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Antioxidative Properties and Flavonoids Contents of Matured *Citrus* Peel Extracts

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Abstract We assessed various antioxidant activities, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2,2'-azino-bis 3-ethylbenz-thiazoline-6-sulfonic acid (ABTS) radical, and reactive oxygen species (ROS) radical scavenging effect, along with antioxidant capacity, of soybean oil with added *Citrus* species peel extracts (CPEs). These extract oils showed higher radical scavenging effects than grape fruit seed extract, the natural antioxidant agent, did. When CPEs were added to soybean oil, they showed peroxide value (POV) and acid value (AV) increasing inhibition effects. Furthermore, none of the CPEs showed any cytotoxicity over the tested concentration range of 0.01-100 ppm. The major flavonoid contents of *Citrus junos*, as determined by HPLC, were naringin (7.5 μg/mg) and neohesperidin (7.5 μg/mg), and those of *Citrus unshiu* were narirutin (3.13 μg/mg) and hesperidin (1.97 μg/mg). However, the aglycone form was not found. This study showed that CPEs might be a potent source of natural antioxidant, without any toxic effects.

Keywords: *Citrus* species extract, antioxidant activity, flavonoid, cytotoxicity, natural antioxidant

Introduction

Flavonoids are produced in all plants, particularly in the outer tissues and leaves, almost exclusively as glycosides (1). *Citrus* peel has many phenolic classes including benzoic acids (gallic, protocatechuic, chlorogenic, *p*-hydroxybenzoic, vanillic, and syringic), cinnamic acids (caffeic, *p*-coumaric, ferulic, sinapinic, and *t*-cinnamic), flavanone glycoside (hesperidin, naringin, and rutin), flavonol (myricetin, quercetin, and kaempferol), flavanone (hesperitin and naringenin), flavone (luteolin), and phloridzin (2). Approximately 60 species structures of poly-phenolic compound have been reported (3). Nevertheless, the composition and concentration in each *Citrus* flavonoid vary. For example, flavonoids concentration in *Citrus* was decreased during maturation (4), and composition varied according to harvest time and species (5).

Because of their useful health effects, flavonoids have continuously attracted considerable research attention (6). Potentially antioxidant and anti-carcinogenic properties of flavonoids have been described by many researchers (7-13). Phenolic compounds are now associated with a low risk of developing degenerative diseases, such as cancer, diabetes, cardiovascular and neurological diseases. In a remarkable study, Reshef *et al.* (14) reported on the correlation between high flavonoid intake and low incidence of hypertension. Ryu (15) reported the antioxidant activity of flavonoids on the oxidation of human low-density lipoprotein. It has been also reported that flavonoids prevent anti-platelet (16), anti-allergic, and anti-inflammatory activities (17, 18), and that ROS scavenging effect or metal ion chelating are related to these diseases mechanisms (19).

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In this study, we investigated the antioxidant activity of *Citrus* species peel extracts (CPEs) using the scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2,2'-azino-bis 3-ethylbenz-thiazoline-6-sulfonic acid (ABTS) radical, and reactive oxygen species (ROS), as well as the antioxidant capacity of extracts in oil. Additionally, the toxicity grade of CPEs was researched by cell toxicity test and flavonoids, which are known to be the major antioxidant compounds. The CPE Contents were analyzed by HPLC.

Materials and Methods

Materials *Citrus unshiu* was purchased from the national agricultural cooperative federation market (Deajung, Jeju, Korea), *Citrus junos* from a fruit farm (Deajung), and grapefruit seed extract (GFSE) from Pangawa Sciences Inc. (Mississauga, Ontario, Canada)

Preparation of CPEs and the determination of its ethanol extracts Peel of *Citrus* species was reduced to a fine powder in a mixing mill after lyophilizing. The oil was removed by placing 100 g of dried peel in a Soxhlet extractor overnight with 2 L hexane. Then, the peel was dried at 15°C in an evaporated chamber after filtration through Whatman No.1 filter paper. The oil-removed *Citrus* peel with remaining hexane was stored at -20°C in a cold chamber. *Citrus* peel sample for extract was thawed at 35°C in a chamber.

For water and ethanol extract, 10 g of *Citrus* peel sample was sonicated for 30 min with either 200 mL water or 200 mL of 80%(v/v) ethanol and was extracted at either 90°C for 3 hr or 30°C for 6 hr. The residues were re-extracted, on average 3 times, until the extraction solvents became colorless, after which the extracts were filtrated over Whatman No. 1 filter paper and centrifuged at 5400× g for 30 min. The filtrates were frozen and lyophilized at

5 μm Hg pressure at -50°C in a lyophilizator. Extracts were placed in a plastic bottle, and then stored at -20°C until use. CPEs were thawed in a chamber at 35°C prior to use.

Free radical scavenging activity of CPEs *in vitro*

DPPH assay Antioxidant capacity was assessed using modified Blois' methods (20). Initially, 80 μmol of DPPH was dissolved in absolute alcohol for 12 hr before the reaction was started when the samples were added to the DPPH solution. Reaction was performed in a dark place for 30 min. The reducing reaction of DPPH with antioxidants was determined with a UV-VIS spectrophotometer (UVIKON 860; Kantron, Switzerland) at 517 nm.

Trolox equivalent antioxidant capacity (TEAC) assay ABTS radicals were generated by the incubation of 7 mmol ABTS with 2.5 mmol potassium persulfate in the dark at room temperature for 12-16 hr, and then diluted to 60 μmol using a molar extinction coefficient of ABTS+ at 734 nm. The ABTS+ solution in water (2.5 mL) was mixed with the tested compound (25 μL), and 5 min later, the absorbance of ABTS+ was measured at 734 nm. The reaction of ABTS with antioxidants was determined spectrophotometrically at 734 nm.

At each concentration, assays were performed in triplicate and TEAC values were calculated from the slope of the plot of (A_0/A_i)-1 versus the compound concentration at (A_0/A_i)-1 = 1, where, A_0 and A_i are the absorbances in the absence and presence of the tested compounds, respectively.

ROS scavenging effect of CPEs *in vitro*

Superoxide anion radical ($\cdot O_2^-$) scavenging effect The non-enzymatic generation of superoxide anions (21) was measured in samples which contained 20 μmol phenazine methosulfate (PMS), 78 μmol β-nicotinamide adenine dinucleotide (NADH), and 50 μmol nitroblue tetrazolium (NBT) in 0.1 mol phosphate buffer at pH 7.4. After 3 min of incubation at room temperature, the color was read at 540 nm against blank samples which did not contain PMS. The influence of non-enzymatic reduction of NBT was measured using a micro plate reader.

Hydroxyl radical ($\cdot OH-$) scavenging effect In accordance with the modified Halliwell's method (22), hydroxyl radicals produced by Fenton reaction cause damage to the structure of deoxyribose. The total solution, 2 mL, was composed of 20 mmol potassium phosphate buffer (pH 7.4), 2.8 mmol deoxyribose, 1.42 mmol H_2O_2, 100 μmol ascorbic acid, 100 μmol EDTA, samples, and 20 μmol $FeSO_4$. The reaction solution was incubated at 35°C for 1 hr and then boiled at 100°C for 12 min after adding 1%(v/v) thiobarbituric acid 1 mL and 2.8%(v/v) trichloroacetic acid 1 mL solution. Then the reaction was stopped at 0°C, and the hydroxyl radical scavenging effect was determined by using a UV-VIS spectrophotometer at 517 nm.

Hydrogen peroxide (H_2O_2) scavenging effect Hydrogen peroxide was detected by measuring the formation of brown color (recorded at 436 nm) in reaction mixtures of 1 mL final volume containing 150 mmol potassium phosphate buffer at pH 7.4, 0.2%(v/v) guaiacol solution (100 μL of pure guaiacol liquid in 50 mL of H_2O), and 10 μL of Sigma type IV horseradish peroxidase (1,500 U/mL in the same phosphate buffer) (23). The rate of absorbance change at 436 nm was proportional to the concentration of 10 mmol H_2O_2 added. Substances to be tested for their reaction with H_2O_2 were incubated at concentrations up to 10 mmol H_2O_2 for 30 min at room temperature. Samples were taken and assayed for remaining H_2O_2 by using peroxidase system (24).

Hypochlorous acid (HOCl) scavenging effect HOCl scavenging activity by chlorination of taurine assay was measured according to the method of Weiss (25). Sodium hypochlorite (100 mL of 60 mmol) was added to 100 μL taurine (150 mmol) and 100 mL of phosphate buffer solution (PBS) tissue solution was added in a final volume of 1 mL PBS at pH 7.4. The absorbance was measured at 350 nm. HOCl was prepared by diluting an aliquot of NaOCl with 0.1 mol PBS at pH 7.0.

Antioxidant capacity of extracts in oil

Change in peroxide value (POV) Extract at 100 ppm was added to oil samples which were then incubated at 30°C in the dark for 10 min after the addition of 25 mL of chloroform-acetic acid-ethanol (2:3:1, v/v/v). Next, 1 mL of saturated potassium iodide and 30 mL of distilled water were added to 1 g of oil sample. Finally, POV was analyzed by 1/100 N sodium thiosulfate ($Na_2S_2O_3$) titration. For the blank value, oil was substituted with H_2O in the titration. For 1/100 N sodium thiosulfate solution correction, 1/100 N potassium dichromate (K_2CrO_7) was used as the standard solution to determine the correction value.

Change in acid value (AV) Extract at 100 ppm was added to oil samples which were then incubated at 30°C. Free fatty acid was decomposed in 20-40 mL of benzene-ethanol (2:1, v/v). Finally, free fatty acid content was analyzed by titration using 1/10 N KOH-EtOH after phenolphthalein solution had been added to the oil samples. For the blank value, oil was substituted with H2O in the titration. For 1/10 N KOH-EtOH solution correction, potassium-phthalate monobasic was used as the standard solution to determine the correction value.

Analysis of the flavonoid contents of *Citrus* peel extracts Amberlite XAD-2 resin was suspended in methanol and then packed in a column (2.5×30 cm) after removal of floating Amberlite XAD-2 resin. The column was washed with 3 column volumes of deionized water. The resin was equilibrated by 30%(v/v) isopropyl alcohol. After loading the sample, phenolic compounds were eluted with step gradients of 250 mL of 0, 30, 70, and 100%(v/v) isopropyl alcohol. Flavonoid contents of each fraction were analyzed by HPLC (pump 510, UV 486, Auto-sampler 717 PLUS). Reverse-phase separations were carried out using a Waters Bondapak^{TM} C_{18}, 12Å 10 μmol, and dimensionS 3.9×300 mm (Waters, Milford, MA, USA). The column temperature was 25°C. The sample temperature in the auto-sampler was 18°C. Two solvents, A (Acetonitrile) and B (water:acetic acid (96:4, v/v)) were used in a linear gradient. The gradient elution showed the following profile: 0-5 min 95-80% B solvent, 5-65 min 80-70% B solvent. Total running time was 50 min and the flow-rate was 1.0 mL/min. Sample injection volume was

10 L. The flavonoids were detected at 280 nm and identified according to the retention times and UV spectra of the standards.

Cell toxicity of extracts HepG-2 cells, the human hepatocellular carcinoma cell line, were cultured in a T-flask with Rosewell Park Memorial Institute (RPMI) 1640 containing 10% heat-inactivated fetal bovine serum (FBS), 2 mmol L-glutamine, and 1% penicillin at 37°C under 5% CO_2 atmosphere. To measure the cell toxicity of the extracts, cell proliferation assay was performed using water-soluble tetrazolium (WST-1; the sodium salt of 4-[3-(4 iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfate) reagent. The cells (2×10^5) were seeded in 96-well plates and incubated for 24 hr at 37°C under 5% CO_2 atmosphere. The cells in each well were treated with various concentrations of extracts for another 24 hr at the same conditions. After treatment with WST-1, the absorbance was detected at 440 nm by micro-plate reader.

Results and Discussion

Free radical scavenging activity of *Citrus* peel extracts *in vitro* The antioxidant activity of the extracts was attributed to their hydrogen donating ability (26). The total antioxidant capacity of the extracts was detected according to the level of hydrogen donation by using the stable free radicals $DPPH^+$ and $ABTS^+$. The capacity of hydrogen donation represents how well it reacts to the radical. Structurally, the flavonoids have phenolic groups which serve as a source of readily available 'H' atoms such that the radicals subsequently produced can be delocalized over the flavonoids structure (26). As these compounds produce H^+ easily, they can readily react with the radicals. Therefore, antioxidant capacity, as generally distinguished, means a high hydrogen donation capacity.

Table 1 presents comparison results of control values of the solvent (DMSO, H_2O) which dissolved the samples. In addition, the antioxidant activities of the extracts were compared with those of well-known antioxidants, BHT, GFSE, and Trolox (in ABTS assay). CPEs were less effective than the antioxidant BHT at scavenging DPPH radicals, although in extracts at 100 ppm concentration, the scavenging effect of CPEs on DPPH radicals increased dramatically.

The scavenging activities of stable radical ABTS were expressed as the TEAC values, which can be illustrated for the determination of antioxidant activity. TEAC is the millimolar concentration of a Trolox solution containing the antioxidant capacity equivalent to a 1.0 mmol solution of the used compounds. As shown in Table 2, the TEAC values of CPEs were higher than those of GFSE. Especially, the TEAC value of *C. junos* peel ethanol extract (CJE) at 1000 ppm was about 1.8 times higher than that of GFSE at 1000 ppm.

The data obtained revealed that certain compounds in *Citrus* fruits are free radical scavengers and primary antioxidants that react with free radicals, which may be attributed their proton donating ability.

ROS scavenging effect of CPEs *in vitro* Experiments were done *in vitro* to define the scavenging effects of superoxide anion radical, hydroxyl radical, hydrogen peroxide, and hypochlorous acid as the most representative ROS.

Table 1 shows the superoxide anion radical scavenging

Table 1. The free radical and ROS[1)] scavenging effect of *Citrus* peel extracts

Compound[2)]		Scavenging effect (%)				
		DPPH	$\cdot O_2^-$	$\cdot OH^-$	H_2O_2	HOCl
CJE	10ppm	7.67±1.29[3)]	22.42±6.53	49.01±6.79	11.83±4.68	7.92±2.72
	100ppm	22.09±1.53	33.94±1.84	55.56±1.37	25.76±1.44	26.23±4.06
CJW	10ppm	3.43±3.09	24.03±5.17	52.46±2.50	14.19±1.07	3.56±2.49
	100ppm	11.29±3.93	43.16±3.79	61.61±5.91	25.82±0.91	3.89±0.37
CUE	10ppm	2.03±1.87	10.0±5.00	39.70±5.63	14.46±4.26	5.68±2.62
	100ppm	15.67±2.49	29.09±1.57	54.76±6.29	21.18±2.36	12.50±2.76
CUW	10ppm	0.48±1.78	20.70±0.61	49.13±3.77	13.15±5.35	2.61±0.95
	100ppm	10.92±4.26	35.85±0.93	56.52±5.75	23.61±3.53	3.55±1.47
GFSE	10ppm	0.51±2.23	13.77±2.83	5.00±5.00	7.53±0.86	5.23±3.93
	100ppm	26.80±1.70	39.27±3.26	37.90±1.82	22.90±3.66	17.40±4.11
Ascorbic acid	10ppm	85.37±0.76	18.86±5.31	32.32±3.57	62.36±2.24	1.00±1.88
	100ppm	86.12±0.17	22.37±2.28	36.98±6.12	90.85±1.52	5.26±1.07
BHT	10ppm	32.78±4.23	2.39±4.93	27.45±3.40	7.05±1.80	12.68±0.92
	100ppm	75.04±4.60	14.43±7.27	53.17±2.75	31.99±4.59	26.29±7.25

[1)]Reactive oxygen species.
[2)]CJE, *C. junos* peel ethanol extract; CJW, *C. junos* peel water extract; CUE, *C. unshiu* peel ethanol extract; CUW, *C. unshiu* peel ethanol extract; GFSE , Grapefruit seed extract; BHT, Butylated hydroxyl toluene.
[3)]Data are mean±SD (n=3).

Table 2. Antioxidant capacity of *Citrus* peel extract (1000 ppm) using TEAC value[1)]

Compounds, 1.0 mg/mL	TEAC (mmol)
Citrus junos peel ethanol extract	0.26±0.01[2)]
Citrus junos peel water extract	0.23±0.01
Citrus unshiu peel ethanol extract	0.22±0.01
Citrus unshiu peel water extract	0.21±0.01
Grapefruit seed extract	0.21±0.01
Ascorbic acid	1.04±0.01
Butylated hydroxyl toluene	0.82±0.02

[1)]TEAC : millimolar concentration of a Trolox solution having the antioxidant capacity equivalent to 1.0 mg/mL solution of *Citrus* peel extract and other compounds.
[2)]Data are mean±SD (n=3).

activity of CPEs. *C. junos* peel water extract (CJW) at 100 ppm had the highest activity, 43.16±3.79%, followed by *C. unshiu* water extract (CUW). In addition, the scavenging effects of most of the CPEs at 10 ppm were about 20% and their activities were 1.5 times greater than those of GFSE.

Hydroxyl radical scavenging activities of the water extracts were higher than those of the ethanol extracts (Table 1). In addition, the activities of CPEs were higher than those of GFSE and BHT. Especially, the scavenging effect of *C. junos* was the highest at around 50%.

In the case of hydrogen peroxide quenching activity, the extracts at 10 and 100 ppm concentrations had average inhibition activities of 13.39 and 24.09%, respectively. The extracts did not exhibit particularly high activity, and all activities of the extracts and BHT were similar. The activities of the extracts and controls are shown in Table 1.

Hypochlorous acid (HOCl) is a powerful, two-electron, oxidizing and chlorinating agent generated by activated neutrophils (25). Neutrophil-mediated injury may also be important in toxicology when an initial insult is followed by an inflammatory response. The production of HOCl by myeloperoxidase-activated neutrophils at the site of inflammation can contribute to tissue damage and have important effects on proteins and enzymes (27). Myeloperoxidase has the unique property of converting chloride to HOCl. For instance, HOCl may rapidly inactivate 1-antiproteinase and antioxidant enzymes, activate collagenase and gelatinase, and deplete antioxidant vitamins. In this assay, extracts tested over a concentration range of 10-100 ppm were able to react directly with HOCl and to protect taurine against chlorination. As shown in Table 1, the activity of 100 ppm CJE, 26.23±4.06%, was the highest of all the extracts. Eventually, the non-free radical scavenging effect of the extracts was low.

Antioxidant capacity of CPEs in oil Generally, the principal route of the deterioration of fat is through oxidative rancidity, which takes place at the double bond in the triglyceride molecule (28). In fat deterioration, the first initiating step is the formation of free fatty acids, which are susceptible to oxygen attack in the presence of light, resulting in the formation of many organic compounds and free fatty acids which are responsible for the development of rancidity and off-flavors in fatty food materials (29). Production of free fatty acids and increase in POV are the best predictors of fat deterioration, which could be used to monitor the extent of fat spoilage.

Addition of CPEs changed POV and AV of the soybean oil system. Differences in POV and AV were observed between the control and the CPEs-treated oil. The decrease in POV and AV clearly indicated that autoxidation of oil was inhibited in the presence of CPEs at 100 ppm concentration. As shown in Fig. 1, ethanol peel extracts of *C. junos* and *C. unshiu* have high antioxidant capacity.

Analysis of the flavonoid contents of *Citrus* spp. peel extracts The major antioxidant material is the phenolic compound in CPEs. Many researchers found antioxidant activities in the extracts of edible and non-edible plant materials due to the presence of phenolic compounds (30). The main phenolic constituents of *Citrus* peel are flavanone and flavone glycosides. John (31) found that the antioxidant property was observed in orange peel ultrafiltered molasses due to the presence of phenols, including numerous flavones, flavanones, polymethoxylated flavones, hydroxycinnamates, and other miscellaneous phenolic glycosides and amines. Flavonoid glycosides, were found in *Citrus* fruits. These compounds could be structural isomers, which contain rhamnose and glucose bound in either 1-2 (neohesperidoside) or 1-6 (rutinoside) positions (32). Belajová and Suhaj (33) separated and confirmed naringin, hesperidin, and neohesperidin in *Citrus* juices using HPLC. Jeong *et al.* (34) isolated narirutin from ethyl acetate fraction which showed strong antioxidative activity. John and Karel (35) reported that the flavanone glycosides occur in the highest concentrations, along with hesperidin, the main flavonoid in orange peel. Bocco *et al.* (36) reported that lemon peel was rich in neoeriocitrin, naringin, and neohesperidin and that sour orange was a very interesting source of naringin and neohesperidin. As Table 3 shows, the major components of *C. junos* were naringin and neohesperidin, and those of *C. unshiu* (CU) were narirutin and hesperidin. Aglycones such as naringenin, hesperitin, and quercetin were not found because most flavonoids exist in the glycoside-form which is stable and undamaged in the natural world, due to the

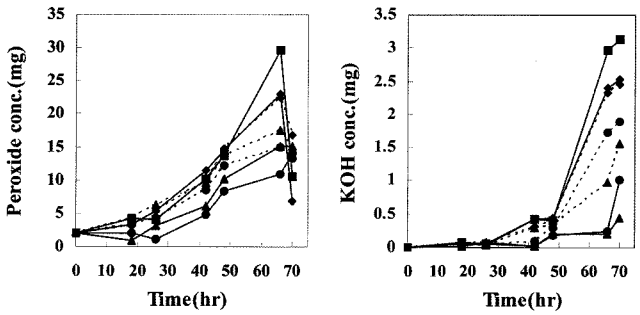

Fig. 1. Increasing inhibition effect of *Citrus* peel extracts as determined by POV and AV. *C. junos* peel ethanol extract (-●-), *C. junos* peel water extract (--●--), *C. unshiu* peel ethanol extract (-▲-), *C. unshiu* peel ethanol extract (--▲--), N,N-dimethyl-formamide (-◆-), Soy Oil (-■-).

Table 3. Flavonoid contents of *Citrus* peel extracts[1] 100 mg

Flavonoid (mg)	CJE	CUE	CJW	CUW
Rutin	0.414±0.013[2]	0.051±0.026	0.274±0.103	0.044±0.009
Narirutin	0.11±0.059	0.313±0.076	0.089±0.054	0.189±0.056
Naringin	0.75±0.042	0.034±0.016	0.589±0.096	0.032±0.044
Hesperidin	0.046±0.026	0.197±0.029	0.045±0.031	0.055±0.034
Neohesperidin	0.752±0.05	0.01±0.007	0.63±0.088	0.005±0.003

[1]CJE, *C. junos* peel ethanol extract; CJW, *C. junos* peel water extract; CUE, *C. unshiu* peel ethanol extract; CUW, *C. unshiu* peel ethanol extract; GFSE , Grapefruit seed extract; BHT, Butylated hydroxyl toluene.
[2]Data are mean±SD (n=3).

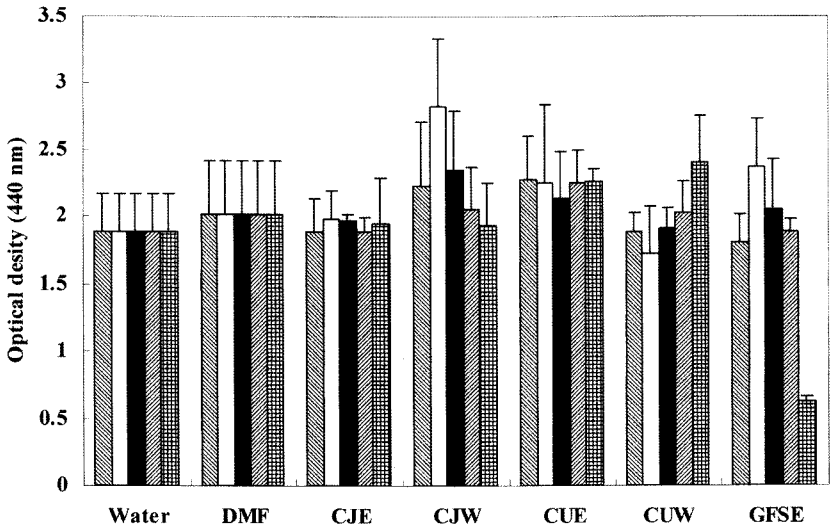

Fig. 2. Cell toxicity of *Citrus* peel extracts. DMF, *N,N*-dimethylformamide; CJE, *C. juno s*peel ethanol extract; CJW, *C. junos* peel water extract; CUE, *C. unshiu* peel ethanol extract; CUW, *C. unshiu* peel ethanol extract; GFSE, Grapefruit seed extract (▨, 0.01 ppm; □, 0.1 ppm; ■, 1 ppm; ▨, 10 ppm; ▦, 100 ppm).

strength of the glycoside linkages in the flavonoids and sugars (37). However, quercetin, naringenin, and hesperitin certainly do exist in *Citrus* and each has a different kind of flavonoid (38).

Comparison of antioxidant activities with the flavonoid contents in CPEs showed that the free radicals, ROS scavenging activities, and decreasing POV rate were all more effective in *C. junos*, which is rich in neohesperidin and naringin, while the decreasing AV rate was higher in *C. unshiu*, which was rich in narirutin and hesperidin.

However, Bocco *et al.* (39) revealed that flavanones could only explain from 36 to 83% of the antioxidant activity of the peels. Bocco *et al.* (36) also reported that there was no clear relationship between the antioxidant power and the glycosylated flavanone concentration of an extract. It is well known that each flavonoid derivative has different biological activities (32, 35). Therefore, further studies are needed on the characterization of individual flavonoids in citrus peels to elucidate their different antioxidant mechanisms and to confirm the existence of possible synergism.

Cytotoxicity test of extracts The negative control was 1% N,N-dimethyl formamide (DMF) or H_2O and the positive control was GFSE in this assay. It has been shown that HepGe-2, which is the most commonly tested cell line in research for toxicity mechanisms, is useful for toxic evaluations (25). In Fig. 2, none of the CPEs showed any cell cytotoxicity over the tested concentration range, but 100 ppm of GFSE showed the highest cell toxicity. Therefore, CPEs may not exhibit any cell toxicity at meaningful concentrations and may be used as antioxidant materials even at higher concentrations than GFSE.

In several antioxidant assays, CPEs showed higher antioxidant activity and ROS scavenging effect than GFSE did. In addition, when CPEs were added to soybean oil, they showed a high antioxidant capacity in the oil. In the cell toxicity test, none of the CPEs showed any cell cytotoxicity over the tested concentration range, whereas 100 ppm of GFSE showed a high cell toxicity. CPEs can therefore be used as food additives even at high concentration because their extracts did not show any toxicity in the tested cell line. The major antioxidant materials of CPEs were flavonoid glycosides.

The recovery of the flavonoids in *Citrus* represents an economically important source of these naturally occurring compounds, and the results presented in this paper demonstrate that CPEs have potential as natural antioxidants and functional food additives in the food industry, without any associated toxic effects.

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