Seasonal Effect of Ultraviolet Irradiation on Polymethoxyflavone and Hesperidin Content in Ponkan and Tachibana Flavedo

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Abstract. Ponkan (*Citrus reticulata*) and Tachibana (*Citrus tachibana*) contain large amounts of polymethoxyflavones (PMFs). To produce highly functional food with high potential, it is important to develop technologies to increase their PMF content. Nobiletin content of the flavedo changed in harvested ‘Ohta Ponkan’ fruits after ultraviolet (UV)-C irradiation and PMF content increased for only the first 2 days following irradiation. The effect of UV-C or UV-B irradiation on PMF content at each harvest time (UV-C, July to December; UV-B, July to September) was observed in Ponkan and Tachibana. We found that UV-C had the greatest impact earlier in the season. The effects of UV-B irradiation were similar to UV-C results. Therefore, it is beneficial to harvest early and extract PMFs from fruit 24 hours after UV-C irradiation.

Flavonoids, including nobiletin, are major secondary metabolites in citrus fruits and have many functions, such as defense against both pathogen attack and solar UV radiation (Moriguchi et al., 2001). Molecular studies suggest that flavonoid synthesis is induced by light irradiation, fungal elicitors, UV radiation, interactions with microorganisms, and wounding (Jackson et al., 1992). PMFs exist almost exclusively in the citrus genus, particularly in the peel of citrus fruits. Among citrus cultivars, Shiikuwasha (*Citrus depressa* Hayata) and Tachibana (*Citrus tachibana* Tanaka) are known to contain large amounts of PMFs, including nobiletin and tangeretin, which have been studied for their ability to suppress tumor formation. Nobiletin, which is a component of the herbal medicine “chinpi” (the dried peel of a mandarin), has been shown to inhibit the accumulation of amyloid β , which has been shown to reduce memory impairment in mice, and have a preventive effect on Alzheimer’s disease. Nobiletin-rich bark is more effective than nobiletin alone, and it has been shown that several naturally occurring active ingredients work to enhance the anti-dementia effect (Yamakuni and Kawahata, 2015). Furthermore, nobiletin may be useful as a novel sunscreen reagent to be applied for protection

against photoinflammation and photoaging because it inhibits the UV-B-induced production of prostaglandin (Tanaka et al., 2004).

In citrus fruits, the composition and quantities of compounds such as carotenoids, flavonoids, and terpenoids have been widely studied for various citrus species (Li et al., 2006; Manthey and Grohmann, 2001; Nishiura et al., 1971). Research especially focuses on flavonoids in special product species in each production area (Inafuku-Teramoto et al., 2011; Miyake, 2006; Miyake et al., 1998; Nogata et al., 2006). Ichinokiyama et al. (2012) reported that the new citrus fruit ‘Niihime’ contained more eriocitrin, narirutin, neoponcirin, and sinensetin than Tachibana or Shiikuwasha, and that the total content of seven flavonoids in the fruit of ‘Niihime’ were higher than in Tachibana. However, ‘Niihime’ contained significantly less nobiletin (65%) than Shiikuwasha, and a similar amount as Tachibana. The flavedo of Tachibana has the highest nobiletin content of the three species. Ponkan (*Citrus reticulata* Blanco) also contains a large amount of PMFs in its flavedo, and PMFs in the extract of Ponkan are used for cellular antioxidant activity and pharmacokinetic study (Yuen et al., 2014). In Shizuoka Prefecture, in central Japan, the early-growing cultivar Ohta Ponkan is widely produced. Numazu City, Shizuoka Prefecture, is the northern limit of Tachibana’s natural habitat in Japan, and in recent years, Tachibana trees have been planted there to process the fruit. For effective utilization of functional components, it is important to note that changes in flavonoid content of the flavedo occur during fruit ripening and that flavonoid content is

high in immature fruits (Inafuku-Teramoto et al., 2011; Yamaga et al., 2020). Theoretically, technology could increase flavonoid content in harvested fruits; however, there have been few reports that technology was able to increase PMF content during cultivation or at postharvest in Ponkan or Tachibana fruits, citrus varieties with high PMF contents. To produce highly functional food with high potential, it is important to develop technology to increase PMF content that will lead to efficient extraction and processing of raw materials. In addition, if functional foods can be developed using the effects of nobiletin, it is expected that alternative therapies for Alzheimer’s disease, as mentioned previously, will progress.

UV induces the resistance of plants to pathogens by producing phenylpropanoid derivatives such as flavonoids and other phenolic compounds (Ballaré, 2014). Ruiz et al. (2016) reported that flavanones-dihydroflavonols, flavonols, and anthocyanins were increased in flavedo of mature postharvest lemons by short-term artificial UV-B exposure. Also, we showed that UV-C irradiation induced the production of scoparone as a defensive measure against fungal development in the flavedo tissue of satsuma mandarin fruits (Yamaga and Nakamura, 2019). Therefore, we tried to gain the basic knowledge for developing a technology to increase flavonoid efficiently by using UV light in a cultivar with high PMF content. The aim of this study was to clarify the effect of UV-C or UV-B irradiation on PMFs (nobiletin, tangeretin, sinensetin) and hesperidin content of the flavedo at each harvest period (UV-C, July to December; UV-B, July to September). Hesperidin is a kind of flavanone and has been reported to cause allergic reactions, decrease blood pressure (Mitsuzumi, 2011), and exhibit high antioxidative activity (Miyake, 2017).

Materials and Methods

Dairy changes in nobiletin contents of flavedo in harvested ‘Ohta Ponkan’ fruits after UV-C irradiation (Expt. 1). ‘Ohta Ponkan’ were grown and harvested at Shizuoka Prefectural Agriculture and Forestry Research Institute Fruit Tree Research Center (Shizuoka, Japan) in Aug. 2016. Harvested fruits were transported immediately to the laboratory. The fruits were treated by 72 kJ·m⁻² (irradiation time; 60 min) UV-C irradiation as described later in this section. Nonirradiated fruit served as a control. These experiments were repeated four times (replications) using five fruits per replication. Irradiated and nonirradiated fruits were kept in the dark at 20 °C for 7 d. Nobiletin was extracted at 1, 2, 3, and 7 d after UV-C irradiation.

Seasonal changes in PMFs and hesperidin contents of flavedo in ‘Ohta Ponkan’ and Tachibana fruits after UV-C or UV-B irradiation (Expt. 2). ‘Ohta Ponkan’ fruits were grown and harvested in production fields in Shizuoka city from 2017 to 2018. The trees were 15 years old, and Karatachi [*Poncirus trifoliata* (L.) Raf.] was used for rootstock. The beginning of flowering date in ‘Ohta Ponkan’ trees was 15 May 2017 and 1 May 2018.

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Tachibana fruits were grown and harvested in production fields in Numazu City from 2017 to 2018. The trees were 12 years old and Karatachi was used for rootstock. The beginning of flowering date in Tachibana trees was 13 May 2017 and 1 May 2018. These samplings were done at the middle of each month. Harvested fruits were transported immediately to the laboratory. The average fruit weight for experiments are indicated in Table 1. The fruits harvested in 2017 were treated with 108 kJ·m⁻² (irradiation time; 90 min) or 54 kJ·m⁻² (45 min) UV-C. The irradiation doses were set to be greater or lesser than those of Expt. 1 in consideration of those results. Nonirradiated fruit served as control; 270 kJ·m⁻² (180 min) or 90 kJ·m⁻² (60 min) UV-B exposure was conducted in July, August, and September in 2018 according to the results of the UV-C irradiation in 2017. These experiments were repeated four times (replications) by using three fruits per replication. Irradiated and nonirradiated fruits were kept in the dark at 20 °C. Flavonoids were extracted at 1 d after ultraviolet irradiation, considering results of Expt. 1.

Ultraviolet irradiation. UV-C irradiation was performed using two lamps (GL-15; Toshiba Corporation, Tokyo, Japan). The peak wavelength emitted by the lamp was 254 nm. The fruits were placed 18 cm from the irradiation source, with the pedicel facing up, as described previously (Yamaga and Nakamura, 2019). UV-B irradiation was performed with two lamps (YGRFX21711GL; Panasonic Corporation, Osaka, Japan). The peak wavelength emitted by the lamp was 280 nm. The fruits were placed 15 cm from the irradiation source. UV irradiance was measured using UV radiometer sensors (UVX-25, UVX-31; Funakoshi, Tokyo, Japan).

Fig. 1. Changes in nobiletin content of flavedo in 'Ohta Ponkan' fruits after 72 kJ·m⁻² UV-C irradiation. **Significant difference at $P < 0.01$ by Welch's t test. FW = fresh weight; UV = ultraviolet.

PMF extraction and analysis. We extracted PMFs (including nobiletin) and hesperidin at 1 d after UV-C irradiation as described by Yamaga and Nakamura (2019). Flavedo tissue, which is located between the pedicel and the lateral part of harvested fruits was excised with a knife and extracted using 1:1 (v:v) mixture of dimethylsulfoxide (DMSO) and methanol and placed in an ultrasonic tank for 60 min. The extraction was repeated twice. The extraction was centrifuged for 10 min at 3500 rpm, the supernatant was collected and passed through a 0.2-μm filter into the sampling bottle. The elution schedule consisted of an initial time of 20 mM H₃PO₄ followed by a gradient with increasing concentration of 1:1 (v:v) mixture of acetonitrile and methanol from 22% to 84% for 44 min at the flow rate of 1 mL·min⁻¹. The column was set at 44 °C; 50-μL aliquots from these solutions were injected into high-performance liquid chromatography (pumps: PU-2089 Plus, automatic sampler: AS-2051 Plus, photo diode array detector: MD-2015 Plus; JASCO Corporation., Tokyo, Japan) equipped with a C18 column (YMC-Pack Pro 3 μm, 3.0 mm I.D. × 100 mm) and an auto-injection system. The detector was set to measure spectra from 220 to 400 nm and to monitor the eluent at 274 nm (PMFs) and 338 nm (hesperidin). Flavonoid standards (nobiletin, tangeretin, sinensetin, hesperidin) were prepared by using reagents from the Fujifilm Wako Chemical Corporation (Osaka, Japan).

Statistical analysis. Statistical analyses were performed using the statistical computing program R (version 3.3.0). The data of Expt. 1 were analyzed using the Welch's t test. The data of Expt. 2 were analyzed using the Tukey's multiple range test, with the significance level set at $P < 0.05$.

Results

Daily changes in nobiletin contents of flavedo in harvested 'Ohta Ponkan' fruits after UV-C irradiation (Expt. 1). Daily changes in nobiletin contents of flavedo in 'Ohta Ponkan' fruits after UV-C irradiation or nonirradiation that were harvested in Aug. 2016 are shown in Fig. 1. The nobiletin contents at 1 d after UV-C irradiation were higher than in the control. The significant difference continued 2 d after irradiation, but no significant difference was noted at 3 and 7 d after irradiation.

Seasonal changes in PMFs and hesperidin contents of flavedo in 'Ohta Ponkan' and Tachibana fruits after UV-C or UV-B irradiation (Expt. 2). In 'Ohta Ponkan' fruits (harvested in August and September), UV-C-irradiated fruits had higher nobiletin content

than the control (Table 2). UV-C 108 kJ·m⁻² treatment had the highest nobiletin content in the August group, and UV-C 54 kJ·m⁻² treatment had the highest nobiletin content in the September group. On the other hand, the nobiletin contents were not increased by UV-C irradiation in the fruits harvested from October to December. Tangeretin and sinensetin content measurements showed increases similar to nobiletin when exposed to UV-C irradiation. In contrast, hesperidin contents were decreased when subjected to UV-C irradiation in the August and September fruits, when the PMF contents were increased. In Tachibana fruits that were harvested from July to September, UV-irradiated fruits had higher nobiletin content than controls (Table 3). UV-C 108 kJ·m⁻² treatment produced the highest content during these harvest periods. Tachibana that were harvested in October and November did not increase when submitted to UV-C irradiation. In December, UV-C-treated fruits again saw a significant increase in nobiletin content. Other PMFs in Tachibana fruits were significantly increased by UV-C irradiation for the same months that nobiletin content increased. Hesperidin content was decreased by UV-C irradiation in the fruits harvested in July and August. In both Ponkan and Tachibana fruits, the PMF and hesperidin contents were gradually decreased as the traditional harvest period approached.

'Ohta Ponkan' fruits that were irradiated with UV-B 90 kJ·m⁻² and UV-B 270 kJ·m⁻² exhibited higher PMF content than controls in July, but in August or September (Table 4). Tachibana fruits that were harvested from July to September exhibited significantly increased PMF contents after UV-B irradiation (Table 5). UV-B 270 kJ·m⁻² treatment elicited the highest PMF contents during these harvest periods.

Discussion

UV-C and UV-B irradiation are known to enhance levels of antioxidants and enzymes in other horticultural crops, such as broccoli, peach, pepper, and tomato (Castagna et al., 2013; Costa et al., 2006; Gonzalez-Aguilar et al., 2004; Vicente et al., 2005). Also, Ruiz et al. (2016) showed that UV-B triggered reactive oxygen species production on flavedo almost immediately (1 h after treatment) increased and progressively decreased, and the antioxidant activity of the flavedo increased significantly at 1 d after UV-B treatment. Our results (Expt. 1) indicate that UV-C induces the accumulation of nobiletin that is produced in the flavonoid pathway (Moriguchi et al., 2001) of the flavedo in Ponkan fruit within 24 h of irradiation. Ballester et al. (2013) reported that the maximum expression of messenger RNA from phenylpropanoid and flavonoid genes in the flavedo and albedo in infected 'Navelina' oranges was observed 48 h post inoculation, when the first symptom of decay started to appear. These results support their studies in that PMFs accumulate rapidly. In addition,

Table 1. Average weight of a fruit used for experiments.

Cultivar	Yr	Avg fruit wt for experiments (g)					
		July	Aug.	Sept.	Oct.	Nov.	Dec.
Ohta Ponkan	2017	15.2	28.2	65.3	104.5	140.6	169.5
	2018	20.6	39.0	94.3	— ^z	—	—
Tachibana	2017	1.3	5.5	7.3	9.9	13.1	13.8
	2018	2.0	5.4	8.5	—	—	—

^z = not done.

Table 2. Seasonal changes of irradiation effect with ultraviolet (UV)-C on polymethoxyflavones and hesperidin content in 'Ohta Ponkan' fruits.

Nobiletin (mg/100 g FW)						
Treatment	July	Aug.	Sept.	Oct.	Nov.	Dec.
UV-C 108 kJ·m ⁻²	647 ± 38	529 ± 8.5 a ^z	518 ± 9.2 b	333 ± 7.4	306 ± 12	268 ± 12
UV-C 54 kJ·m ⁻²	586 ± 34	456 ± 11 b	596 ± 7.6 a	322 ± 16	308 ± 8.5	264 ± 19
Control	642 ± 12	406 ± 13 c	404 ± 16 c	299 ± 12	311 ± 20	252 ± 11
Significance	NS	**	**	NS	NS	NS
Tangeretin (mg/100 g FW)						
Treatment	July	Aug.	Sept.	Oct.	Nov.	Dec.
UV-C 108 kJ·m ⁻²	494 ± 17	333 ± 4.9 a	375 ± 5.6 b	261 ± 7.3	177 ± 29	217 ± 7.5
UV-C 54 kJ·m ⁻²	427 ± 21	302 ± 6.3 b	420 ± 6.4 a	250 ± 14	241 ± 7.6	196 ± 12
Control	464 ± 9.2	276 ± 8.0 c	345 ± 11c	247 ± 8.9	237 ± 9.7	208 ± 8.7
Significance	NS	**	**	NS	NS	NS
Sinensetin (mg/100 g FW)						
Treatment	July	Aug.	Sept.	Oct.	Nov.	Dec.
UV-C 108 kJ·m ⁻²	36 ± 2.5	32 ± 0.6 a	31 ± 0.6 b	21 ± 0.4	16 ± 2.0	16 ± 0.8
UV-C 54 kJ·m ⁻²	32 ± 2.1	27 ± 0.7 b	35 ± 0.4 a	20 ± 1.0	19 ± 0.7	16 ± 1.1
Control	34 ± 0.8	23 ± 0.8 c	27 ± 0.9 c	18 ± 0.8	18 ± 1.4	15 ± 0.8
Significance	NS	**	**	NS	NS	NS
Hesperidin (mg/100 g FW)						
Treatment	July	Aug.	Sept.	Oct.	Nov.	Dec.
UV-C 108 kJ·m ⁻²	4717 ± 265	2103 ± 28 b	856 ± 20 c	508 ± 14	332 ± 44	343 ± 35
UV-C 54 kJ·m ⁻²	4613 ± 317	2009 ± 67 b	968 ± 11 b	481 ± 25	374 ± 15	302 ± 17
Control	4141 ± 325	2467 ± 65 a	1070 ± 17 a	483 ± 14	389 ± 23	318 ± 24
Significance	NS	**	**	NS	NS	NS

^zDifferent letters indicate the significant differences at $P < 0.05$ by Tukey's multiple range test.

Data are expressed as the mean ± SE (n = 5). FW = fresh weight.

NS, **Nonsignificant or significant at $P < 0.01$ by one-way analysis of variance.

Table 3. Seasonal changes of the effect with ultraviolet (UV)-C irradiation on polymethoxyflavones and hesperidin content in Tachibana fruits.

Nobiletin (mg/100 g FW)						
Treatment	July	Aug.	Sept.	Oct.	Nov.	Dec.
UV-C 108 kJ·m ⁻²	1212 ± 20 a ^z	862 ± 24 a	561 ± 6.7 a	396 ± 15	233 ± 10	212 ± 8.5 a
UV-C 54 kJ·m ⁻²	1081 ± 18 b	838 ± 18 b	541 ± 11a	323 ± 17	251 ± 32	190 ± 5.1 b
Control	1128 ± 19 b	650 ± 22 c	428 ± 8.9 b	368 ± 13	234 ± 8.9	166 ± 8.1 b
Significance	**	**	**	NS	NS	**
Tangeretin (mg/100 g FW)						
Treatment	July	Aug.	Sept.	Oct.	Nov.	Dec.
UV-C 108 kJ·m ⁻²	797 ± 12 a	453 ± 12 a	303 ± 3.8 a	215 ± 9.5	156 ± 28	122 ± 4.8 a
UV-C 54 kJ·m ⁻²	702 ± 12 b	416 ± 11 b	293 ± 6.8 a	187 ± 7.8	137 ± 17	110 ± 2.3 ab
Control	779 ± 12 b	357 ± 11 c	236 ± 5.2 b	218 ± 7.1	129 ± 4.2	98 ± 5.2 b
Significance	**	**	**	NS	NS	**
Sinensetin (mg/100 g FW)						
Treatment	July	Aug.	Sept.	Oct.	Nov.	Dec.
UV-C 108 kJ·m ⁻²	116 ± 2.0 a	83 ± 2.4 a	55 ± 0.5 a	37 ± 1.8	23 ± 1.1	21 ± 0.8 a
UV-C 54 kJ·m ⁻²	103 ± 1.7 b	80 ± 1.7 a	53 ± 1.0 a	33 ± 1.7	25 ± 1.1	19 ± 0.5 ab
Control	107 ± 1.8 b	63 ± 2.6 b	41 ± 1.2 b	37 ± 1.5	24 ± 0.9	16 ± 0.7 b
Significance	**	**	**	NS	NS	**
Hesperidin (mg/100 g FW)						
Treatment	July	Aug.	Sept.	Oct.	Nov.	Dec.
UV-C 108 kJ·m ⁻²	2249 ± 21 b	786 ± 16 b	920 ± 13	347 ± 11	442 ± 31	445 ± 20
UV-C 54 kJ·m ⁻²	2174 ± 64 b	808 ± 24 b	977 ± 27	416 ± 42	323 ± 86	464 ± 26
Control	2437 ± 39 a	932 ± 24 a	992 ± 21	374 ± 17	479 ± 33	432 ± 32
Significance	**	**	NS	NS	NS	NS

^zDifferent letters indicate the significant differences at $P < 0.05$ by Tukey's multiple range test.

Data are expressed as the mean ± SE (n = 5). FW = fresh weight.

NS, **Nonsignificant or significant at $P < 0.01$ by one-way analysis of variance.

Ruiz et al. (2016) reported microscopically observing that UV-B irradiation produced cell wall thickening (epidermis, collenchyma, and parenchyma) and phenolic deposits were observed in flavedo inside the vacuoles of both collenchyma and parenchyma cells after UV-B irradiation. Our results indicate that the metabolic and anatomic reactions may be

changed in flavedo tissue by changing the harvest time. The reason for decreasing PMF after increasing with UV-C irradiation could not be clearly elucidated; however, it is thought that subsequent metabolic pathways are involved. PMF accumulation by UV irradiation is a transient stress-tolerant response, such as changes in enzyme activity for scavenging

reactive oxygen species (Kaewsuksaeng et al., 2011; Yamauchi, 2013), and derivatives that produced quickly may be easier to metabolize.

Flavonoids in citrus are accumulated in tissues during the early period of young fruits, before major cell elongation has occurred. Our present study (Expt. 2) indicates that the accumulation of PMFs and flavanone

Table 4. Effect of ultraviolet (UV)-B irradiation on polymethoxyflavones and hesperidin content in 'Ohta Ponkan' fruits.

Treatment	Nobiletin (mg/100 g FW)			Tangeretin (mg/100 g FW)		
	July	Aug.	Sept.	July	Aug.	Sept.
UV-B 270 kJ·m ⁻²	563 ± 15 a [*]	607 ± 33	476 ± 11	442 ± 10 a	433 ± 17	378 ± 8.9
UV-B 90 kJ·m ⁻²	575 ± 14 a	586 ± 27	436 ± 10	451 ± 11 a	413 ± 5.2	317 ± 5.9
Control	433 ± 5.8 b	564 ± 27	432 ± 37	356 ± 4.9 b	402 ± 15	334 ± 29
Significance	**	NS	NS	**	NS	NS

Treatment	Sinensetin (mg/100 g FW)			Hesperidin (mg/100 g FW)		
	July	Aug.	Sept.	July	Aug.	Sept.
UV-B 270 kJ·m ⁻²	35 ± 1.1 a	40 ± 2.5	31 ± 0.66	2863 ± 42 a	1284 ± 45	649 ± 59
UV-B 90 kJ·m ⁻²	35 ± 0.82	39 ± 2.2	28 ± 0.69	2710 ± 108 a	1371 ± 54	592 ± 9.3
Control	26 ± 0.52 b	38 ± 2.0	28 ± 2.5	2648 ± 123 b	1367 ± 82	550 ± 3.5
Significance	**	NS	NS	NS	NS	NS

^{*}Different letters indicate the significant differences at $P < 0.05$ by Tukey's multiple range test.

Data are expressed as the mean ± SE (n = 5). FW = fresh weight.

NS, **Nonsignificant or significant at $P < 0.01$ by one-way analysis of variance.

Table 5. Effect of ultraviolet (UV)-B irradiation on polymethoxyflavones and hesperidin content in Tachibana fruits.

Treatment	Nobiletin (mg/100 g FW)			Tangeretin (mg/100 g FW)		
	July	Aug.	Sept.	July	Aug.	Sept.
UV-B 270 kJ·m ⁻²	1096 ± 18 a [*]	1047 ± 25 a	670 ± 18 a	699 ± 13 a	563 ± 15 a	493 ± 76 a
UV-B 90 kJ·m ⁻²	855 ± 31 b	997 ± 24 a	649 ± 26 ab	569 ± 19 b	527 ± 9.2 a	348 ± 14 ab
Control	845 ± 18 b	847 ± 8.3 b	583 ± 15 b	549 ± 7.2 b	452 ± 4.1 b	303 ± 7.1 b
Significance	**	**	*	**	**	*

Treatment	Sinensetin (mg/100 g FW)			Hesperidin (mg/100 g FW)		
	July	Aug.	Sept.	July	Aug.	Sept.
UV-B 270 kJ·m ⁻²	111 ± 2.0 a	111 ± 2.7 a	72 ± 1.9 a	1477 ± 40	584 ± 25	479 ± 18
UV-B 90 kJ·m ⁻²	86 ± 3.4 b	106 ± 2.6 a	69 ± 3.1 ab	1454 ± 78	602 ± 23	422 ± 23
Control	87 ± 1.8 b	90 ± 0.83 b	62 ± 1.6 b	1407 ± 94	636 ± 15	514 ± 21
Significance	**	**	*	NS	NS	NS

^{*}Different letters indicate the significant differences at $P < 0.05$ by Tukey's multiple range test.

Data are expressed as the mean ± SE (n = 5). FW = fresh weight.

NS, *, **Nonsignificant or significant at $P < 0.05$ or 0.01, respectively, by one-way analysis of variance.

by UV-C irradiation are more likely to be promoted early in the harvest period, rather than later. Moriguchi et al. (2001) indicated that expressions of flavonoid biosynthesis related genes: chalcone synthase (CHS), chalcone isomerase (CHI), and flavanone 3-hydroxylase (F3H) in the flavedo of *Citrus unshiu* fruits were found in high levels during the early developmental stage (26 and 40 d after flowering). The flavedo continues cell division until considerably late in the growth stage of fruits (Kuraoka and Kikuchi, 1961), the signals of flavonoid biosynthetic genes (CitCHS1, CitCHS2, CitCHI, and CitF3H) are detectable in the flavedo during the later developmental stage, unlike the RNA accumulation patterns in the albedo and juice sacs. On the other hand, hesperidin content is higher in the albedo (Ichinokiyama et al., 2012). The change of hesperidin content in albedo subjected to UV irradiation might differ from irradiated flavedo. In addition, our results indicate that the reaction with UV irradiation in the fruit harvested at an earlier stage was more sensitive than those harvested later. Gene expression encoding enzymes such as O-methyl transferase, chalcone synthase, and flavone synthase in the flavedo may experience a greater increase when exposed to UV treatment in its early stage (immature fruit). On the other hand, hesperidin was decreased by UV-C irradiation in Ponkan and Tachibana fruit with increased PMFs by UV-C. Changing composition of

these phenolic compounds is due to various stress responses (Ortuño et al., 2011). It is considered that gene expression such as Cit4'OMT and Cit8'OMT, which related this metabolism has changed (Oikawa et al., 2015). Ruiz et al. (2016) described that flavones and flavanols (final products of the flavonoid pathway) increased more markedly than flavanones, which are their precursors, when subjected to UV-B irradiation. In addition, the PMFs act more as antifungal agents against *Penicillium digitatum* than the flavanones, nobiletin is the most effective compound in reducing the radial growth of the fungus, followed by hesperidin and naringin (Ortuño et al., 2006). Ortuño et al. (2006) reported that the cell walls of *P. digitatum* cultured in the presence of nobiletin are thicker than those of untreated controls; furthermore, a smaller cytoplasmic density was observed in the nobiletin treatment. Although natural UV radiation (including UV-B) induces phenylpropanoids, mainly in epidermal cells of sun-exposed organs such as leaves and fruits, UV-C is not included in sunlight. UV-C may have different effects on flavonoid production compared with UV-B; therefore, it is necessary to clarify these differences. UV-B irradiation for 'Ohta Ponkan' fruits in August and September did not increase PMF content, whereas UV-C irradiation did. In addition, these results may be related to the climate changes from summer to autumn, and the differences of fruit growth rate in the survey years.

Comparing the citrus cultivars, *C. tachibana* fruits have a greater PMF content than *C. reticulata* fruit. Also, *C. tachibana* has a longer harvest time, allowing for the increased effect of PMF content through UV-C irradiation. UV-C irradiation may have a more active effect on the expression of the relevant genes in citrus cultivars that produce a large amount of PMFs. Oikawa et al. (2015) showed that although expressions of flavonoid biosynthesis related gene in Ponkan fruits were higher compared with those of other citrus varieties, a few related gene expressions were lower in August than other months.

Fruit thinning that reduces the number of fruit sets is conducted to reduce the burden on trees and promote fruit development; however, these thinned fruits are left in the orchards. We showed that an earlier harvesting time of July, August, or September could be more efficient times for extraction and utilization of flavonoids. Flavonoid content in thinned fruits is higher than fruits that are harvested at the traditional commercial harvest time (Kawahara et al., 2019). Effective use of thinned fruits as PMF resources would not only improve human health but produce additional profits for farmers too. These flavonoids have been found to be stable even under heat treatment conditions in acidic solution, and they could be used in foods (Miyake et al., 1998). Using the technology indicated in this study to increase the PMF content of citrus peel, we can facilitate food development that uses nobiletin.

In addition, it is expected that evidence as a functional component of PMFs will be established (Birt et al., 2001; Sato et al., 2002) and alternative therapies for the prevention of Alzheimer's disease will be developed (Yamakuni et al., 2008, 2010).

In satsuma mandarin, the presence of β -cryptoxanthin, which is a carotenoid that is correlated with soluble solid (sugar) content (Hamasaki and Ooshiro, 2003), guarantees that it is a food with function (Hisanaga et al., 2018). On the other hand, PMFs are mostly contained in the flavedo, and relationships between PMFs and rind color or substances that can be easily quantitated have not been clarified. Flavonoids are components that decrease with fruit ripening unlike carotenoids, development of technology to estimate PMF contents without damaging fruits is expected in the future. In conclusion, our results indicate that it will be important to harvest during early periods such as August and September and to extract PMFs from the fruits 24 h after UV-C irradiation.

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