NOTE

Antioxidative Activity of Capsorubin and Related Compounds from Paprika (*Capsicum annuum*)

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Abstract : Capsorubin and related compounds, capsanthin, capsanthin 3,6-epoxide and cycloviolaxanthin isolated from paprika (*Capsicum annuum*) inhibited the oxidation of methyl linolate in solution initiated by 2,2′-azobis(2,4-dimethyl vareronitrile) (AMVN). The antioxidative activities decreased in the order of capsorubin> capsanthin 3,6-epoxide> capsanthin> cycloviolaxanthin>β-carotene.

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Key words : *Capsicum annuum*, carotenoid, antioxidative activity, capsorubin, capsanthin, capsanthin 3,6-epoxide, cycloviolaxanthin

1 Introduction

The ripe fruit of paprika (*Capsicum annuum*) is a good source of carotenoid pigments and is used widely as a vegetable and food colorant. The red carotenoids are mainly capsanthin (**1**), capsorubin (**2**), capsanthin 3,6-epoxide (**3**) and their carotenoids account for 30-80% of the total carotenoids in fully ripe fruit (1-4).

On the other hand, a number of studies have showed that not only β-carotene (5-8) but also some xanthophylls such as zeaxanthin, canthaxanthin, astaxanthin, etc. possess antioxidative activity (7-13). Recently, Matsufuji *et al.* (14) reported that capsanthin (**1**) and the fatty acid esters possess effective antioxidative activity. In the present study, we investigated the antioxidative activity of capsorubin (**2**) and related compounds, capsanthin (**1**), capsanthin 3,6-epoxide (**3**) and cycloviolaxanthin (**4**) isolated from paprika, *C. annuum* on free radical-oxidation of methyl linolate in solution.

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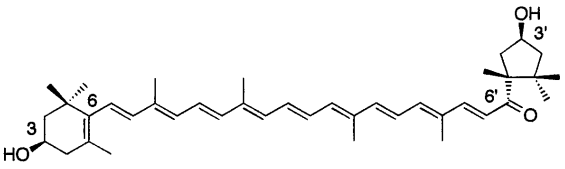
2 Experimental

2·1 Material

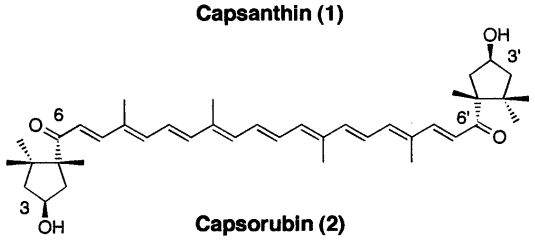
Capsanthin, capsorubin, capsanthin 3,6-epoxide and cycloviolaxanthin were isolated from paprika, *C. annuum* according to our routine methods (15). These carotenoids were identified by UV-VIS, ^{1}H-, ^{13}C-NMR and EI-MS spectral data (16). β-carotene and 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) were purchased from Wako Chemical Co. Methyl linolate, supplied by Sigma Chemical Co., was further purified by silica gel column chromatography to remove any peroxides.

2·2 Measurement of Antioxidative Activity

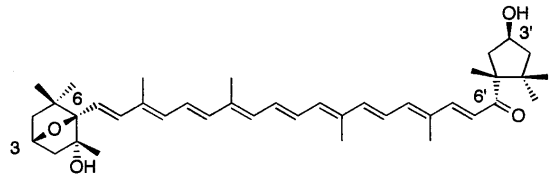
According to the methods described by Terao (7), carotenoids were dissolved in MeOH at a concentration of 2 mM (final concentration of 167 μM in reaction mixture). The sample solution, 0.1 mL, was



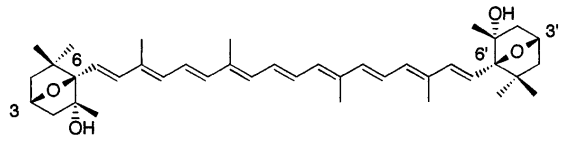
Capsanthin (1)



Capsorubin (2)



Capsanthin 3,6-epoxide (3)



Cycloviolaxanthin (4)

Scheme 1

added to 1 mL of 0.1M methyl linolate solution [*n*-hexane/2-propanol (1/1, vol/vol)], and the solution was incubated at 37°C for 5 min. As the control, MeOH alone was used instead of the sample solution. The oxidation reaction was then initiated by adding 0.1 mL of 100 mM *n*-hexane solution of AMVN, and the mixture was incubated with air at 37°C. At regular intervals, the oxidative reaction products, linolate hydroperoxides, were quantified by high performance liquid chromatography (HPLC).

2·3 HPLC

HPLC was performed with a Hitachi L-6000 intelligent pump and L-4250 UV-VIS dictator. The following HPLC conditions were employed for the quantitative analysis of methyl linolate hydroperoxides : column : LiChrosorb Si 100 (5 μm particle size) (4.6×250 mm) (Merck) ; solvent system : 2-propanol/*n*-hexane (1/99, vol/vol) ; flow rate : 1 mL/min ; and detection : 235 nm.

3 Results and Discussion

The antioxidative activity of capsorubin (**2**) and related compounds, **1**, **3** and **4** was monitored by measuring the accumulation of methyl linolate hydroperoxides during the incubation of methyl linolate with AMVN as a radical initiator. **Figure 1** shows the effects of **1**, **2**, **3**, **4** and β-carotene at 2 mM (final

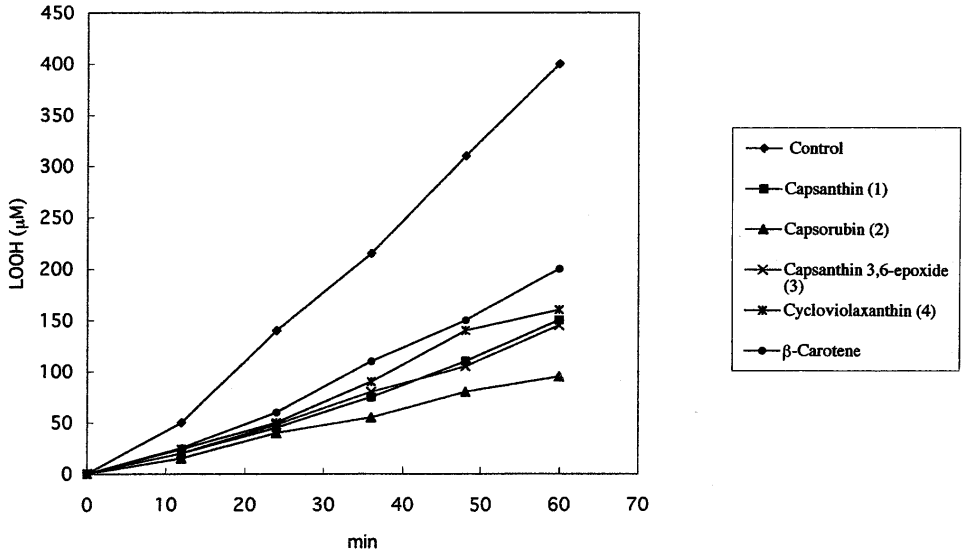


Fig. 1 Formation of methyl linolate hydroperoxides in the oxidation of methyl linolate initiated by AMVN in the presence of capsanthin (1), capsorubin (2), capsanthin 3,6-epoxide (3), cycloviolaxanthin (4) and β-carotene. The reaction system consisting of methyl linolate (83.4 mM), carotenoid (167 μM) and AMVN (8.3 mM) in a mixture of *n*-hexane/2-propanol (1/1, vol/vol) : 1 mL, *n*-hexane : 0.1 mL and methanol : 0.1 m, at 37°C.

concentration at 167 μM) on AMVN induced oxidation of methyl linolate. In the control group (absence of carotenoid), hydroperoxides from methyl linolate increased linearly with reaction time. The addition of carotenoids suppressed the production of hydroperoxides (inhibition ratios after 60 min oxidation: capsorubin, 78% : capsanthin 3,6-epoxide, 65% ; capsanthin, 62% ; cycloviolaxanthin, 60% ; β-carotene, 50%).

Several investigations have reported that the antioxidant effects of carotenoids depend on the number of conjugated double bonds, the polyene chain structure and functional groups (7-15). Recently, Matsufuji *et al.* (14) reported that capsanthin (**1**), which possesses a conjugated carbonyl group at the 6′ position in the polyene chain, was a more potent antioxidant than β-carotene and the radical scavenging ability of capsanthin was not influenced by esterification, thus, the antioxidant ability was due to the polyene chain, especially the conjugated carbonyl group (14).

In the present investigation, capsorubin (**2**), having the conjugated carbonyl groups at the 6,6′ positions in the polyene chain, exhibited more potent activity than that of **1**. This result also suggested that the antioxidative activity of carotenoids depends on the number of conjugated double bonds in the polyene chain. Capsanthin 3,6-epoxide (**3**), capsanthin (**1**) and cycloviolaxanthin (**4**) showed almost the same activity.

In conclusion, capsorubin (**2**) and related carotenoids, **1**, **3** and **4** isolated from paprika were the more effective antioxidants than β-carotene in peroxyl radical-dependent lipid peroxidation.

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