and slope of the plots of \( [\text{AO}_2^-]_1 \) vs \( [A]^1 \) at various concentrations of quencher (Q) are \( K^\cdot \text{dam K}^{-1} \cdot (k_q Q + k_{qX} Q^2/Q) + k_p/k_p \) respectively. The intercepts of the plots are independent of the concentration of quencher (quercetin), and the slopes are dependent on the concentration of quencher (Foote, 1979).

**Quenching mechanism and kinetics of quercetin on erythrosine photosensitized oxidation of palm oil in methylene chloride model system**

The effect of Q; 0.25 x 10^{-3}, 0.50 x 10^{-3}, 0.75 x 10^{-3} and 1.0 x 10^{-3} M quercetin on the peroxide value of 0.01, 0.02, 0.03, and 0.04 M palm oil in methylene chloride containing 100 ppm erythrosine under fluorescent light are shown in Figure 4. The plot of \( [\text{AO}_2^-]_1 \) vs \( [A]^1 \) for different levels of quercetin is shown in Figure 4.

To determine the singlet oxygen quenching rate \( (k_q + k_{qX}) \) of quercetin, the slope/intercept ratio vs [quercetin, Q] was plotted in Figure 5. The linear regression equation of the plot/intercept ratio vs [Q] of Figure 2 was \( Y = 26800 X + 0.07 \), and the correlation coefficient \( (R^2) \) was 0.98. Foote (1979) reported that the slope of the plot of slope/intercept

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**Table 1.** The intercept and slopes of the plots of regression lines of Figure 4 and Figure 6 to determine the quenching mechanism and rate of quercetin and on the erythrosine photosensitized oxidation of palm oil and linoleic acid in methylene chloride model system.

<table>
<thead>
<tr>
<th>System</th>
<th>Conc. Quercetin (x10^{-3} M)</th>
<th>Intercept (I)</th>
<th>Slope (S)</th>
<th>S/I</th>
</tr>
</thead>
<tbody>
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<td>4.78</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>65</td>
<td>8.03</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>65</td>
<td>12.72</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>65</td>
<td>16.41</td>
<td>0.25</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>1.00</td>
<td>65</td>
<td>22.24</td>
<td>0.34</td>
</tr>
</tbody>
</table>

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**Figure 4.** Effect of quercetin on peroxide formation of palm oil in a solvent methylene chloride containing 100 ppm erythrosine during 2 hr under 4000 lux fluorescent.
ratio vs. [Q] is $k + k_mQ/k_q$. The value of total single oxygen quenching rate constant ($k + k_mQ$) of quercetin is slope $k_q$. Because the slope of the plot for quercetin (Fig. 5) was $26800M^{-1}$, and $k_q$ was $1.6 \times 10^4 M^{-1}s^{-1}$, the total quenching rate constant ($k + k_mQ$) was $(26800 \times 1.6 \times 10^3) = 4.3 \times 10^7 M^{-1}s^{-1}$.

The rate constants for single oxygen quenching by 2-tocopherol have been reported as $2.5 \times 10^7 M^{-1}s^{-1}$ in chlorophyll photosensitized oxidation of soybean oil in methylene chloride, $2.6 \times 10^7 M^{-1}s^{-1}$ on methylene blue photosensitized oxidation of methyl linolate in alcohol (Jung and Min, 1991). Hust, et al. (1982) reported since solvents affect the decay rate ($k_q$) of single oxygen, the quenching rate may vary in different solvent systems.

![Figure 5. The plot of slope/intercept of the plots (1/ hydroxide vs. 1/palm oil, shows Fig. A) vs. the concentration of quercetin.](image)

**Quenching mechanism and kinetics of quercetin on erythrose photo sensitized oxidation of linoleic acid**

The effect of $0.25 \times 10^{-5}, 0.50 \times 10^{-5}, 0.75 \times 10^{-5}$ and $10^{-5} M$ quercetin on the peroxide value of $0.01, 0.02, 0.03$, and $0.04 M$ linoleic acid in methylene chloride containing 100 ppm erythrose under fluorescent light are shown Figure 6.

![Figure 6. Effect of quercetin on peroxide formation of linoleic acid in a solvent methylene chloride containing 100 ppm erythrose during 1 hr under 4000 lux fluorescent](image)

The linear regression line for the plot of $([AO]_0)^{-1}$ vs. $[A]_0$ without quercetin (Fig. 6) was $Y = 2.25 X + 25$, where $Y = ([AO]_0)^{-1}$ and $X = [A]_0$. The slope/intercept ratio of the regression line was 0.09. Froode (1979) showed that the slope/intercept ratio of the regression line for the oil without quercetin is $k/k_q$. The $k$ value is in a solvent methylene chloride is $1.1 \times 10^7 M^{-1}s^{-1}$ (Safarzadeh and Haddad, et al., 1981). Because the single oxygen oxidation rate ($k_q$) of linoleic acid is $k$/slope, then $k_q = 1.1 \times 10^7/0.09 = 1.2 \times 10^7 M^{-1}s^{-1}$ in a solvent methylene chloride. This present value ($k_q$) for methyl linolate was close to the one reported previously in pyridine. Doleides, et al., (1974) reported that the single oxygen oxidation rates of methyl oleate, methyl linolate, and methyl linolenate were $0.67 \times 10^5, 1.3 \times 10^5$ and $1.9 \times 10^5 M^{-1}s^{-1}$ in pyridine, respectively.

For the calculation of the ratios of slope/intercept of the plots in Figure 6, the average intercept value (25) of the line plots was used. The intercepts (b), slopes (S) and $S/b$ of quercetin from Figure 6 are shown Table 1.

To determine the singlet oxygen quenching rate ($k + k_mQ$) of quercetin, the slope/intercept ratio vs. [Q] of Figure 7 was plotted in Figure 7. The linear regression equation of the plot/intercept ratio vs. [Q] of Figure 7 was $Y = 26400 X + 0.09$, and the correlation coefficient ($R^2$) was 0.699. Froode (1979) reported that the slope of the plot of slope/intercept...
ratio vs. [Q] is \((k_2 + k_{\text{obs}})k_p\). The value of total singlet oxygen quenching rate constant \((k_2 + k_{\text{obs}})\) of quercetin is slope \(x\). Because the slope of the plot for quercetin (Fig.7) was 26400 M\(^{-1}\), and \(k_2\) was 1.2 \times 10^9 M\(^{-1}\)s\(^{-1}\), the total quenching rate constant \((k_2 + k_{\text{obs}})\) was (26400 x 1.2 x 10^9) = 3.2 \times 10^18 M\(^{-1}\)s\(^{-1}\).

Figure 7. The plot of slope/intercept of the plots (1/ hydroperoxide vs. 1/linolic acid, shown Fig. 6) vs. the concentration of quercetin.

CONCLUSION

The total singlet oxygen quenching rate constant of quercetin on erythrosine photosensitized oxidation of palm oil and linoleic acid in methylene chloride were 4.3 \times 10^9 M\(^{-1}\)s\(^{-1}\) and 3.2 \times 10^9 M\(^{-1}\)s\(^{-1}\) respectively. The intercepts were the same for different levels of quercetin, but the slope of the plots increased as the concentration of quercetin increased, indicating that quercetin quenched singlet oxygen only to reduce photosensitized oxidation of oils by the singlet oxygen quenching mechanism but not by the excited triplet sensitizer quenching mechanism. This present kinetic value for singlet oxygen quenching ability of quercetin is consistent with its antiphotooxidative activity. That is, quercetin, which had a stronger singlet oxygen activity, also had a stronger antioxidative activity in photosensitized oxidation of oil than did tocopherol.

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64