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Reaction of Paprika Carotenoids, Capsanthin and Capsorubin, with Reactive Oxygen Species

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Supporting Information

ABSTRACT: The reaction of paprika carotenoids, capsanthin and capsorubin, with reactive oxygen species (ROS), such as superoxide anion radical ($\cdot O_2^-$), hydroxyl radical ($\cdot OH$), and singlet oxygen ($^1O_2$), was analyzed by LC/PDA ESI-MS and ESR spectrometry. Capsanthin formed both the 5,6-epoxide and 5,8-epoxide by reaction with $\cdot O_2^-$ and $\cdot OH$. Furthermore, capsanthin also formed 5,6- and 5,8-endoperoxide on reaction with $^1O_2$. The same results were obtained in the case of capsanthin diacetate. On the other hand, capsorubin showed higher stability against these ROS. Capsorubin formed 7,8-epoxide on reaction with $\cdot O_2^-$ and $\cdot OH$ and 7,8-endoperoxide on reaction with $^1O_2$.

KEYWORDS: *capsanthin, capsorubin, capsanthin epoxide, capsanthin endoperoxide, reactive oxygen species*

INTRODUCTION

Paprika (*Capsicum annuum*) is a good source of carotenoids and used widely as a vegetable and food colorant. The red carotenoids are mainly capsanthin and capsorubin,[1−4] along with capsanthin 3,6-epoxide[5] and capsanthone[6] as minor components (Figure 1), and their carotenoids account for 30−80% of the total carotenoids in fully ripe fruits.[1−4] These carotenoids showed excellent antioxidative[7−10] and anticancer[11−14] activities. Recently, Maeda et al. reported that these carotenoids showed effects to prevent and improve obesity-related insulin resistance.[15] Furthermore, it was reported that capsanthin could take up peroxynitrite into its molecule by the formation of nitrocapsanthin.[16]

It is known that carotenoids inhibit singlet oxygen ($^1O_2$) through physical quenching and chemical reactions.[17] For example, β-carotene formed endoperoxides, epoxides, and apocarotenoids by the reaction with $^1O_2$.[18−21] Very recently, we investigated reaction of astaxanthin with $^1O_2$, superoxide anion radical ($\cdot O_2^-$), and hydroxyl radical ($\cdot OH$), using LC/PDA ESI-MS and ESR spectrometry.[22] As the results, we found that astaxanthin formed astaxanthin endoperoxides on reaction with $^1O_2$ and formed astaxanthin epoxides on reaction with $\cdot O_2^-$ and $\cdot OH$.[22]

However, there have been few reports on the reaction of capsanthin and capsorubin with reactive active oxygen species (ROS). In order to clarify the chemical scavenging mechanism of these carotenoids with ROS, we investigated reaction products of these paprika carotenoids with $\cdot O_2^-$, $\cdot OH$, and $^1O_2$ using LC/PDA ESI-MS and ESR spectrometry. This paper reports the scavenging effect of $\cdot O_2^-$, $\cdot OH$, and $^1O_2$ with capsanthin and capsorubin by ESR spin-trapping analysis and the reaction products of these paprika carotenoids with these ROS. Furthermore, the reaction mechanisms of ROS with capsanthin and capsorubin are discussed.

MATERIALS AND METHODS

Chemicals. Hematoporphyrin, riboflavin, and hydrogen peroxide were purchased from Wako Pure Chemicals (Osaka, Japan). 2,2,6,6-Tetramethyl-4-piperidone (TMPD) was purchased from Aldrich (Milwaukee, WI, USA). 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) was purchased from Labotec (Tokyo, Japan). β-Carotene, zeaxanthin, capsanthin, and capsorubin were prepared from paprika according to the method described previously.[9,10,12] Astaxanthin was prepared from *Paracoccus carotinifaciens*.[23] Capsanthin diacetate and astaxanthin diacetate were prepared from capsanthin and astaxanthin, respectively, by acetylation with acetic anhydride in pyridine at room temperature. *syn*- and *anti*-Capsanthin 5,6-epoxide and capsanthin 5,8-epoxide were prepared from capsanthin by epoxydation with *m*-chloroperbenzoic acid as described below.

Apparatus. The ESR spectra were recorded at room temperature on a JEOL JES-RFR30 spectrometer (Tokyo, Japan). The LC/MS and MS/MS analysis of carotenoids was carried out using a Waters Xevo G2S Q TOF mass spectrometer (Waters Corporation, Milford, CT, USA) equipped with an Acquity UPLC system. The ^1H NMR (500 MHz) spectra were measured with a Varian UNITY INOVA 500 spectrometer (Agilent Technologies, Santa Clara, CA, USA) in $CDCl_3$ with TMS as an internal standard.

Reaction of Capsanthin with ROS. According to the method described previously,[22] after the addition of 200 μL of 8 mM H_2O_2 solution to 200 μL of 22 μg/mL capsanthin CH_3CN solution, $\cdot OH$ was generated by UV-A irradiation

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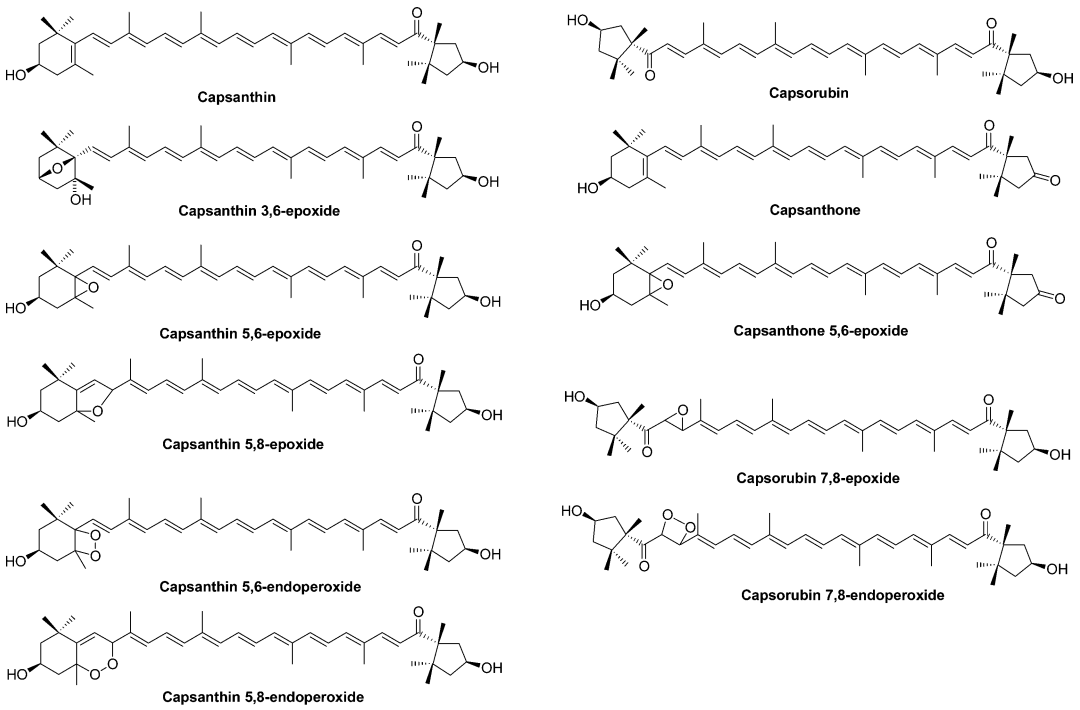

Figure 1. Structures of carotenoids in this study.

using SUPERCURE-203S (SAN-Ei ELECTORIC) at room temperature. In a similar manner to that described above, $\cdot O_2^-$ and $^1O_2$ were generated by the addition of 200 μL of 25 μM of riboflavin or 200 μL of 250 μM hematoporphyrin, respectively, to 200 μL of 22 μg/mL of capsanthin CH_3CN solution. At regular intervals of UV-A irradiation, reaction products were analyzed by LC/PDA-ESI-MS.

ESR Spin-Trapping Analysis. ESR spectra were recorded at room temperature on a JEOL JES-RFR30 spectrometer (Tokyo, Japan) using an aqueous quartz flat cell (Labotec, Tokyo, Japan). DMPO was used as a $\cdot O_2^-$ and ·OH-trapping agent, and TMPD was used as $^1O_2$, respectively. The $\cdot O_2^-$ and ·OH were generated by addition of either the 100 μL of 25 μM of riboflavin, or 100 μL of 8 mM H_2O_2 solution and 10 μL of 250 mM DMPO to 100 μL of 14 nmol/mL capsanthin CH_3CN solution by UV-A irradiation. In a similar manner described above, $^1O_2$ was generated by the addition of both the 100 μL of 250 μM hematoporphyrin and 10 μL of 500 mM TMPD to 100 μL of 14 nmol/mL capsanthin CH_3CN solution by UV-A irradiation. ESR spectra were started simultaneously to measure after UV-A irradiation. The all spin-trapped ESR spectra were monitored between the third and fourth signals from the low magnetic field due to the external standard, Mn(II) doped MnO. As the similar manner described above, reaction of capsanthin acetate and capsorubin (14 nmol/mL of both in CH_3CN solution) with ROS were also performed.

Analysis of Reaction Products of Capsanthin with ROS using LC/MS and MS/MS. The electro-spray ionization (ESI) time-of-flight (TOF) MS spectra were acquired by scanning from m/z 100 to 1,500 with a capillary voltage of 3.2 kV, cone voltage of 40 eV, and source temperature of 120 °C. Nitrogen was used as a nebulizing gas at a flow rate of 30 L/h. MS/MS spectra were measured with a quadrupole-TOF MS/MS instrument with argon as a collision gas at a collision energy of 30 V. UV−visible (UV/vis) absorption spectra were recorded from 200 to 600 nm using a photodiode-array detector (PDA). An Acquity 1.7 μm BEH UPLC C18 column (Waters Corporation) was used as a stationary phase and 90% MeOH as a mobile phase, at a flow rate of 0.2 mL/min for the HPLC system. In a similar manner to that described above, reaction of capsanthin diacetate (27 μg/mL in CH_3CN solution) and capsorubin (34 μg/mL in CH_3CN solution) with ROS were performed.

Capsanthin Diepoxide. ESI TOF MS m/z 639.4217 ($M+Na^+$, calcd for $C_{40}H_{56}O_5Na$, 639.4025) ; UV/vis 405−420 nm (in 90% MeOH).

Capsanthin 5,6-Epoxide. ESI TOF MS m/z 623.4080 ($M+Na^+$, calcd for $C_{40}H_{56}O_4Na$, 623.4076) ; UV/vis 463 nm (in 90% MeOH).

Capsanthin 5,8-Epoxide. ESI TOF MS m/z 623.4080 ($M+Na^+$, calcd for $C_{40}H_{56}O_4Na$, 623.4076) ; UV/vis 448 nm (in 90% MeOH).

Capsanthone 5,6-Epoxide. ESI TOF MS m/z 621.3920 ($M+Na^+$, calcd for $C_{40}H_{54}O_4Na$, 621.3920) ; UV/vis 463 nm (in 90% MeOH).

Capsanthin Diendoperoxide. ESI TOF MS m/z 671.3937 ($M+Na^+$, calcd for $C_{40}H_{56}O_7Na$, 671.3948) ; UV/vis 446 nm (in 90% MeOH).

Capsanthin 5,6-Endoperoxide. ESI TOF MS m/z 639.4056 ($M+Na^+$, calcd for $C_{40}H_{56}O_5Na$, 639.4025); UV/vis 463 nm (in 90% MeOH).

Capsanthin 5,8-Endoperoxide. ESI TOF MS m/z 639.4003 ($M+Na^+$, calcd for $C_{40}H_{56}O_5Na$, 639.4025); UV/vis 448 nm (in 90% MeOH).

Capsanthin Diacetate Diepoxide. ESI TOF MS m/z 723.4258 ($M+Na^+$, calcd for $C_{44}H_{60}O_7Na$, 723.4237) ; UV/vis 405−420 nm (in 90% MeOH).

Capsanthin Diacetae 5,6-Epoxide. ESI TOF MS m/z 707.4280 ($M+Na^+$, calcd for $C_{44}H_{60}O_6Na$, 707.4288); UV/vis 463 nm (in 90% MeOH).

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Capsanthin Diacetate 5,8-Epoxide. ESI TOF MS *m/z* 707.4280 (M+Na$^+$, calcd for $C_{44}H_{60}O_6Na$, 707.4288); UV/vis 448 nm (in 90% MeOH).

Capsanthin Diacetate Diendoperoxide. ESI TOF MS *m/z* 755.4145 (M+Na$^+$, calcd for $C_{44}H_{60}O_9Na$,755.4135) ; UV/vis 446 nm (in 90% MeOH).

Capsanthin Diacetate 5,6-Endoperoxide. ESI TOF MS *m/z* 723.7229 (M+Na$^+$, calcd for $C_{44}H_{60}O_7Na$, 723.4237); UV/vis 463 nm (in 90% MeOH).

Capsanthin Diacetate 5,8-Endoperoxide. ESI TOF MS *m/z* 723.7229 (M+Na$^+$, calcd for $C_{44}H_{60}O_7Na$, 723.4237); UV/vis 448 nm (in 90% MeOH).

Capsorubin 7,8-Epoxide. ESI TOF MS *m/z* 639.4003 (M+Na$^+$, calcd for $C_{40}H_{56}O_5Na$, 639.4025); UV/vis 424 nm (in 90% MeOH).

Capsorubin 7,8-Endoperoxide. ESI TOF MS *m/z* 665.3962 (M+Na$^+$, calcd for $C_{40}H_{56}O_6Na$, 655.3975); UV/vis 424 nm (in 90% MeOH).

Preparation of Capsanthin 5,6-Epoxides and 5,8-Epoxides from Capsanthin by Epoxydation with *m*-Chloroperbenzoic Acid. According to the method described by Khachik et al.,[24] *m*-chloroperbenzoic acid, 3.6 mg in 2 mL dichlorometane, was added to a solution of capsanthin, 2 mg in 2 mL dichlorometane. The solution stood for 20 min at room temperature in darkness. After the time, the reaction mixture was partitioned with 5% solution of $NaHCO_3$ and ether. The ether layer was removed and evaporated. The reaction products were submitted to preparative HPLC. HPLC separation provided *syn*-capsanthin 5,6-epoxide (0.8 mg) and *anti*-capsanthin 5,6-epoxide (0.4 mg) and capsanthin diepoxides (0.2 mg mixtures). These spectral data were identical with those of published data by Deli at al.[25] Capsanthin 5,8-epoxides were prepared by epoxide-furanoxide rearrangement[26] with HCl from capsanthin 5,6-epoxides. Preparative HPLC was performed on a Shimadzu LC-6AD with a Shimadzu SPD-6AV spectrophotometer (Shimadzu Corporation, Kyoto, Japan) set at 450 nm. The columns used were a 250 × 10 mm i.d., 10 μm Cosnosil 5C18-II (Nacalai Tesque, Kyoto, Japan) and a 250 × 10 mm i.d., 10 μm Cosnosil 5SL-II (Nacalai Tesque, Kyoto, Japan). Spectral data of *syn*-capsanthin 5,6-epoxide, *anti*-capsanthin 5,6-epoxide, and capsanthin 5,8-epoxides are available in the Supporting Information.

RESULTS AND DISCUSSION

Scavenging Effect of ROS with Capsanthin and Capsorubin. Figure 2 shows the scavenging effect of $^1O_2$ (A) and ·OH (B) with capsanthin and capsorubin. Astaxanthin, astaxanthin diacetae, β-carotene, and zeaxanthin were used as positive controls. It was reported that capsanthin and capsorubin quenched $^1O_2$[7,10] and inhibited lipid peroxidation caused by free radicals.[8,9] In the present investigation, the ESR study also revealed that capsanthin, capsanthin acetate, and capsorubin quenched not only $^1O_2$ but also scavenged ·OH. The order of quenching activity for $^1O_2$ is astaxanthin diacetate > capsorubin > astaxanthin > capsanthin diacetate > capsanthin > β-carotene > zeaxanthin. It was reported that the $^1O_2$ quenching activity of carotenoids depends on the number of conjugated polyenes, polyene chain structures, and functional groups, especially conjugated carbonyl groups.[7,10,27] It was also reported that acetylation of the hydroxyl group improved carotenoid stability.[22] Astaxanthin and its acetate have 13 conjugated double bonds including two conjugated carbonyl groups. Capsorubin has 11 conjugated double bonds including two conjugated carbonyl groups. Capsanthin and its acetate have 11 conjugated double bonds including one conjugated carbonyl group. On the other hand, β-carotene and zeaxanthin have 11 conjugated double bonds without a conjugated carbonyl group. The results of the present investigation are markedly consistent with this hypothesis.

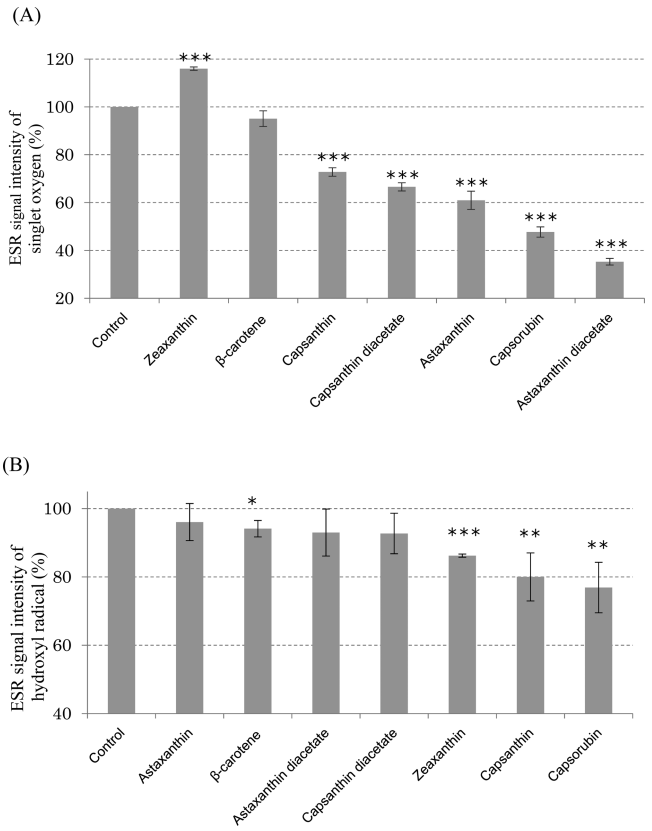

Figure 2. Quenching effect on $^1O_2$ (A) and scavenging effect on ·OH (B) with capsanthin, capsanthin diacetate, and capsorubin. The vertical axis shows the relative rate (%) of the ESR signal intensity due to singlet oxygen (A) and hydroxyl radical (B) compared with the control group (100%). Astaxanthin, astaxanthin diacetate, β-carotene, and zeaxanthin were used as positive controls. Significance compared with the control group: * $p < 0.05$, $p < 0.01$, $p < 0.001$.

On the other hand, capsanthin and capsorubin showed excellent scavenging activity for ·OH. Contrary to $^1O_2$, astaxanthin and its acetate hardly showed scavenging activity for ·OH. The presence of the 3-hydroxy-κ-end group with a conjugated carbonyl group, which is a characteristic structural moiety of paprika carotenoids, may enhance the quenching activity of ·OH.

Reaction Products of Capsanthin with $\cdot O_2^-$ and ·OH. Structures of reaction products of capsanthin with ROS are shown in Figure 1. Figure 3 shows HPLC chromatograms of reaction products of capsanthin with $\cdot O_2^-$ by UV-A irradiation of riboflavin solution. After 1 min of UV-A irradiation, reaction products assigned as capsanthin 5,6-epoxide and capsanthin 5,8-epoxide were detected on HPLC. On UV-A irradiation, the amount of these reaction products was increased and amount of capsanthin was decreased time-dependently. After 6 min of UV-A irradiation, the HPLC peak of capsanthin completely disappeared and the amount of reaction products reached a maximum. Then, the reaction products were markedly decreased and the reaction solution was bleached.

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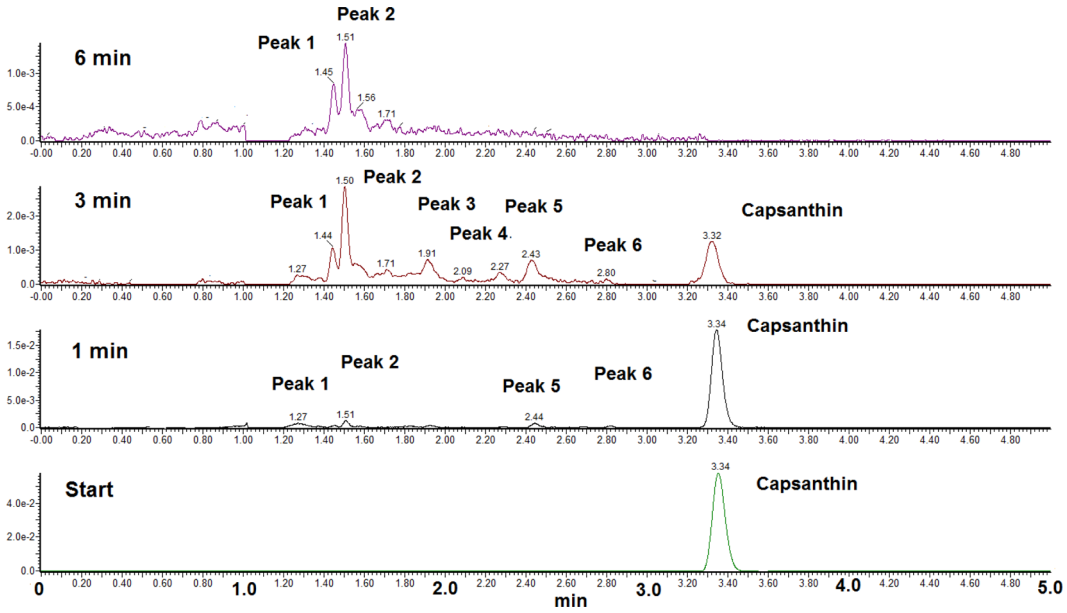

Figure 3. HPLC chromatograms of reaction products of capsanthin with superoxide anion radical. Peaks 1 and 2: capsanthin diepoxide; peak 3: capsanthone 5,6-epoxide; peak 4, *syn*-capsanthin 5,6-epoxide; peak 5: *anti*-capsanthin 5,6-epoxide; and peak 6: capsanthin 5,8-epoxide. HPLC condition ACQUITY UPLC system (Waters); column: BEH Shield RP18 (1.7 μm, 2.1 × 100 mm); mobile phase: 90% MeOH; column temperature: 40 °C; flow rate: 0.2 mL/min; detection: 450 nm.

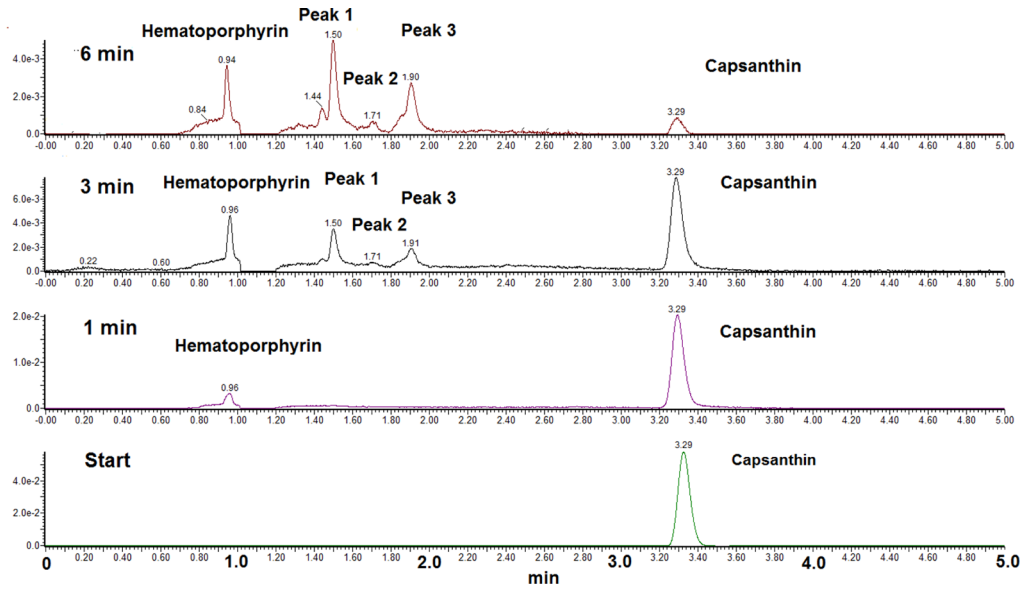

Figure 4. HPLC chromatograms of reaction products of capsanthin with singlet oxygen. Peak 1: capsanthin diendoperoxide and peaks 2 and 3: capsanthin 5,6-endoperoxide. HPLC conditions were described in Figure 3.

The structures of reaction products were assigned to capsanthin diepoxides (peaks 1 and 2), capsanthone 5,6-epoxide (peak 3), capsanthin 5,6-epoxide (peaks 4 and 5), and capsanthin 5,8-epoxide (peak 6) based on the ESI-TOF MS, MS/MS, and UV/vis spectral data.

Both reaction products of peaks 4 and 5 showed the molecular formula of $C_{40}H_{56}O_4$, which corresponded to the molecular formula of capsanthin epoxide. The MS/MS spectrum of sodium adduct ions $[M+Na]^+$ of these compounds showed the characteristic product ions $[M+Na-92]^+$ (elimination of toluene moiety from polyene chain) and $[M+Na-106]^+$ (elimination of xylene moiety from polyene chain), which were characteristic product ions of carotenoids in EI and ESI MS.[28,29] Both compounds showed an absorption maximum at 463 nm. These spectral data were in agreement with those of capsanthin 5,6-epoxide.[25,30] In order to confirm the structure of these reaction products, capsanthin 5,6-epoxides were prepared from capsanthin by epoxydation with *m*-chloroperbenzoic acid, as shown in Supporting Information. Peaks 4 and 5 were identical with semisynthetic *syn*-capsanthin 5,6-epoxide and *anti*-capsanthin 5,6-epoxide, respectively, on comparison with retention times in HPLC, MS, MS/MS, and UV/vis spectra data. Therefore, the structures of reaction products of peaks 4 and 5 were identified as capsanthin 5,6-epoxides.

Reaction products of peak 6 showed the molecular formula of $C_{40}H_{56}O_4$, consistent with the molecular formula of

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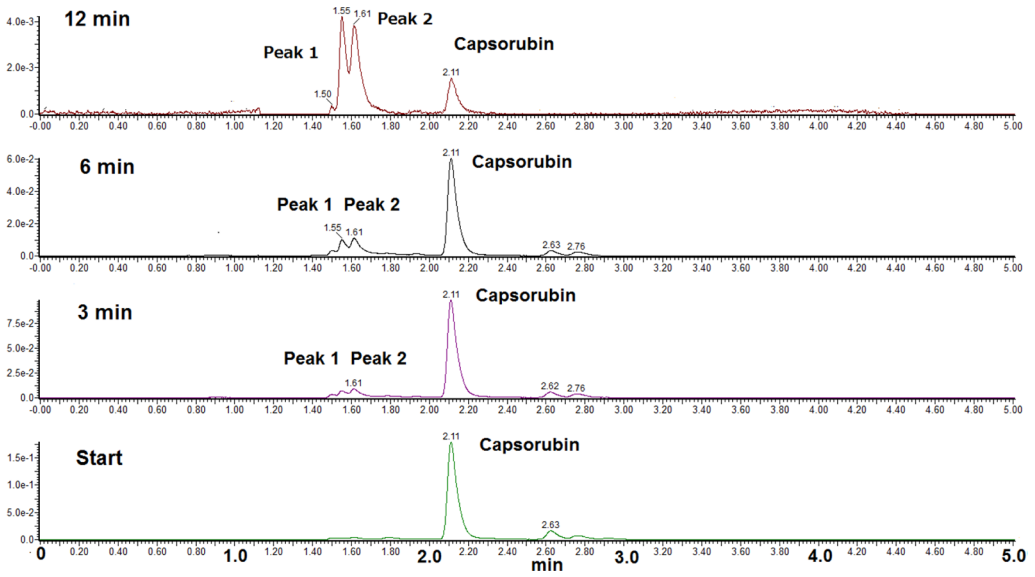

Figure 5. HPLC chromatograms of reaction products of capsorubin with superoxide anion radical. Peaks 1 and 2: capsorubin epoxides. HPLC conditions were described in Figure 3.

capsanthin epoxide. Its absorption maximum was at 448 nm. The hypsochromic shift of about 15 nm from capsanthin 5,6-epoxide suggested that these compounds were furanoid rearrangement products of capsanthin 5,6-epoxide,[26,31] namely capsanthin 5,8-epoxide. These compounds were identical to semisynthetic capsanthin 5,8-epoxides prepared from capsanthin 5,6-epoxides based on acid catalysis on comparison of the retention times of HPLC and MS, MS/MS, and UV/vis spectral data.

Both reaction products of peaks 1 and 2 showed the molecular formula of $C_{40}H_{56}O_5$, which corresponded to the molecular formula of capsanthin diepoxide. These absorption maxima were at 405−420 nm. Therefore, these compounds were assigned to capsanthin diepoxide. Epoxidation positions in capsanthin could not be determined.

Peak 3 showed the molecular formula of $C_{40}H_{54}O_4$. Its absorption maximum was at 463 nm. These data suggest that this compound is a didehydro derivative of capsanthin 5,6-epoxide. Therefore, this reaction product was tentatively identified as capsanthone 5,6-epoxide. This compound was suggested to form from capsanthin 5,6-epoxide through oxidation of the hydroxy group at C-3′. Superoxide anion radical might oxidize the hydroxy group in carotenoids to ketone.

As well as $\cdot O_2^-$, the reaction of capsanthin with ·OH hydroxyl radical generated by UV-A irradiation in hydrogen peroxide solution provided capsanthin diepoxide, capsanthone 5,6-epoxide, capsanthin 5,6-epoxide, and capsanthin 5,8-epoxide.

Reaction Products of Capsanthin with $^1O_2$. Figure 4 shows the HPLC chromatograms of the reaction products of capsanthin with $^1O_2$ by UV-A irradiation in hematoporphyrin solution. After 3 min of UV-A irradiation, reaction products assigned as capsanthin diendoproxide and capsanthin 5,6-endoperoxide were detected in HPLC. The amounts of these reaction products were increased and amount of capsanthin was decreased with UV-A irradiation time-dependently. After 6 min of UV-A irradiation, the amount of the reaction products reached a maximum. Then, the reaction products were markedly decreased and the reaction solution was bleached. Both reaction products of peaks 2 and 3 showed the molecular formula of $C_{40}H_{56}O_5$ and an absorption maximum at 463 nm. These spectral data indicated that the dioxetane moiety was attached at the double bond of C-5 to C-6 in capsanthin. Therefore, the structure of these compounds was assigned as capsanthin 5,6-endoperoxide. Compounds of peak 2 and 3 were suggested to be stereoisomers of C-5 and C-6. Reaction products of peak 1 showed the molecular formula of $C_{40}H_{56}O_7$, which indicated that two more oxygen atoms were attached to capsanthin 5,6-endoperoxide. Its absorption maximum was at 446 nm. The hypsochromic shift of the UV/vis spectrum of 15 nm from capsanthin 5,6-endoperoxide comprised with the loss of one conjugated double bond from capsanthin 5,6-endoperoxide by attaching a dioxetane moiety.[26,31] Therefore, the structure of this compound was tentatively assigned as capsanthin diendoperoxide.

Reaction Products of Capsanthin Diacetate with $\cdot O_2^-$, ·OH, and $^1O_2$. Results similar to those for capsanthin were obtained in the case of capsanthin diacetate with these ROS. Namely, capsanthin diacetate epoxides were obtained on reaction with ·OH and $\cdot O_2^-$, while capsanthin diacetate endoperoxides were produced on reaction with $^1O_2$. It was found that oxidation products of capsanthin diacetate were more stable than those of free capsanthin. Acetylation might protect the oxidation hydroxy groups of capsanthin from ROS and inhibit rapid degradation of the capsanthin molecule.

Reaction Products of Capsorubin with $\cdot O_2^-$ and ·OH. Figure 5 shows the HPLC chromatograms of the reaction products of capsorubin with $\cdot O_2^-$. Reaction products assigned as capsorubin epoxide were increased with UV-A irradiation time-dependently. After 12 min of UV-A irradiation, the amount of reaction products reached a maximum. Then, the reaction solution was bleached. The major reaction products (peaks 1 and 2) showed the molecular formula of $C_{40}H_{56}O_5$, which corresponded to the formula of capsorubin epoxide. The epoxidation position of capsorubin in these compounds was estimated from the UV/vis absorption spectra. These compounds showed an absorption maximum at 424 nm. The hypsochromic shift of the UV/vis spectrum of 45 nm from capsorubin indicated that these compounds lacked two

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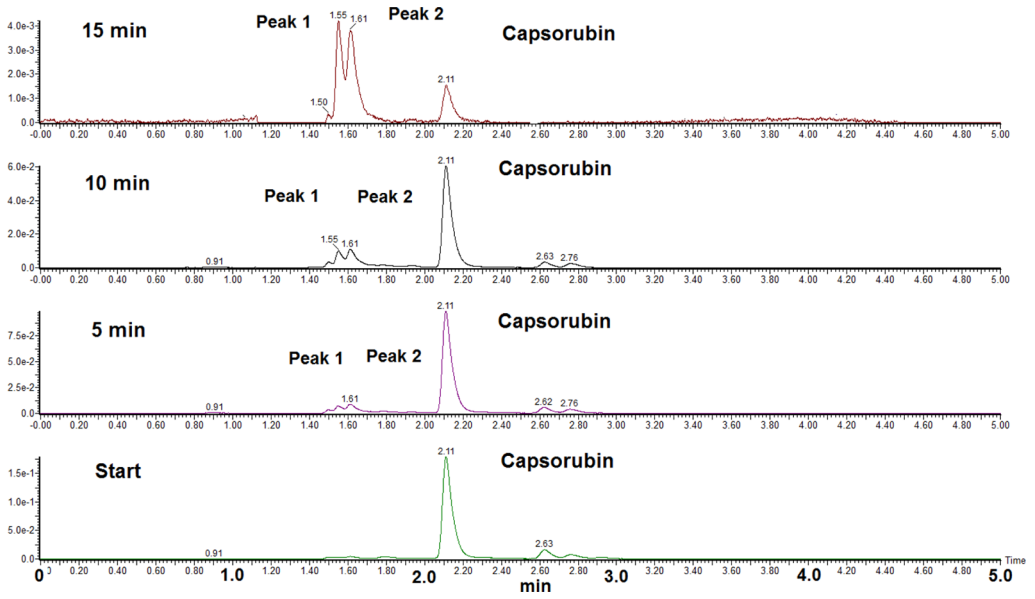

Figure 6. HPLC chromatograms of reaction products of capsorubin with singlet oxygen. Peaks 1 and 2: capsorubin endoperoxides. HPLC conditions were described in Figure 3.

conjugated double bonds (double bond of a carbonyl group at C-6 and a double bond at C-7 to C-8 in the polyene chain) from 11 conjugated double bonds of capsorubin by epoxidation.[31] Therefore, the epoxidation position of these compounds was assigned to a double bond at C-7 to C-8.

Reaction Products of Capsorubin with $^1O_2$. Figure 6 shows the HPLC chromatograms of the reaction products of capsorubin with $^1O_2$. Contrary to capsanthin and astaxanthin,[22] capsorubin hardly formed oxidation products on exposure to $^1O_2$. The compound showed the molecular formula of $C_{40}H_{56}O_6$, which corresponded to the molecular formula of capsorubin endoperoxide, being slightly generated after 10 min of UV-A irradiation. After 15 min of UV-A irradiation, capsorubin and its reaction product were markedly decreased. Capsorubin showed greater stability on exposure to singlet oxygen than β-carotene, capsanthin, or astaxanthin.[22] Therefore, capsorubin showed a strong quenching effect for $^1O_2$.

Previously, we investigated the reaction of astaxanthin and its acetate with $\cdot O_2^-$, ·OH, and $^1O_2$ by LC/PDA ESI-MS and ESR spectrometry.[22] As the results, astaxanthin endoperoxides were identified as major reaction products of astaxanthin with $^1O_2$, and astaxanthin epoxides were found to be major reaction products of astaxanthin with $\cdot O_2^-$ and ·OH. The same results in this study were obtained in the case of astaxanthin acetate. The ESR spin-trapping study revealed that astaxanthin and its acetate take up superoxide anion radical by the formation of their epoxide through peroxide and oxide radicals.[22]

In the present investigation, we found that capsanthin and capsorubin quenched not only $^1O_2$ but also scavenged ·OH by the ESR spin-trapping study. Furthermore, we found that capsanthin formed capsanthin epoxides by the reaction with $\cdot O_2^-$ and ·OH and also formed capsanthin endoperoxides by the reaction with $^1O_2$. Contrary to astaxanthin, capsanthin formed epoxide and endoperoxide more effectively and stably. It is well-known that $\cdot O_2^-$ and $^1O_2$ are electrophilic. Thus, they react with the electron-rich double bond in carotenoids. The electron density of the double bond at C-5 (5′) to C-6 (6′) in the β-end group of astaxanthin is suggested to be lower than in parts of double bonds because of the presence of the electron withdrawn effect of the carbonyl group at C-4 (4′). On the other hand, the electron density of the double bond at C-5 to C-6 in the β-end group of capsanthin is suggested to be higher than that of astaxanthin. Therefore, $\cdot O_2^-$ and $^1O_2$ might facilely attack the double bond at C-5 to C-6 in capsanthin and form 5,6-epoxide and 5,6-endoperoxide, respectively. Then, these 5,6-epoxide and 5,6-endoperoxides are converted to the corresponding 5,8-epoxide and 5,8-endoperoxide by acid catalysis.[22] The mechanism of epoxide ring formation in capsanthin with ·OH can be explained as follows: A hydroxyl radical was attached at C-5 of capsanthin. Then, an epoxide ring was formed by intramolecular homolytic substitution reaction.[22] Capsorubin showed more stability against the attack of ROS, especially $^1O_2$. Capsorubin has a long linear conjugated double bond system in its molecule. Capsorubin might exclusively quench $^1O_2$ through a physical quenching system. Therefore, capsorubin showed the strongest quenching effect on $^1O_2$ among the carotenoids.[10] Therefore, we conclude that the reaction of carotenoids with ROS might depend on their structure, such as the conjugated polyene system and functional groups.[32,33]

In conclusion, capsanthin and capsorubin formed their epoxides and endoperoxides on reaction with $\cdot O_2^-$, ·OH, and $^1O_2$. The results indicate that capsanthin and capsorubin could trap $\cdot O_2^-$ and ·OH by the formation of their epoxides. Furthermore, it was found that capsanthin and capsorubin could quench $^1O_2$ through not only a pysical quenching system but also chemical direct reaction by the formation of their endoperoxide.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b01706.

ESI TOF MS and MS/MS data of reaction products of capsanthin, capsanthin acetate, and capsorubin. UV/vis, ESI TOF MS, MS/MS, and ^1H NMR data of syn- and anticapsanthin 5,6-epoxide. UV/vis, ESI TOF MS, and MS/MS data of capsanthin 5,8-epoxide. Preparation

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scheme of capsanthin 5,6-epoxides and capsanthin 5,8-epoxides from capsanthin by *m*-chloroperbenzoic acid (PDF)

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Notes

The authors declare no competing financial interest.

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