Isolation of *C*-Glucosylflavone from Lemon Peel and Antioxidative Activity of Flavonoid Compounds in Lemon Fruit

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Two antioxidative two *C*-glucosylflavones were isolated from the peel of lemon fruit (*Citrus limon* B_{URM}. f.). They were identified as 6,8-di-*C*-β-glucosyldiosmin (LE-B) and 6-*C*-β-glucosyldiosmin (LE-C) by UV, IR, FAB-MS, ^{1}H NMR, and ^{13}C NMR analyses. The antioxidative activities of LE-B, LE-C, and flavonoid compounds (eriocitrin, diosmin, hesperidin, and narirutin) in lemon fruit were examined using linoleic acid autoxidation, the liposome oxidation system, and the low-density lipoprotein (LDL) oxidation system. LE-B and LE-C showed antioxidative activity in these autoxidation systems but exhibited weaker activity than eriocitrin, its eriodictyol of its aglycon. Eriocitrin and its metabolites by intestinal bacteria (eriodictyol, 3,4-dihydroxyhydrocinnamic acid, and phloroglucinol) exhibited stronger antioxidative activity than α-tocopherol in the LDL oxidation system and had approximately the same activity as (−)-epigallocatechin gallate. Eriocitrin and its metabolites are powerful antioxidants using an in vitro oxidation model for heart disease.

Keywords: *Lemon fruit; Citrus limon; antioxidant; eriocitrin; flavonoid; 6,8-di-C-β-glucosyldiosmin; 6-C-β-glucosyldiosmin*

INTRODUCTION

Lemon fruit (*Citrus limon*) is used in various foods, for example, soft drinks, alcoholic drinks, and jams. Lemon fruits contain a number of nutrients such as citric acid, ascorbic acid, minerals, and flavonoids. Flavonoid compounds are widespread in the plant kingdom and comprise a large group of naturally occurring compounds found in all vascular plants. They are present in *Citrus* as well as in other fruits, vegetables, nuts, seeds, grains, tea, and wine, and the average Western diet contains ≈1 g of mixed flavonoids (Kuhnan, 1976). Flavonoids that exhibit beneficial effects on capillary permeability and fragility were once known as vitamin P. The flavonoids in citrus fruit have been investigated regarding their physiological function (Middleton and Kandaswami, 1994; Matsubara et al., 1985). Recently, we have been involved in the isolation of antioxidative compounds from lemon fruit and have identified eriocitrin (eriodictyol 7-rutinoside) from a flavonoid glycoside (Miyake et al., 1997a). It was found to be present mainly in lemon and lime fruit among the citrus fruits.

Lipid peroxidation is known to be one of the major factors in deterioration during the storage and processing of food. In addition, it is thought that lipid peroxidation is strongly associated with aging, carcinogenesis, and atherosclerosis (Steinberg et al., 1989; Yagi, 1987; Harman, 1982; Cutler, 1984). Recently, it was reported that dietary antioxidants may offer effective protection from peroxidative damage in living systems and may play an important role in prevention of carcinogenesis and in extending the life span of animals (Cutler, 1984, 1992; Osawa et al., 1990). Therefore, much attention has been focused on natural antioxidants (Miyake et al., 1997a; Tuda et al., 1994; Osawa et al., 1992). These compounds are expected to provide antioxidative activity in vivo and protection from peroxidative damage in living systems related to aging and carcinogenesis. It is important to determine how the antioxidants in food are metabolized in vivo and how antioxidant metabolites function in a living system. We have reported the metabolic mechanism of eriocitrin by human intestinal bacteria (Miyake et al., 1997b).

In this study, we attempted to isolate antioxidative flavonoid compounds other than eriocitrin in lemon fruit and to compare the antioxidative activities of the flavonoid compounds in the lemon fruit.

MATERIALS AND METHODS

Chemicals. Linoleic acid, egg lecithin, and α-tocopherol were purchased from Wako Pure Chemical Industries, Ltd., Japan. Flavonoid compounds (HPLC grade) were purchased from Funakoshi, Ltd., Japan. The low-density lipoprotein (LDL) was purchased from Sigma Chemical Co., St. Louis, MO. Eriocitrin was purified from lemon peel (*Citrus limon* B_{URM}. f.) using preparative HPLC (Miyake et al., 1997a). The purity of eriocitrin was >99.0% from HPLC analysis.

Isolation of Antioxidant from Lemon Fruit. The peel of lemons (1.50 kg) obtained from 20 lemon fruits was chopped (5 mm × 5 mm) in a homogenizer and extracted with methanol (3 L) at room temperature for 3 days. The extract was filtered through a cloth to remove the peel and was concentrated in vacuo. The peel extract (110 g) was then dissolved in water (300 mL). The solution was chromatographed on a Cosmosil 75C 18-OPN ODS column (Nakalai Tesque, Inc., Kyoto, Japan; column size ∅ 37 × 500 mm). The column was washed with 2 L of water and successively eluted with 20% methanol/water, 40% methanol/water, and methanol (2 L each). The 40% methanol/water portion was concentrated in vacuo, and 6.00 g was obtained after drying. This sample was dissolved in methanol, and preparative high-performance liquid chromatography (HPLC) was carried out using a YMC-Pack ODS column (YMC Co., Ltd., Kyoto, Japan; column size ∅ 20 × 250 mm; particle size 5 μm) with a UV spectrophotometric detector (280 nm) and 40% methanol as the solvent at a flow rate of 10

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mL/min under room temperature. The antioxidative LE-B (52.0 mg) and LE-C (15.8 mg) were isolated from the lemon peel. The purity of LE-B and LE-C was >99.0% from analysis of HPLC.

Determination of *C*-Glucosyldiosmin (LE-B and LE-C) in Lemon Fruit. Lemon fruit (Eureka varieties) was separated into the peel and juice by hand-squeezing the fruit. The lemon fruit from a California producer was used. The peel (5.0 g) was homogenized and extracted with 100 mL of methanol at 37 °C for 4 days. The juice was removed from the pulp by filtration. LE-B and LE-C in the extract of the peel and filtered juice were determined by HPLC analysis. HPLC (LC-10A, Shimadzu Co., Ltd., Kyoto, Japan) was carried out using a Shim-pack CLC-ODS(M) (column size ∅ 4.6 × 250 mm; particle size 5 μm) at 40 °C with a UV detector (λ = 280 nm). The solvent system contained 70% of a 5% acetic acid solution and 30% methanol. The flow rate was 1.0 mL/min.

Instrumental Analysis. UV−vis absorption spectra were recorded on a spectrophotometer with a Hitachi U-2000 in methanol. IR spectra were recorded on a FT/IR-8200RC (Shimadzu Co.) with KBr. The fast atom bombardment mass spectra (FAB-MS) were recorded on a JEOL JMS-DX-705L with 1 N HCl/glycerol as the mounting matrix. ^{1}H NMR and ^{13}C NMR spectra were obtained using a JEOL JNM-EX-400 NMR instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) in dimethyl-*d* sulfoxide containing tetramethylsilane (TMS) as the internal standard.

Antioxidative Assay by Linoleic Acid Autoxidation Model. Antioxidative activity was evaluated using the linoleic acid system (Osawa and Namiki, 1981). Each sample was added to a solution mixture of linoleic acid (0.13 mL), 99.0% ethanol (10 mL), and a 50 mM sodium phosphate buffer (pH 7.0, 10 mL), and the volume was adjusted to 25 mL with distilled water. The reaction mixture in a sealed conical flask was incubated at 50 °C for 7 days in the dark. After incubation, the hydroperoxide from linoleic acid was determined by using the thiocyanate method (Mitsuda et al., 1966). An aliquot (200 μL) of the reaction mixture was mixed with 75% ethanol (9.4 mL), 30% ammonium thiocyanate (200 μL), and 20 mM $FeCl_2$ (200 μL), and the absorbance of this colored solution was measured at 500 nm. A control containing no added sample represents 100% lipid peroxidation. Antioxidants of 50 and 100 μM were used for this experiment. α-Tocopherol was used as the standard sample.

Antioxidative Assay of Liposome System. Egg lecithin (100 mg) was sonicated in a sonicator with a 10 mM phosphate buffer (pH 7.4). The resulting multilamellar vesicles (MLV) were sonicated in a cup-horn-type sonicator (ultrasonic disruptor UD-201, Tomy Seiko Co., Ltd., Tokyo, Japan) at 120 W for 20 min, by which process small unilamellar vesicles (SUV) were obtained. The SUV solution (10 mg of liposome/mL) and 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH) with a phosphate buffer (pH 7.4) and the antioxidants were mixed to produce a final concentration of 1 mg of liposome/mL, 2 mM AAPH, and 1 mM phosphate buffer (pH 7.4). The samples and standard antioxidant (α-tocopherol) were dissolved in dimethyl sulfoxide and added to the reaction mixture. The final concentrations of antioxidants were 100 μM. After incubation at 37 °C for 6 h, 1 mL of 2.0 M TCA/1.7 M HCl and 2 mL of 0.67% thiobarbituric acid (TBA) solution were added to stop the reaction. The quantity of TBA reactive substance (TBARS) was determined at 532 nm. A control containing no added sample represents 100% lipid peroxidation. Antioxidants were used for this experiment. α-Tocopherol was used as the standard sample.

Antioxidative Assay of Low-Density Lipoprotein (LDL) System. LDL was dialyzed against 10 mM phosphate-buffered saline (PBS) for 3 days to exclude EDTA. The solution of LDL (100 μg of protein/mL) in 10 mM PBS was incubated at 37 °C with 5 μM $CuSO_4$ in the absence or presence of 0.5 μM antioxidant. Antioxidants were dissolved in dimethyl sulfoxide and added to the reaction mixture. α-Tocopherol and butylated hydroxyanisole (BHA) were used as the standard sample. LDL was oxidized, and the conjugated diene formation was measured by determining the absorbance increase at 234 nm of the LDL solution. The absorbance was measured every 10 min for 10 h using a Hitachi U-2000 spectrophotometer, and the results are expressed as lag time (minutes) of the absolute absorbance at 234 nm.

Table 1. UV−Vis, IR, FAB-MS, and ^{1}H NMR Spectral Data for LE-B and LE-C Isolated from Lemon Fruit

	LE-B	LE-C
UV λ_{max} (nm)	s[a] 243 (log ε = 4.31)	s 243 (log ε = 4.28)
	s 254 (log ε = 4.33)	s 254 (log ε = 4.27)
	273 (log ε = 4.36)	271 (log ε = 4.28)
	342 (log ε = 4.36)	342 (log ε = 4.32)
IR ν_{max} (cm^{-1})	3360, 1628, 1443, 1366, 1273, 1080	3418, 1624, 1439, 1366, 1269, 1080
FAB-MS (*m/z*)	625 [M + H]$^+$	463 [M + H]$^+$
^{1}H NMR (δ)	7.66 (dd, m, H6′)	7.55 (dd, *J* = 2, 9, H6′)
	7.52 (d, *J* = 1.5, H2′)	7.43 (d, *J* = 2, H2′)
	7.07 (d, *J* = 9, H5′)	7.07 (d, *J* = 9, H5′)
	6.77 (s, H3)	6.75 (s, H3)
	3.88 (s, OCH_3)	6.51 (s, H8)
		3.87 (s, OCH_3)

[a] Shoulder of UV peak.

Table 2. ^{13}C NMR Spectral Data for LE-B and LE-C Isolated from Lemon Fruit (δ)

LE-B				LE-C			
aglycon		sugar		aglycon		sugar	
C	δ	C	δ	C	δ	C	δ
C2	163.3	6-C1″	77.3	C2	162.9	6-C1″	8.4
C3	102.7	C2″	5.1	C3	103.0	C2″	72.5
C4	181.7	C3″	72.0	C4	181.4	C3″	70.1
C5	158.1	C4″	70.6	C5	160.2	C4″	69.7
C6	108.8	C5″	82.3	C6	108.4	C5″	81.1
C7	162.8	C6″	62.1	C7	162.8	C6″	61.0
C8	107.0	8-C1‴	79.3	C8	93.1		
C9	154.7	C2‴	75.7	C9	155.7		
C10	104.7	C3‴	72.9	C10	103.0		
C1′	122.9	C4‴	71.1	C1′	122.4		
C2′	112.8	C5‴	82.3	C2′	112.4		
C3′	146.3	C6‴	62.2	C3′	146.3		
C4′	150.7		150.7	C4′	150.7		
C5′	111.5		111.7	C5′	111.7		
C6′	118.7		118.2	C6′	118.2		
OCH_3	55.3			OCH_3	55.3		

RESULTS AND DISCUSSION

Characterization of Antioxidant Isolated from Lemon Fruit. UV−vis absorption λ_{max}, IR spectrum, FAB-MS, and ^{1}H NMR spectral data of LE-B and LE-C are shown in Table 1. The ^{13}C NMR spectral data of these isolated antioxidants are shown in Table 2. LE-B and LE-C could likely be identified as 6,8-di-*C*-β-glucosyldiosmin and 6-*C*-β-glucosyldiosmin, because the spectral properties obtained from LE-B and LE-C are also consistent with those of the literature data for the UV−vis absorption λ_{max} and ^{1}H NMR (Kumamoto et al., 1985; Gentili and Horowitz, 1968). Given agreement with the literature data, the data for the IR spectrum, FAB-MS, and ^{13}C NMR spectra, LE-B and LE-C were identified as 6,8-di-*C*-β-glucosyldiosmin and 6-*C*-β-glucosyldiosmin. The structures of these antioxidants are shown in Figure 1. It has been reported that 6,8-di-*C*-β-glucosyldiosmin and 6-*C*-β-glucosyldiosmin were isolated from lemon peel (Gentili and Horowitz, 1968) and have a hypotensive effect (Kumamoto et al., 1985). However, their antioxidative effect has never been reported. We determined the antioxidative function of 6,8-di-*C*-β-glucosyldiosmin and 6-*C*-β-glucosyldiosmin in lemon fruit.

The distribution of LE-B and LE-C in lemon fruit was examined by HPLC analysis. LE-B and LE-C are enriched in the peel, respectively, 216 and 34.8 ppm.

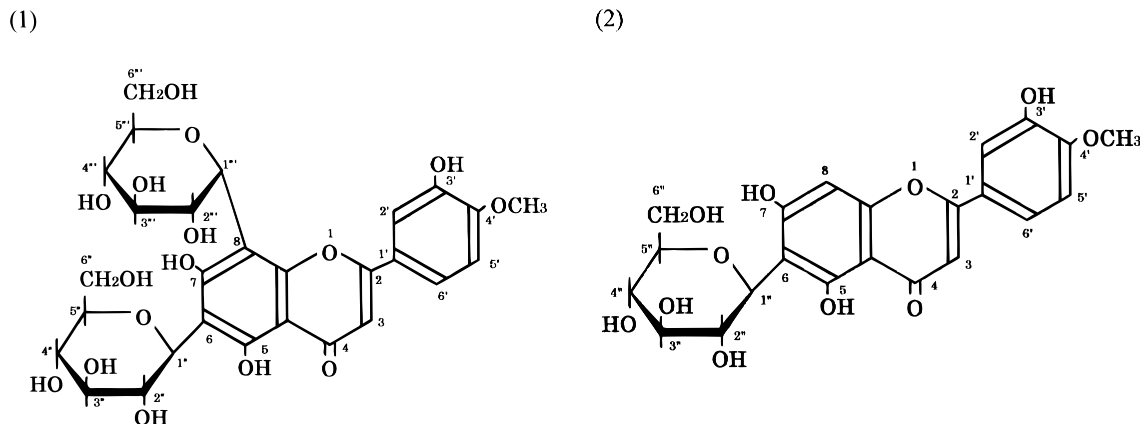

Figure 1. Chemical structures of *C*-glucosyldiosmins isolated from lemon peel extract: (1) 6,8-di-*C*-β-glucosyldiosmin (LE-B); (2) 6-*C*-β-glucosyldiosmin (LE-C).

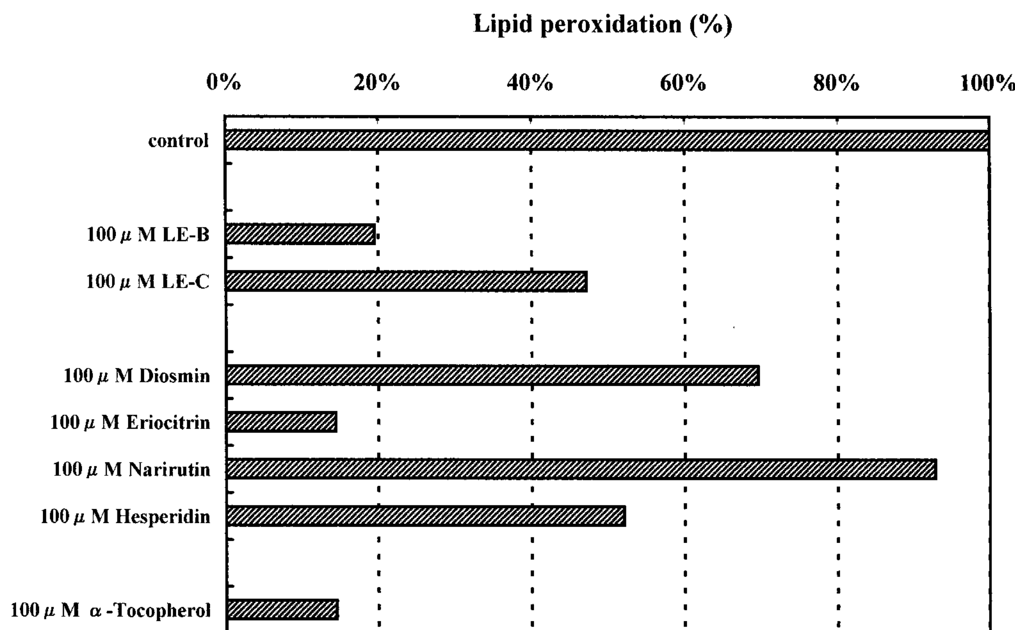

Figure 2. Antioxidative activity of LE-B, LE-C, the flavonoid glycosides in lemon fruit, and α-tocopherol in the linoleic acid system as measured by the thiocyanate method. A control containing no added samples represents 100% lipid peroxidation. Antioxidants of 100 μM were used for this experiment; details are given under Materials and Methods. Values in the figure are the average of duplicates.

LE-B was present at 15.4 ppm, and little LE-C was present in the juice; these compounds were not detected in the seeds.

Antioxidative Activity Using the Linoleic Acid System. The flavonoid glycosides of eriocitrin (eriodictyol β-7-rutinoside), narirutin (naringenin β-7-rutinoside), diosmin (diosmetin β-7-rutinoside), and hesperidin (hesperetin β-7-rutinoside) had been reported to exist in lemon fruit (Mouly et al., 1994; Park et al., 1983). We had reported that the antioxidative activity of eriocitrin in the linoleic acid system was similar to that of α-tocopherol (Miyake et al., 1997a). A comparison of the antioxidative activities of LE-B, LE-C, and the flavonoid glycosides in lemon fruit was carried out using the linoleic acid system as shown in Figure 2. LE-B and LE-C possessed antioxidative activity but were shown to have lower activity than eriocitrin or α-tocopherol. These results suggested that eriocitrin had the strongest activity of the flavonoid glycosides in lemon fruit because of its containing adjacent dihydroxy groups on the B-ring, and LE-B and LE-C had lower activities because they did not. The antioxidative activities between LE-B, LE-C, and diosmin having the same aglycon (diosmetin) were different. We suspected that diosmin was shown to have lower activity than LE-B and LE-C because diosmin linked with rutinose at the 7-OH position of the A-ring.

Antioxidative Activity Using the Liposome System. A major target of free radical damage is the cellular membrane, which contains abundant unsaturated lipids (Aust and Sringen, 1982); rising lipid peroxidation is strongly associated with aging and carcinogenesis. Liposomes have been used extensively as cellular models for in vitro lipid peroxidation studies. Therefore, we used the liposome system for evaluating antioxidants as simple in vitro cellular models. Figure 3 shows the antioxidative activities of LE-B, LE-C, the flavonoid compounds in lemon fruit, and their aglycons (diosmetin, eriodictyol, hesperetin, and naringenin) in the liposome system in which lipid peroxidation is induced by AAPH. As shown, LE-B and LE-C show an inhibitory effect against the formation of malondialdehyde but were shown to have lower activity than α-tocopherol. Eriocitrin and eriodictyol, its aglycon, had stronger antioxidative activity than α-tocopherol and were the strongest of the flavonoid compounds in lemon

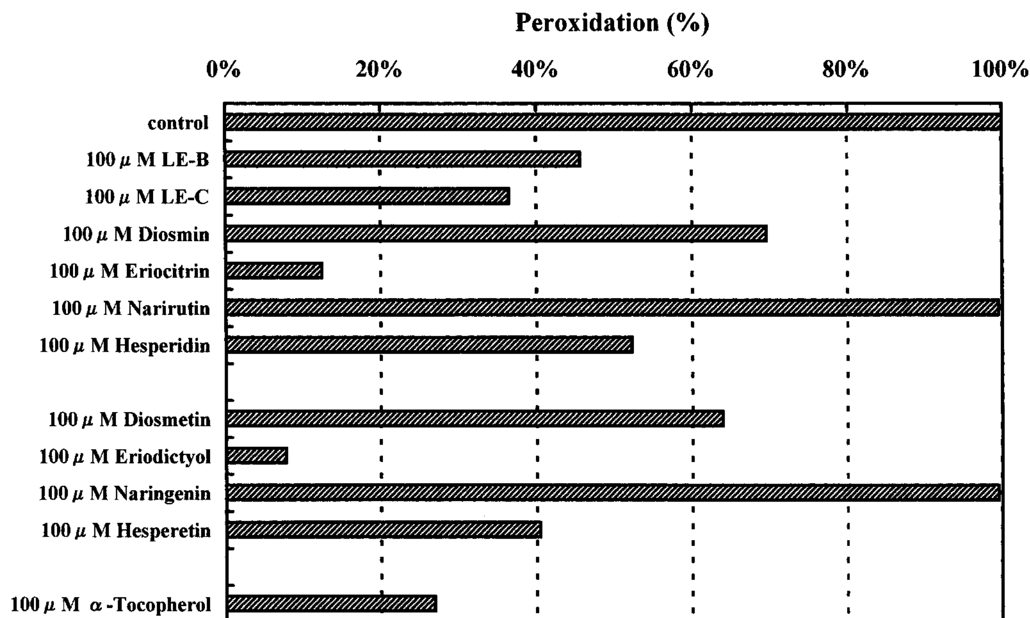

Figure 3. Antioxidative activity of LE-B, LE-C, the flavonoid glycosides in lemon fruit, and α-tocopherol in the liposome acid system. A control containing no added samples represents 100% lipid peroxidation. Antioxidants of 100 μM were used for this experiment; details are given under Materials and Methods. Values in the figure are the average of duplicates.

Table 3. Antioxidative Activity for Inhibitory Effects of Lemon Flavonoid Compounds on Cu^{2+}-Mediated Conjugated Diene Formation in LDL

	lag time (min)		lag time (min)
flavonoid glycosides		control	10
LE-B	40		
LE-C	30	α-tocopherol	20
diosmin	20	BHA	30
eriocitrin	450	3,4-DHCA	360
hesperidin	20	phloroglucinol	340
naringin	20	EGCg	430
flavonoids			
diosmetin	110		
eriodictyol	430		
hesperetin	20		
naringenin	20		

fruit. The compounds that contained adjacent dihydroxyl groups on the B-ring, such as eriocitrin and eriodictyol, showed high antioxidative activity. The antioxidative activity of the aglycons of flavonoid glycosides, diosmetin, eriodictyol, hesperetin, and naringenin, was stronger than that of the flavonoid glycosides, LE-B, LE-C, diosmin, eriocitrin, hesperidin, and naringin. These aglycons are more hydrophobic, indicating that they have higher affinity with the liposome and show stronger antioxidative activity than the respective flavonoid glycosides.

Antioxidative Activity Using the LDL System. Oxidative modified LDL, which is taken up by scavenger receptors, is suggested to be involved in the development of atherosclerosis (Steinberg et al., 1989). Many flavonoids have been found to inhibit LDL oxidation in vitro by cupric ion (Vinson et al., 1995; Miura et al., 1994). We examined the effect on Cu^{2+}-mediated oxidative modification of LDL of the antioxidative flavonoid compounds in lemon fruit.

The effect of the flavonoid compounds on the conjugated diene formation in LDL that had been incubated with 5 μM $CuSO_4$ is shown in Table 3. In the absence of the flavonoid compounds (control), the lag time was 10 min. The lag time of control was shorter relative to the result of Miura et al. (1995), which lag time of control was 15 min. The purchased LDL may more easily receive greater oxidation, but the antioxidative activity of these flavonoids seemed to be still valid because they are relative to a control. In the presence of 0.5 μM eriocitrin and eriocitrin metabolites by human intestinal bacteria, which include eriodictyol, 3,4-DHCA, and phloroglucinol (Miyake et al., 1997b), prolongations in lag time of more than 340 min were observed. (−)-Epigallocatechin gallate (EGCg) of a green tea component was reported to be the best antioxidant, giving the longest lag time of Cu^{2+}-mediated LDL oxidation (Vinson et al., 1995) and having the strongest activity of the cathechins (Miura et al., 1994). Eriocitrin (lag time = 450 min) and eriodictyol of eriocitrin metabolites (lag time = 430 min) had approximately the same activity of EGCg (lag time = 430 min). The activities of 3,4-DHCA, phloroglucinol, and eriocitrin metabolites were weaker than that of EGCg, but they had stronger antioxidative activities than did LE-B, LE-C, and other flavonoid compounds. Eriocitrin and eriocitrin metabolites are powerful antioxidants, as is EGCg, using an in vitro oxidation model for heart disease. These results indicated that eriocitrin and eriocitrin metabolites were effective antioxidants for disease of oxidative stress, and they seemed to be concerned with the health benefits of eating citrus fruits.

ABBREVIATIONS USED

LE-B, 6,8-di-*C*-β-glucosyldiosmin; LE-C, 6-*C*-β-glucosyldiosmin; 3,4-DHCA, 3,4-dihydroxyhydrocinnamic acid; EGCg, (−)-epigallocatechin gallate; BHA, butylated hydroxyanisole; TBA, thiobarbituric acid; TBARS, TBA reactive substance.

LITERATURE CITED

Aust, S. D.; Sringen, B. A. The role of ion in enzymatic lipid peroxidation. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: Orlando, FL, 1982; Vol. 5, Chapter 1.

Cutler, R. G. Antioxidants, aging, and longevity. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: Orlando, FL, 1984; Vol. 6; pp 371−423.

Cutler, R. G. Genetic stability and oxidative stress: common mechanism in aging and cancer. In *Free Radicals and Aging*; Emerit, I., Chance, B., Eds.; Birkhauser Verlag: Basel, Switzerland, 1992; pp 31−46.

Gentili, B.; Horowitz, R. M. Flavonoids of citrus. IX. Some new *C*-glycosylflavones and a nuclear magnetic resonance method for differentiating 6- and 8-*C*-glycosyl isomers. *J. Org. Chem.* **1968**, *33*, 1571−1577.

Harman, D. The free-radical theory of aging. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: Orlando, FL, 1982; Vol. 5, Chapter 8.

Kuhnan, J. The Flavonoids. A class of semi-essential food components; their role in human nutrition. *World Rev. Nutr. Diet* **1976**, *24*, 117−191.

Kumamoto, H.; Matsubara, Y.; Iizuka, K.; Okamoto, K.; Yokoi, K. Structure and hypotensive effect of flavonoid glycosides in lemon peelings. *Nippon Nogeikagaku Kaishi* **1985**, *59*, 667−682.

Matsubara, Y.; Kumamoto, H.; Iizuka, Y.; Murakami, T.; Okamoto, K.; Miyake, H.; Yokoi, K. Structure and hypotensive effect of flavonoid glycosides in *Citrus unshiu* peeling. *Agric. Biol. Chem.* **1985**, *49*, 909−914.

Middleton, E.; Kandaswami, C. Potential Health-Promoting Properties of Citrus Flavonoids. *Food Tecnol.* **1994**, *48*, 115−119.

Mitsuda, H.; Yasumoto, K.; Iwami, K. Antioxidative action of indole compounds during the antioxidation of linoleic acid. *Eiyo to Shokuro* **1966**, *19*, 210−214.

Miura, S.; Watanabe, J.; Sano, M.; Tomita, T.; Sono, M.; Tomita, I. The inhibitory effect of tea polyphenols (flavan-3-ol derivatives) on Cu^{2+} mediated oxidative modification of low-density lipoprotein. *Biol. Pharm. Bull.* **1994**, *17*, 1567−1572.

Miura, S.; Watanabe, J.; Sano, M.; Tomita, T.; Osawa, T.; Hara, Y.; Tomita, I. Effect of various natural antioxidants on the Cu^{2+}-mediated oxidative modification of low-density lipoprotein. *Biol. Pharm. Bull.* **1995**, *18*, 1−4.

Miyake, Y.; Yamamoto, K.; Osawa, T. Isolation of eriocitrin (eriodictyol 7-rutinoside) from lemon fruit (*Citrus limon Burm. f.*) and its antioxidative activity. *Food Sci. Technol. Int. Tokyo* **1997a**, *3*, 84−89.

Miyake, Y.; Yamamoto, K.; Osawa, T. Metabolism of antioxidant in lemon fruit (*Citrus limon* B_{URM}. f.) by human intestinal bacteria. *J. Agric. Food Chem.* **1997b**, *45*, 3738−3742.

Mouly, P. P.; Arzouyan, C. R.; Gaydou, E. M.; Estienne, J. M. Differentiation of citrus juices by factorial discriminant analysis using liquid chromatography of flavanone glycosides. *J. Agric. Food Chem.* **1994**, *42*, 70−79.

Osawa, T.; Namiki, M. A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves. *Agric. Biol. Chem.* **1981**, *45*, 735−739.

Osawa, T.; Namiki, M.; Kawakishi, S. Role of dietary antioxidants in protection against oxidative damage. In *Antimutagenesis and Anticarcinogenesis Mechanisms*; Kuroda, Y., Shankel, D. M., Waters, M. D., Eds.; Plenum Publishing: New York, 1990; pp 139−153.

Osawa, T.; Katsuzaki, H.; Hagiwara, Y.; Hagiwara, H.; Shibamoto, T. A novel antioxidant isolated from young green barley leaves. *J. Agric. Food Chem.* **1992**, *40*, 1135−1138.

Park, G. L.; Avery, S. M.; Byers, J. L.; Nelson, D. B. Identification of bioflavonoids from citrus. *Food Technol.* **1983**, *37*, 98−105.

Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witzum, J. L. Beyond cholesterol; modification of low-density lipoproteins. *New Engl. J. Med.* **1989**, *320*, 915.

Tuda, T.; Watanabe, M.; Ohshima, K.; Norinobu, S.; Choi, S. W.; Kawakishi, S.; Osawa, T. Antioxidative activity of anthocyanin pigments cyanidin 3-*O*-β-D-glucoside and cyanidin. *J. Agric. Food Chem.* **1994**, *42*, 2407−2410.

Vinson, J. A.; Dabbagh, Y. A.; Serry, M. M.; Jang, J. Plant flavonoids, especially tea flavanol, are powerful antioxidants using an *in vitro* oxidation model for heart disease. *J. Agric. Food Chem.* **1995**, *43*, 2800−2802.

Yagi, K. Lipid peroxides and human disease. *Chem. Phys. Lipids* **1987**, *45*, 337−341.

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