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RESEARCH NOTE

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Changes in β-Cryptoxanthin Content of *Setoka* Fruits Ripened in Greenhouse Cultivation

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Abstract *Setoka* (*Tangor Norin* No. 8) is a superior tangor cultivar cultivated in a greenhouse on Jeju Island, and its β-cryptoxanthin content was determined during the ripening season (September 2005 to March 2006). The β-cryptoxanthin content of the peel of *Setoka* fruits was higher than that of the flesh. Also, the β-cryptoxanthin content in both peel and flesh gradually increased through the ripening season from the beginning of pigmentation, and then decreased slightly late in the ripening season. The β-cryptoxanthin content in the peel of *Setoka* fruits varied throughout the season with values of 0.02 (Sep), 0.67 (Nov.), 2.27 (Dec.), 2.88 (Jan.), 2.27 (Feb.), and 2.13 mg% (Mar.). The β-cryptoxanthin content in *Setoka* fruit flesh increased throughout the ripening season with values of 0.05 (Sep.), 0.22 (Nov.), 0.57 (Dec.), 0.80 (Jan.), and 0.91 mg% (Feb).

Keywords: β-cryptoxanthin, *Setoka* fruits, HPLC, peel, flesh

Introduction

Citrus fruit trees are the most widely cultivated fruit trees in the world (1), and *Citrus* fruits have been used as a valuable ingredient for oriental medicine and functional foods (2-4). Among *Citrus* cultivars, *Setoka* was produced from a cross between Kuchinitsu No.37 (*Kiyomi* × *Encore* No.2) and *Murcott*. It was registered as *Tangor Norin* No.8 and released as a superior citrus cultivar in 1998 (5). Since *Setoka* was introduced from Japan, the growing area of *Setoka* on Jeju Island in Korea has rapidly increased. The demand for new technology in the local cultivation of the *Setoka* cultivar has increased during its short history of cultivation on Jeju Island. Generally, changes in cultivation technology affect the physico-chemical properties of *Setoka* fruits. Although the analyses of functional components such as carotenoids are necessary, no such studies have been reported with regard to the ripening of *Setoka* fruits.

Carotenoids are natural pigments that have been shown to play vital physiological roles as well as contributing to food quality (6). Recently, there has been considerable interest in dietary carotenoids with respect to their potential in alleviating chronic diseases in humans (7). Carotenoids present in fruits and vegetables are widely believed to protect human health by preventing certain cancers (8, 9) and functioning as antioxidants to quench oxygen radicals (10). Various natural carotenoids, being an important biological precursor of vitamin A, have been associated with the reduced risk of cardiovascular disease (11). Some epidemiological studies have confirmed an inverse relationship between carotenoid consumption and the development of some cancers (12, 13).

More than 600 carotenoids have been identified in foods, and most nutritional research has been focused on β-carotene, lycopene, lutein, and β-crypto-xanthin (14). β-Cryptoxanthin is present in human serum and tissues and plays a protective role against human disease (15, 16). β-Cryptoxanthin has been demonstrated to have anti-breast cancer activity and inhibitory effects on osteoclast-like cell formation in mouse marrow cultures *in vitro* (17, 18) and the development of colon carcinoma in rats (15).

β-Cryptoxanthin has received recent attention as a target carotenoids because of its powerful biological activities regarding human health. To evaluate the nutritive and biological value of food carotenoids, concentrations need to be determined quantitatively (6). There has been particular emphasis on obtaining more accurate data on the concentrations of various carotenoids in foods for evaluating various health and nutritional benefits (19). In addition, the accurate characterization of the association between carotenoid intake and various chronic diseases is also necessary (14).

In general, the accumulation of β-carotenoids in *Citrus* fruits can vary with the cultivar type and cultural environment, including climate and cultivation conditions. Quantitation of the content of individual carotenoids in *Citrus* fruits has become increasingly important, and a variety of separation and quantization procedures have been used in their study (1). To evaluate the *Setoka* as a source of β-cryptoxanthin it is necessary to ascertain the compositional difference between the peel and flesh of *Setoka* fruits cultivated in a greenhouse on Jeju Island. The comparison of functional components will provide valuable information for producing *Setoka* fruits fortified with higher β-cryptoxanthin content. In this study, the β-cryptoxanthin contents were determined for the peel and flesh of *Setoka* fruits harvested in a greenhouse during the

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6 month ripening season.

Materials and Methods

Materials *Setoka* fruits cultivated in a greenhouse on Jeju Island were harvested from September 2005 to March 2006. The peel and flesh from *Setoka* fruits were separated and sliced. Each part of *Setoka* fruits was stored in a -70°C deep freezer. The frozen samples were thawed in a refrigerator before carotenoid extraction. β-Cryptoxanthin as a standard and butylated hydroxy toluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Crystalline carotenoids were obtained from Extrasynthese (Genay, France). Other chemicals used were of high performance liquid chromatography (HPLC) or analytical grade. Thin layer chromatography (TLC) plates were purchased from Merck Co. (Silica gel 60 F254; Whitehouse Station, NJ, USA). A Bondapak™ C18 reverse phase column (3.9×300 mm, particle size 10 μm) was obtained from Waters Co. (Milford, MA, USA).

Extraction of carotenoids The extraction of carotenoids was performed using the method reported by Heo *et al.* (20). Ten g of peel (or 100 g of flesh) was mixed with 60 mL of 40% methanol containing 1 g of $MgCO_3$ using a juice mixer (LG, Korea). The supernatant was obtained by centrifugation at 7,600×g for 10 min at 10°C. The residue was mixed with 140 mL of an acetone/methanol mixture (7/3) with 0.1% BHT and stirred at 170 rpm at 10 for 1 hr. The extract was collected by vacuum filtration. This process was repeated to recover carotenoid pigments until the residue turned colorless. Filtrate containing carotenoid pigments was transferred to a 1 L of separating funnel and mixed thoroughly with 150 mL of distilled water, 250 mL ethyl ether and 100 mL of 10% NaCl. After standing for 1 hr the top phase containing carotenoid pigments was collected and concentrated using a vacuum evaporator (Eyera, Tokyo, Japan) at 35°C. Crude carotenoids were dissolved in 20 mL of ethyl ether containing 20% methanolic KOH. Saponification was performed at room temperature in the dark for 2 hr at 22°C. The saponified sample was subsequently partitioned by mixing with 20 mL of saturated NH_4Cl and 50 mL of ethyl ether in a separating funnel, and collecting the organic layer. The aqueous layer was mixed with diethyl ether, and then combined with the organic layer and washed several times with distilled water. The organic layer was concentrated to dryness using a rotary evaporator at 35°C. The saponified samples were dissolved in 10 mL of methyl tert-butyl ether (MTBE)/methanol (1/1, v/v) containing 1% BHT and filtered through a 0.45 μm PTFE filter (Micro Filtration System, Ventura, CA, USA).

Chromatography The carotenoids extracted from peel or flesh of *Setoka* fruit were analyzed using silica gel TLC plates (Silica gel 60 F254; Merck). For the separation of pigment compounds, a hexane/acetone (3/1, v/v) mixture was used as the mobile solvent. The separation of components was carried out for 30 min in darkness at room temperature. β-Cryptoxanthin as a standard carotenoid was used for determining the retention factor (R_f) value.

The quantitative separation of carotenoids was also performed by HPLC equipped with a liquid chromatogram (LC-10AD VP; Shimadzu, Kyoto, Japan), UV/Vis detector (SPD-10A VP; Shimadzu), and a Bondapak™ C_{18} reverse phase column. The column temperature was maintained at 35°C. As the mobile phase, HPLC-grade methanol, water and analytical grade MTBE were mixed to create a gradient ranging from (95:1:4) to (25:71:4) for 13 min. Each solvent was filtered through a 0.5 μm PTFE membrane filter (Advantec MFS, Inc., Dublin, CA, USA) and then passed through a solvent degasser (DGU-14A; Shimadzu). Each sample was injected onto the column with an automatic sampler (SIL-20A; Shimadzu) equipped with a sample loop (20 μL). Each operation was performed for 35 min with a 1 mL/min flow rate, and then peak responses were determined by measuring the absorbance at 445 nm. A 5 mg sample of β-cryptoxanthin used as a reference was dissolved in 25 mL of methyl tert-butyl ether containing 1% BHT and methanol (1:1, v/v). The concentrations of carotenoid standards ranged from 0.1-0.5 μg/mL. The linearity of the calibration between the concentration of β-cryptoxanthin and the absorbance was determined. The retention time of β-cryptoxanthin was used for the identification of β-cryptoxanthin from the extract of *Setoka* fruits. Duplicate samples were injected for each extract for HPLC analysis.

Results and Discussion

TLC analysis of carotenoids in *Setoka* Crude carotenoids were extracted from the peel and flesh of *Setoka* fruits grown in a greenhouse on Jeju Island. The composition of crude carotenoids prepared by solvent extraction was analyzed by TLC. As shown in Fig. 1 and 2, crude carotenoids extracted from peel were composed of several carotenoid compounds, including β-cryptoxanthin with an R_f value of 0.38. It was reported that β-cryptoxanthin from *Citrus* fruits produced in Jeju Island has an R_f value of 0.39 (21). It was expected that β-carotene would be separated with an R_f value of 0.9. Crude carotenoids containing β-carotene were the only dominant fraction in extract from *Setoka* fruit peel that were harvested at the beginning of pigmentation (Fig. 1). On the other hand, the crude carotenoid content of the flesh of *Setoka* fruits was very low (Fig. 2). The β-cryptoxanthin content of the flesh and peel was greatly increased as the harvest season progressed from September to January. However, β-cryptoxanthin in the peel of *Setoka* fruits decreased after January. Crude carotenoids containing β-cryptoxanthin in the peel were greatly reduced in fruits harvested in March (Fig. 1). As shown in Fig. 2, β-cryptoxanthin in the flesh did not decrease until March. β-Carotene in the peel of *Setoka* fruits was synthesized during the early pigmentation season (September) and then greatly reduced during ripening. In contrast, the β-carotene content of the flesh remained relatively constant during the late ripening season beginning in January (Fig. 2).

Analysis of β-cryptoxanthin by HPLC To determine the β-cryptoxanthin content quantitatively, HPLC analysis was performed. The retention time of β-cryptoxanthin was about 21 min. β-Cryptoxanthin in the peel increased in

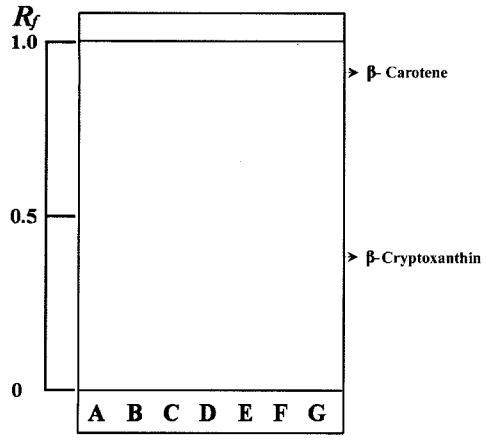

Fig. 1. TLC chromatograms of β-cryptoxanthin standard and carotenoids from peel of *Setoka* fruits. A, β-Cryptoxanthin standard; B, September; C, November; D, December; E, January; F, February; G, March.

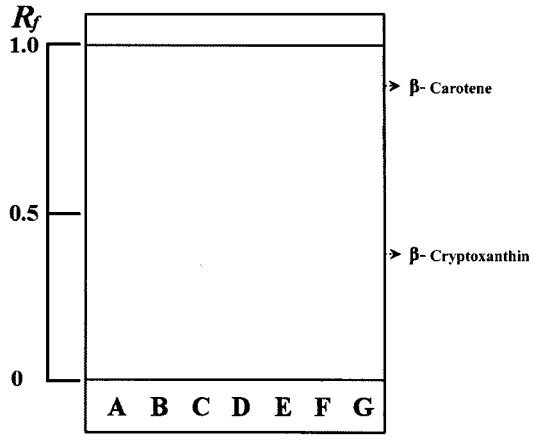

Fig. 2. TLC chromatograms of β-cryptoxanthin standard and carotenoids from flesh of *Setoka* fruits. A, β-Cryptoxanthin standard; B, September; C, November; D, December; E, January; F, February; G, March.

Setoka fruits harvested from September through January, and decreased thereafter as shown in Fig. 3. However, β-cryptoxanthin in the flesh increased up through February and then decreased (Fig. 4). Simultaneously, β-cryptoxanthin in the crude carotenoid fraction was more concentrated as shown in the chromatograms of various carotenoid components (Fig. 3 and 4). Based on these results, it was considered that crude carotenoids in the peel contained xanthophyll-like compounds with a retention time of 4-10 min, along with β-carotene and lutein with retention times of 26 and 10 min, respectively. As shown in Fig. 3, the peak of β-carotene detected in the peel disappeared during the harvesting season, however the peak of β-carotene in the flesh appeared late in the ripening season (Fig. 4).

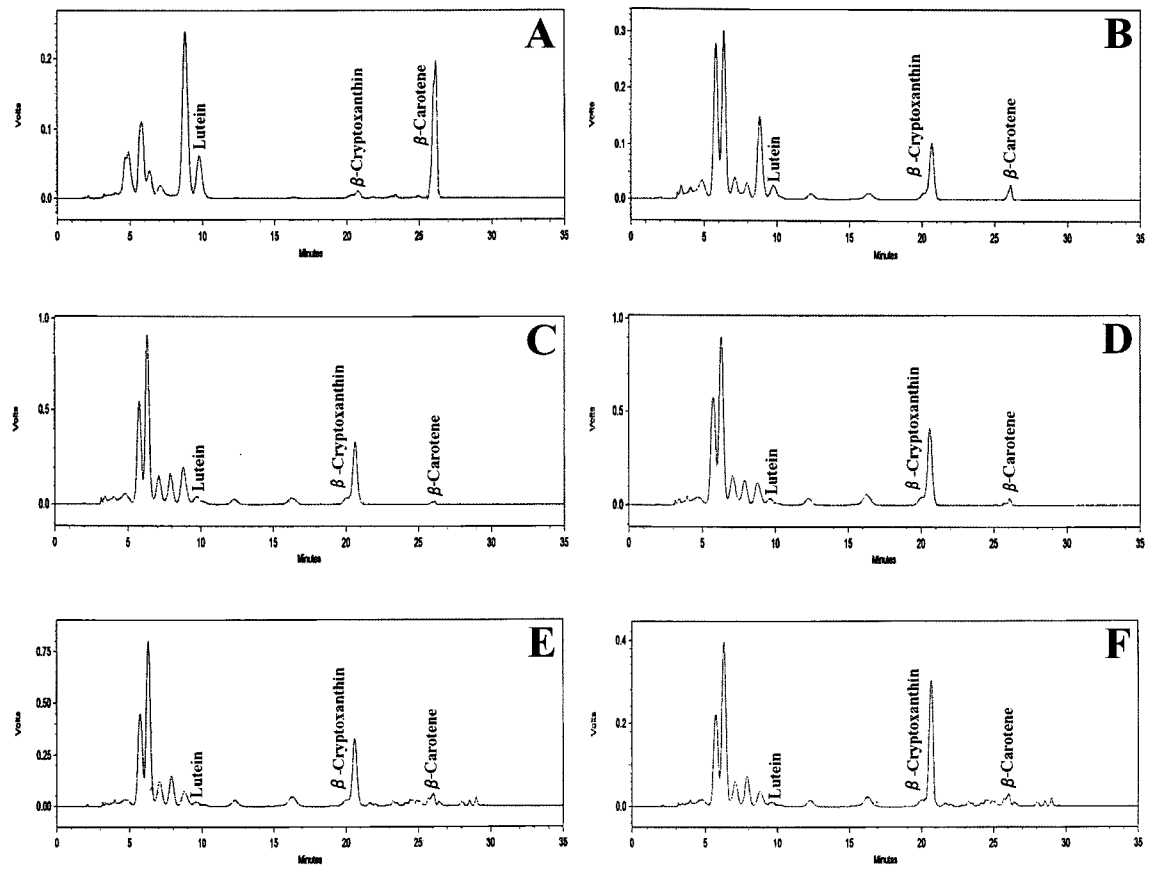

Fig. 3. HPLC elution profiles of carotenoid pigments extracted from peel of *Setoka* fruits. A, September; B, November; C, December; D, January; E:, February; F, March.

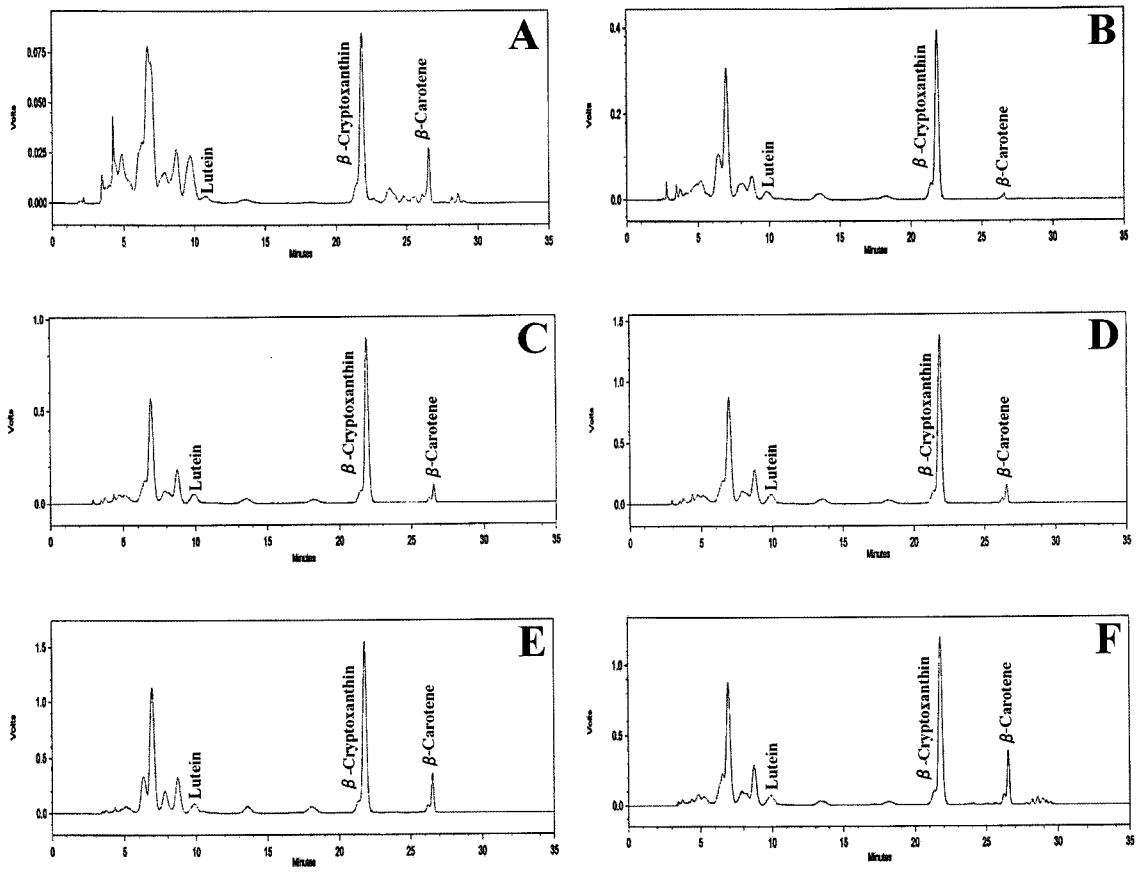

Fig. 4. HPLC elution profiles of carotenoid pigments extracted from flesh of *Setoka* fruits. A, September; B, November; C, December; D, January; E, February; F, March.

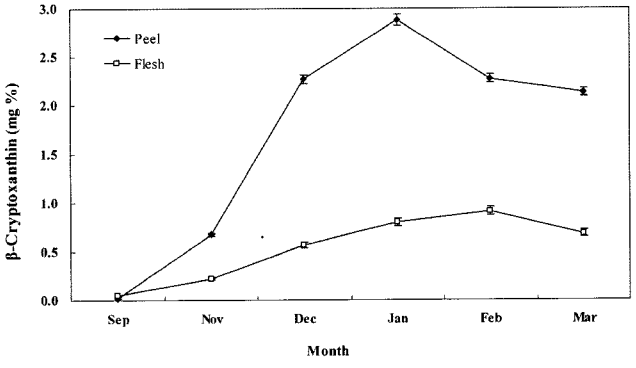

Fig. 5. β-Cryptoxanthin content from peel and flesh of *Setoka* fruits according to the harvesting season.

These results coincide with the patterns of TLC analysis.

As shown in Fig. 5, the β-cryptoxanthin content varied greatly in both peel and flesh of *Setoka* fruits depending on the month of harvest. In *Setoka* fruits grown in a greenhouse, the β-cryptoxanthin contents of the peel were 0.02 (Sep.), 0.67 (Nov.), 2.27 (Dec.), 2.88 (Jan.), 2.27 (Feb.), and 2.13 mg% (Mar.). β-Cryptoxanthin contents of the flesh showed a similar pattern of increase with values of 0.05 (Sep.), 0.22 (Nov.), 0.57 (Dec.), 0.80 (Jan.), 0.91 (Feb.), and 0.68 mg% (Mar.). The β-cryptoxanthin content of *Setoka* fruit peel was higher than that of flesh during the harvesting season.

It has been previously reported that the β-cryptoxanthin content of the peel of *Citrus* fruits is higher than that of the flesh (1, 21). The β-cryptoxanthin content in the peel and flesh of *Citrus* fruits is also greatly affected by the time of harvest (20). Specifically, it was concluded that the β-cryptoxanthin contents of both peel and flesh increased as the *Setoka* fruits matured. This suggested that the synthesis of β-cryptoxanthin was initiated earlier in the peel which was directly exposed to sunlight, followed by synthesis in the flesh. The β-cryptoxanthin contents of both the peel and flesh of *Setoka* fruits were reduced late in the harvesting season. During the harvesting season the reduction of β-cryptoxanthin content began earlier in the peel than in the flesh. The β-cryptoxanthin content was greatly increased in the flesh of *Setoka* fruits grown in a greenhouse and harvested in February (Fig. 5). This suggested that the harvesting of *Setoka* fruits in February is quite reasonable for maximizing the β-cryptoxanthin content.

Considering the higher content of β-cryptoxanthin in the peel of *Setoka* fruits, it is necessary to utilize the peel effectively as a functional ingredient. Generally, the β-cryptoxanthin contents vary according to *Citrus* cultivar. It was reported that the amount of β-cryptoxanthin ranged from 0.3 to 2.1 mg% in the peel of domestic *Citrus* cultivars (19). *Miyagawa wase* showed the highest β-cryptoxanthin content to be 5.26 mg% in the peel and 0.78

mg% in flesh (2). However, the β-cryptoxanthin contents of lemon and grapefruit were considerably lower than those found in other *Citrus* cultivars, containing below 0.1 mg% in both peel and flesh (19). The accumulation of β-cryptoxanthin could be affected by various environmental conditions such as temperature and sunlight. It is expected that improvements in the culture environment will increase the levels of β-cryptoxanthin in *Setoka* fruits. The basic evaluation of β-cryptoxanthin content from *Setoka* fruits cultivated in a greenhouse on Jeju Island will provide important information to improve and utilize the *Setoka* cultivar in the future.

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