Effect of Maturation Stage and Storage Temperature and Duration on β-Cryptoxanthin Content in Satsuma Mandarin (Citrus unshiu Marc.) Fruit

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In satsuma mandarin fruit (Citrus unshiu Marc.), β-cryptoxanthin is a major carotenoid and an important quality component in the juice sacs. The stage of maturity and storage conditions of satsuma mandarin fruit shipped to markets varies. However, the effects of maturation stage, storage temperature and duration on changes in β-cryptoxanthin content have not been fully studied. In the present study, fruits were harvested at different maturation stages, and changes in β-cryptoxanthin content in the juice sacs were investigated during storage at temperatures from 5 to 20°C. At 20°C, in fruit harvested while β-cryptoxanthin is still being accumulated on the tree, the content continued to increase following 15 days of storage. However, in the fruit without β-cryptoxanthin accumulation on the tree, the content did not increase. At 10°C, in the fruit accumulating β-cryptoxanthin on the tree, the content continued to increase after 14 days’ storage, whereas in the fruit without β-cryptoxanthin accumulation, the content did not increase after 14 days’ storage but increased after 30 days’ storage. At 8°C, the increase in content was also observed in the fruit when stored for 80 days. In contrast, at 5°C, the content did not change notably at any maturation stage regardless of the experimental period. The changes in the carotenoid content and gene expression suggest that carotenoid accumulation during on-tree maturation continues after harvest at 20°C, but not at 5°C. The present results suggest that β-cryptoxanthin content will not decrease at a range from 5 to 20°C irrespective of the maturation stage. Moreover, at 8°C and above, the β-cryptoxanthin content will gradually increase, and the rate will be more rapid in fruit harvested when carotenoid accumulation is in progress on the tree.

Key Words: β-cryptoxanthin, carotenoid, maturation stage, satsuma mandarin, storage temperature.

Introduction

Before consumption, postharvest citrus fruit is exposed to various temperatures (low to high) in storage rooms, on store shelves, and during transportation (Grierson and Miller, 2006; Kader, 2002). Postharvest temperature affects citrus fruit quality (e.g., color, taste, and flavor), as well as quality components such as carotenoids, sugars, acids, and volatiles (Alquézar et al., 2008; Ladaniya, 2008; Obenland et al., 2011; Tietel et al., 2012).

Carotenoids are important quality components in citrus fruit and contribute to the color of the peel and pulp (Gross, 1987). In addition, some carotenoids, such as β-carotene and β-cryptoxanthin, are precursors of vitamin A, which serve as human nutrients, and reduce the risk of chronic diseases (Olson, 1989; Yamaguchi, 2012).

The postharvest temperature effect on carotenoid accumulation has been widely studied in the peel of citrus fruit (Alquézar et al., 2008; Barry and Van Wky, 2006; Van Wky et al., 2009; Wheaton and Stewart, 1973). However, studies on carotenoids in the edible juice sacs of the fruit have been limited and conducted for only a few cultivars and storage conditions. Recent studies have reported that in the pulp of grapefruit and oranges, the carotenoid content in fruit stored at 11–20°C was higher than that in fruit stored below 5°C (Carmona et al., 2012; Chaudhary et al., 2012, 2014, 2017). Previously, we reported that in ‘Aoshima’ satsuma mandarin fruit harvested at the fully-colored stage, the carotenoid content in the juice sacs did not change notably during storage.
storage at 5 or 20°C for three weeks (Matsumoto et al., 2009).

The satsuma mandarin (C. unshiu. Marc.) has many cultivars with different maturity seasons: very early ripening (mature in late September to October), early ripening (October to November), mid-ripening (November), and late ripening (late November to December) (Hodgson, 1967; Iwamasa, 1976). The harvest time of these cultivars is generally determined on the basis of peel color and maturity index (total soluble solid/acidity), but differs among production areas and growers (Grierson, 2006; Iwamasa, 1976). For example, some very early ripening cultivars are harvested at the stage of color break in several production areas, but at the fully colored stage in others. In addition, late ripening cultivars are normally stored, but storage temperatures and durations differ among growers, shipping cooperatives, and so on. Thus, the maturation stage and storage conditions of satsuma mandarin fruit shipped to markets vary. It is thought that these factors affect changes in the carotenoid content after harvest. However, in satsuma mandarin fruit, the effects of maturation stage and storage temperature and duration on carotenoid content have not yet been fully studied.

In 2015, the system of Foods with Function Claims was established by the Consumer Affairs Agency in Japan. Satsuma mandarin fruit was registered as the first fresh product with function claims based on β-cryptoxanthin as a functional substance (Maeda-Yamamoto, 2017), and the function claims are labeled on the packaging by several agricultural cooperatives. Thus, it is important to understand factors affecting the β-cryptoxanthin content of the fruit shipped to market.

The aim of the present study was to investigate the effects of postharvest temperatures, storage duration, and maturation stage on changes in β-cryptoxanthin content in the juice sacs of satsuma mandarin fruit. The temperature effect included a range from 5 to 20°C, assuming storage at cold or ambient temperature and room temperature (e.g., store shelf). Since the satsuma mandarin has many cultivars, the experiments were conducted using several cultivars to examine variations in these effects. In experiment 1, the effect of temperature (5°C and 20°C) and maturation stage on carotenoid accumulation and gene expression related to carotenoid biosynthesis during storage for 15 days was examined in ‘Aoshima’ as an experimental model. In experiment 2, varietal differences in carotenoid accumulation at 20°C storage for 14 days were examined in 10 satsuma mandarin cultivars. In experiment 3, the effect of an intermediate temperature ranging from 5 to 20°C, and the combined effect of maturation stage and prolonged storage duration were examined in ‘Nichinan No. 1’ as an experimental model. In experiment 4, temperature effects during long-term storage were examined in ‘Aoshima’ late ripening cultivars.

Materials and Methods

Experiment 1: Effect of Temperature on carotenoid accumulation in ‘Aoshima’ satsuma mandarin harvested at different stages of maturation

To examine the effect temperature on carotenoid content and its biosynthesis at different maturation stages, ‘Aoshima’ satsuma mandarin (C. unshiu. Marc.) fruits were used as an experimental model. The fruit was harvested at different maturation stages, Oct. 5 (148 days after flowering (DAF), dark green), Oct. 20 (163 DAF, light green), Nov. 4 (178 DAF, color break), Nov. 19 (193 DAF, orange-yellow), Dec. 10 (214 DAF, fully colored), Dec. 24 (228 DAF, overripe), and Jan. 9 (244 DAF, overripe) from a tree at the Institute of Fruit Tree and Tea Science, Citrus Research Division, Okitsu (Shizuoka, Japan). Fruit that were uniform in size and color were selected. The fruit were divided into 3 groups and stored in the dark for 15 days at 5 and 20°C under 85–90% relative humidity with continuous ventilation. After 0 and 15 days storage, three to six fruits per treatment were collected, and three samples per treatment were prepared by blending one or two fruit. The juice sacs were separated from the fruit, immediately frozen in liquid nitrogen, and stored at −80°C until use (Matsumoto et al., 2009). The changes in carotenoid content and gene expression related to carotenoid biosynthesis in the juices sacs of each sample were analyzed as described below.

Experiment 2: Varietal differences in β-cryptoxanthin accumulation after storage at 20°C

The fruit of 10 satsuma mandarin cultivars with different maturity seasons were harvested at the commercial harvest date. Harvest dates were as follows: Oct. 9 for very early ripening cultivars (‘Nichinan No. 1’, ‘Iwasaki’, ‘Dowaki’, and ‘Ueno-wase’), Nov. 7 for early ripening cultivars (‘Okitsu-wase’ and ‘Miyagawase’), Nov. 19 for mid-ripening cultivars (‘Nankan No. 20’ and ‘Seto’), and Nov. 27 for late ripening cultivars (‘Otsu No. 4’ and ‘Aoshima’). Fruit were almost fully colored; however, the peel had some green areas. The fruit was stored for 14 days at 20°C under 85–90% relative humidity. After 0 and 14 days storage, six fruits per treatment were collected, and three samples per treatment were prepared as described above. In addition, at 14 days after storage, the fruit stored on the tree were harvested. The average temperature in the field during on-tree storage for 14 days was calculated based on the AMeDAS (Automated Meteorological Data Acquisition System) equipment in the field at our institute. The average temperatures were as follows: 18°C for very early ripening, 14°C for early ripening, 13°C for mid-ripening, and 10°C for late ripening cultivars, respectively. The changes in carotenoid content in the juice sacs of each sample were analyzed as described below.
Experiment 3: Effect of intermediate temperature ranging from 5 to 20°C and combined effect of maturation stage and prolonged storage duration on β-cryptoxanthin accumulation in ‘Nichinan No. 1’ satsuma mandarin

To examine effect of intermediate temperature ranging from 5 to 20°C and the combined effect of maturation stage and prolonged storage duration on β-cryptoxanthin accumulation, ‘Nichinan No. 1’ was used as an experimental model. The fruit was harvested in early- and late-October. The fruit was divided into four groups and stored in the dark for 30 days at 5, 10, 15 and 20°C under 85–90% relative humidity with continuous ventilation. After 0, 14 and 30 days storage, at least three fruits per tree were collected and three samples per treatment were prepared as described above. The changes in carotenoid content in the juice sacs of each sample were analyzed as described below.

Experiment 4: Effect of temperature on β-cryptoxanthin accumulation during long-term storage of ‘Aoshima’ satsuma mandarin fruit

To examine temperature-effect on β-cryptoxanthin accumulation during long-term storage, the fruit of ‘Aoshima’, a late ripening cultivar, was harvested from a tree in early December. The fruit was divided into three groups and stored without pre-storage conditioning in the dark for 90 days at 5, 10, and 20°C under 85–90% relative humidity with continuous ventilation. After 0, 14, 30, 60, and 90 days storage, six fruits per treatment were collected, and three samples per treatment were prepared as described above. In another year, to examine the temperature-effect during long-term storage and the effect of the intermediate temperatures of 5 to 10°C, a large-scale experiment was conducted. The fruit of ‘Aoshima’ was harvested from three different trees in early December. The fruit was conditioned at ambient temperature until the weight was reduced by 5% to prevent fruit decay during long-term storage. After pre-storage conditioning, the fruit was divided into three groups and stored in the dark for 80 days at 5, 8, and 10°C under 85–90% relative humidity with continuous ventilation. After 0 and 80 days storage, at least three fruits per tree were collected and blended, and three samples derived from three different trees were prepared as described above. A total of nine fruits were used. The changes in carotenoid content in the juice sacs of each sample were analyzed as described below.

Carotenoid analysis

In experiment 1, carotenoids, phytoene, ζ-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, 9-cis-violaxanthin, and all-trans-violaxanthin in the juice sacs were extracted and analyzed according to a previously described method (Matsumoto et al., 2009). The carotenoid content of each sample was analyzed using HPLC (Agilent 1100; Agilent Technologies, Palo Alto, CA, USA) with a 250 mm × 4.6 mm i.d., 5 µm, YMC Carotenoid C30 column (YMC Europe GMBH, Dinslaken, Germany) with a ternary gradient elution of water, methanol, and MTBE pumped at a flow rate of 1 mL·min⁻¹ and photodiode array detection (Kato et al., 2004). In experiments 2, 3, and 4, conventional analysis of β-cryptoxanthin was performed according to a previously described method. Briefly, β-cryptoxanthin was extracted with ethanol and analyzed using HPLC with binary gradient elution (Kumagai et al., 2016). The carotenoids were identified by comparing the retention times and spectra with authentic standards or literature data (Kato et al., 2004). Carotenoid content was determined by reference to the standard curve prepared for each carotenoid.

Total RNA extraction and real-time quantitative RT-PCR

Total RNA was extracted from the flavedo and juice sacs according to a previously reported method and cleaned up with on-column DNase digestion (Ikoma et al., 1996). The reactions of reverse transcription (RT) were performed with purified RNA and a random hexamer using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA) (Kato et al., 2006). The gene expression analyses for PSY (phytoene synthase), PDS (phytoene desaturase), ZDS (ζ-carotene desaturase), LCYb (lycopene-β-cyclase), HYb (β-ring hydroxylase), ZEP (zeaxanthin epoxidase), and NCED (nine-cis-epoxycarotenoid dioxygenase) were performed according to a previously reported method (Kato et al., 2006). TaqMan MGB probes and sets of primers for CitPSY, CitPDS, CitZDS, CitLCYb, CitHYb, CitZEP, CitNCED3, and CitNCED5 (previously described as CitNCED2), designed on satsuma mandarin cDNAs, have been described in previous studies (Ikoma et al., 2016). TaqMan real-time PCR was carried out with TaqMan Universal PCR Master Mix (Applied Biosystems) according to the manufacturer’s instructions. As an endogenous control, the TaqMan Ribosomal RNA Control Reagents VIC Probe (Applied Biosystems) was used. The levels of gene expression were analyzed with ABI PRISM 7000 Sequence Detection System Software (Applied Biosystems) and normalized with the results of 18S ribosomal RNA. Real-time quantitative RT-PCR was performed in three replicates for each sample.

Statistical data analysis

The statistical significance of the results was analyzed using Tukey’s HSD test at the 5% level using the application software JMP 8 (JMP release 8.0; SAS Institute Inc., Cary, NC, USA).
**Results and Discussion**

**Experiment 1: The effect of temperature and maturation stage on carotenoid accumulation in the juice sacs of ‘Aoshima’ satsuma mandarin**

To examine the effect of temperature and maturation stage on carotenoid accumulation, fruit was harvested at 7 different stages of maturation from Oct. 5 to Jan. 9 and stored at 5 or 20°C for 15 days. The carotenoid content was analyzed before and after storage. Seven carotenoids, phytoene, ζ-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and violaxanthin, were quantified. The total carotenoid content was the sum of them. The violaxanthin content was the sum of 9-cis-violaxanthin and all-trans-violaxanthin.

During on-tree maturation, the total carotenoid content and β-cryptoxanthin, a major carotenoid in the juice sacs (60–70% of the total carotenoids), linearly increased from Oct. 5 to Nov. 19 or Dec. 10, and then remained at similar levels until Jan. 9 (Fig. 1). At 20°C, in the fruit harvested before Nov. 4, the content of total carotenoids and β-cryptoxanthin significantly increased (1.4- to 2-fold) at 15 days after harvest, and the increase in content was similar to that in fruit stored on the tree for 15 days (“before storage” for 15 days later, Fig. 1). To be more specific, the β-cryptoxanthin content of the fruit harvested on Oct. 20 and stored until Nov. 4 (10.9 μg·g⁻¹) at 20°C was similar to that of the fruit harvested on Nov. 4 (10.5 μg·g⁻¹). In contrast, in fruit harvested after Nov. 19, the increase in the β-cryptoxanthin content after harvest at 20°C was small and statistically insignificant. At 5°C, the increase in β-cryptoxanthin content was small and statistically insignificant across all maturation stages (Fig. 1).

Likewise, β-carotene and zeaxanthin significantly increased after harvest at 20°C, and the increase was greater in the early than the later stage of maturation (Fig. 1). For example, in fruit harvested before Nov. 4, β-carotene increased 2.1- to 5.9-fold after harvest at 20°C, whereas after Nov. 19, the content increased only 1.2- to 1.6-fold (Fig. 1). At 5°C, the change in content after harvest was small and not statistically significant at most maturation stages. Similar changes were observed in the juice sacs of the early ripening cultivar ‘Miyagawa-wase’ (data not shown).

These results showed that in satsuma mandarin fruit

**Fig. 1.** Changes in carotenoid content before and after storage at 5°C and 20°C in the juice sacs of ‘Aoshima’ harvested at different maturation stages. Different letters indicate significant differences among samples harvested on the same day at P < 0.05 by Tukey’s HSD test. Content is expressed on a fresh-weight basis, μg·g⁻¹. Days after flowering (DAF) were as follows: Oct. 5 (148 DAF), Oct. 20 (163 DAF), Nov. 4 (178 DAF), Nov. 19 (193 DAF), Dec. 10 (214 DAF), Dec. 24 (228 DAF), and Jan. 9 (244 DAF). The values are the mean ± SE of three replicates.
harvested at the stage in which β-cryptoxanthin continuously accumulates on the tree, the content in the juice sacs continued to increase at 20°C for 15 days after harvest and that the increased content was similar to that in fruit left on the tree. In contrast, in fruit harvested at the stage in which accumulation of β-cryptoxanthin stagnates on the tree, the increase in the content was negligible at 20°C for at least 15 days (Fig. 1). These results suggest that the effects of temperature on the accumulation of β-cryptoxanthin in the juice sacs was maturation stage-dependent and that the accumulation on the tree would continue in postharvest fruit at 20°C, but not at 5°C, for at least 15 days after harvest.

Recent studies have shown that storage temperature affect the carotenoid content in the pulp of several citrus varieties, and the higher content during storage tended to be maintained at 10–20°C compared to below 5°C (Carmona et al., 2012; Chaudhary et al., 2012, 2014, 2017; Matsumoto et al., 2009). In satsuma mandarin fruit harvested in early December, we previously reported that the carotenoid content in the juice sacs did not clearly increase at 20°C, but the content was higher at 20°C than at 5°C for three weeks (Matsumoto et al., 2009). The present results are consistent with these previous findings.

To confirm our hypothesis that β-cryptoxanthin accumulation on the tree continues after harvest at 20°C, but not at 5°C, the gene expression of carotenoid metabolic enzymes was analyzed in the fruit harvested on Nov. 4, Dec. 10, and Dec. 24. Gene expressions for CitPSY (phytoene synthase), CitPDS (phytoene desaturase), CitZDS (ζ-carotene desaturase), CitLCYb (lycopene-β-cyclase), CitHYb (β-ring hydroxylase), CitZEP (zeaxanthin epoxidase), CitNCED3 (nine-cis-epoxy-carotenoid dioxygenase), and CitNCED5 examined in the present study are considered to contribute to carotenoid accumulation during citrus fruit maturation and after harvest (Alquézar et al., 2008; Ikoma et al., 2016; Kato et al., 2004, 2006; Matsumoto et al., 2009). CitNCED5 isolated from the satsuma mandarin was previously described as CitNCED2 (Ikoma et al., 2016).

In the fruit harvested on Nov. 4, the gene expression of CitPSY, CitZDS, and CitLCYb, related to β-cryptoxanthin biosynthesis, did not notably change after storage at 20°C (Fig. 2). During on-tree maturation from Nov. 4 to Dec. 10, these changes were also negligible (Black bars in Fig. 2). The gene expression of CitHYb, CitZEP, and CitNCED5, related to β-cryptoxanthin catabolism, decreased both after storage at 20°C of the fruit harvested on Nov. 4 and during on-tree maturation from Nov. 4 to Dec. 10 (Fig. 2). The content of β-cryptoxanthin increased both during storage at 20°C and on-tree maturation (Fig. 1). In contrast, at 5°C, gene expression related to β-cryptoxanthin biosynthesis (CitPSY, CitPDS, CitZDS, and CitLCYb) decreased during the storage of fruit harvested on Nov. 4, but did not clearly change during on-tree maturation from Nov. 4 to Dec. 10 (Fig. 2). The gene expression of CitHYb did not change at 5°C, but decreased during on-tree maturation. The β-cryptoxanthin content did not change at 5°C, but increased during on-tree maturation (Fig. 1). Thus, in the fruit harvested on Nov. 4 and stored at 20°C, changes in the gene expression and β-cryptoxanthin content after harvest were similar to those during on-tree maturation from Nov. 4 to Dec. 10; however, they were different when stored at 5°C.

In the fruit harvested on Dec. 10, the β-cryptoxanthin content and most of the gene expression did not change notably after storage at 20°C (Figs. 1 and 2). These changes were also small during on-tree maturation from Dec. 10 to Dec. 24 (Fig. 2). In contrast, at 5°C, gene expression related to β-cryptoxanthin synthesis did not notably change after harvest; however, the gene expression of CitNCED3 and CitNCED5, related to β-
cryptoxanthin catabolism, markedly increased (Fig. 2). The increased expression of CitNCED3 and CitNCED5 after storage at 5°C was not observed during on-tree maturation from Dec. 10 to Dec. 24. Thus, the present results support our hypothesis that carotenoid biosynthesis during on-tree maturation continues after harvest at 20°C, but not at 5°C, as the pattern of changes in the gene expression and β-cryptoxanthin content during on-tree maturation were similar to those at 20°C after harvest, but not at 5°C.

Previous studies also reported that in ‘Navelina’ oranges, changes in the carotenoid content and expression of carotenoid biosynthetic genes during storage at 12°C were similar to those occurring naturally during the maturation of citrus fruit (Carmona et al., 2012; Rodrigo and Zacarías, 2007). The results are consistent with our hypothesis that carotenoid biosynthesis continues after harvest at normal temperatures, but not at low temperatures. Similar results were also reported in postharvest watermelons; the results showed that carotenoid biosynthesis in watermelons continued long after harvest, and carotenoid biosynthesis was enhanced during storage at 21°C, but inhibited at 5°C (Perkins-Veazie and Collins, 2006).

**Experiment 2: Varietal differences in temperature-responsive β-cryptoxanthin accumulation at 20°C**

To examine whether the increase in β-cryptoxanthin content at 20°C is also observed in other cultivars with different maturity seasons, the fruits of 10 different satsuma mandarin cultivars were harvested at their commercial harvest times and stored at 20°C for 14 days (Fig. 3). In all the very early ripening cultivars (‘Nichinan No. 1’, ‘Iwasaki’, ‘Dowaki’, and ‘Ueno-wase’), the content significantly increased (1.1- to 1.5-fold) at 14 days after harvest, and the increase in content was similar to that of fruit stored on the tree (Fig. 3). In all the early ripening (‘Okitsu-wase’ and ‘Miyagawa-wase’) and mid-ripening cultivars (‘Nankan No. 20’ and ‘Seto’), changes in the content after harvest or on the tree were small or negligible (Fig. 3). In the late-ripening cultivars, the change was negligible in ‘Aoshima’, but in ‘Otsu No. 4’, the content significantly increased (1.5-fold) after harvest. These results suggest that the effects of β-cryptoxanthin accumulation after storing at 20°C are different among satsuma mandarin cultivars, probably because the progress of β-cryptoxanthin accumulation on the tree at harvest time is different among cultivars.

**Experiment 3: Effect of intermediate temperature (5 to 20°C) and combined effect of maturation stage and prolonged storage duration on β-cryptoxanthin accumulation in ‘Nichinan No. 1’ satsuma mandarin**

To examine the effect of intermediate temperature between 5 and 20°C and the combined effect of maturation stage and prolonged storage duration on β-
but increased at 12°C (Carmona et al., 2012). In ‘Star Ruby’ grapefruit, β-carotene and lycopene levels in the juice of fruit stored at 11°C were higher than that in fruit stored at 2°C after 16 weeks of storage, but no significant differences were observed after four and eight weeks of storage (Chaudhary et al., 2014). These results are consistent with our present result that at approximately 10°C, prolonged storage increased carotenoid content, and the content at 10°C and above was higher than that at 5°C.

**Experiment 4: Effect of temperature on β-cryptoxanthin accumulation during long-term storage of ‘Aoshima’ satsuma mandarin fruit**

‘Aoshima’, a late ripening cultivar, are usually stored at low or ambient temperature for one to three months. Thus, the effects of temperature on β-cryptoxanthin accumulation during storage were examined at 5, 10, and 20°C for 90 days. The fruit was harvested in the normal harvest season of early December, during which carotenoid accumulation on the tree stagnates, and stored without pre-storage conditioning. At 5°C, changes in the β-cryptoxanthin content were negligible for 90 days after harvest (Fig. 5). At 10°C, changes in content were negligible at 14 days after harvest, but the content increased 1.3-fold at 30 days after harvest and was maintained until 90 days after harvest (Fig. 5). At 30 and 90 days after harvest, the content in the fruit stored at 10°C was higher than that at 5°C. Similar changes were observed at 20°C, but many fruits had decayed by 60 days after harvest (Fig. 5).

To reduce decay during storage, pre-storage conditioning and low-temperature storage below 10°C is desirable (Hasegawa and Iba, 1983, 1984). In addition, recent studies reported that storage at 8°C was desirable to maintain the flavor quality of some mandarin cultivars (Obenland et al., 2011; Tietel et al., 2012). Thus, to examine the effect of temperatures between 5 and 10°C on β-cryptoxanthin accumulation, ‘Aoshima’ was harvested in early December and pre-storage conditioning was conducted before the fruit was stored at 5, 8, and 10°C for 80 days. After 80 days storage, changes in the β-cryptoxanthin content were negligible at 5°C, but the content increased 1.3-fold at 8 and 10°C (Fig. 6).

These results showed that in ‘Aoshima’ satsuma mandarin fruit stored at conditions ranging from 5 to 10°C, the β-cryptoxanthin content does not decrease for at least 90 days of storage. Moreover, the present results suggest that at 8°C and above, the β-cryptoxanthin content would increase after harvest even if the fruit was harvested at the stage in which β-cryptoxanthin accumulation stagnates on the tree. Another recent study on ‘Aoshima’ satsuma mandarin fruit also reported that the β-cryptoxanthin content in the pulp was maintained during storage at 8°C for 4 months (Hamasaki and Yamaga, 2017).

In conclusion, the results of the present study have shown that, in the juice sacs of satsuma mandarin fruit, the β-cryptoxanthin content does not decrease under postharvest conditions between 5 and 20°C, irrespective of the maturation stage. The present results suggest that

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**Fig. 4.** Changes in β-cryptoxanthin content before and after storage at 5, 10, 15, and 20°C in the juice sacs of ‘Nichinan No. 1’ harvested in early October (A) and late October (B). Different letters indicate significant differences among the storage temperatures at $P < 0.05$ by Tukey’s HSD test. Content is expressed on a fresh-weight basis, μg·g$^{-1}$. The values are the mean ± SE of three replicates.

**Fig. 5.** Changes in β-cryptoxanthin content before and after storage at 5, 10, and 20°C in the juice sacs of ‘Aoshima’ harvested in early December. Different letters indicate significant differences among the samples before and after storage at different temperatures at $P < 0.05$ by Turkey’s HSD test. Content is expressed on a fresh-weight basis, μg·g$^{-1}$. The values are the mean ± SE of three replicates.

**Fig. 6.** Changes in β-cryptoxanthin content before and after storage at 5, 8, and 10°C for 80 days in the juice sacs of ‘Aoshima’ harvested in early December. Different letters indicate significant differences among the samples before and after storage at different temperatures at $P < 0.05$ by Turkey’s HSD test. Content is expressed on a fresh-weight basis, μg·g$^{-1}$. The values are the mean ± SE of three replicates.
the β-cryptoxanthin content gradually increases after harvest at 8°C and above, and that the increase rate is maturation stage-dependent. The rate of increase in the β-cryptoxanthin content will be more rapid in fruit harvested when carotenoid accumulation is in progress on the tree.

**Literature Cited**


