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Antioxidant Activity of Premature Mandarin (*Citrus unshiu*) and Its Effect on the Lipid Oxidation of Horse Oil-in-Water Emulsion

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Antioxidant Activity of Premature Mandarin (*Citus unshiu*) and Its Effect on the Lipid Oxidation of Horse Oil-in-Water Emulsion

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ABSTRACT

Mandarin is mainly used as a fresh product or juice in the food industry. During harvesting and processing mandarins, by-products including premature mandarins and mandarin peels are occurred and discarded. However, mandarin by-products are known to contain high concentrations of flavonoids, polyphenols, and organic acids with health functions. In this study, the antioxidant activity of premature and mature mandarin extracts and their potentials as functional food materials were investigated. The total phenolic and flavonoid contents (TPC, TFC) were measured and the flavanone compounds were analyzed by HPLC for identification and quantification of antioxidant active ingredients. The DPPH and $ABTS^{+}$ radical, hydrogen peroxide, and hydroxyl radical scavenging activity, superoxide dismutase activity, and reducing power were measured for *in vitro* antioxidant activities. Additionally, to investigate the effect of premature mandarin on the lipid oxidation, premature and mature mandarin peel extracts, α-tocopherol, and Trolox were added to horse-oil-in-water (HO/W) emulsions, which were stored at 40 °C for 30 days thiobarbituric acid reactive substances (TBARS) were measured. The TPC and TFC of premature mandarin peel extract (PMP), mature mandarin peel extract (MP), premature mandarin juice (PMJ), and mature mandarin juice (MJ) were 94.04, 79.15, 37.05, and 31.20 mg GAE/g and 43.99, 23.51, 4.20, and 0.09 mg QE/g, respectively. The flavanone compounds in PMP, MP, PMJ, and MJ were identified only as glycoside form flavanones, hesperidin and narirutin with total amounts of 5.33 to 128.16 mg/g. The *in vitro* antioxidant activities of mandarin peels (PMP and MP) were higher than those of mandarin juices (PMJ and MJ). When compared to the premature and mature mandarins, *in vitro* antioxidant activities of premature were higher than mature. Especially, the mandarin peel extracts

(PMP and MP) were particularly active in scavenging hydrogen-containing radicals. For the lipid oxidation of HO/W emulsions, α-tocopherol at 0.2 mg/mL acted as a pro-oxidant to promote lipid oxidation while mandarin peel extracts at 0.5 and 1 mg/mL and Trolox at 0.2 mg/mL acted as antioxidants to inhibit lipid oxidation. These results confirmed the potential of premature and mature mandarin peels, by-products of mandarin, for the functional food and cosmetics materials.

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1. Introduction

Mandarin, widely distributed in the tropics, is one of the most consumed fruits in the world because of their attractive color, pleasant flavors, aroma, and phytochemicals, such as phenolic acids, flavonoids, carotenoids, and vitamin C (Chen et al., 2020; Zou et al., 2016). Among 1,800 species of mandarins, *Citrus unshiu* is the most cultivated in Jeju, Korea (Carbonell-Caballero et al., 2015; Kim and Lim, 2020a). About 50% of mandarins are manufactured for mandarin juices and approximately 50-60% of the processed mandarins are discarded as wastes. These wastes mostly include peel, pulp, and premature and damaged fruits of mandarins (Negro et al., 2016). Among them, mandarin peels and premature mandarins are known to contain more health functional compounds than edible parts and mature mandarins (Choi et al., 2019; Li et al., 2009; Park et al., 2020). As functional compounds of mandarin, flavonoids are known and hesperidin, naringin, rutin, nobiletin, and tangeretin are mostly founded flavonoids in mandarin (Huang et al., 2020). These flavonoids compounds were reported to show various bioactivities, such as radical scavenging, antioxidant, and anti-inflammatory properties (Choi et al., 2019; Yi et al., 2017; Zou et al., 2016). Thus, mandarin flavonoids are one of the most important source of bioactive compounds and possibly decrease the generation of reactive oxygen species (ROS) and inhibit lipid peroxidation when exert their antioxidant capacities (Yi et al., 2017).

Nowadays, along with changes in lifestyle and dietary patterns, modern people are paying attention to health and antioxidants to prevent aging and lifestyle-related diseases. Excessive accumulation of ROS including superoxide anion radical, hydroxyl radical, hydrogen peroxide, singlet oxygen, and alkoxy radicals causes oxidative stress. The ROS has high reactive and unstable, so it can promote many diseases, including cancer, cardiovascular and

neurodegenerative diseases, and aging (Lee et al., 2020; Yang et al., 2018). Antioxidants can prevent or reduce the generation of ROS and in food industry antioxidants are used to prevent autoxidation during food processing and storage. Butylated hydroxyl anisole (BHA) and butylated hydroxytoluene (BHT), synthetic antioxidants, have been used as an antioxidant in foods to inhibit lipid oxidation processes. However, the use of these synthetic antioxidants has been associated with potentially toxic and carcinogenic effects, which has led to some restraint in its use (Agregán et al., 2017; Lee et al., 2020). For this reason, consumers are demanding natural plant-based antioxidant alternatives to replace synthetic antioxidants.

As the demand for the usage of a natural antioxidant has increased, this study was intended to utilize premature mandarin which is mostly discarded during harvest of mandarin. Thus, the bioactive compounds of premature mandarins were analyzed and the *in vitro* antioxidant activities were measured along with the comparison of mature mandarins. To be used in the food, cosmetics, and pharmaceutical industries, premature and mature mandarin peel extracts were added to the HO/W emulsion to compare the efficiency of lipid oxidation with existing used antioxidants during storage.

2. Materials and Methods

2.1. Materials and chemicals

The premature mandarin (harvested from August to September, 2019) and the mature mandarin (harvested in December, 2019) were purchased from a local farm and market (Jeju, Korea).

Methanol and isopropyl alcohol was purchased from Burdick & Jackson Co. (Muskegon, MI, USA) and J. T. Baker (Philipsburg, NJ, USA), respectively. Trichloroacetic acid (TCA) and isooctane were purchased from DaeJung Chemical&Metals Co., Ltd. (Siheung, Korea). Hesperidin, hesperetin, narirutin, naringenin, Folin-Ciocalteu reagents, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), peroxidase, iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), ethylenediaminetetraactic acid (EDTA), 2-deoxyribose, 2-thiobarbituric acid (TBA), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate (ferrozine), iron (II) chloride ($FeCl_3$), potassium ferric cyanide, ferric chloride ($FeCl_3$) , sorbitan monostearate (Span 60), polyethylene glycol sorbitan monostearate (Tween 60), α-tocopherol, barium chloride, iron(II) sulfate heptahydrate, 1,1,3,3-tetraethoxypropane (TEP), butyl alcohol (1-butanol), and cumene hydroperoxide were obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used in the current study were of analytical grade.

2.2. Preparation of premature and mature mandarin peel extract

Peels of premature and mature mandarins were separated and dried at 80℃ for 9 h. The dried peels were ground into a 40 mesh size using a blender

(SMX-8000EMT, Shinil, Seoul, Korea). The peel powder was mixed with methanol as a ratio of 1:10 (w/v) and extracted by reflux extraction at 50℃ for 24 h. The extract was filtered through a filter paper (Whatman No. 2, Whatman, Maidstone, UK) and methanol was evaporated using a rotary evaporator (SB-1200, EYELA, Shanghai, China) under partial vacuum at 40℃. The concentrates were dissolved with distilled water and freeze-dried to obtain premature mandarin peel extract (PMP) and mature mandarin peel extract (MP). For the measurement of *in vitro* antioxidant activities, the PMP and MP powder were stocked in DMSO at a concentration of 10 mg/mL and the stock solution was diluted accordingly.

2.3. Preparation of premature and mature mandarin juice

Premature and mature mandarin fruits after peeling off were ground using a blender (SMX-8000EMT, Shinil) to prepare mandarin juice. The juices were centrifuged at 2700 × g for 20 min (centrifuge, A32010, Labogene) and the supernatant were filtered through a filter paper (Whatman No. 2, Whatman). The juices were stored at -80℃ and then freeze-dried to obtain premature and mature mandarin juice powder (PMJ and MJ, respectively). For the analysis of *in vitro* antioxidant activities, the PMJ and MJ were stocked as described in the preparation of PMP and MP.

2.4. Determination of total phenolic and flavonoid contents

Total phenolic contents (TPC) of PMP, PMJ, MP, and MJ were determined by Folin-Ciocalteau method of Lee et al. (2020). In brief, 100 μL of a sample was mixed with 1.5 mL of distilled water and 100 μL of 2 N Folin-Ciocalteu reagent in a test tube. After 30 sec, 300 μL of 20% sodium carbonate was added and mixed. And the mixtures were incubated at room temperature for

1 h in the dark. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (OPTIZEN 2120UV, Mecasys, Daejeon, Korea) and the result was expressed as gallic acid equivalent (mg GAE/g of sample).

Total flavonoid contents (TFC) was measured by the method of Yi et al. (2014). Two hundred μL of the PMP, PMJ, MP, and MJ was mixed with 800 μL of ethyl alcohol and 60 μL of 5% $NaNO_2$ in a test tube and incubated at room temperature. After 5 min, the mixture was reacted with 60 μL of 10% $AlCl_3$ and allowed to stand for 5 min. Four hundred μL of 1 M NaOH and 500 μL of distilled water were added to the mixture. The absorbance was measured at 415 nm using a UV-Vis spectrophotometer (Mecasys). The total flavonoid content was expressed as quercetin equivalent (mg QE/g of sample).

2.5. Analysis of flavanone compounds by HPLC

Flavanone compounds of the mandarin peel extracts and juices were analyzed by high performance liquid chromatography (HPLC, Agilent 1260 series, Agilent Technologies, Santa Clara, CA, USA) with a diode array detector (G7115A DAD WR, Agilent Technologies) and a Pursuit C18 column (250×4.6 mm, 5 μm, Agilent Technologies) (Sun et al., 2010). Elution was performed at the flow rate of 1.0 mL/min with mobile phase consisting of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) for 0-10 min A : B = 78 : 22; 10-35 min A : B = 39 : 61; and 35-40 min A : B = 0 : 100%. The injection volume was 20 μL and chromatograms were recorded under the detection wavelength of 280 nm. The chromatographic peaks of flavanone compounds were recognized by comparing their spectra and retention times and the flavanone contents were quantified based on the calibration curves of hesperidin, hesperetin, narirutin, and naringenin.

2.6. *In vitro* antioxidant activity

2.6.1. *DPPH radical scavenging activity*

DPPH (1,1-diphenyl-2-pucrylhydrazyl) radical scavenging activity was measured by the method of Lee et al. (2020). The change of absorbance was measured exactly at 517 nm using a microplate reader (Epoch^{TM}, BioTek Instruments INC., Winooski, VT, USA) after 30 min by adding 70 μL mandarin extract or each flavanone compound into 140 μL of 1 mM DPPH solution.

2.6.2. *$ABTS^{+}$ radical scavenging activity*

$ABTS^{+}$ radical scavenging activity was determined according to the method described by Sung et al. (2018) with some modifications. The $ABTS^{+}$ radical solution was prepared by 7 mM ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) aqueous solution with 2.45 mM aqueous solution of potassium persulfate in equal quantities and allowed them to react at room temperature in the dark for 16 h. Then, 180 μL of $ABTS^{+}$ radical solution was mixed with 20 μL of mandarin extract or each flavanone compounds. The absorbance was measured at 734 nm using a microplate reader (BioTek Instruments).

2.6.3. *Hydrogen peroxide scavenging activity*

Hydrogen peroxide scavenging activity was determined using the method of Lee et al. (2020) with some modifications. Briefly, 100 μL of mandarin extract or each flavanone compounds, 20 μL of hydrogen peroxide, and 100 μL of 0.1 M phosphate buffer (pH 7.4) were mixed together and then incubated at 37℃

for 5 min. After the incubation was done, 30 μL of 1.25 mM ABTS and 40 μL of peroxidase (1 unit/mL) were added to the mixture and incubated again at 37℃ for 10 min. The absorbance was measured at 405 nm using a microplate reader (BioTek Instruments).

2.6.4. *Hydroxyl radical scavenging activity*

Hydroxyl radical scavenging activity was measured according to a slightly modified method of Lee et al. (2020). Hydroxyl radical was generated by Fenton reaction in the presence of $FeSO_4 \cdot 7H_2O$. Two hundred μL of mandarin extract or flavanone compound (distilled water was used as a blank) was placed to a test tube containing 200 μL of 10 mM $FeSO_4 \cdot 7H_2O$, 10 mM EDTA, 10 mM 2-deoxyribose, 10 mM hydrogen peroxide, and 1 mL of 0.1 M phosphate buffer solution (pH 7.4) and incubated at 37℃ for 2 h. After taking out, 1 mL of the mixture, 1 mL of 2.8% TCA and 0.4% TBA solution were added and put it in a boiling water bath for 10 min. The tube was cooled down to room temperature and the absorbance was measured at 532 nm using UV-Vis spectrophotometer (Mecasys).

All radical scavenging activities were calculated as followed:

$$\text{Radical scavenging activity}\,(\%) = \left[\frac{(A_{sample} - A_{control})}{A_{sample}}\right] \times 100 \qquad (1)$$

2.6.5. *Superoxide dismutase activity*

Superoxide dismutase activity of mandarin extracts and flavanone compounds were measured according to the manufacturer protocol of the SOD Assay kit (Dojindo Molecular Technologies, Inc., Tokyo, Japan). Premature and mature mandarin extracts and flavanone compounds were added to

96-well plates, reacted with reagents in the kit, and the absorbance was measured at 450 nm using a microplate reader (BioTek Instruments).

2.6.6 *Reducing power*

Reducing power of the mandarin extracts and flavanone compounds were determined by the method of Lee et al. (2020). One milliliter of PMP, PMJ, MP, MJ, or flavanone compounds, 0.1 M phosphate buffer (pH 6.6), and 1% potassium ferricyanide were mixed together. The mixture was incubated at 50℃ for 20 min. After incubation, 1mL of 10% TCA was added. After taking 2 mL of the supernatant from the reaction solution, 2 mL of distilled water and 400 μL of 0.1% ferric chloride were mixed and left to react at room temperature in the dark for 10 min. The absorbance of the solution was measured at 700 nm using an UV-Vis spectrophotometer (Mecasys). The reducing power of the extracts and flavanone compounds was compared as the absorbance at 700 nm.

2.7. Preparation of horse oil-in-water emulsions

To determine the effect of the premature and mature mandarin peel extract as an antioxidant on the lipid oxidation, horse-oil-in-water emulsion was prepared. First, horse oil was extracted from horse fatty meats (Jeju, Korea) using a vacuum low temperature extractor (Cosmos-660, Kyungseo E&P, Incheon, Korea) by the method of Cho and Kim (2020). The horse oil-in-water (HO/W) emulsion were prepared by mixing 30% of horse oil with 15% of blended surfactant (Span 60 and Tween 60, HLB value 12) and 55% of ultrapure water (Park, 2020). As an antioxidant, α-tocopherol (α-T) and Trolox (Trolox) at 0.2 mg/mL, MP and PMP at 0.5 mg/mL (MP 500 and

PMP 500), and MP and PMP at 1 mg/mL (MP 1000 and PMP 1000) were added to the HO/W emulsion. The HO/W emulsion without an antioxidant was used as a control.

When prepared 100 g of the HO/W emulsion, the blended surfactant and antioxidants were firstly dissolved in horse oil (oil phase). The temperature of oil phase and water phase was adjusted to 60℃. The oil phase was homogenized with a homogenizer (T25, Ika, Staufen, Germany) at 10,000 rpm for 1 min. Then, 55 mL ultrapure water was poured to the homogenized oil phase for 5 min to manufacture an emulsion. The emulsion was stirred at room temperature for 20 min and poured into a 50 mL test tube. For lipid oxidation, they were stored at 40℃ in the dark for 30 days.

2.8. Measurement of lipid oxidation products of HO/W emulsion

Lipid oxidation of the HO/W emulsions added with different antioxidants was determined by the measurement of thiobarbituric acid reactive substances (TBARS) (McDonald and Hultin, 1987). TBA solution was prepared by mixing 150 g of trichloroacetic acid (TCA), 3.75 g of TBA, 17.6 mL of 12 N HCl, and 829 mL of distilled water. First, 1 g of the emulsion was mixed with 2 mL of TBA solution at high speed three time for 10 s each. Then, the mixtures were reacted in boiling water (above 90℃) for 15 min. And they were cooled down to room temperature in a bath filled with cold water and centrifuged at 2,700 × g for 15 min. The absorbance of the supernatant was measured at 532 nm using a microplate reader (BioTek Instruments). To quantify the amount of TBARS concentrations in the HO/W emulsions, a standard curve was produced using 1,1,3,3-tetrathoxypropane (TEP) and TBARS were expressed as μmol/kg oil

2.9. Statistical analysis

All analyses were performed in triplicate. The results were exhibited as means ± standard deviation. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Significant differences were considered at $p<0.05$.

3. Results and Discussion

3.1. Total phenolic and flavonoid contents of premature and mature mandarin extracts

Total phenolic (TPC) and flavonoid contents (TFC) of premature mandarin peel methanol extract (PMP), mature mandarin peel methanol extract (MP), premature mandarin juice (PMJ), and mature mandarin juice (MJ) are shown in Table 1. The TPC and TFC of PMP, MP, PMJ, and MJ were 94.04, 79.15, 37.05, and 31.20 mg GAE/g and 43.99, 23.51, 4.20, and 0.09 mg QE/g, respectively. The peel extracts contained higher amounts of TPC and TFC than juice whether the mandarin was premature or mature. When compared to TPC and TFC of premature and mature mandarin, premature mandarin contained greater amounts than mature did although TPC of PMJ and MJ was not significantly different. Mostly, mandarin peels contained higher TPC and TFC than juices. In previous studies, TPC and TFC of mandarin peels were reported higher than those of mandarin juice (Che et al., 2020; Xi et al., 2017). Because of high TPC and TFC, mandarin peels could be used as a source of natural antioxidant. In the results of TPC and TFC, premature mandarin contained higher than mature mandarin. During the maturing stage of mandarins, the oxidation of polyphenol by polyphenol oxidase would lead to the reduction of TPC (Dong et al., 2019). Dong et al. (2020) reported that TPC and TFC of lemon peel was the highest in August and gradually decreased in maturing stage. The TPC in one of citrus, Yuzu was also the highest in August and lowest in December (Moon et al., 2014). The results of the current study showed that mandarin peels have higher TPC and TFC than juice and premature mandarins had higher TPC and TFC than mature mandarins.

Table 1. Total phenolic and total flavonoid contents of premature and mature mandarin peel and juice

Mandarin extract[1)]	Total phenolic contents (mg GAE/g)	Total flavonoid contents (mg QE/g)
PMP	94.04±6.25[2)a3)]	43.99±3.73[a]
MP	79.15±2.38[b]	23.51±0.81[b]
PMJ	37.05±4.16[c]	4.20±1.04[c]
MJ	31.20±3.29[c]	0.09±0.46[d]

1) PMP: premature mandarin peel methanol extract, MP: mature mandarin peel methanol extract, PMJ: premature mandarin juice, MJ: mature mandarin juice

2) Each value is mean±standard deviation

3) Means with different letters (a, b et al.) in a column are significantly different at $p<0.05$.

3.2. Flavanone compounds of premature and mature mandarin extracts

Flavanone compounds, such as hesperidin, hesperetin, narirutin, and naringenin, which are mainly found in mandarins, were determined in premature and mature mandarin peel extract and juice and shown in Fig. 1 and Table 2. Among flavanone compounds, only hesperidin and narirutin, a glycoside form of flavanone, were detected in PMP, MP, PMJ, and MJ. Hesperidin in mandarin peel extract and juice was highly detected compared to narirutin. Total flavanones contents of PMP, MP, PMJ, and MJ were 128.16, 86.12, 10.32, and 5.33 mg/g, respectively. Mandarin peel extracts contained much greater amounts of flavanone compounds than juice whether mandarin was premature or mature, which were similar to the results of TPC and TFC (Table 1). Hesperidin and narirutin were more detected in premature mandarin than mature. The narirutin concentration in mandarin was usually high in the early stage of growing (Multari et al., 2020) and hesperidin was also detected in high of premature mandarin (Choi et al., 2007). In addition, hesperidin was present in high of mandarin peels while narirutin was detected in low (Kim and Kim, 2016). According to Chen et al. (2019), hesperidin was the most abundant flavonoid compound present in mandarin peels and flavonoids contents were different depending on the production area of mandarin. Kim and Lim (2020) reported that major flavonoids of mandarin peels were hesperidin and narirutin and minor flavonoids were sinensetin, nobiletin, and tangeretin. Eriocitrin, hesperidin, and diosmin were mainly found in lemon (Dong et al., 2019). While low quantities of naringin were found in lemon, sweet orange, and lime, hesperidin was found in high (Khan and Dangles, 2014). These results indicated that hesperidin was the main flavonoid compound in citrus fruits and the amount and type of flavanones were depended on the species, parts, and growing stage of mandarins.

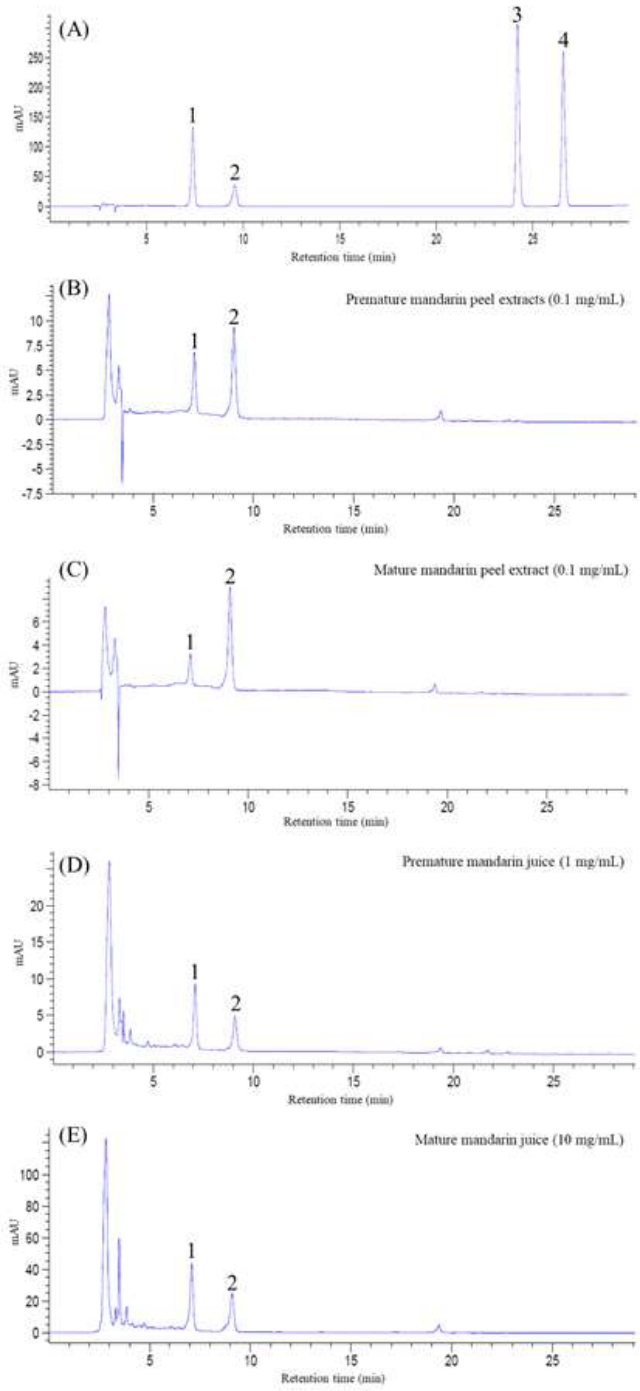

Fig. 1. HPLC of (A) flavanone compounds, (B) premature mandarin peel extract, (C) mature mandarin peel extract, (D) premature mandarin juice, and (E) mature mandarin juice (1. narirutin, 2. hesperidin, 3. naringenin, and 4. hesperetin).

Table 2. Flavanone compounds identified in premature and mature mandarin peel and juice

Mandarin extract[1)]	Flavanone compound (mg/g)				
	Hesperidin	Hesperetin	Narirutin	Naringenin	Total
PMP	76.81±7.32[2)a3)]	-[4)]	51.35±3.96[a]	-	128.16±11.29[a]
MP	62.12±4.28[b]	-	24.00±1.49[b]	-	86.12±5.58[b]
PMJ	3.48±0.20[c]	-	6.84±0.30[c]	-	10.32±0.50[c]
MJ	2.25±0.01[c]	-	3.08±0.01[c]	-	5.33±0.01[c]

1) PMP: premature mandarin peel methanol extract, MP: mature mandarin peel methanol extract, PMJ: premature mandarin juice, MJ: mature mandarin juice

2) Each value is mean±standard deviation.

3) Means with different letters (a, b et al.) in a column are significantly different at $p<0.05$.

4) '-'means not detected.

3.3. *In vitro* antioxidant activities of premature and mature extracts and flavanone standards

In vitro antioxidant activities including DPPH radical, $ABTS^+$ radical, hydrogen peroxide, hydroxyl radical scavenging, superoxide dismutase activity, and reducing power of the PMP, MP, PMJ, and MJ at 1 mg/mL and flavanone standard compounds, hesperidin, hesperetin, narirutin, and naringenin at 0.1 mg/mL were shown in Table 3.

The DPPH radical scavenging activity of the PMP, MP, PMJ, and MJ was 30.39, 27.67, 26.52, and 16.74%, respectively. The flavanone standard compounds of hesperidin, hesperetin, narirutin, and naringenin showed 13.65, 24.95, 4.32, and 5.34% of DPPH radical scavenging activity. The $ABTS^+$ radical scavenging activity of the PMP, MP, PMJ, and MJ was determined to 24.91, 24.03, 8.77, and 5.82%, respectively, and that of hesperidin, hesperetin, narirutin, and naringenin was 19.49, 36.63, 2.99, and 12.12%, respectively. The DPPH and $ABTS^+$ radical scavenging activities of premature mandarins were higher than mature mandarins; however, there were no significant difference in $ABTS^+$ radical scavenging activity of PMP and MP ($p>0.05$). The mandarin peels had higher radical scavenging activities than mandarin juices did, but there were no significant differences in DPPH radical scavenging in PMJ and MP ($p>0.05$). When comparing DPPH and $ABTS^+$ radical scavenging activities of flavanone standards, hesperetin, an aglycone form of hesperidin was the highest. And naringenin showed higher radical scavenging activity than narirutin, a glycoside. Glycosylated flavanones, such as hesperidin and narirutin, made reduce antioxidant activity (Di Mazo et al., 2005; Wang et al., 2018).

Hydrogen peroxide, one of reactive oxygen species (ROS), is generated from various cellular processes by oxygen molecules and relatively unreactive non-radical; however, it causes oxidative stress because it can be changed

into deleterious product, hydroxyl radical (• OH) by Fenton reaction in the presence of Fe^{2+}. Therefore, scavenging hydrogen peroxide by antioxidants can prevent harmful reactions initiated by hydroxyl radical (Boligon et al., 2014; Lee et al., 2019). Hydrogen peroxide scavenging activities of mandarin peel extract and juice were in the range of 44.90 to 98.20 % (Table 3). The hydrogen peroxide scavenging activity of PMP (81.52%) was lower than that of MP (93.24%) and PMJ (51.39%) was higher than MJ (44.90%), which exhibited higher scavenging activity in mandarin peel extracts than those in juice. Hydrogen peroxide scavenging activities of flavanone standard components were in 98.20 to 61.49% and those of hesperidin and hesperetin were higher than narirutin and naringenin. The contents of hesperidin and narirutin separated from the premature mandarin peels were more correlated with hydrogen peroxide scavenging activity than those with DPPH and $ABTS^{+}$ radical scavenging activity (Kim and Lim, 2020b). Similarly, in the current study, hydrogen peroxide scavenging activity of hesperidin and narirutin was higher than DPPH and $ABTS^{+}$ radical scavenging activities.

Hydroxyl radical produced by the Fenton reaction is known as the most reactive and damaging ROS to cause oxidative stress to cellular biomolecules, such as DNA, protein, and lipid (Boligon et al., 2014; Lee et al., 2019). To scavenge hydroxyl radical, very high concentrations of antioxidants are may be required (Boligon et al., 2014). Hydroxyl radical scavenging activities of mandarin peel extracts (PMP and MP) and flavanone standard components were determined to over 90%; however, those of mandarin juice were very low, 14.35 (PMJ) and 16.84% (MJ). The hydroxyl radical scavenging activities of hesperidin, hesperetin, narirutin, and naringenin were as high as the mandarin peel extracts, which contained high concentrations of hesperidin and narirutin. The correlation between phenol content separated from premature mandarin pomace and hydroxyl radical scavenging activity was reported to be greater than 0.9 (Hayat et al., 2010). Additionally, the correlation between

hesperidin and narirutin contents separated from mandarin peel and hydroxyl radical scavenging activity was 0.940 and 0.787 showing high correlation with hesperidin contents (Kim and Lim, 2020b). Therefore, it is thought that mandarin peel extracts rich in hesperidin and narirutin could effectively scavenge hydroxyl radicals.

Superoxide dismutase (SOD) converts superoxide anion into molecular oxygen and hydrogen peroxide to prevent the action of superoxide anion in the body (Spagnol et al., 2019). The SOD activity of mandarin peel extracts was 44.31 (PMP) and 34.09% (MP) and that of mandarin juice was 11.05 (PMJ) and 4.87% (MJ). The mandarin peels showed higher SOD activity than mandarin juices did. When compared to those of premature and mature mandarins, premature showed higher SOD activity than mature. Among flavanone compounds, hesperetin presented the highest SOD activity, 33.93% while other three components ranged in 25.34-26.69%. The SOD activities of flavanone compounds were not significantly different except for hesperetin. Kim et al. (2009) study showed a high correlation between TPC and superoxide anion radical scavenging activity. In addition, Kim and Lim (2020b) study reported a high correlation between hesperidin and narirutin contents and superoxide anion scavenging activity. In this study, high SOD activity in the mandarin peel extracts, which had high TPC and high concentrations of hesperidin and narirutin were exhibited. As shown the results of previous studies and current study, the SOD activity of the premature mandarin peel extract possibly has a high correlation with TPC, hesperidin, or narirutin concentration.

Reducing power, which measures the transition of Fe^{3+} complex to Fe^{2+} form by reducers, indicates the electron donating capacity of the antioxidant (Sharma et al., 2018). Reducing power of mandarin peel extracts (PMP and MP, 0.93 and 0.82) were much high compared to mandarin juices (PMJ and MJ, 0.38 and 0.23) and flavanone standard components (0.19-0.34) (Table 3).

Similar to our results, Guimarães et al. (2010) reported mandarin peels gave better reducing power than mandarin juices. Kim and Lim (2020b) reported that the correlation between hesperidin or narirutin contents and the reducing power was the lowest among *in vitro* antioxidant activities. Similarly, in this study, among flavanone compounds hesperidin showed the highest reducing power, but its activity was lower than the results of other *in vitro* antioxidant activities.

Mandarin peel extracts and juice, and flavanone compounds showed very high scavenging activity for hydrogen-containing radicals (hydrogen peroxide and hydroxyl radical). However, nitrogen-containing radical scavenging (DPPH and $ABTS^{+}$ radical, and reducing power of ($Fe(CN)_6^{3-}$)) and SOD activity were relatively low. In addition, the Fe^{2+} ion chelating activity was not be detected (data not shown). The main structural features required for efficient radical scavenging of flavonoids could be summarized: (1) an ortho-dihydroxy (catechol) structure, (2) 2,3-double bond in conjugation with a 4-oxo function in the C ring, and (3) hydroxyl groups at positions 3 and 5 (Croft, 2006; Procházková et al., 2011). The presence of catechol in the B ring in hesperidin allowed it to have higher antioxidant activity than narirutin (Kim and Lim, 2020; M'hiri et al., 2017). The structure of flavonoids for metal ion chelating is the catechol moiety in the B ring, 3-hydroxyl and 4-oxo groups in the heterocyclic ring C, and 4-oxo and 5-hydroxyl groups between C and A rings (Pietta, 2000; Procházková et al., 2011). Because of the features of flavonoid structure, the metal chelating efficiencies of premature and mature mandarin peel extracts and flavanone compounds were very limited (data not shown). These results indicated that premature and mature mandarin peel extracts as antioxidants more defended hydrogen-containing radicals than nitrogen-containing radicals, superoxide anion, reducing power, or metal chelating.

Table 3. *In vitro* antioxidant activities of premature and mature mandarin peel and juice, and flavanone compounds

		In vitro antioxidant activity[1)]					
		DPPH radical scavenging activity (%)	$ABTS^{+}$ radical scavenging activity (%)	Hydrogen peroxide scavenging activity (%)	Hydroxyl radical scavenging activity (%)	SOD activity (%)	Reducing power (Abs)
Mandarin extract	PMP	30.39±1.70$^{a2)3)}$	24.91±0.73^{b}	81.52±1.00^{c}	92.74±0.23^{a}	44.31±1.11^{a}	0.93±0.05^{a}
	MP	27.67±0.99^{b}	24.03±0.77^{b}	93.24±0.43^{b}	92.13±1.24^{ab}	34.07±1.31^{b}	0.82±0.03^{b}
	PMJ	26.52±1.53^{b}	8.77±0.72^{e}	51.39±2.56^{f}	14.35±1.83^{d}	11.05±0.67^{d}	0.38±0.02^{c}
	MJ	16.74±0.53^{c}	5.82±1.08^{f}	44.90±0.26^{g}	16.84±2.26^{c}	4.87±1.40^{e}	0.23±0.02^{e}
Flavanone compound	Hesperidin	13.65±1.03^{d}	19.49±1.38^{c}	98.20±0.17^{a}	90.97±1.23^{ab}	25.34±0.68^{c}	0.26±0.03^{d}
	Hesperetin	24.95±2.01^{b}	36.63±0.67^{a}	96.41±0.43^{a}	90.07±1.03^{b}	33.83±1.33^{b}	0.34±0.04^{c}
	Narirutin	4.32±0.39^{e}	2.99±0.07^{g}	74.64±1.02^{d}	91.78±0.78^{ab}	25.58±1.00^{c}	0.19±0.01^{e}
	Naringenin	5.34±0.37^{e}	12.12±0.12^{d}	61.49±0.63^{e}	92.16±0.67^{ab}	26.69±0.44^{c}	0.20±0.01^{e}

[1)] The concentration of mandarin extracts and flavanone compounds was 1 mg/mL and 0.1 mg/mL, respectively.

[2)] PMP: premature mandarin peel methanol extract, MP: mature mandarin peel methanol extract, PMJ: premature mandarin juice, MJ: mature mandarin juice

[3)] Each value is mean±standard deviation

[4)] Means with different letters (a, b et al.) in a row are significantly different at $p<0.05$.

3.4. The IC_{50} of *in vitro* antioxidant activity in premature and mature mandarin extracts

The half maximal inhibitory concentrations (IC_{50}) for DPPH and $ABTS^+$ radical, hydrogen peroxide, and hydroxyl radical scavenging activities of premature and mature mandarin extracts were shown in Table 4. The IC_{50} values of DPPH radical scavenging activity for PMP, MP, PMJ, and MJ were 4.06, 4.22, 4.16, and 6.25 mg/g, respectively. There were no significant differences in the IC_{50} of DPPH radical scavenging activity in the PMP, PMJ, and MP (p>0.05). The IC_{50} values of $ABTS^+$ radical scavenging activity were 5.12, 5.49, 7.99, and 13.06 mg/g for PMP, MP, PMJ, and MJ, respectively, which were similar to the tendencies of $ABTS^+$ radical scavenging activities at 1 mg/mL (Table 3). The mandarin peels showed lower IC_{50} than mandarin juices that means mandarin peels scavenged more effectively $ABTS^+$ radicals. When compared to the IC_{50} of hydrogen peroxide scavenging activity, they were very low range, 0.47, 0.47, 0.91, and 1.69 mg/g for PMP, MP, PMJ, and MJ, respectively. Mandarin juices were needed to twice as much as mandarin peel extracts to inhibit hydrogen peroxide. For hydroxyl radical scavenging activity, the IC_{50} values of premature and mature peel extracts (PMP and MP) were 3.87 and 16.93 μg/g, which were very low concentrations. The PMP was the highest activity in hydroxyl radical scavenging activity and the MJ was the lowest, which meant that mandarin peel extracts were highly efficient to scavenge hydroxyl radicals.

Overall, the ROS (e.g., hydrogen peroxide, hydroxyl radical, and superoxide anion radical) scavenging activities of premature mandarin peel extracts were significantly higher than mature mandarin peel extracts and mandarin juices. Moulehi et al. (2012) reported that premature bitter orange and mandarin showed higher DPPH radical scavenging activity than matures. Hwang et al. (2020) also reported that DPPH and $ABTS^+$ radical scavenging activities of

blueberry showed higher values in the premature stage than in the mature stage. With the results of previous studies and the current study, premature mandarin possibly possessed high antioxidant activities compared to mature one.

Table 4. Half maximal inhibitory concentration (IC_{50}) of premature and mature mandarin peel extract and juice for radical scavenging activity

Mandarin extract[1)]	IC_{50} (mg/g)			
	DPPH radical scavenging activity	$ABTS^{+}$ radical scavenging activity	Hydrogen peroxide scavenging activity	Hydroxyl radical scavenging activity
PMP	4.06±0.02[2)a3)]	5.12±0.07[a]	0.47±0.02[a]	3.87±0.00(μg/g)[4)a]
MP	4.22±0.10[a]	5.49±0.06[b]	0.47±0.01[a]	16.93±0.43(μg/g)[a]
PMJ	4.16±0.12[a]	7.99±0.05[c]	0.91±0.01[b]	10.19±0.30[b]
MJ	6.25±0.18[b]	13.06±0.14[d]	1.69±0.04[c]	51.48±1.80[c]

[1)] PMP: premature mandarin peel methanol extract, MP: mature mandarin peel methanol extract, PMJ: premature mandarin juice, MJ: mature mandarin juice

[2)] Each value is mean±standard deviation

[3)] Means with different letters (a, b et al.) in a row are significantly different at $p<0.05$.

[4)] The IC_{50} of hydroxyl radical scavenging activity of mandarin peel extract was expressed as μg/g because the concentration was low.

3.4. Effect of premature and mature mandarin peel extracts on lipid oxidation of HO/W emulsions

To investigate that premature and mature mandarin peel extracts acted as antioxidants on the lipid oxidation, the TBARS of the HO/W emulsion added with PMP and MP at 0.5 mg/mL or 1 mg/mL during storage at 40 °C for 30 days in the dark were determined and shown in Fig. 2. The HO/W emulsions added with different types of antioxidants showed the particle size of 3.07 to 9.16 μm immediately after preparation and they were remained to the size of 3.17 to 8.61 μm after storage of 30 days (data not shown). Osborn and Akoh (2004) reported that the particle size of emulsion was not significantly affected by the lipid oxidation. It indicated that the lipid oxidation of HO/W emulsion occurred during storage but not changed to its particle size. The TBARS of the HO/W emulsions added with α-tocopherol, Trolox, PMP, and MP were increased during storage of 30 days. When stored for 30 days, the TBARS values of the HO/W emulsion with different antioxidants were in the range of 15.68 to 77.84 μmol/kg oil. Among all treatments, the TBARS of the HO/W emulsion with α-tocopherol at 0.2 mg/mL were remained high and the emulsion with Trolox low during storage. The HO/W emulsions with MP and PMP had lower TBARS values than the control. The MP and PMP at either 0.5 or 1.0 mg/mL was tended to act as an antioxidant similar to Trolox at 0.2 mg/mL. Compared to MP, the TBARS of the HO/W emulsion with PMP were high. Overall, while MP and PMP acted as antioxidants in the O/W emulsion system and inhibited lipid oxidation, α-tocopherol at 0.2 mg/mL, which is known as a common antioxidant, acted as a pro-oxidant.

Lipid oxidation is an inevitable chemical reaction in lipid-contained products during production and storage. The loss of nutritional value, reduction of shelf-life, and the changes in color, aroma, and texture are attributed to the lipid oxidation (Agregán et al., 2017; Gim et al., 2018; Noon et al., 2020).

Lipid oxidation is deteriorative to not only bulk oil system but also O/W emulsions which mostly occur at the interfaces of water and oil in O/W emulsions (McClements and Decker, 2000). Adding antioxidants is one of the practical strategies to prevent and inhibit lipid oxidation (Gim et al., 2018). The type and concentration of antioxidants are known to be an important factor in determining whether they possess antioxidant or pro-oxidant activity (Noon et al., 2020). Especially, for the O/W emulsion, the antioxidants possess amphiphilic characteristics and properly located to the surface of oil and water (McClements and Decker, 2000; Kim et al., 2012). All mandarin flavonoids are known to work as antioxidants in hydrophilic environments and narirutin is known to act as a pro-oxidant in hydrophobic environments (Finotti and Di Major, 2003). Hydrophobic and hydrophilic antioxidants inhibit lipid oxidation more effectively in O/W emulsion systems and bulk oil, respectively (Kim et al., 2012). However, in this study, α-T promoted oxidation more than Trolox. Park et al. (2018) reported that horse oil contains 9.5 mg/kg of α-T. Park (2020) reported that higher concentrations of α-T acted as a pro-oxidant in HO/W emulsion. Therefore, in this study, α-T present in horse oil increased the concentration of a-T in the final manufactured HO/W emulsion, and it is thought that acted as a pro-oxidant.

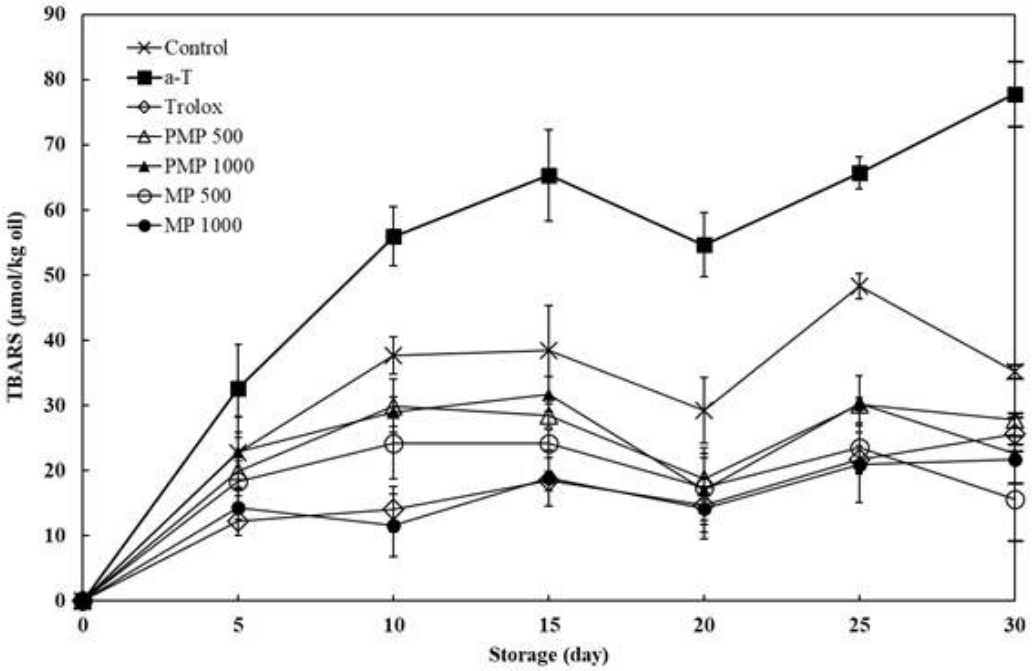

Fig. 2. Effects of premature and mature mandarin peel on the TBARS in HO/W emulsions during storage at 40℃ for 30 days.

4. Conclusions

In this study, *in vitro* antioxidant activity of premature and mature mandarin extracts were investigated. The TPC and TFC of mandarin peel extracts and juices ranged from 31.20 to 94.04 mg GAE/g and 0.09 to 43.99 mg QE/g, respectively. The flavanone contents of PMP, MP, PMJ, and MJ were quantified to 128.16, 86.12, 10.32, and 5.33 mg/g, respectively. Hesperidin and narirutin, a glycoside form of hesperetin and naringenin, were mainly identified in premature and mature mandarin peel extracts and juices and hesperetin and naringin were not detected. The nitrogen-containing (DPPH and $ABTS^{+}$) radicals scavenging activity of mandarin extracts was highest in premature mandarin peel extract and the lowest in mature mandarin juice. The hydrogen-containing radicals (hydrogen peroxide and hydroxyl radical) scavenging activity of mandarin extracts and flavanone compounds were higher than other *in vitro* antioxidant activities because the flavanone compounds present in mandarin had a high effect on scavenging hydrogen-containing radicals. The SOD activity of mandarin extracts was higher than nitrogen-containing radicals scavenging activity, but lower than hydrogen-containing radicals scavenging activity. The SOD activity was no significant difference of flavanone compounds except from hesperetin. The reducing power of mandarin peel extracts were higher than mandarin juices, premature mandarins were higher than mature mandarins, and hesperidin and hesperetin were higher than narirutin and naringenin. The *in vitro* antioxidant activities is expected to be highly correlated with total phenolic and flavonoid contents and flavanone concentrations.

To confirm the effectiveness of the premature and mature mandarin peel extracts, which showed the highest antioxidant activity among mandarin extracts, it was added to a HO/W emulsion and its oxidation stability was

investigated. The TBARS of HO/W emulsion added with PMP and MP were higher than Trolox at 5 days, however lower than Trolox on the 30 days. The PMP and MP acted as antioxidants to suppress lipid oxidation. The HO/W emulsion added α-T at 0.2 mg/mL was shown the highest TBARS during storage, α-T was acted pro-oxidant.

In conclusion, premature mandarin and mature mandarin peels, which are by-products of mandarin, can be a rich source of bioactive compounds and used as a natural antioxidant in the food, cosmetics, and pharmaceutical industries.

국문요약

본 연구에서는 제주산 풋귤과 완숙귤 추출물의 *in vitro* 항산화 활성을 평가하였다. 귤 껍질 추출물(PMP와 MP)과 주스(PMJ와 MJ)의 총 폴리페놀 및 플라보노이드 함량은 31.20-94.04mg GAE/g, 0.09-43.99mg QE/g이었다. PMP, MP, PMJ, MJ의 flavanone 함량은 각각 128.16, 86.12, 10.32, 5.33mg/g로 정량화되었다. 풋귤과 완숙귤에서는 배당체 형태인 hesperidin과 narirutin이 검출되었으며, 비배당체 형태인 hesperetin과 naringenin는 검출되지 않았다. DPPH와 $ABTS^{+}$ 라디칼 소거 활성은 PMP에서 가장 높았고 MJ에서 가장 낮았다. Hesperidin은 narirutin 보다 높은 소거 활성을 보였으며, 배당체 flavanone보다 비배당체 flavanone이 더 높은 소거 활성을 보였다. 풋귤, 완숙귤, flavanone화합물의 hydrogen peroxide와 hydroxyl 라디칼 소거 활성은 다른 *in vitro* 항산화 활성보다 높았다. 풋귤과 완숙귤 추출물의 SOD 활성은 DPPH와 $ABTS^{+}$ 라디칼 소거 활성보다 높았지만 hydrogen peroxide와 hydroxyl 라디칼 소거 활성보다는 낮았다. Hesperetin을 제외한 flavanone 화합물의 SOD 활성은 유의적인 차이가 없었다. PMP와 MP의 환원력은 PMJ와 MJ보다 높았으며, 풋귤이 완숙귤보다 높은 환원력을 보였고, hesperidin과 hesperetin이 narirutin과 naringenin 보다 높았다. 결론적으로 *in vitro* 항산화 활성은 귤의 총 폴리페놀 및 플라보노이드 함량과 flavanone 농도와 높은 상관관계가 있을 것으로 예상된다.

풋귤과 완숙귤 추출물의 천연 항산화제로서의 효능을 확인하기 위해 *in vitro* 항산화 활성이 높았던 껍질 추출물(PMP와 MP)을 HO/W 에멀션에 첨가하여 지질 산화 안정성을 조사하였다. .

저장 초기에는 PMP와 MP가 첨가된 HO/W 에멀션의 TBARS는 Trolox보다 높았으나, 30일 저장 후에는 α-T, Trolox가 첨가된 HO/W 에멀션과 Control보다 TBARS가 낮았다. 따라서 PMP와 MP는 지질 산화를 억제하는 항산화제 역할을 했다. 0.2 mg/mL 농도의 α-T가 첨가된 HO/W 에멀션은 저장 기간 중 가장 높은 TBARS를 보였으며, α-T는 HO/W에서 pro-oxidant로 작용하였다. 동일한

농도의 Trolox를 첨가한 HO/W 에멀션은 α-T가 첨가된 HO/W 에멀션보다 지질 산화 억제 효과가 높았다.

결론적으로 귤을 생산하고 가공하는 과정에서 발생되는 풋귤과 완숙귤 껍질은 기능성 화합물의 풍부한 공급원이 될 수 있으며, 높은 항산화 활성을 나타내어 식품, 화장품, 제약 산업에서 천연 항산화제로 사용될 수 있는 가능성을 확인하였다.

REFERENCES

Agregán R, Lorenzo JM, Munekata PE, Dominguez R, Carballo J, Franco D. Assessment of the antioxidant activity of Bifurcaria bifurcata aqueous extract on canola oil. Effect of extract concentration on the oxidation stability and volatile compound generation during oil storage. Food Res. Int. 99: 1095-1102 (2017)

Barauskaite D, Gineikiene J, Fennis BM, Auruskeviciene V, Yamaguchi M, Kondo N. Eating healthy to impress: How conspicuous consumption, perceived self-control motivation, and descriptive normative influence determine functional food choices. Appetite. 131: 59-67 (2018)

Boligon AA, Machado MM, Athayde ML. Technical evaluation of antioxidant activity. Med Chem. 4: 517-522 (2014)

Carbonell-Caballero J, Alonso R, Ibañez V, Terol J, Talon M, Dopazo J. A phylogenetic analysis of 34 chloroplast genomes elucidates the relationships between wild and domestic species within the genus Citrus. Mol. Biol. Evol. 32: 2015-2035 (2015)

Chen Q, Wang D, Tan C, Hu Y, Sundararajan B, Zhou Z. Profiling of Flavonoid and Antioxidant Activity of Fruit Tissues from 27 Chinese Local Citrus Cultivars. Plants. 9: 196 (2020)

Chen X, Ding Y, Forrest B, Oh J, Boussert SM, Hamann MT. Lemon yellow# 15 a new highly stable, water soluble food colorant from the peel of Citrus limon. Food Chem. 270: 251-256 (2019)

Cho MJ, Kim HJ. Effects of rendering and α-tocopherol addition on the oxidative stability of horse fat. Food Sci. Biotechnol. 29: 169-177 (2020)

Choi MH, Kim KH, Yook HS. Antioxidant and antibacterial activity of premature mandarin. J. Korean Soc. Food Sci. Nutr. 48: 622-629 (2019)

Choi SY, Ko HC, Ko SY, Hwang JH, Park JG, Kang SH, Han SH, Yun SH,

Kim SJ. Correlation between flavonoid content and the NO production inhibitory activity of peel extracts from various citrus fruits. Biol. Pharm. Bull. 30: 772-778 (2007)

Croft KD. The Chemistry and Biological Effects of Flavonoids and Phenolic Acids a. Ann. N. Y. Acad. Sci. 854: 435-442 (1998)

Di Majo D, Giammanco M, La Guardia M, Tripoli E, Giammanco S, Finotti E. Flavanones in Citrus fruit: Structure - antioxidant activity relationships. Food Res. Int. 38: 1161-1166 (2005)

Dong X, Hu Y, Li Y, Zhou Z. The maturity degree, phenolic compounds and antioxidant activity of Eureka lemon [Citrus limon (L.) Burm. f.]: A negative correlation between total phenolic content, antioxidant capacity and soluble solid content. Sci. Hortic-Amsterdam. 243: 281-289 (2019)

Finotti E, Di Majo D. Influence of solvents on the antioxidant property of flavonoids. Nahrung. 47: 186-187 (2003)

Gim SY, Jung J, Kwon Y, Kim MJ, Kim G, Lee J. Effects of chitosan and collagen containing α-tocopherol on the oxidative stability in bulk oil and oil-in-water emulsion. Food sci. Biotechnol. 27: 947-956 (2018)

Guimarães R, Barros L, Barreira JC, Sousa MJ, Carvalho AM, Ferreira IC. Targeting excessive free radicals with peels and juices of citrus fruits: grapefruit, lemon, lime and orange. Food and Chem. Toxicology. 48: 99-106 (2010)

Hayat K, Zhang X, Farooq U, Abbas S, Xia S, Jia C, Zhong F, Zhang J. Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. Food Chem. 123: 423-429 (2010)

Huang X, Zhu J, Wang L, Jing H, Ma C, Kou X, Wang H. Inhibitory mechanisms and interaction of tangeretin, 5-demethyltangeretin, nobiletin, and 5-demethylnobiletin from citrus peels on pancreatic lipase: Kinetics, spectroscopies, and molecular dynamics simulation. Int. J. Biol. Macromol. 164: 1927-1938 (2020)

Hwang H, Kim YJ, Shin Y. Assessment of Physicochemical Quality, Antioxidant Content and Activity, and Inhibition of Cholinesterase between Unripe and Ripe Blueberry Fruit. Foods. 9: 690 (2020)

Khan MK, Dangles O. A comprehensive review on flavanones, the major citrus polyphenols. J. Food Compos. Anal. 33: 85-104 (2014)

Kim DS, Lim SB. Semi-Continuous Subcritical Water Extraction of Flavonoids from Citrus unshiu Peel: Their Antioxidant and Enzyme Inhibitory Activities. Antioxidants. 9: 360 (2020a)

Kim DS, Lim SB. Extraction of flavanones from immature Citrus unshiu pomace: process optimization and antioxidant evaluation. Sci. Rep-UK. 10: 1-13 (2020b)

Kim JH, Kim MY. The potential use of citrus juice waste as sources of natural phenolic antioxidants. J. App. Pharm. Sci. 6: 202-205 (2016)

Kim YD, Ko WJ, Koh KS, Jeon YJ, Kim SH. Composition of flavonoids and antioxidative activity from juice of Jeju native citrus fruits during maturation. J. Nutr. Health. 42: 278-290 (2009a)

Kim YD, Mahinda S, Koh KS, Jeon YJ, Kim SH. Reactive oxygen species scavenging activity of Jeju native citrus peel during maturation. J. Korean Soc. Food Sci. Nutr. 38: 462-469 (2009b)

Lee JH, Kim HJ, Jee Y, Jeon YJ, Kim HJ. Antioxidant potential of Sargassum horneri extract against urban particulate matter-induced oxidation. Food Sci. Biotechnol. 29:855-865 (2020)

Li S, Pan MH, Lo CY, Tan D, Wang Y, Shahidi F, Ho CT. Chemistry and health effects of polymethoxyflavones and hydroxylated polymethoxyflavones. J. Funct. Foods. 1: 2-12 (2009).

M'hiri N, Irina I, Cédric P, Ghoul M, Boudhrioua N. Antioxidants of Maltease orange peel: comparative investigation of the efficiency of four extraction methods. J. Appl. Pharm. Sci, 7: 126-135 (2017).

McClements DJ, Decker EA. Lipid oxidation in oil in water emulsions:

Impact of molecular environment on chemical reactions in heterogeneous food systems. J. Food Sci. 65: 1270-1282 (2000)

McDonald RE, Hultin HO. Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of flounder skeletal muscle. J. Food Sci. 52: 15-21 (1987)

Moon SH, Assefa AD, Ko EY, Park SW. Comparison of flavonoid contents and antioxidant activity of yuzu (Citrus junos Sieb. ex Tanaka) based on harvest time. Kor. J. Hort. Sci. Technol. 33:283-291(2015)

Moulehi I, Bourgou S, Ourghemmi I, Tounsi MS. Variety and ripening impact on phenolic composition and antioxidant activity of mandarin (Citrus reticulate Blanco) and bitter orange (Citrus aurantium L.) seeds extracts. Ind. Crop. Prod. 39: 74-80 (2012)

Multari S, Licciardello C, Caruso M, Martens S. Monitoring the changes in phenolic compounds and carotenoids occurring during fruit development in the tissues of four citrus fruits. Food Res. Int. 134: 109228 (2020)

Negro V, Mancini G, Ruggeri B, Fino D. Citrus waste as feedstock for bio-based products recovery: Review on limonene case study and energy valorization. Bioresource Technol, 214: 806-815 (2016)

Noon J, Mills TB, Norton IT. The use of natural antioxidants to combat lipid oxidation in O/W emulsions. J. Food Eng. 281: 110006 (2020).

Osborn HT, Akoh CC. Effect of emulsifier type, droplet size, and oil concentration on lipid oxidation in structured lipid-based oil-in-water emulsions. Food Chem. 84: 451-456 (2004)

Park YH. Emulsion characteristics and oxidative stability of horse oil-in-water emulsion as impacted by HLB system and α-tocophrol addition. MS thesis, Jeju National Universitiy, Jeju, Korea (2020)

Park B, Choi JW, Kim SH, Yun YR, Jung JH. Antioxidant activity of premature mandarin vinegar according to harvest period and raw material conditions. Korean J. Food Sci. Technol. 52: 333-341 (2020)

Park GH, Lee SH, Kim HY, Jeong HS, Kim EY, Yun YW, Nam SY, Lee BJ. Comparison in antioxidant effects of four citrus fruits. J. Fd. Hyg. Safety. 26: 355-360 (2011)

Park, Y. H., Cho, M. J., & Kim, H. J. (2018). Effects of α-, ɣ-, and δ-tocopherol on the oxidative stability of horse fat. Korean Journal of Food Science and Technology, 50(3), 267-273.

Pietta PG. Flavonoids as antioxidants. J. Nat. Prod. 63: 1035-1042 (2000)

Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 82: 513-523 (2011)

Sharma S, Saxena DC, Riar CS. Changes in the GABA and polyphenols contents of foxtail millet on germination and their relationship with in vitro antioxidant activity. Food Chem. 245: 863-870 (2018)

Shin Y, Ryu JA, Liu RH, Nock JF, Watkins CB. Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity, and inhibition of cell proliferation of strawberry fruit. Postharvest Biol. Tec. 49: 201-209 (2008)

Spagnol CM, Assis RP, Brunetti IL, Isaac VLB, Salgado HRN, Corrêa MA. *In vitro* methods to determine the antioxidant activity of caffeic acid. Spectrochim. Acta. A. 219: 358-366 (2019)

Sun Y, Wang J, Gu S, Liu Z, Zhang Y, Zhang X. Simultaneous determination of flavonoids in different parts of Citrus reticulata 'Chachi'fruit by high performance liquid chromatography－photodiode array detection. Molecules. 15: 5378-5388 (2010)

Sung HM, Seo YS, Yang EJ. Anti-oxidant and anti-inflammatory activities of hot water extract obtained from Geranium thunbergii using different extraction temperatures and times. J. Korean Soc. Food Sci. Nutr. 47: 1006-1013 (2018).

Wang TY, Li Q, Bi KS. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian J. Pharm. Sci. 13: 12-23 (2018)

Xi W, Lu J, Qun J, Jiao B. Characterization of phenolic profile and antioxidant capacity of different fruit part from lemon (Citrus limon Burm.) cultivars. J. Food Sci. Technol. 54: 1108-1118 (2017)

Yang CS, Ho CT, Zhang J, Wan X, Zhang K, Lim J. Antioxidants: Differing meanings in food science and health science. J. Agr. Food Chem. 66: 3063-3068 (2018)

Yi L, Ma S, Ren D. Phytochemistry and bioactivity of Citrus flavonoids: a focus on antioxidant, anti-inflammatory, anticancer and cardiovascular protection activities. Phytochem. Rev. 16: 479-511 (2017)

Zou Z, Xi W, Hu Y, Nie C, Zhou Z. Antioxidant activity of Citrus fruits. Food Chem. 196: 885-896 (2016)

감사의 글

학부 2학년부터 실험실 생활을 시작해 석사과정을 무사히 마치게 되어 감사의 글을 올리게 되었습니다. 4년간의 실험실 생활은 많은 배움과 경험을 얻을 수 있었으며, 많은 성장을 할 수 있는 계기가 되었습니다. 제 주변에서 항상 많은 가르침과 격려를 보내주신 분들께 감사의 마음을 전하고자 합니다.

먼저 많이 부족했던 학부 시절부터 저를 항상 믿어주시고, 이끌어주시고, 격려해주셔서 본 학위 논문이 완성 될 수 있게 지도해주신 제가 가장 존경하는 김현정 교수님께 감사드립니다. 또한 바쁘신 와중에도 논문 심사와 조언을 주신 고영환 교수님, 천지연 교수님께 진심으로 감사드립니다. 학부생부터 대학원까지 많은 가르침을 주신 임상빈 교수님, 박은진 교수님께도 감사드립니다.

실험실 생활을 하면서 정말 많은 도움을 주신 효진언니, 현수언니, 주희언니, 윤형오빠에게 감사드리며, 1년간 제 옆에서 항상 도와준 대동이한테 너무 고맙고 남은 시간동안 많은 것을 배우길 바랍니다. 1년 6개월 동안 함께 대학원 생활을 하면서 동생을 항상 챙겨주시고 도와주신 유리언니와 화영언니에게도 진심으로 감사드립니다. 유용한 정보와 응원으로 많은 도움을 주신 지아 조교선생님, 호정 조교선생님, 진우 조교선생님에게 감사드립니다. 또한 학석사연계과정을 같이 보낸 하영이에게 감사드립니다. 덕분에 학교생활을 즐겁게 할 수 있었으며, 하영이가 언제나 잘 되길 바랍니다. 많은 조언과 도움을 주신 동신오빠에게도 감사드립니다.

타지 생활을 하는 저를 항상 응원해주고, 모든 것에 마음을 써주고, 내편이 되어준 지현이, 정양이, 소연이, 지연이, 원희, 정민, 제원이에게 사랑하고 고마운 마음을 전합니다. 그리고 석사과정을 지내고 있어 묵묵히 응원을 해주는 은지, 혜원언니에게도 고마운 마음을 전하고, 항상 응원합니다.

마지막으로 공부하는 동안 언제나 딸의 결정을 응원해준 엄마와 원하는 공부를 할 수 있게 도와주신 아빠, 누나의 발전을 위해 충고의 말을 해주는 내 동생 호영이, 항상 건강하라고 말씀해주시고 걱정해주신 가족들에게 감사의 마음을 전하며, 이 논문을 바칩니다.

감사합니다.