Original Article
J. Korean Wood Sci. Technol. 2022, 50(4): 272-282
https://doi.org/10.5658/WOOD.2022.50.4.272

Journal of the Korean Wood Science and Technology
pISSN: 1017-0715 eISSN: 2233-7180
http://www.woodj.org

Antioxidant Activities of Essential Oils from *Citrus* × *natsudaidai* (Yu. Tanaka) Hayata Peels at Different Ripening Stage

Jiyoon YANG[1] · Won-Sil CHOI[2] · Su-Yeon LEE[1] · Minju KIM[3] · Mi-Jin PARK[1,†]

ABSTRACT

The essential oil extracted from *Citrus* × *natsudaidai* (Yu. Tanaka) Hayata peels is known to have various biological properties. However, the chemical composition of essential oil is influenced by the ripening stages of fruits, which then affects related biological activities. This study investigates the antioxidant activities of essential oils extracted from *Citrus* × *natsudaidai* peels at different ripening stages (immature, mature, and overripe). The essential oils were extracted using the hydro-distillation method. As a result of gas chromatography-mass spectrometry (GC-MS) analysis, d-limonene was dominant and was increased as matured. However, γ-terpinene was decreased. The antioxidant properties and their total phenolic content (TPC) were influenced by the ripening stages. The TPC was highest in the immature stage of essential oil (1,011.25 ± 57.15 mg GAE/100 g). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was excellent in the immature stage (EC_{50} = 15.91 ± 0.38 mg/mL). 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity was superior in overripe stage (EC_{50} = 20.43 ± 0.37 mg/mL). The antioxidant activity measured using ferric reducing antioxidant power (FRAP) assay showed higher values for the essential oils in immaturity (1,342.37 ± 71.07 mg Fe^{2+}/100 g). Comprehensively, the essential oil in the immature stage showed the best antioxidant activity. Finally, knowing the chemical composition and antioxidant activity at different ripening stages will provide data for selecting the right fruit.

Keywords: *Citrus* × *natudaidai*, amanatsu, essential oil, antioxidant, fruit ripening

1. INTRODUCTION

Citrus spp. is a large genus of flowering plants belonging to the Rutaceae family. Citrus fruits such as lemons, grapefruits, limes, oranges, tangerines, and mandarins are the most widely cultivated fruits in the world, with production increasing every year as consumer demand increases (Khan *et al*., 2021). This is because citrus fruits provide important nutrients such as vitamin C and other health-promoting compounds, including its distinctive flavonoids, which are beneficial for human health (Liu *et al*., 2012). Therefore, they provide several nutri-

Date Received May 24, 2022, Date Revised June 13, 2022, Date Accepted July 8, 2022
[1] Division of Forest Industrial Materials, Department of Forest Products and Industry, National Institute of Forest Science, Seoul 02455, Korea
[2] National Instrumentation Center for Environmental Management, Seoul National University, Seoul 08826, Korea
[3] Citrus Research Institute, Jeju 63607, Korea
[†] Corresponding author: Mi-Jin PARK (e-mail: lionpmj@korea.kr, https://orcid.org/0000-0002-7748-3886)
© Copyright 2022 The Korean Society of Wood Science & Technology. This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

tional and health benefits and are consumed as fruits or processed products such as peels, pulps, juice, and other commercialized extracts, and used as additives in the food industry. For example, bitter orange extracts are used as additives in several beverages, desserts, sweets, drinks, and ice creams. Thus, *Citrus* has a substantial commercial value.

Biologically important active constituents of secondary metabolites are reported in this genus, such as limonoids, coumarins, carotenoids, alkaloids, polyphenols, and essential oils, which can be used in food, cosmetic and pharmaceutical industries (Lv *et al*., 2015). Among them, essential oils have various biological activities such as antioxidant, antibacterial, anti-anxiety, anti-inflammatory, anti-obesity, chemoprotective, and anti-tumor (Dosoky and Setzer, 2018; Ham *et al*., 2020; Jeong *et al*., 2017; Lee *et al*., 2021; Yang *et al*., 2019). Due to their biological properties, *Citrus* essential oils are widely used in the food preservation, perfume, pharmacological, and cosmetic industries (Palazzolo *et al*., 2013).

Citrus × *natsudaidai* (Yu. Tanaka) Hayata (natsudaidai) is a popular fruit with medical benefits in many countries. It contains numerous bioactive compounds such as vitamin C and flavonoids. Many flavonoids such as hesperidin, neohesperidin, naringin, nobiletin, tangeretin, and auraptene are obtained from natsudaidai, and have been studied for pharmacological properties such as anti-inflammatory, anticarcinogenic, and antioxidant, cytotoxic effects (Ahn *et al*., 2021; Nakayama *et al*., 2011; Yamaura *et al*., 2012). Treatment with *C.* × *natsudaidai* extract significantly attenuated the increase in ear edema and improved dermatitis scores. Additionally, the increase in serum d-ROM was attenuated by *C.* × *natsudaidai* extract. In another study, following the administration of 300 mg/kg acetaminophen, all mice died within 6 h. However, pretreatment with *C.* × *natsudaidai* extract (300 and 1,000 mg/kg) improved the survival rate to 16.7% and 33.3%, respectively, at 24 h. These results indicate that *C.* × *natsudaidai* has a relaxation effect on inflammatory.

However, it has been found that the ripening stages of fruits have influenced the chemical composition and related activities of the essential oil (El-Gamal and Ahmed, 2017; Ella Nkogo *et al*., 2022; Harhar *et al*., 2019; Manurung *et al*., 2019). Jonas's study investigated the influence of the fruit maturity stage on the physicochemical properties of Jatropha seed oil and the produced biodiesel (Jonas *et al*., 2020). This investigation revealed that free fatty acid content and peroxide value of seed oil vary as Jatropha fruits mature from green to brown dry. The free fatty acid content in Jatropha seed oil increases continuously with seed maturity. Similarly, the peroxide value of Jatropha seed oil increases gradually with fruit maturity. Results from this investigation have revealed that using seed oil from the early stages of fruit maturity can improve the quality of seed oil and hence of derived biodiesel in contrast to the final maturity stage. Therefore, further studies on the antioxidant activity of essential oils according to ripening stages are required to make optimal use of these fruits.

The study investigates the chemical components and antioxidant activities of essential oils extracted from *C.* × *natsudaidai* peels during fruit ripening. The purpose of these results is to understand the phytochemical changes that occur during the ripening stage of *Citrus* × *natsudaidai* and to recommend the best harvesting time for bioactive compounds extraction. Finally, the presented results can serve various industries as guidelines for high-quality pharmaceuticals and foods.

2. MATERIALS and METHODS

2.1. Material

Citrus × *natsudaidai* (Yu. Tanaka) Hayata peels used in this study were collected from Citrus Research Institute (National Institute of Horticultural and Herbal Science) in Jeju Island, Korea. The samples were collected

thrice (March, September, and December) according to ripening stages.

2.2. Extraction of the essential oil

To extract the essential oil from the *Citrus* × *natsu-daidai* peel with that of moisture content from 80.06% to 81.37%, we used the previously used extraction method (Yang *et al*., 2021a). *C.* × *natsudaidai* peels (2 × 2 cm) were hydro-distillated at atmospheric pressure using a Clevenger-type apparatus. A 10 L round-bottom flask containing 1.0 kg of peels was placed in a digital heating mantle (MS-DM 608, MTOPS®, Yangju, Korea), and 5 L of distilled water was poured into it, which was then connected to the Clevenger-type apparatus. The peel was extracted for 30 h. The collected essential oils were dried over anhydrous sodium sulfate (98.5%, Cat No. 834605125, Samchun, Seoul, Korea) and filtered using a 0.45 μm membrane disk filter. The essential oils were transferred to dark vials and stored at 4℃ for further analysis. The yield of the essential oils was calculated using Equation (1):

$$\text{Essential oil yield (\%(w/w))} = \frac{\text{Mass of the essential oil obtained (g)}}{\text{Mass of the oven-dry matter (g)}} \times 100 \quad (1)$$

2.3. GC-MS analysis

To identify the volatile components therein, the essential oils during fruit ripening were analyzed using GC-MS equipped with a VF-5MS capillary column (Table 1). These analysis conditions were based on our prior research (Yang *et al*., 2021b).

The components of essential oils according to maturity stage were identified on the basis of the peaks with the highest spectral matching when the S/N ratio reached ≥ 50 in total-ion chromatography using the NIST library

Table 1. GC/MS operating conditions

Column	VF-5MS capillary column (60 m × 0.25 mm, 0.25 μm; Agilent Technologies, Santa Clara, CA, USA)		
GC oven	50℃, hold 5 min 10℃/min to 65℃, hold 30 min 5℃/min to 210℃, hold 10 min 20℃/min to 325℃, hold 10 min		
Carrier gas	He (1 mL/min, 25 psi)		
Linear velocity	19.8 cm/s		
Injection mode	Split 1:20		
Injection temperature	250℃		
MS parameters		FID parameters	
MS ionization mode	EI	FID temperature	300℃
Scan time	0.2 s	Hydrogen flow	35.0 mL/min
Mass range	35-550 amu	Air flow	350.0 mL/min
Ion source temperature	270℃	Make up flow	40.0 mL/min
Interface temperature	250℃		

search program (version 11; National Institute of Standards and Technology, Gaithersburg, MD, USA). The Kovats retention index (KI) values of the individual compounds were determined based on the comparison of their relative retention times with an *n*-alkanes mixture (C_8-C_{20}, Cat No. 04071, Merck KGaA, Darmstadt, Germany) using the VF-5MS column. The essential oils' components were assigned based on the comparison of their calculated KI values to literature values (e.g., NIST Chemistry WebBook).

2.4. Determination of total phenolic content (TPC)

The TPC of the essential oils was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965). Using a standard curve as a reference, the TPC was determined and represented as mg gallic acid equivalents (mg GAE/100 g).

Ethanol (≥ 99.5%, Cat No. 459836, Merck KGaA) was used to dilute the essential oils to be analyzed. Afterward, in a 10 mL volumetric flask, 500 μL of oil was added to 2.5 mL of 2N Folin-Ciocalteu reagent (eCat No. F9252, Merck KGaA) alongside 2 mL Na_2CO_3 (7.5%, Cat No. 222321, Merck KGaA). After incubation for 60 min at 37℃, the absorbance versus prepared blank was read at 760 nm. The standard reference curve for gallic acid was made for the following concentrations: 1, 2, 4, 6, 8, and 10 mg/mL, respectively. The correlation coefficient and regression equation were determined and expressed in mg GAE/100 g. All measurements were performed in triplicates.

2.5. DPPH free-radical scavenging capacity

The free-radical scavenging assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH, Cat No. D9132, Merck KGaA) was used to examine the antioxidant properties of the essential oils. The measurement was based on the method described in (Sayuti *et al*., 2018). The essential oils were prepared by diluting 100 mg of peel oil in 1 mL of ethanol, producing a concentration of 1 mg/mL. The stock solution was sonicated to ensure sample homogeneity. Six other concentrations were prepared at 1, 2, 4, 6, 8, and 10 mg/mL, diluted from the 100 mg/mL stock solution. Approximately 1 mL of 0.2 mM solution of DPPH in ethanol was each added into six series of prepared concentrations (1, 2, 4, 6, 8, and 10 mg/mL) of the sample solution (4 mL). The solution was vigorously mixed and allowed to stand at room temperature for 30 minutes in the dark, after which its absorbance was measured spectrophotometrically at 517 nm using a Micro-plate spectrophotometer. Ethanol was used as blank (only ethanol) and negative control (4 mL ethanol mixed with 1 mL DPPH), while ascorbic acid was used as the standard.

2.6. ABTS radical scavenging assay

The scavenging activity of $ABTS^+$ was conducted to determine the radical scavenger activity. The ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), Cat No. 10102946001, Merck KGaA] radical scavenging activity of the essential oils was determined using Re *et al*. (1999) method. $ABTS^+$ was generated by reacting an $ABTS^+$ aqueous solution (7 mM) with $K_2S_2O_8$ (2.45 mM, final concentration, Cat No. 216224, Merck KGaA) in the dark for 16 h and adjusting the absorbance of 734 nm to 0.7 ± 0.02 with ethanol. The sample with different concentrations (1, 2, 4, 6, 8, and 10 mg/mL) was diluted with 0.2 mL of the required amount to react with the $ABTS^+$ solution (2 mL). After 30 min, the absorbance was measured at 734 nm. All measurements were conducted in triplicate.

2.7. Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of the samples was

determined using the ferric reducing capacity of plasma FRAP assay by Fernandes *et al.* (2016) as a measure of antioxidant capacity. The analysis was based on the compound's reducing power. A potential antioxidant can reduce the ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The latter forms a blue complex (Fe^{2+}/TPTZ) that promotes absorption at 593 nm. The FRAP reagent was prepared by mixing a solution of acetate buffer (300 mM, pH 3.6), 10 mM TPTZ (≥ 98%, Cat No. T1253, Merck KGaA) in 40 mM HCl (1 M, Cat No. H1202, TCI, Tokyo, Japan), and 20 mM $FeCl^{3+}$ (97%, Cat No. 236489, Merck KGaA) at 10:1:1 (v/v/v). Reagent and sample solution were added to each well and mixed thoroughly. After 30 min, absorbance was measured at 593 nm. A standard curve was generated using different concentrations of Trolox. All solutions were used on the day of manufacture. The results were expressed as mg Trolox equivalent/100 g. Analysis was conducted in triplicate for each essential oil.

2.8. Statistical analysis

The results of the tests were repeated in triplicate, and results were presented as mean ± standard deviations. Statistical analysis was conducted using R x64 (ver.4.0.3).

3. RESULTS and DISCUSSION

3.1. Yield and chemical composition

The yield of the essential oils largely varied with the ripening stages. Among the essential oils, immaturity showed the highest yield (5.88 ± 0.23%) followed by overripe (3.62 ± 0.12%) and maturity (2.06 ± 0.09%). According to a previous study, the yield of immature *C.* × *natsudaidai* was 0.6%, and that of matured *C.* × *natsudaidai* peel was 1.7% (Baik *et al*., 2008; Oh *et al*., 2007). Despite the same ripening stage, the essential oil was extracted using the steam distillation method for only 6 hours, resulting in a big difference in yield. This difference in yield is due to the extraction time (about 30 h).

The chemical composition of the essential oils during fruit maturation was identified by GC-MS. As shown in Table 2, the chemical composition at different stages of ripening was identified by GC-MS.

As a result, the proportion of the constituents in the essential oil was also different. D-Limonene was the major constituent in all oil samples (86.44% to 90.45%), followed by γ-terpinene and β-myrcene ranged between 4.75% to 6.32% and 1.09% to 1.30%, respectively. According to Vekiari *et al.* (2002), harvesting time is a critical parameter influencing the chemical compositions of the essential oil significantly. As *C.* × *natsudaidai* matured, the contents of β-pinene, cymene, and d-limonene were increased. On the other hand, the contents of α-terpinene, γ-terpinene, terpinolene, linalool, β-terpineol, terpinene-4-ol, α-terpineol, and octanal, were gradually decreased. Dugo *et al.* investigated the seasonal variation of the chemical composition of the essential oil extracted from two cultivars, Sicilian mandarin (*Citrus* × *deliciosa* Tenore. cv Avana and Tardivo di Ciaculli), and reported an increase of limonene content from September to February (from immature stage to mature stage) (Dugo *et al*., 2011). In contrast to the behavior observed from October to February, limonene content decreased at the overripe stage (from February to March). This result showed a similar tendency to our study result. According to Dugo *et al*., limonene is compensated by the other constituents of the monoterpene hydrocarbons, so it decreases sharply in March when each monoterpene hydrocarbon exhibits the same decreased behavior. Furthermore, it was found that the yield and composition of these essential oils were different depending on soil properties (Moretti *et al*., 1998).

3.2. Total phenolic content (TPC)

Phenolic compounds are related to several biological

Table 2. Chemical composition of the essential oils extracted from *Citrus* × *natsudaidai* peels during fruit ripening (only components with S/N > 50)

Classification	Compound name[1)]	Immaturity	Maturity	Overripe	KI[2)]
Monoterpene hydrocarbons	α-Pinene	0.57	0.46	0.52	926
	β-Pinene	0.17	0.21	0.21	970
	β-Myrcene	1.17	1.09	1.30	986
	α-Terpinene	0.17	0.10	0.08	1,024
	Cymene	0.15	0.21	0.36	1,033
	D-Limonene	86.44	90.34	90.45	1,041
	β-Ocimene	0.27	0.19	0.23	1,057
	γ-Terpinene	6.32	5.03	4.75	1,068
	Terpinolene	0.67	0.37	0.34	1,089
Oxygenated monoterpenes	Linalool oxide	0.26	0.11	0.12	1,078
	Linalool	0.32	0.17	0.11	1,099
	β-Terpineol	0.41	0.13	0.10	1,146
	Terpinen-4-ol	0.25	0.15	0.11	1,179
	α-Terpineol	2.16	0.77	0.61	1,194
Sesquiterpene hydrocarbons	β-Elemene	-	0.09	0.06	1,395
Oxygenated sesquiterpenes	(+)-Nootkatone	-	-	0.14	1,828
Organic compound	Octanal	0.15	0.04	-	1,003
Unknown compound		0.49	0.52	0.44	

[1)] Compounds are listed in order of their elution from a VF-5MS column.
[2)] Retention index experimentally determined on VF-5MS column with reference to n-alkanes (C_8–C_{20}).
KI: Kovats retention index.

roles, such as activities of antioxidant free radical scavenging (Salar and Seasotiya, 2011). We were determined to compare the TPC of essential oils according to the ripening stages.

The results, as shown in Table 3 indicated that TPC in all essential oils was seen to be significantly different amounts. Among them, the highest TPC was found in immaturity (1011.25 ± 57.15 mg/100 g) followed by overripe (961.76 ± 124.55 mg/100 g) and maturity (895.77 ± 69.99 mg/100 g). One-way ANOVA analysis was performed to prove whether TPCs of essential oils according to the ripening stage were significantly diffe-

Table 3. Total phenolic content in essential oils during fruit ripening

Maturity / Stage properties	Immaturity	Maturity	Overripe
mg GAE/100 g	1,011.25 ± 57.15	895.77 ± 69.99	961.76 ± 124.55

rent. As a result, the ripening stages do not make significant differences in TPC (P = 0.4503). The TPC contents decreased in the ripening stage but increased in overripe. That can be related to a shift in the biosynthetic pathway of secondary metabolites toward the production of essential oils in the plant. When the plant encounters oxidative stress due to a sudden change of temperature in summer and early winter, the biosynthetic pathway of secondary metabolites in the plant is directed toward the production of essential oils rather than phenolic compounds (Boveiri Dehsheikh *et al*., 2019). These phytochemical compounds support bioactivity in medicinal plants and are thus responsible for the antioxidant activities of the essential oils extracted from *C.* × *natsudaidai* peels according to ripening stages (Altemimi *et al*., 2017).

3.3. DPPH radical scavenging ability

In this analysis, the abilities of the test compounds to donate hydrogen atoms or electrons were measured spectrophotometrically. Table 4 shows the results of the DPPH inhibition assay using different essential oils and control (Ascorbic acid).

It showed that all the essential oils can reduce the pink-colored free radical to yellow-colored diphenyl picrylhydrazine, indicating that these essential oils possessed DPPH radical scavenging activity. The EC_{50} values of DPPH free-radical scavenging capacity were 15.91 ± 0.38, 16.98 ± 0.40, and 16.21 ± 0.32 mg/mL for immaturity, maturity, and overripe, respectively. The mechanism of the DPPH assay depends on the structural conformation of the antioxidant. Some compounds react very quickly with DPPH·, reducing the number of DPPH· molecules equal to the hydroxyl groups (Bondet *et al*., 1997). In Table 2, the proportion of oxygenated terpene components in the essential oil at the immaturity period is higher than in other ripening stages. In particular, the proportion of α-terpineol was high at 2.16% in the immaturity stage, but it significantly reduced as the maturation stage progressed. The proportion of other oxygenated monoterpene-based components also gradually decreased as the *C.* × *natsudaidai* matured. This was consistent with the DPPH radical scavenging ability. Also, this tendency was consistent with the change in the proportion of monoterpene components (α-pinene, β-ocimene, and linalool oxide). Choi *et al.* (2000) were investigated thirty-four species of citrus essential oils and their components for radical scavenging activities using DPPH assay (Choi *et al*., 2000). *Citrus ichangensis, Citrus* × *aurantiifolia*, and *Citrus* limon showed higher radical-scavenging activities than other citrus cultivars. These excellent radical scavenging activities were found to be γ-terpinene and terpinolene. Therefore, the high radical scavenging ability in the immature stage is due to the higher proportion of γ-terpinene and terpinolene than in other stages.

3.4. ABTS radical scavenging assay

Although the DPPH· free radical is ubiquitously used to estimate the potential free radical scavenging activity of natural products, the $ABTS^{+}$ free radical is commonly used when issues of solubility of interference arise, and the use of DPPH based assay becomes inappropriate (Medini *et al*., 2011). In Table 5, the results of ABTS

Table 4. DPPH radical scavenging effects of the essential oils extracted from *Citrus* × *natsudaidai* peels according to ripening stages

	Immaturity	Maturity	Overripe	Ascorbic acid
DPPH EC_{50} (mg/mL)	15.91 ± 0.38	16.98 ± 0.40	16.21 ± 0.32	0.064 ± 0.002

DPPH: 2,2-diphenyl-1-picrylhydrazyl.

Table 5. ABTS radical scavenging effects of essential oils extracted from *Citrus* × *natsudaidai* peels according to ripening stages

	Immaturity	Maturity	Overripe	Ascorbic acid
ABTs EC_{50} (mg/mL)	22.43 ± 0.45	26.82 ± 0.48	20.43 ± 0.37	0.066 ± 0.001

ABTS: 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid).

radical scavenging activity of peel essential oils according to ripening stages.

In the determination of ABTS radical scavenging capacity, the EC_{50} values of immaturity (22.43 ± 0.45 mg/mL), maturity (26.82 ± 0.48 mg/mL), and overripe (20.43 ± 0.37 mg/mL) were much higher than those of ascorbic acid (0.066 ± 0.001 mg/mL). The overripe stage showed the highest ABTS radical scavenging activity compared to the other stages. For ABTS, no correlation between EC_{50} and total phenols was found. However, the antioxidant activity was low in the maturity stage, as shown in the DPPH results. The ABTS radical scavenging activity was similar to the change of β-myrcene, and it is judged that β-myrcene affects the ABTS radical scavenging activity. In a previous study, rosemary essential oil had great free radical scavenging capacity; it was confirmed that myrcene contributed significantly to the free radical scavenging ability of rosemary essential oil (Ojeda-Sana *et al*., 2013). *In vivo* β-myrcene demonstrate antioxidant effect (Bonamin *et al*., 2014). The antioxidant effects of orally administered β-myrcene against ethanol-induced gastric ulcers in male Wistar rats were confirmed. The β-myrcene showed antioxidant enzyme activity from the glutathione reductase system as evidenced by the decreased activity of superoxide dismutase (SOD) and increased levels of glutathione peroxidase, glutathione reductase, and total glutathione in gastric tissue. Future studies investigating antioxidant activity of β-myrcene, need to conduct appropriate dosage levels that have therapeutic effects in humans.

3.5. Radical scavenging ability of essential oils using ferric reducing antioxidant power (FRAP)

The FRAP assay, which provides fast reproducible results, measures the reducing potentials of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to produce colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ). The results of the FRAP assay using different essential oils are shown in Table 6.

According to ripening stages, most essential oils showed reducing power from 815.21 ± 29.39 mg Fe (Ⅱ)/100 g in maturity stage to 1,417.68 ± 0.00 mg Fe (Ⅱ)/100 g in the overripe stage. The highest reducing power (FRAP) was shown in overripe and the reducing power in immaturity is comparable but less than the reducing power in overripe. This FRAP away tendency was similar as shown in the ABT results. In a previous study, the antioxidant activities of different 15 species flavonoids were measured by the FRAP assay (Firuzi *et al*., 2005). As a result, quercetin, fisetin, myricetin, and

Table 6. Radical scavenging effects of the essential oils extracted from *Citrus* × *natsudaidai* peels according to ripening stages

Stage properties \ Maturity	Immaturity	Maturity	Overripe
mg Fe(Ⅱ)/100 g	1,342.37 ± 71.07	815.21 ± 29.39	1,417.68 ± 0.00

kaempferol were the highest reducing power. It has been shown that hydroxyl groups and especially catechol moiety, 3-OH, and 2,3-double bonds are the most important factors in determining high antioxidant activity. The proportion of β-myrcene is a constituent that shows a similar tendency compared to reducing power among the components in essential oils for each maturation stage. Since β-myrcene has a 2,3-double bond structure, it is considered a component is contributing to the high antioxidant activity of essential oils in the overripe.

4. CONCLUSIONS

This study provides useful information on the ripening stages of the quality and antioxidant activities of essential oil extracted from *C.* × *natsudaidai* peels. Our results revealed that the ripening stages affect the essential oil's constituents and antioxidant activities. The total polyphenol contents are closely connected to the antioxidant activity. The relationship between polyphenol contents and antioxidant activity was observed in the results of this study. The essential oil in the immaturity stage was more susceptible to accelerated oxidation than the maturity and overripe stages. Therefore, understanding the chemical composition and antioxidant activities according to the ripening stages can help producers in selecting the best time to harvest plants and produce plant products richer in the required compounds for use in the pharmaceutical and food industries.

CONFLICT of INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENT

This research was funded by the National Institute of Forest Science grant number 'FP0701-2021-01-2021'.

REFERENCES

Ahn, C., Yoo, Y.M., Park, M.J., Ham, Y., Yang, J., Jeung, E.B. 2021. Cytotoxic evaluation of the essential oils from Korean native plant on human skin and lung cells. Journal of the Korean Wood Science and Technology 49(4): 371-383.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., Lightfoot, D.A. 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 6(4): 42.

Baik, J.S., Kim, S.S., Lee, J.A., Oh, T.H., Kim, J.Y., Lee, N.H., Hyun, C.G. 2008. Chemical composition and biological activities of essential oils extracted from Korean endemic citrus species. Journal of Microbiology and Biotechnology 18(1): 74-79.

Bonamin, F., Moraes, T.M., dos Santos, R.C., Kushima, H., Faria, F.M., Silva, M.A., Junior, I.V., Nogueira, L., Bauab, T.M., Souza Brito, A.R.M., da Rocha, L.R.M., Hiruma-Lima, C.A. 2014. The effect of a minor constituent of essential oil from *Citrus aurantium*: The role of β-myrcene in preventing peptic ulcer disease. Chemico-Biological Interactions 212: 11-19.

Bondet, V., Brand-Williams, W., Berset, C. 1997. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. LWT-Food Science and Technology 30(6): 609-615.

Boveiri Dehsheikh, A., Mahmoodi Sourestani, M., Boveiri Dehsheikh, P., Vitalini, S., Iriti, M., Mottaghipisheh, J. 2019. A comparative study of essential oil constituents and phenolic compounds of Arabian lilac (*Vitex trifolia* var. *purpurea*): An evidence of season effects. Foods 8(2): 52.

Choi, H.S., Song, H.S., Ukeda, H., Sawamura, M. 2000. Radical-scavenging activities of citrus essential oils and their components: Detection using 1,1-diphenyl-2-picrylhydrazyl. Journal of Agricultural and Food Chemistry 48(9): 4156-4161.

Dosoky, N.S., Setzer, W.N. 2018. Biological activities and safety of *Citrus* spp. essential oils. International Journal of Molecular Sciences 19(7): 1966.

Dugo, P., Bonaccorsi, I., Ragonese, C., Russo, M., Donato, P., Santi, L., Mondello, L. 2011. Analytical characterization of mandarin (*Citrus deliciosa* Ten.) essential oil. Flavour and Fragrance Journal 26(1): 34-46.

El-Gamal, S.M.A., Ahmed, H.M.I. 2017. Influence of different maturity stages on fruit yield and essential oil content of some Apiaceae family plants B: Fennel (*Foeniculum vulgare* Mill.). Journal of Plant Production 8(1): 127-133.

Ella Nkogo, L.F., Bopenga Bopenga, C.S.A., Ngohang, F.E., Mengome, L.E., Aboughe Angone, S., Edou Engonga, P. 2022. Phytochemical and anti-termite efficiency study of *Guibourtia tessmanii* (*harms*) J. Léonard (Kévazingo) bark extracts from Gabon. Journal of the Korean Wood Science and Technology 50(2): 113-125.

Fernandes, R.P.P., Trindade, M.A., Tonin, F.G., Lima, C.G., Pugine, S.M.P., Munekata, P.E.S., Lorenzo, J.M., de Melo, M.P. 2016. Evaluation of antioxidant capacity of 13 plant extracts by three different methods: Cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. Journal of Food Science and Technology 53(1): 451-460.

Firuzi, O., Lacanna, A., Petrucci, R., Marrosu, G., Saso, L. 2005. Evaluation of the antioxidant activity of flavonoids by “ferric reducing antioxidant power” assay and cyclic voltammetry. Biochimica et Biophysica Acta (BBA)-General Subjects 1721(1-3): 174-184.

Ham, Y., Yang, J., Choi, W.S., Ahn, B.J., Park, M.J. 2020. Antibacterial activity of essential oils from Pinaceae leaves against fish pathogens. Journal of the Korean Wood Science and Technology 48(4): 527-547.

Harhar, H., Gharby, S., El Idrissi, Y., Pioch, D., Matthäus, B., Charrouf, Z., Tabyaoui, M. 2019. Effect of maturity stage on the chemical composition of argan fruit pulp. Oilseeds and Fats, Crops and Lipids 26: 15.

Jeong, M.J., Yang, J., Choi, W.S., Kim, J.W., Kim, S.J., Park, M.J. 2017. Chemical compositions and antioxidant activities of essential oil extracted from *Neolitsea aciculata* (Blume) Koidz leaves. Journal of the Korean Wood Science and Technology 45(1): 96-106.

Jonas, M., Ketlogetswe, C., Gandure, J. 2020. Effect of fruit maturity stage on some physicochemical properties of Jatropha seed oil and derived biodiesel. ACS Omega 5(23): 13473-13481.

Khan, U.M., Sameen, A., Aadil, R.M., Shahid, M., Sezen, S., Zarrabi, A., Ozdemir, B., Sevindik, M., Kaplan, D.N., Selamoglu, Z., Ydyrys, A., Anitha, T., Kumar, M., Sharifi-Rad, J., Butnariu, M. 2021. *Citrus* genus and its waste utilization: A review on health-promoting activities and industrial application. Evidence-Based Complementary and Alternative Medicine 2021: 2488804.

Lee, S.Y., Lee, D.S., Cho, S.M., Kim, J.C., Park, M.J., Choi, I.G. 2021. Antioxidant properties of 7 domestic essential oils and identification of physiologically active components of essential oils against *Candida albicans*. Journal of the Korean Wood Science and Technology 49(1): 23-43.

Liu, Y., Heying, E., Tanumihardjo, S.A. 2012. History, global distribution, and nutritional importance of citrus fruits. Comprehensive Reviews in Food Science and Food Safety 11(6): 530-545.

Lv, X., Zhao, S., Ning, Z., Zeng, H., Shu, Y., Tao, O., Xiao, C., Lu, C., Liu, Y. 2015. Citrus fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. Chemistry Central Journal 9(1): 68.

Manurung, H., Sari, R.K., SyafII, W., Cahyaningsih, U., Ekasari, W. 2019. Antimalarial activity and phytochemical profile of ethanolic and aqueous extracts of bidara laut (*Strychnos ligustrina* Blum) wood. Journal of the Korean Wood Science and Technology 47(5): 587-596.

Medini, H., Elaissi, A., Larbi Khouja, M., Piras, A., Porcedda, S., Falconieri, D., Marongiu, B., Chemli, R. 2011. Chemical composition and antioxidant activity of the essential oil of *Juniperus phoenicea* L. berries. Natural Product Research 25(18): 1695-1706.

Moretti, M.D.L., Peana, A.T., Passino, G.S., Solinas, V. 1998. Effects of soil properties on yield and composition of *Rosmarinus officinalis* essential oil. Journal of Essential Oil Research 10(3): 261-267.

Nakayama, N., Yamaura, K., Shimada, M., Ueno, K. 2011. Extract from peel of *Citrus natsudaidai* alleviates experimental chronic allergic dermatitis in mice. Pharmacognosy Research 3(3): 155-159.

Oh, H.J., Ahn, H.M., Kim, S.S., Yun, P.Y., Jeon, G.L., Ko, Y.H., Riu, K.Z. 2007. Composition and antimicrobial activities of essential oils in the peel of citrus fruits. Journal of Applied Biological Chemistry 50(3): 148-154.

Ojeda-Sana, A.M., van Baren, C.M., Elechosa, M.A., Juárez, M.A., Moreno, S. 2013. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. Food Control 31(1): 189-195.

Palazzolo, E., Laudicina, V.A., Germanà, M.A. 2013. Current and potential use of citrus essential oils. Current Organic Chemistry 17(24): 3042-3049.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26(9−10): 1231-1237.

Salar, R.K., Seasotiya, L. 2011. Free radical scavenging activity, phenolic contents and phytochemical evaluation of different extracts of stem bark of *Butea monosperma* (Lam.) Kuntze. Frontiers in Life Science 5(3-4): 107-116.

Sayuti, M., Supriatna, I., Hismayasari, I.B., Budiadyani, I.G.A., Yani, A., Siti Asma, S. 2018. Antioxidant potentials and fatty acid composition of extracts *Sterculia tragacantha* Lindl. Leaves from Raja Ampat west Papua province Indonesia. International Research Journal of Pharmacy 9(10): 58-63.

Singleton, V.L., Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16(3): 144-158.

Vekiari, S.A., Protopapadakis, E.E., Papadopoulou, P., Papanicolaou, D., Panou, C., Vamvakias, M. 2002. Composition and seasonal variation of the essential oil from leaves and peel of a Cretan lemon variety. Journal of Agricultural and Food Chemistry 50(1): 147-153.

Yamaura, K., Nakayama, N., Shimada, M., Ueno, K. 2012. Protective effects of natsumikan (*Citrus natsudaidai*) extract on acetaminophen-induced lethal hepatotoxicity in mice. Pharmacognosy Research 4(4): 234-236.

Yang, J., Choi, W.S., Jeung, E.B., Kim, K.J., Park, M.J. 2021a. Anti-inflammatory effect of essential oil extracted from *Pinus densiflora* Sieb. et Zucc. wood on RBL-2H3 cells. Journal of Wood Science 67(1): 52.

Yang, J., Choi, W.S., Kim, J.W., Lee, S.S., Park, M.J. 2019. Anti-inflammatory effect of essential oils extracted from wood of four coniferous tree species. Journal of the Korean Wood Science and Technology 47(6): 674-691.

Yang, J., Choi, W.S., Kim, K.J., Eom, C.D., Park, M.J. 2021b. Investigation of active anti-inflammatory constituents of essential oil from *Pinus koraiensis* Sieb. et Zucc. wood in LPS-stimulated RBL-2H3 cells. Biomolecules 11(6): 817.