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Methanolic extract of *Coptis japonica* Makino reduces photosensitized oxidation of oils

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Abstract

Antioxidative activities of methanolic extracts of 47 plants on the methylene blue-sensitized or chlorophyll-sensitized photooxidations of linoleic acid were studied to select the plant species which possess the strong antioxidative activity in sensitized photooxidation of oil. The methanol extract of *Coptis japonica* Makino showed the strongest antioxidative activity in both methylene blue-sensitized and chlorophyll-sensitized photooxidations of linoleic acid. The study on the extracting solvent effects indicated that the antiphotooxidative components in the *Coptis japonica* Makino possess strong polar property, and are easily extracted with highly polar solvent (methanol). The methanol extract of *Coptis japonica* Makino was fractionated into three fractions (ethyl ether fraction, ethyl acetate fraction and butanol fraction) by liquid–liquid partitioning fractionation. Among the tested fractions, the butanol fraction showed strongest antioxidative activity in both chlorophyll-sensitized and methylene blue-sensitized photooxidation of linoleic acid. The butanol fraction also significantly inhibited the photooxidation of model food emulsion (50% soybean oil emulsion). The treatment with 0.3% (w/w) butanol fraction resulted in 64.9% inhibition of photooxidation of the model food emulsion during 60 h fluorescent light illumination.

1. Introduction

Oils, amino acids, proteins, vitamins (ascorbic acid, retinyl palmitate, ergocalciferol, carotenoids, tocopherols), cholesterol, limonene, and conjugated terpenes in various types of foods are very susceptible to photooxidation during storage under light, especially when photosensitizers such as chlorophylls and riboflavin are present in the systems (Jung, Lee & Kim, 1998; Jung, Yoon, Lee & Min, 1998; King & Min, 1998; Koryck-Dahl & Richardson, 1978). Photooxidation occurs through Type I or II reaction pathways. Type I photosensitized reaction involves the formation of superoxide anion and other radicals due to the transfer of hydrogen atoms or electrons by interaction of triplet sensitizer with molecular oxygen or other components. The type II process involves the generation of singlet oxygen by the energy transfer from an excited triplet sensitizer to a triplet oxygen. The photochemical processes in the food system are dependent on the types and concentration of sensitizers, and substrates in the system.

Chlorophylls, myoglobin derivatives, and riboflavin are reportedly efficient photochemical sensitizers for the formation of both singlet oxygen and/or superoxide anion radicals in various foods (Berliner, Badley, Heinhelz, Min & Ogaka, 1994; Koryck-Dahl & Richardson, 1978; Whang & Peng, 1988a,b). Thus, the foods containing these sensitizers deteriorate easily under light-illuminated conditions. The effective radical scavengers, such as BHA and BHT, do not possess antioxidative properties in the photosensitized oxidation of oils due to their lack of ability in scavenging singlet oxygen (Carlsson, Suprunchik & Siles, 1976; Chan, 1977; Clements, Van DenEngh, Frost & Hoogenhout, 1973). There are few antioxidants that can be used for the protection of foods from the photosensitized oxidation.

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These antioxidants are ascorbic acid, ascorbyl palmitate, carotenoids and tocopherols (Jung, Choe & Min, 1991; Jung, Kim & Kim, 1995; Jung, Lee & Kim 1998; Jung & Min, 1991; Jung, Yoon, et al. 1998; Lee, Jung & Kim, 1997; Lee & Min, 1991). Thus, the need for novel antioxidants for effective reduction of photosensitized oxidation of food components is obvious, and academia and industry continue to look for novel natural antioxidants.

The objectives of this work were (1) to select the herbal plant species which exhibit especially strong antioxidative activity in the chlorophyll or methylene blue sensitized photooxidation of linoleic acid by screening the antiphotooxidative activity with methanolic extracts of 47 Oriental herbal plants, (2) to study the antioxidative activity of fractions (by liquid-liquid partitioning), obtained from the methanolic extract of the plant species, which showed the strongest antioxidative activity in the sensitized photooxidation of linoleic acid, and (3) to study the effects of the most active fraction on the photooxidation of model food emulsion during fluorescent light illumination.

2. Materials and methods

2.1. Materials

Forty-seven different kinds of dried herbal plants were purchased from local Oriental herbal stores in Seoul, Korea. The herbal plants and plant parts used in this research are listed in Table 1. Linoleic acid, α-tocopherol, tween 80, span 80, chlorophyll b, methylene blue and BHA were purchased from Sigma Co. (St. Louis, MO). Soybean oil without additives was obtained from Samryp Oil Co. (Yangsan, Korea).

2.2. Extraction

Freshly ground oriental herbal plants (5g each) were extracted with 50 ml of methanol, ethyl acetate or ethyl ether for 24 h at 4°C. The extracted solutions were filtered through filter paper to obtain the particle-free plant extracts.

2.3. Fractionation of Coptis japonica Makino methanol extract

Distilled and demineralized water was first added to methanolic extract of *Coptis japonica* Makino and the solution was concentrated using a rotary vacuum evaporator at 30°C. The aqueous extract was fractionated by successively extracting with ethyl ether, ethyl acetate and butanol, and these fractions were designated as ethyl ether fraction, ethyl acetate fraction and butanol fraction, respectively.

2.4. Screening of antiphotooxidative activities with methanolic extracts of plants in the methylene blue-sensitized photooxidation of linoleic acid in methanol

A mixture of 1.5 ml methanolic extracts, 2.5 ml of 0.3M linoleic acid, 1 ml of methylene blue solution (20 μg/ml methanol) was, in triplicate, prepared in a 30 ml-capacity serum bottle. The serum bottles were air-tightly sealed with Teflon-lined rubber septa and aluminium caps, and then were randomly placed in a light storage box for 5 h under fluorescent illumination condition (described in detail previously by Jung et al., 1995). The light intensity at the sample level was 3300 lux. To minimize the possible radical chain reaction, the light storage box was placed in a 4°C working cooler. The temperature within the light box was 7 ± 1°C during the experiment. The oxidation of linoleic acid was determined by measuring the peroxide values according to the AOCS Official Method (1990).

2.5. Protective activity of methanolic extracts of the 10 selected plants in the chlorophyll-sensitized photooxidation of linoleic acid in a solvent mixture of methanol and benzene

Based on the results from screening experiments, we selected 10 species which had strong antioxidative properties (++, +++, ++++, Table 1) in methylene blue-sensitized photooxidation of linoleic acid. To study the protective activities of the methanolic extracts of these 10 selected species in the chlorophyll-sensitized photooxidation of linoleic acid in a solvent mixture (methanol and benzene, 1:4, v/v), a mixture of 1.0 ml methanolic extracts, 2.5 ml of 0.3 M linoleic acid in a solvent mixture of methanol and benzene (1:4, v/v), 1.5 ml of chlorophyll b solution (20 mg/ ml solvent mixture of methanol and benzene, 1:4, v/v) was, in triplicate, prepared in a 30 ml-capacity serum bottle, and the samples were stored in a light storage box for 5 h as described before. The oxidation of the linoleic acid was determined by measuring peroxide values according to the AOCS Official Method (1990).

2.6. Extracting solvent effects on the antioxidative activity of Coptis japonica Makino extract in the chlorophyll-sensitized photooxidation of linoleic acid

Methanol, ethyl acetate and ethyl ether were used as extracting solvents to prepare the *Coptis japonica* Makino extracts as described previously. Then, the solvents were removed using a rotary vacuum evaporator at 40°C. The obtained dried-extracts (1 g) were redissolved with 10 ml of a solvent mixture (methanol/benzene, 1:4, v/v). A mixture of 1.0 ml extract solution, 2.5 ml of 0.3 M linoleic acid in a methanol and benzene mixture (1:4, v/v), and 1.5ml chlorophyll b solution (20

Table 1
Antioxidative activity of methanolic extracts of selected plants on the methylene blue sensitized photooxidation of linoleic acid in methanol during 5 h storage under fluorescent light

No.	Botanical name	Pant part	Activity[a]	PVs/PVc[a]
1.	*Aectum lappa* L	Root & seed	-	0.83
2.	*Aconitium rocyanum* Raymund	Root	-	1.11
3.	*Akebia quinata* Decne	Root	-	1.08
4.	*Anthriscus sylvestris* Hoffman	Root	-	1.08
5.	*Asarum heterotropoids* Maekawa	Whole plant	-	1.10
6.	*Atractyloides ovata* Thunb	Root & caulis	-	0.88
7.	*Atractylides lyrata* S. & Z.	Root & caulis	-	1.48
8.	*Brassica Cernaua* Forbes et Hensl	Seed	-	0.95
9.	*Broussonetia* Kozinoki Sieb.	Seed	-	0.88
10.	Canabis sativus L.	Stem	-	1.46
11.	*Chelidonium majus* L. v. *asiaticum*	Whole plant	-	1.94
12.	*Cimicifuga heracleifolia* Komarov	Root & leaf	-	0.87
13.	*Cnidum officinale* Makino	Aerial tuber	-	1.51
14.	*Coptis japonica* Makino	Root	++++	0.22
15.	*Crataegus curneata* S.	Fruit	+	0.74
16.	*Crystanthenum indicum* L.	Flower & seed	-	0.91
17.	*Cuscuta japonica* Choicy	Seed	-	0.91
18.	*Epimedium koreanum* Nakai	Stem & leaf	-	1.88
19.	*Eugennia caryopylla* T.	Bud	++++	0.29
20.	*Evadia rutaecarpa* Benth	Seed	-	0.86
21.	*Foropyium esculentum* Moench	Seed	-	1.08
22.	*Glycirrhiza glabra* L.	Root	++	0.64
23.	*Gossypium indicum* Lam.	Seed	-	1.08
24.	*Julans regia* L.	Seed	++	0.59
25.	*Lithospermum offinale* L.	Root	+	0.76
26.	*Machilus rimosa v. thumbergii*	Cortex	+++	0.49
27.	*Maganolia kobus* A.P. DE Candolle	Flower	-	1.18
28.	*Morus alba* L.	Leaf	-	1.18
29.	*Morus bombycis* Koitzumi	Cortex	-	0.96
30.	*Nelumbo nucifera* G.	Seed	+	0.71
31.	*Nelumbo* G.	Flower	++	0.58
32.	*Palupara cordata* Busk	Whole plant	-	2.80
33.	*Panax Ginseng*	Root	-	1.1
34.	*Polygonum multiflorium* Thunber	Root	-	1.06
35.	*Prunus mume* S. & Z.	Flower	-	1.35
36.	*Pueraria hirsuta* Matsum	Root	++	0.69
37.	*Pseudocydonia sinensis* Schneider	Fruit	++	0.57
38.	*Raphanus Sativus* L.	Seed & root	-	1.02
39.	*Rheum undulatum* L.	Root & caulis	-	1.52
40.	*Sanguisorba officinalis v. coreana*	Stem	+	0.75
41.	*Scutellaria baicalensis Geogie*	Root	++	0.56
42.	*Sorphora angustifloia*	Root	-	0.87
43.	*Sorphora japonica* L.	Flower	-	0.94
44.	*Thesium chinensis* Turc.	Whole plant	-	1.76
45.	*Ulmas japonica* Sarg	Cortex	+++	0.37
46.	*Vitex rotundifolia* L.	Fruit	-	1.08
47.	*Zinger offinale* Rosc.	Root	+	0.74

[a] The level of antioxidant activity of plants on the methylene blue-sensitized photooxidation of linoleic acid was arbitrarily divided into 4 categories by calculating the ratio of peroxide value (PVs) of sample containing plant extract to peroxide value (PVc) of control after 5 h storage under fluorescent light (++++: PVs/PVc <0.3; +++ : $0.3 <$ PVs/PVc < 0.5; ++: $0.5 <$ PVs/PVc < 0.7; +: $0.6 <$ PVs/PVc < 0.8 ; -: $0.8 <$ PVs/PVc).

mg/ml methanol and benzene mixture, 1:4, v/v) in 30 ml serum bottles was placed in the light storage box (3300 lux) for 5 and 10 h as described before.

2.7. *Protective activity of butanol fraction of* Coptis japonica *Makino methanolic extract in the chlorophyll-sensitized or methylene blue-sensitized photooxidations of linoleic acid*

A mixture of 1 ml butanol fraction solution in methanol, 2.5 ml of 0.3 M linoleic acid in methanol, and 1.5 ml of chlorophyll b solution (20 μg/ml methanol) was prepared in a 30 ml capacity serum bottle to study the effect of butanol fraction of *Coptis japonica* Makino methanol extract on the chlorophyll-sensitized photooxidation of linoleic acid. A mixture of 1 ml butanol fraction solution in methanol, 2.5 ml of 0.3 M linoleic

acid in methanol, and 1.5 ml of methylene blue solution (13.3 μg/ml methanol) was also prepared in a serum bottle to study the effects of butanol fraction of *Coptis japonica* Makino methanolic extract on the methylene blue-sensitized photooxidation of linoleic acid. The prepared sample bottles were placed in the light storage box for 10 h. The number of prepared sample bottles pre-treatments was 10. Sample bottles were taken out, two at a time, per treatment, every 2 h to determine the peroxide contents in the samples.

2.8. Protective activity of butanol fraction of Coptis japonica Makino methanolic extract in the photooxidation of soybean oil emulsion

An oil-in-water emulsion system (50% soybean oil) was prepared by mixing 500 g soybean oil, 25 g tween 80, 25 g span 80, and 450 g demineralized water with a homogenizer at 10,000 rpm for 5 min. Then, calculated amounts of dried butanol fraction (0, 0.1, 0.2 and 0.3%, w/w) were added to the 200 g portion of the prepared emulsions in a 500 ml beaker. The samples were sonicated for 60 s with a Fisher sonic dismembrator (Model 300) to prepare the fine oil-in-water emulsion and to thoroughly disperse the dried butanol fraction into the emulsion system. The homogenized soybean emulsions (2.5 g each), treated with or without butanol fraction of *Coptis japonica* Makino methanolic extract, were transferred to 30 ml-capacity serum bottles and stored in a light storage box for 60 h. The number of prepared sample bottles per treatment was 10. Sample bottles were taken out two at a time per treatment every 12 h to determine the peroxide values in the samples.

2.9. Statistical analysis

The experiments were carried out in duplicate or triplicate and the statistical analysis was done using the Statistical Analysis System (SAS Institute, Inc., 1990). Duncan's multiple range test was used to ascertain treatment effect on the photooxidation of linoleic acid or soybean oil. Significance of difference was defined at $p < 0.05$.

3. Results and discussion

3.1. Screening of antiphotooxidative activities with methanolic extracts of herbal plants in the methylene blue-sensitized photooxidation of linoleic acid

Antioxidative activities of methanolic extracts of 47 herbal plants on the methylene blue-sensitized photooxidation of linoleic acid in methanol during 5 h fluorescent light illumination are shown in Table 1. Methylene blue was used in this system as a photosensitizer for the production of singlet oxygen in the presence of light. Ten herbal plant species (Nos. 14, 19, 22, 24, 26, 31, 36, 37, 41, 45), out of 47 tested species, showed more than 30% inhibition (++, +++, or ++++) of linoleic acid photooxidation. These results indicated that some of the tested species were rich in natural antioxidants in photosensitized oxidation of oils, and that the qualities and quantities of the antioxidants in methanolic extracts of the plants seemed to be very different. The tested 47 plant species were selected for this research because these plants showed strong antioxidative activity in autooxidation system in the previous work in our laboratory (Kim, Kim, Kim, Oh & Jung, 1994). The result indicated that autooxidation and photooxidation had different reaction mechanisms, and that the antioxidative components might not be effective in one system, even if the components had strong antioxidative activity in another system.

Among the tested species in the present study, *Coptis japonica* Makino (No. 14) and *Eugennia caryopylla* T. (No. 19) showed specially strong antioxidative activities, showing 88.5 and 81.1% inhibition of methylene blue-sensitized photooxidation of linoleic acid, respectively. The result indicates that these methanolic extracts have great potential for the protection of numerous foods from light-induced deterioration. Even though many research papers have reported on the antioxidative activities of plant extracts in the autooxidation or thermal oxidation of oils, no attempt has been previously made to select plant species with strong anti-photooxidative properties.

3.2. Protective activities of 10 selected plant extracts in the chlorophyll-sensitized photooxidation of linoleic acid

Since it was found that 10 plant extracts possessed strong antioxidative activity in the methylene blue-sensitized photooxidation of linoleic acid, we decided to continue to find whether these 10 plants extracts also exert strong antioxidative activity in the chlorophyll b-sensitized photooxidation of linoleic acid (Table 2). Chlorophylls and their derivatives are other types of effective sensitizer for the production of singlet oxygen under light illumination. Cholorophyll b was used as a photosensitizer in this system since chlrophyll b was a more effective sensitizer than chlorophyll a (Endo, Usuki & Kaneda, 1984). The results showed that Eugennia caryopylla T. (No. 19) did not show strong antioxidative activity in the chlorophyll b-sensitized photooxidation of linoleic acid of methanol, showing only 23% inhibition of linoleic acid oxidation. Note that the methanolic extracts of *Eugennia caryopylla* T. (No. 19) showed 81% inhibition of linoleic acid oxidation in the methylene blue-sensitized photooxidation system. *Coptis japonica* Makino (No. 14) showed very strong antioxidative activity on the chlorophyll b-sensitized

Table 2
Effects of the methanolic extract of selected plant extracts on the chlorophyll b-sensitized photooxidation of linoleic acid in a solvent mixture (methanol : benzene, 1:4, v/v) during 5 h storage under fluorescent light (3300 lux)

No.	Botanica Name	PV	PVs/PVc[a,b]
	Control	136.3	1.00*a*
14.	*Coptis japonica* Makino	26.7	0.20*e*
19.	*Eugennia caryopyllata* T.	103.0	0.76*c*
22.	*Glycirrhiza glabra* L.	115.1	0.84*b*
24.	*Julans regia* L	133.3	0.98*a*
26.	*Machilus rimosa* v. *thumbergii*	108.0	0.79*c*
31.	*Nelumbo nucifera* G.	109.5	0.80*b*
36.	*Pueraria hirsuta* Matsum	128.7	0.94*ab*
37.	*Pseudocydonia sinensis* Schneider	121.8	0.89*b*
41.	*Scutellaria baicalensis* George	119.3	0.88*b*
45.	*Ulmas japonica Sarg*	86.5	0.63*d*

[a] The ratio of peroxide value (PVs) of sample containing plant methanolic extract to peroxide value (PVc) of control after 5 h storage under fluorescent light.
[b] Means with the different italicized letters are significantly different at $p < 0.05$.

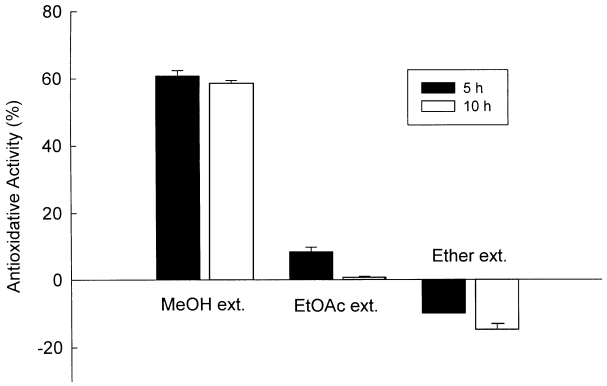

Fig. 1. Effects of 0.2% methanol extract, ethyl acetate extract or ethyl ether extract of *Coptis japonica* Makino on the chlorophyll b-sensitized photooxidation of linoleic acid in a solvent mixture of methanol and benzene (1:4, v/v) under fluorescent light illumination (3300 lux) for 5 and 10 h at 7±1°C. The chlorophyll concentration in this experimental system was 6 μg/ml. MeOH ext., EtOAc ext., and ether ext in the figure represent methanol extract, ethyl acetate extract and ethyl ether extract, respectively.

photooxidation of linoleic, resulting in 80% inhibition of linoleic acid. This result indicated that *Coptis japonica* Makino (No. 14) can exert its especially strong antioxidative activity on both methylene blue-sensitized and chlorophyll b-sensitized photooxidations of oil. The treatment of *Ulmas japonica* Sarg. (No. 45) also showed strong antioxidative activity in the chlorophyll sensitized photooxidation of linoleic acid, resulting in 47% inhibition of chlorophyll b-sensitized photooxidation of linoleic acid. Since numerous types of foods naturally contain chlorophylls, the antioxidative properties in the chlorophyll sensitized photooxidation are greatly important. Thus, *Coptis japonica* Makino was selected for the further study in this research.

3.3. *Extracting solvent effects on the antioxidative activity of* Coptis japonica *Makino extract in the chlorophyll-sensitized photooxidation of linoleic acid*

The extracting solvent effects on the antioxidative activity of *Coptis japonica* Makino extract in the chlorophyll-sensitized oxidation of linoleic acid in a solvent mixture of methanol and benzene (1:4, v/v) during 5 h fluorescent light illumination were also studied (Fig. 1). The concentration of the treated extract was 0.2% (w/v, based on the total solution volume) in this system. The methanolic extract of *Coptis japonica* Makino resulted in 61 and 59% inhibition of chlorophyll b-sensitized-photooxidation of linoleic acid after 5 and 10 h fluorescent light illumination, respectively. However, the ethyl ether extract and ethyl acetate extract showed little or no protective activity in the chlorophyll b-sensitized photooxidation. The antiphotooxidative activity of *Coptis japonica* Makino was greatly dependent on the polarity of the extracting solvents. As the polarity of the extracting solvent increased, the antioxidative activity of the extract also increased. This result indicated that the antiphotooxidative component(s) in the *Coptis japonica* Makino has(have) strong polar properties, and are easily extracted with highly polar solvent (methanol).

3.4. *Protective activity of ethyl ether, ethyl acetate, and butanol fraction of* Coptis japonica *Makino methanolic extract in the chlorophyll-sensitized photooxidation of linoleic acid*

The methanolic extract of *Coptis japonica* Makino was fractionated into 3 fractions (ethyl ether fraction, ethyl acetate fraction, and butanol fraction) by subsequent liquid–liquid partitioning extraction. Table 3 showed the effects of diethyl ether, ethyl acetate and butanol fractions on the chlorophyll b-sensitized photooxidation of linoleic acid in methanol. The result

Table 3
Effects of 0.1% (w/v) ethyl ether, ethyl acetate, or butanol fractions of *Coptis japonica* Makino on the chlorophyll b-sensitized photooxidation of linoleic acid in methanol after 5 hr storage during fluorescent light illumination

Fraction	PV	PVs/PVc[a,b]
Control	51.7	1.00*a*
Ethyl ether fraction	46.2	0.89*b*
Ethyl acetate fraction	57.1	1.10*a*
Butanol fraction	25.8	0.50*c*

[a] The ratio of peroxide value (PVs) of sample containing plant extract (0.1%) to peroxide value (PVc) of control after 5 h storage under fluorescent light.
[b] Means with the different italicized letters are significantly different at $p < 0.05$.

showed that the butanol fraction showed the strongest antioxidative activity ($p < 0.05$). The treatment with 0.1% butanol fraction inhibited 50% linoleic acid oxidation in the chlorophyll b-sensitized photooxidation system after 5 h fluorescent light illumination (3300 lux). The treatment with 0.1% ethyl ether fraction resulted in 11% inhibition of chlorophyll b-sensitized photooxidation of linoleic acid. However, the treatment with 0.1% ethyl acetate fraction increased the chlorophyll b-sensitized photooxidation of linoleic acid.

3.5. Effects of different concentrations (0, 0.1, 0.15, 0.2 and 0.25%) of butanol fraction of Coptis japonica *Makino methanol extract on the chlorophyll b-sensitized or methylene blue sensitized photooxidation of linoleic acid*

The effects of different concentrations of butanol fraction on the chlorophyll-sensitized photooxidations of linoleic acid during 10 h fluorescent light illumination are shown in Fig. 2. The chlorophyll concentration used was 6 μg/ml in this experimental system. In the chlorophyll-sensitized photooxidation, 0.05% butanol fraction significantly decreased the peroxide formation during 10 h fluorescent light illumination ($p < .05$), resulting in 23.1% inhibition of photooxidation of linoleic acid. And the protective activity increased as the concentration increased. The 0.25% treatment of the butanol fraction resulted in 77.6% inhibition of chlorophyll b-sensitized photooxidation of linoleic acid after 10 h fluorescent light illumination. Duncan's multiple range test showed that each treatment (0.05, 0.1, 0.15, 0.2 and 0.25% butanol fraction) showed a significant difference in peroxide values after 10 h fluorescent light illumination ($p < 0.05$).

The effects of different concentrations of butanol fraction on the methylene blue-sensitized photooxidations of linoleic acid during 10 h fluorescent light illumination are shown in Fig. 3. In this experimental system, the methylene blue concentration was 4 μg/ml. Methylene blue (4 μg/ml) showed much more effective sensitizing activity than chlorophyll b (6 μg/ml). This result was consistent with a previous report (Lee et al., 1997). The butanol fraction, however, showed less protective activity in the methylene-blue sensitized system than in the chlorophyll-sensitized system. The treatment with 0.05% butanol fraction decreased the peroxide formation during the first 2 h illumination, but increased peroxide formation after 4 h illumination (Fig. 3). The treatment with 0.1% butanol fraction decreased the peroxide formation up to 6 h fluorescent light illumination but increased the peroxide formation after 8 h fluorescent light illumination. It seems that the butanol fraction contained both antioxidative and prooxidative components for the photosensitized oxidation. Since methylene blue rapidly increased preoxides,

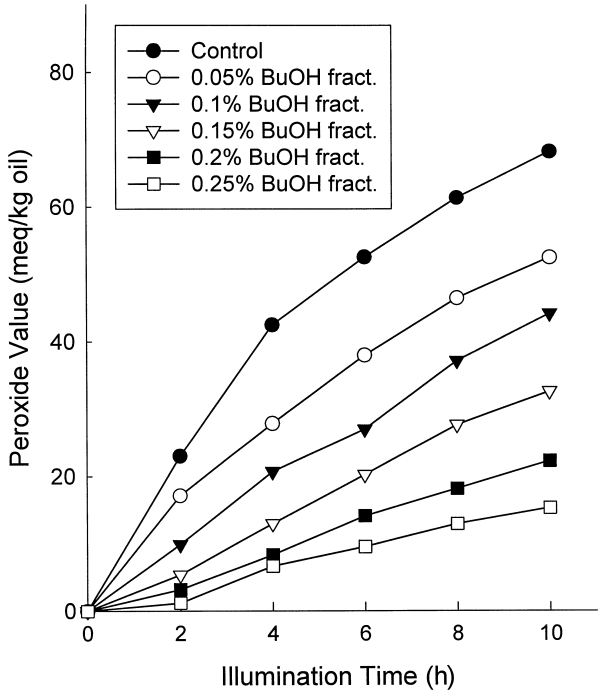

Fig. 2. Effects of 0, 0.05, 0.1, 0.15, 0.2 and 0.25% butanol fraction of *Coptis japonica* Makino methanol extract on the chlorophyll b-sensitized photooxidation of linoleic acid in methanol during 10 h fluorescent light illumination (3300 lux) at 7±1°C. The chlrophyll concentration in this experimental system was 6 μg/ml.

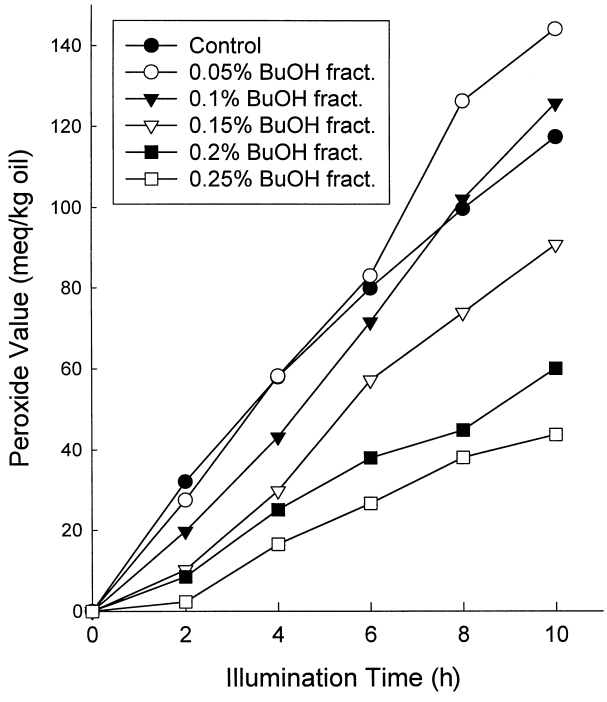

Fig. 3. Effects of 0, 0.05, 0.1, 0.15, 0.2 and 0.25% butanol fraction of *Coptis japonica* Makino methanol extract on the methylene blue-sensitized photooxidation of linoleic acid in methanol during 10 h fluorescent light illumination (3300 lux) at 7±1°C. The methylene blue concentration in this experimental system was 4 μg/mL

the antioxidative component of the butanol fraction was consumed at the faster rate. Thus, one can postulate that the prooxidant activity, with low level of butanol fraction at the later stage, was due to the faster consumption of the antioxidative components in the butanol fraction. However, the treatment with 0.15% or higher concentration greatly decreased the peroxide formation even at the later stage of the photooxidation. The treatments with 0.15, 0.20 and 0.25% butanol fraction showed 22.7, 48.8 and 62.7% inhibition of methylene blue-sensitized photooxidation of linoleic acid after 10 h fluorescent light illumination. A statistical analysis (Duncan's multiple range test) showed that each of the 0.15, 0.2 and 0.25% butanol fraction treatments were significantly different in peroxide value after 10 h light illumination ($p < 0.05$). Since the extraction and the fractionation procedures used in this experiment were not favourable conditions for the retaining ascorbic acid, ascorbyl palmate, tocopherols and carotenoids in the butanol fraction, one can postulate that the active components in the butanol fractions seemed not to be these previously known antiphotooxidants.

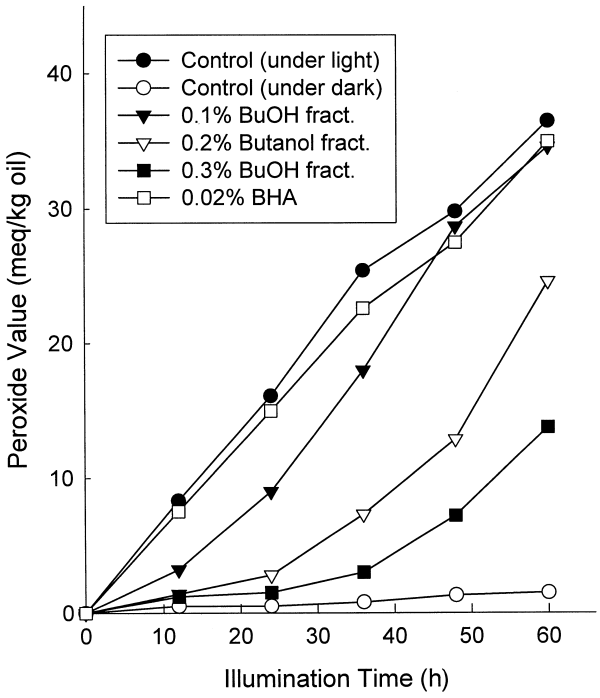

Fig. 4. Effects of 0, 0.1, 0.2 and 0.3% butanol fraction of *Coptis japonica* Makino methanol extract on the photooxidation of model food emulsion (50% soybean oil emulsion) during 60 h fluorescent light illumination (3300 lux) at 7 ± 1°C.

3.6. *Protective activity of butanol fraction of* Coptis japonica *Makino methanol extracts in the photooxidation of oil-in-water emulsion*

Numerous foods are sold in an emulsion state and these emulsion foods contain natura photosensitizers such as chlorophyll and riboflavin. The emulsion foods containing natural photosensitizers are very susceptible to oxidation under sunlight or fluorescent light. The protective activity of the butanol fraction of *Coptis japonica* Makino methanolic extracts on the photooxidation in oil-in-water emulsion (model food emulsion) under fluorescent light (3300 lux) at 7 ± 1°C was studied (Fig. 4). As the illumination time increased, the peroxide value of control (containing no butanol fraction) increased drastically, resulting in a peroxide value of 36.5 meq/kg oil after 60 h fluorescent light illumination at 3300 lux. However, the model food emulsion, protected with aluminium foil from light, did not increase greatly peroxide contents during storage, showing a peroxide value of 1.2 meq/kg oil after 60 h storage. This result indicated that light illumination is required for the oxidation of oil-in-water emulsion under this experimental condition.

The treatment with 0.02% BHA slightly decreased the peroxide formation in the model food emulsion during 60 h fluorescent light illumination. BHA reportedly is an effective free radical scavenger, but does not effectively reduce singlet oxygen oxidation. Thus, the result indicated that the oxidation in this experimental system was not due to the free radical chain reaction, but mostly due to singlet oxygen oxidation. The 0.1% butanol fraction treatment significantly decreased peroxide formation in the emulsion system during the first 36 h light illumination ($p < 0.05$) but did not show a significant effect after 48 h illumination ($p > 0.05$). However, the treatments with 0.2 and 0.3% butanol fraction significantly decreased the peroxide formation during 60 h light illumination ($p < 0.05$). The peroxide values of the emulsions treated with 0.2 and 0.3% butanol fraction were 24.6 and 13.8 meq/kg oil after 60 h fluorescent light illumination, respectively. That is, the treatments with 0.2 and 0.3% butanol fraction inhibited 32.6 and 64.9% photosensitized oxidation of soybean oil emulsion during 60 h fluorescent light illumination.

Since both methylene blue and chlorophyll are well known sensitizers for the production of singlet oxygen in the presence of light (Endo et al., 1984; Whang & Peng, 1988a), it can be assumed that the active components in the butanol fraction had, to at least some extent, a singlet oxygen-quenching ability. In our laboratory, continued research is now being carried out to isolate and identify the active components in the *Coptis japonica* Makino, and to determine the mechanism for the antiphotooxidative activity. The present study provides important information for the discovery of novel antiphotooxidative plant species (*Coptis japonica* Makino). This present result also indicates that the butanol fraction of the *Coptis japonica* methanol extract has great potential as a strong antiphotooxidative additive in the food emulsions.

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