Characteristics of Antioxidative Flavonoid Glycosides in Lemon Fruit

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We investigated the antioxidative flavonoid glycosides in the peel extract of lemon fruit (*Citrus limon*). Six flavanone glycosides: eriocitrin, neoeriocitrin, narirutin, naringin, hesperidin, and neohesperidin, and three flavone glycosides: diosmin, 6,8-di-*C*- β -glucosyldiosmin (DGD), and 6-*C*- β -glucosyldiosmin (GD) were identified by high-performance liquid chromatography (HPLC) analysis. Their antioxidative activity was examined using a linoleic acid autoxidation system. The antioxidative activity of eriocitrin, neoeriocitrin and DGD was stronger than that of the others. Flavonoid glycosides were present primarily in the peel of lemon fruit. There was only a small difference in the content of the flavonoid glycosides of the lemon fruit juice from various sources and varieties. Lemon fruit contained abundant amounts of eriocitrin and hesperidin and also contained narirutin, diosmin, and DGD, but GD, neoeriocitrin, naringin, and neohesperidin were present only in trace amounts. The content of DGD, GD, and eriocitrin was especially abundant in lemons and limes; however, they were scarcely found in other citrus fruits. The content of flavonoid compounds in lemon juice obtained by an in-line extractor at a juice factory was more abundant than that obtained by hand-squeezing. These compounds were found to be stable even under heat treatment conditions (121°C, 15 min) in acidic solution.

Keywords: lemon fruit, *Citrus limon*, antioxidant, flavonoid glycoside

Lemons (*Citrus limon*) are used in various food preparations such as soft drinks, alcoholic drinks, and jams, and they are one of the most popular citrus fruits in the world. Lemons, which contain a number of nutrients such as citric acid, ascorbic acid, minerals, and flavonoids, have been known for a long time as typical healthy foods. 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. Flavonoid compounds that exhibit beneficial effects on capillary permeability and fragility were once known as vitamin P; they have been investigated regarding their physiological functions, such as hypotensive, antiallergic, anti-inflammatory, antiviral, anticancer, and anticarcinogenic properties (Bracke *et al.*, 1994; Middleton & Kandaswami, 1994; Mabry & Ulubelen, 1980).

It has been reported that lemon juice has desmutagenic and antimutagenic effects (Achiwa *et al.*, 1991; Jain *et al.*, 1987) in concern with the prevention of carcinogenesis. Recently, we have been involved in the isolation of antioxidative flavonoid glycosides from lemon fruit and have identified eriocitrin (eriodictyol-7-rutinoside), a flavanone glycoside (Miyake *et al.*, 1997a), and 6,8-di-*C*- β -glucosyldiosmin (DGD) and 6-*C*- β -glucosyldiosmin (GD), *C*-glucosyl flavones (Miyake *et al.*, 1997b). Lipid peroxidation is known as one of the major factors which causes deterioration of foods during storage and processing. It is also suspected to be strongly associated with aging and carcinogenesis (Yagi, 1987; Cutler, 1984; Harman, 1982). Recently, it was reported that natural antioxidants contained in dietary plants may play

an important role in the prevention of carcinogenesis and in extending the life span of animals and may offer effective protection against peroxidative damage in living systems (Jacob, 1995; Cutler, 1992; Osawa *et al.*, 1990). Therefore, much attention has been focused on the importance of natural antioxidants.

In this study, we paid great attention to the antioxidative flavonoid glycosides in lemon fruit. We studied the antioxidative activity and the stability after heat treatment of the flavonoid glycosides to examine their properties for use in foods. We also examined for the distribution of flavonoid glycosides in various sources or varieties of lemon fruit in other citrus fruits.

Materials and Methods

Materials Lemons (*Citrus limon* BURM. f.) and the other citrus fruits were purchased from a supermarket. The varieties of lemon, eureka, lisbon, and verna were obtained from the Citrus Research & Education Center (Lake Alfred, Florida, USA). The lemon juice from a juice factory was squeezed using an in-line extractor (FMC Co., Lakeland, Florida, USA).

Chemicals Linoleic acid and α -tocopherol were obtained from Wako Pure Chemical Industries, Ltd. (Osaka). Flavonoid glycosides were obtained from Funakoshi, Ltd. (Tokyo). Eriocitrin, DGD, and GD were purified according to the method of Miyake *et al.* (1997a, b.)

Isolation of antioxidative flavonoid glycosides from peel of lemon fruit The peel of lemon fruit (1.50 kg) was chopped in a homogenizer and extracted with methanol (3 l) at room temperature for 3 days. The extract was concentrated

in vacuo. The concentrated peel extract (110 g) was chromatographed on a Cosmosil 75C 18-OPN ODS column (Nacalai Tesque, Inc., Kyoto, $\phi 37 \times 500$ mm). The column was washed with 2 l of 20% methanol-water and was eluted with 40% methanol-water. The eluate was concentrated *in vacuo*, and the antioxidative flavonoid fraction (6.00 g) was obtained. According to the method of Miyake *et al.* (1997a), the antioxidative flavonoid glycosides were isolated from the antioxidative flavonoid fraction by preparative high-performance liquid chromatography (HPLC). Eriocitrin (1.04 g), neoeriocitrin (2.30 mg), narirutin (20.5 mg), DGD (52.0 mg), and GD (15.8 mg) were isolated. Eriocitrin, DGD, and DG were identified by MS, $^1\text{H-NMR}$, and $^{13}\text{C-NMR}$ analyses (Miyake *et al.*, 1997a, b). Neoeriocitrin and narirutin were identified from the retention time of standard reagents for HPLC analysis. The aglycones of neoeriocitrin and narirutin were prepared by hydrolysis using pectinase according to the method of Miyake *et al.* (1997a). Also, it was confirmed that the retention time of these aglycones was compatible with that of the standard reagent, eriodictyol and naringenin, during HPLC analysis. Other flavonoid glycosides of naringin, hesperidin, neohesperidin, and diosmin were identified from the retention time of standard reagents for HPLC analysis of the antioxidative flavonoid fraction (Fig. 1). HPLC (LC-10A, Shimadzu Co., Ltd., Kyoto) was carried out using a Shim-pack CLC-ODS(M) (4.6×250 mm) with a UV detector ($\lambda = 280$ nm). The solvent system contained 70% of a 5% acetic acid solution and 30% methanol. The flow rate was 1.0 ml/min.

Antioxidative assay Antioxidative activity was evaluated using the linoleic acid system (Osawa & Namiki, 1981). Each sample was added to a mixed solution of linoleic acid (0.13 ml) and 99.0% distilled ethanol (10 ml) with a 50 mM phosphate buffer (pH 7.0, 10 ml), and the volume was adjusted to 25 ml with distilled water. The reaction mixture in a conical flask was incubated at 40°C. At intervals during the incubation, the degree of oxidation was measured using the thiocyanate method (Mitsuda *et al.*, 1966) by recording the absorbance at 500 nm after coloring with FeCl_2 and thiocyanate. α -Tocopherol was used as the positive standard.

Determination of flavonoid glycosides The lemon fruit, from a Californian source, was separated into the peel, juice, and seed in order to determine the location of eriocitrin. The peel was further separated into the flavedo (epicarp), albedo (mesocarp), and pulp vesicles using a knife. Samples (5.0 g) of the subdivided peel and the seed were homogenized and extracted with 100 ml of methanol at 37°C for 4 days. The pulp of the squeezed juice was removed by filtration.

Each citrus fruit was separated into the peel and juice by hand-squeezing the fruit. The peel (5.0 g) was homogenized and extracted with 100 ml of methanol at 37°C for 4 days. The pulp of the squeezed juice was removed by filtration. Flavonoid glycosides in the extract of the peel and in the filtered juice were determined by HPLC analysis according to the previous method.

Stability of flavonoid glycosides by heat treatment at different pH The buffer solutions were at pH 2 and pH 3.5 using a 100 mM sodium citrate-HCl buffer, and at pH 5.0 and pH 7.0 using a 100 mM sodium phosphate buffer. The final

Fig. 1. HPLC profile for the antioxidative flavonoid fraction. ERI: eriocitrin, NeoERI: neoeriocitrin, NAR: narirutin, NAN: naringin, HES: hesperidin, NeoHES: neohesperidin, DIO: diosmin, DGD: 6,8-di- C - β -glucosyldiosmin, GD: 6- C - β -glucosyldiosmin.

concentration of 0.1% flavonoid glycosides was added to the different buffer solutions, and the solutions were heated at 80°C for 30 min or at 121°C for 15 min. The stability of the flavonoid glycosides after heat treatment was determined by the residual ratio of the flavonoid glycosides after heat treatment. The residual ratio of eriocitrin was determined from the residual peak of eriocitrin after heat treatment by HPLC analysis.

Results and Discussion

Antioxidative flavonoid compounds in lemon fruit We previously reported that flavonoid fractions prepared from peel extract or juice using ODS column chromatography exhibited strong antioxidative activity in the linoleic acid autooxidation system (Miyake *et al.*, 1997a). The antioxidants were identified as eriocitrin (eriodictyol β -7-rutinoside), a flavanone glycoside, and as DGD and GD, C -glucosyl flavones (Miyake *et al.*, 1997b). We further isolated another two antioxidative flavonoid glycosides from peel extract. They were identified as neoeriocitrin (eriodictyol β -7-rhamnoglucoside) and narirutin (naringenin β -7-rutinoside). Figure 1 shows the HPLC profile of the antioxidative flavonoid fraction. The other flavonoid compounds were identified as naringin (naringenin β -7-rhamnoglucoside), hesperidin (hesperetin β -7-rutinoside), and diosmin (diosmetin β -7-rutinoside) from the antioxidative flavonoid fraction. We identified nine flavonoid glycosides in the antioxidative flavonoid fraction in the peel extract of the lemon fruit, and their structures are shown in Fig. 2.

The antioxidative activity of the nine flavonoid glycosides was examined using the linoleic acid autooxidation system as

shown in Fig. 3. We previously reported the antioxidative activity of eriocitrin, DGD and GD in the linoleic acid system (Miyake *et al.*, 1996a, b). Eriocitrin had the strongest antioxidative activity among the flavonoid glycosides in lemon fruit. Neeriocitrin and DGD also showed antioxidative activity, but the others had only a weak activity. We expect that the antioxidants in lemon fruit will provide protection against peroxidative damage in living systems in relation to aging and carcinogenesis.

Properties of flavonoid compounds The properties of the flavonoid compounds in lemon fruit were examined for solubility in water or ethanol. These compounds were soluble in ethanol, but GD, hesperidin, neohesperidin, and diosmin were insoluble in water. The taste of these flavonoid glycosides (0.1% water solution) was examined. Naringin, neeriocitrin, and neohesperidin having the sugar chain of neohesperidose (2-*O*- α -L-rhamnosyl- β -D-glucose) were more bitter than narirutin, eriocitrin, and hesperidin having the sugar chain of hesperidose (2-*O*- α -L-rhamnosyl- β -D-glucose).

Fig. 2. Structure of flavonoid compounds in lemon fruit.

Fig. 3. Antioxidative activity of flavonoid glycosides. DGD : 6,8-di-*C*- β -glucosyldiosmin, GD : 6-*C*- β -glucosyldiosmin.

chain of rutinoid (6-*O*- α -L-rhamnosyl- β -D-glucose). These findings suggest that the bitterness of citrus juice is related to the structure of the neohesperidose portion of the flavonoid glycosides.

We examined the stability of flavonoid glycosides under conditions of sterilization because of the importance of this process in the manufacture of beverages and foods. The stability of flavonoid compounds after heat treatment in different buffer solutions of pH 2.0, pH 3.5, pH 5.0, and pH 7.0 was also examined. The heat treatment at 80°C for 30 min assuming the sterilization of yeast or mold was used, and the heat treatment at 121°C for 15 min assuming the sterilization of heat-stable bacteria was used. For all of the flavonoid glycosides, except eriocitrin and neoeriocitrin, more than 90% remained after heat treatment at 80°C for 30 min, and more than 80% remained after heat treatment at 121°C for 15 min. These compounds were fairly stable under the heat treatment conditions. Eriocitrin and neoeriocitrin were unstable in a neutral solution (pH 7.0) at 80°C for 30 min and 121°C for 15 min, these compounds were decreased to about half of the initial content after heating at 80°C for 30 min and almost completely disappeared after heat treatment at 121°C for 15 min. It is suggested that these compounds having strong antioxidative activity changed by oxidation under the previous conditions, as well as ascorbic acid, which is an antiox-

idant and unstable under high temperature in neutral solution. The chemical and physical properties of flavonoid compounds in citrus fruit have been reported (Rouseff, 1980). It has been reported that the A and B rings in the flavonoid molecule are stable and relatively unreactive, and that the central C ring of the central C₃ group is the most reactive portion of the molecule and undergoes a ring opening reaction with a base or acetic anhydride (Hatori, 1962). The decrease in eriocitrin and neoeriocitrin by heat treatment in a neutral solution may be due to such ring opening reaction. The C ring of these compounds having the catechol structure of the adjacent dihydroxy groups on the B-ring seemed to be opened easier than that of other flavonoid compounds. These results suggest that the antioxidative flavonoid compounds such as eriocitrin and neoeriocitrin, which are stable after heat treatment in the acidic condition of pH 3.5–5.0, may be suitable for use in acidic beverages and foods.

Flavonoid compounds in citrus fruit have generally been reported to be stable under storage conditions (Ranganna *et al.*, 1983). In commercial lemon juice, the nine flavonoid compounds were stable during sterilization at 80°C for 30 min and under storage conditions (room temperature for 9 months), and the content of ascorbic acid decreased to 60% under the same conditions (data not shown.) The stability of

Table 1. Distribution of flavonoid compounds in each variety and source of lemon juice.

	Concentration (mg/100 ml)								
	ERI	NeoERI	NAR	NAN	HES	NeoHES	DIO	DGD	GD
Variety									
eureka	15.9	ND ^{a)}	0.5	0.4	5.3	TR ^{b)}	1.4	5.8	TR
lisbon	13.6	ND	1.0	0.8	5.8	TR	1.2	4.4	TR
verna	16.1	ND	0.4	0.5	5.5	ND	TR	3.4	TR
Country of Produce									
California	12.1	TR	0.2	ND	8.9	ND	1.3	4.9	TR
South Africa	11.3	TR	0.3	ND	1.7	ND	0.5	2.4	TR
Australia	16.4	0.1	0.2	ND	5.8	ND	1.1	4.9	0.1
New Zealand	11.0	TR	0.5	ND	3.4	ND	0.6	4.0	0.1
Chile	11.0	0.1	0.2	ND	4.2	ND	0.5	4.6	0.1
Japan	16.0	ND	TR	0.1	6.0	ND	1.0	4.2	0.1

^{a)}not detected.

^{b)}trace: less than 0.1 mg/100 ml.

ERI: eriocitrin, NeoERI: neoeriocitrin, NAR: narirutin, NAN: naringin, HES: hesperidin, NeoHES: neohesperidin, DIO: diosmin, DGD: 6,8-di-*C*- β -glucosyl-diosmin, GD: 6-*C*- β -glucosyldiosmin.

Table 2. Distribution of flavonoid compounds in various locations in lemon fruit.

	Concentration (mg/100 ml)								
	ERI	NeoERI	NAR	NAN	HES	NeoHES	DIO	DGD	GD
Juice ^{a)}	12.1	TR	0.2	ND	8.9	ND	1.3	4.9	TR
Peel	280	1.5	9.4	TR	173	TR	26.2	33.9	5.1
flavedo	113	1.3	3.43	ND	2.5	0.8	40.6	30.3	1.3
albedo	252	0.9	11.8	0.2	227	1.0	9.6	23.6	8.9
pulp vesicles	285	ND ^{b)}	5.8	0.2	70.5	0.2	10.8	12.3	2.7
Seed	TR	ND	TR ^{c)}	ND	ND	ND	TR	ND	ND

^{a)}The content of juice represented with flavonoid compounds (mg) of 100ml juice (mg/100 ml).

^{b)}not detected.

^{c)}trace: less than 0.1 mg/100 g.

ERI: eriocitrin, NeoERI: neoeriocitrin, NAR: narirutin, NAN: naringin, HES: hesperidin, NeoHES: neohesperidin, DIO: diosmin, DGD: 6,8-di-*C*- β -glucosyl-diosmin, GD: 6-*C*- β -glucosyldiosmin.

flavonoid compounds under storage conditions of the lemon juice was greater than that of ascorbic acid.

Determination of flavonoid glycosides in lemon fruit

As shown in Table 1, the content of flavonoid glycosides in the juice of different varieties of lemon fruit (eureka, lisbon, and verna) and in the juice of fruit produced in different countries (California, South Africa, Australia, New Zealand, Chile, and Japan) was examined. It was found that the

content of flavonoid compounds was fairly similar among the varieties and the sources of the lemon fruit.

The content of flavonoid compounds in various parts of the lemon fruit was examined (Table 2). All of the nine flavonoid compounds were found to be more abundant in the peel than in the juice, but only a small amount of these compounds was found in the seed. Eriocitrin and hesperidin existed abundantly in the peel and juice. The content of

Table 3. Content of flavonoid compounds in lemon juice obtained by hand squeezing and factory squeezing.

	Concentration (mg/100 ml)										pH	Acidity
	ERI	NeoERI	NAR	NAN	HES	NeoHES	DIO	DGD	GD	Brix		
Hand squeezing	12.5	0.3	0.3	ND ^{a)}	8.9	ND	2.1	7.2	ND	8.5	2.5	4.8
In-line extractor	19.8	0.3	0.8	0.3	12.2	0.2	3.4	9.7	0.2	8.9	2.4	5.5

^{a)}not detected.

ERI: eriocitrin, NeoERI: neoeriocitrin, NAR: narirutin, NAN: naringin, HES: hesperidin, NeoHES: neohesperidin, DIO: diosmin, DGD: 6,8-di-*C*- β -glucosyl-diosmin, GD: 6-*C*- β -glucosyldiosmin.

Table 4. Distribution of flavonoid compounds in citrus juices.

	Concentration (mg/100 ml)								
	ERI	NeoERI	NAR	NAN	HES	NeoHES	DIO	DGD	GD
<i>Citrus. limon</i> (lemon)	12.1	TR ^{a)}	0.2	ND ^{b)}	8.9	ND	1.3	4.9	TR
<i>C. aurantifolia</i> (lime)	9.7	TR	0.6	ND	10.2	ND	0.3	2.2	0.1
<i>C. sinensis</i> (valencia orange)	0.2	TR	22.9	ND	32.1	ND	ND	ND	ND
<i>C. sinensis</i> (navel orange)	0.3	0.2	14.8	0.3	37.4	ND	ND	ND	ND
<i>C. unshiu</i> (unshiu)	0.3	TR	26.4	TR	30.1	ND	TR	ND	ND
<i>C. iyo</i> (iyo)	ND	ND	10.0	ND	12.3	ND	ND	ND	ND
<i>C. reticulata</i> (ponkan)	0.1	ND	3.6	ND	15.3	ND	ND	ND	ND
<i>C. paradisi</i> (grapefruit, white)	0.1	ND	18.6	390	0.9	2.9	ND	ND	ND
<i>C. paradisi</i> (grapefruit, pink)	0.1	0.2	8.5	27.0	0.5	1.5	ND	ND	ND
<i>C. aurantium</i> (daidai)	ND	8.4	0.6	22.3	ND	12.9	ND	ND	ND
<i>C. hassaku</i> (hassaku)	0.1	0.3	8.6	28.5	1.8	9.7	ND	ND	ND
<i>C. sphaerocarpa</i> (kabosu)	ND	ND	16.4	1.8	2.8	1.1	ND	TR	ND
<i>C. junos</i> (yuzu)	0.1	0.1	15.0	5.4	6.5	3.3	ND	ND	ND

^{a)}trace: less than 0.1 mg/100 ml.

^{b)}not detected.

ERI: eriocitrin, NeoERI: neoeriocitrin, NAR: narirutin, NAN: naringin, HES: hesperidin, NeoHES: neohesperidin, DIO: diosmin, DGD: 6,8-di-*C*- β -glucosyl-diosmin, GD: 6-*C*- β -glucosyldiosmin.

Table 5. Distribution of flavonoid compounds in each variety and source of lemon juice.

	Concentration (mg/100 ml)								
	ERI	NeoERI	NAR	NAN	HES	NeoHES	DIO	DGD	GD
<i>Citrus. limon</i> (lemon)	280	1.5	9.4	TR ^{a)}	173	TR	26.2	33.9	5.1
<i>C. aurantifolia</i> (lime)	94.0	3.7	2.7	0.1	132	ND ^{b)}	10.4	25.3	8.4
<i>C. sinensis</i> (valencia orange)	3.4	ND	115	0.1	277	ND	1.9	ND	ND
<i>C. sinensis</i> (navel orange)	7.4	ND	121	0.7	351	ND	3.6	ND	ND
<i>C. unshiu</i> (unshiu)	9.1	ND	450	1.2	513	ND	ND	ND	ND
<i>C. iyo</i> (iyo)	4.2	ND	284	0.6	394	0.9	2.5	TR	ND
<i>C. reticulata</i> (ponkan)	4.3	2.4	42.3	ND	415	ND	2.3	ND	ND
<i>C. paradisi</i> (grapefruit, white)	2.1	1.0	153	67.3	2.4	3.0	0.8	ND	ND
<i>C. paradisi</i> (grapefruit, pink)	ND	ND	190	1310	2.7	13.3	ND	ND	ND
<i>C. aurantium</i> (daidai)	8.6	215	14.8	835	0.5	489	28.2	ND	TR
<i>C. hassaku</i> (hassaku)	2.1	9.6	108	545	30.4	259	0.5	ND	ND
<i>C. sphaerocarpa</i> (kabosu)	ND	10.3	393	102	190	71.8	ND	ND	ND
<i>C. junos</i> (yuzu)	4.3	3.8	193	87.8	118	75.8	ND	ND	ND

^{a)}trace: less than 0.1 mg/100 g.

^{b)}not detected.

ERI: eriocitrin, NeoERI: neoeriocitrin, NAR: narirutin, NAN: naringin, HES: hesperidin, NeoHES: neohesperidin, DIO: diosmin, DGD: 6,8-di-*C*- β -glucosyl-diosmin, GD: 6-*C*- β -glucosyldiosmin.

eriocitrin in the albedo (mesocarp, white interior of the peel) and pulp vesicles (in part of the enveloping flesh) was more abundant than in the flavedo (epicarp, yellow exterior of the peel), and the hesperidin content was found to be abundant in the albedo. DGD, narirutin, and diosmin existed in various parts of the peel and juice, but only small amounts of neoeriocitrin, naringin, GD, and neohesperidin were detected.

The concentration in the juice of the lemon fruit of ascorbic acid, which is used as an antioxidant in food products and has biofunctional activity *in vivo* (Jacob, 1995), was about 45 mg in 100 ml of juice. Ascorbic acid is an important antioxidant in the juice of the lemon fruit but was unstable under the food processing conditions of heat treatment and food sterilization, and under food storage conditions. The total concentration of the above nine flavonoid compounds, which are stable under conditions of sterilization of foods and beverages, was 37.4 mg in 100 ml of juice and that of eriocitrin, the strong antioxidative flavonoid compound, it was 12.1 mg in 100 ml of juice. The flavonoid compounds seemed to be important antioxidants, as well as ascorbic acid, because they exist in a considerable amount in the juice of the lemon fruit and are stable under normal processing or storage conditions.

The content of flavonoid compounds in lemon juice was examined relative to the method for squeezing the fruit by an in-line extractor or by hand squeezing (as shown in Table 3). The content of eriocitrin and hesperidin in lemon juice obtained by an in-line extractor was greater than that of the juice obtained by hand squeezing. We estimated that the juice obtained by an in-line extractor contained abundant amounts of eriocitrin and hesperidin because pulps, which are parts of the albedo and pulp vesicle in peel, were contaminated in the juice. The in-line extractor method of squeezing fruit is important commercially for the better yield of the juice. Using this method, the lemon fruit was squeezed with higher pressure than by hand squeezing. Because of the pressure on the fruit, parts of the peel, the albedo and pulp vesicle, in which eriocitrin and hesperidin are abundant, were present in the juice as pulp.

Distribution of flavonoid compounds in citrus fruits

The distribution of flavonoid compounds from the peel (Table 4) and juice (Table 5) of citrus fruits was investigated. Eriocitrin, DGD and GD were also shown to exist in limes as well as lemons, but very little was present in the other types of citrus fruits. Little neoeriocitrin was found in lemons, but it was found to be especially rich in daidai. Eriocitrin and GD were also shown to exist in limes as well as in lemons, but very little was present in the other types of citrus fruits. These compounds were found to be especially plentiful in lemon and lime fruits and abundant in the peel obtained as a byproduct from juice factories. These results suggested that effective utilization of the peel in juice products will make

available a greater amount of antioxidative flavonoid compounds.

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