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Effect of Harvesting Season on the β-Cryptoxanthin in Shiranuhi Mandarin Fruit Cultivated in Jeju Island

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Abstract

β-Cryptoxanthin content was determined in Shiranuhi mandarin fruits harvested at monthly intervals from October to February in Jeju Island. Crude carotenoids were extracted from both peel and flesh of Shiranuhi mandarin fruits and analyzed using TLC and HPLC; β-cryptoxanthin was indicated the R_f value of 3.2 and retention time of 23 min, respectively. β-Cryptoxanthin contents in both peel and flesh were increased gradually as the citrus fruits ripened fully until harvesting season (February). According to the harvesting time, β-cryptoxanthin contents in the peel were 0.15 mg% (October), 0.28 mg% (November), 0.38 mg% (December), 1.23 mg% (January), and 1.71 mg% (February). In the flesh, β-cryptoxanthin contents were lower than those of peels, having 0.06 mg% (October), 0.08 mg% (November), 0.19 mg% (December), 0.26 mg% (January), and 0.65 mg% (February). These results demonstrate that β-cryptoxanthin in Shiranuhi mandarin fruits accumulated during ripening of the citrus fruits. In particular, the peels had much higher concentrations of β-cryptoxanthin and have potential for use as a functional ingredient.

Key words: β-cryptoxanthin, Shiranuhi mandarin fruits, HPLC, peel, flesh

INTRODUCTION

Citrus are the most widely cultivated fruit trees in the world (1). A new *Citrus* cultivar by hybridization of [(*Citrus unshiu* Marc × *Citrus sinensis* Osb)(*Citrus reticulata* Blanco)] was created in Japan at 1972, and then was enrolled as *Citrus* cultivar (No. 11) by the Japan Ministry of Agriculture and Forestry in 1984 (2). This new *Citrus* cultivar designated as "Shiranuhi mandarin" was also introduced in Jeju Island 15 years ago. Shiranuhi mandarin fruit produced in Jeju Island has the advantages of a superior sweetness and flavor, bigger size, and easy peeling compared to other *Citrus* cultivars. Generally, the Shiranuhi mandarin cultivar begins color development in the middle of October and then ripens with complete pigmentation after early of December (3). The technology for local cultivation of the Shiranuhi mandarin cultivar needs refinement because of its short history of cultivation in Jeju Island. Recently, the growth and nutritional characteristics of Shiranuhi mandarin cultivar were reported (4). However, analyses of functional components such as carotenoids during ripening are not yet reported.

Carotenoids are isoprenoid molecules that are widespread in nature and are typically seen as pigments in fruits, flowers, birds, and crustaceans (5). Besides their obvious contribution to food quality as natural pigments, they have been shown to play vital physiological roles (6). More than 600 carotenoids have been identified, but most nutrition research has focused on the five carotenoids with the highest known blood concentrations in US. populations: α-carotene, β-carotene, lycopene, lutein, and β-cryptoxanthin (7). Citrus fruits accumulate carotenoids including β-cryptoxanthin, and β-carotene in edible parts (8). Recently, carotenoids have attracted attention for their reported beneficial health effects (9,10), and are associated with reduced risk of chronic degenerative diseases, such as cancer, cardiovascular disease, and age-related eye disease (5). Carotenoids also function as quenchers of singlet oxygen, as antioxidants, in gene activation, and in inflammation and immune processes as modulators of lipoxygenases (11). β-Cryptoxanthin as a source of vitamin A is a known biological active compound, having anticancer properties as well as increasing immunity (12), and antimutagenicity (13). β-Cryptoxanthin has been found to have a unique anabolic effect on bone calcification (14), and anticarcinogenic effects (15).

Recently, as the functions of biological active component in natural foods are being elucidated, the qual-

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itative and quantitative analyses of component has been needed (16). For the assessment of the nutritive and biological value of food carotenoids, the absolute concentrations are needed (6) Carotenoid measurement in natural products involves extraction and chromatography with organic solvents (17). Considerable attention has been directed towards analysis of carotenoid pigments by HPLC (18). HPLC is one of the most powerful methods for analyzing carotenoids in various animal, plant and food materials (10,19). β-Cryptoxanthin from various citrus fruits can be separated quantitatively using HPLC (20). Therefore, the objective of the study was to determine the β-cryptoxanthin content from the peel and flesh of Shiranuhi mandarin fruits harvested during the 5 month in ripening season.

MATERIALS AND METHODS

Materials

Shiranuhi mandarin fruits were cultivated in a greenhouse in Jeju Island and were harvested through November 2003 to February 2004. The peel and flesh from citrus fruits were separated, sliced and stored at -70°C. β-Cryptoxanthin as a standard and butylated hydroxy toluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals used were HPLC or analytical grade. The TLC plate was purchased from Merck Co. (Silica gel 60 F254, Merck, USA). A μBondapak™ C18 reverse phase column (3.9×300 mM, particle size 10 μm) was obtained from Waters Co. (Waters chromatography, Milford, MA, USA).

Extraction of carotenoids

The extraction of carotenoids was performed using the method reported by Ko et al. (20). Approximately 10 g of peel (or 100 g of flesh) was mixed with 70 mL of 40% methanol and 1 g $MgCO_3$ and then homogenized using a juice mixer (LG, Korea). The supernatant was obtained by centrifugation at 7,000 rpm for 10 min at 10°C. The residue was mixed with 140 mL of acetone/methanol mixture (7/3) with 0.1% BHT and stirred at 170 rpm at 10°C 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 into a 1 L of separatory 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 then was concentrated using a vacuum evaporator (EyEra, Japan) at 35°C. Crude carotenoids were saponified by mixing 10 mL of ethyl ether and 10 mL of 20% methanolic KOH for 2 hr at 22°C in a dark room. The saponified sample was subsequently partitioned by mixing 20 mL of saturated NH_4Cl and 50 mL of ethyl ether in a separating funnel, and then the organic layer was collected. The aqueous layer was mixed with diethyl ether, and then the organic layer combined with it and washed several times with distilled water. The organic layer was concentrated to dryness using a rotary evaporator at 30°C. The saponified samples were dissolved in 5 mL of MTBE/methanol (1/1, v/v) containing 1% BHT and then filtered through a Millipore PTFE 0.45 μm filter (Micro Filtration System, CA, USA) before injection to the HPLC. The sample solutions were stored under nitrogen in a dark room, and were diluted to prepare working solutions in the range of 0.1~5.0 μg/ mL.

Chromatography

The carotenoids extracted from peel and flesh of citrus fruit were analyzed using silica gel TLC plates. The pigment compounds were separated using hexane/acetone (3/1, v/v) as the mobile solvent. β-Cryptoxanthin as a standard carotenoid was applied to determine its R_f value. The separation of carotenoids was also performed by HPLC. HPLC equipment included a Spectra-Physics (Spectra-SYSTEM) consisting of a P4000 pump (Spectrahysica Analytical, Inc., CA, USA), and a UV1000 UV/is detector (Spectra-Physics Analytical, Inc., CA, USA). A μBondapak™ C18 reverse phase column (3.9×300 mM particle size 10 μm) was used and column temperature was maintained at 35°C. The mobile phase was HPLC-grade methanol, water and analytical grade methyl tert-butyl ether (MTBE) mixed to yield gradient conditions 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., CA, USA) and then passed through a solvent degasser (A0099-504, Spectra-physics Analytical, Inc.). Each sample was injected onto the column via an automatic sampler (AS1000, Thermo Separation Productions Inc., USA) equipped with a sample loop (20 μL). An operation was performed for 30 min with 1 mL/min flow rate, and then peak responses were determined by measuring absorbance at 445 nm. With a reference, the linearity of the calibration between concentration of β-cryptoxanthin and absorbance was determined. The retention time of β-cryptoxanthin was used for the identification of β-cryptoxanthin from the extract of citrus fruits.

RESULTS AND DISCUSSION

TLC analysis of carotenoids in Shiranuhi mandarin

Crude carotenoids were extracted from peel and flesh of Shiranuhi mandarin fruits grown in Jeju Island and analyzed by TLC. As shown in Fig. 1, several crude

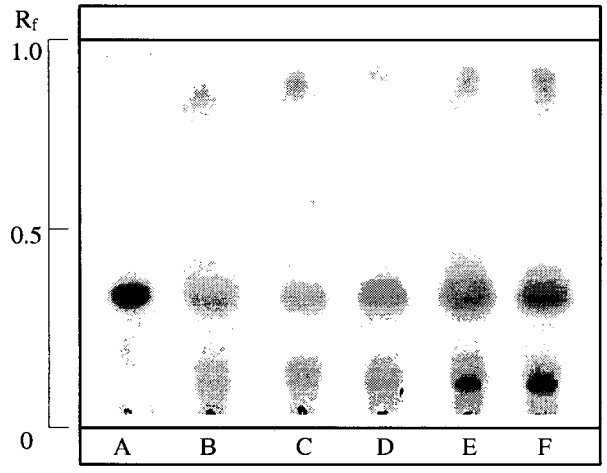

Fig. 1. TLC chromatograms of standard β-cryptoxanthin, and carotenoids from peel of Shiranuhi mandarin fruits. A: standard of β-cryptoxanthin, B: Oct., C: Nov., D: Dec, E: Jan., F: Feb.

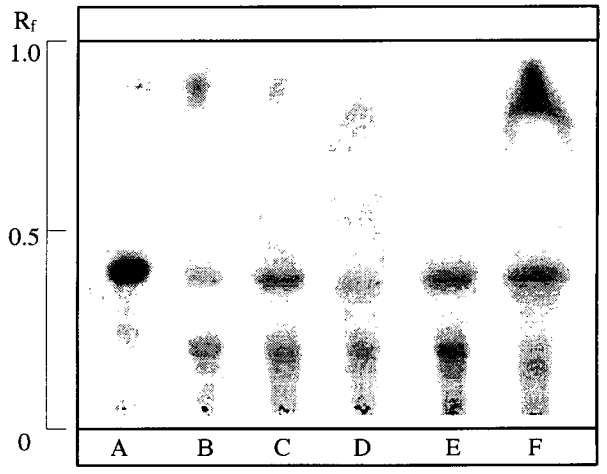

Fig. 2. TLC chromatograms of standard β-cryptoxanthin, and carotenoids from flesh of Shiranuhi mandarin fruits. A: standard of β-cryptoxanthin, B: Oct., C: Nov., D: Dec, E: Jan., F: Feb.

carotenoids were extracted from the peel, including β-cryptoxanthin. The R_f value of the standard β-cryptoxanthin was 0.32. In previous experiment, β-cryptoxanthin from citrus fruits produced in Jeju Island indicated an R_f value of 0.39 (20). The content of β-cryptoxanthin in peel gradually increased as the citrus harvest season progressed from October (coloring season) to February in the next year (harvesting season). Particularly, β-cryptoxanthin content was significantly increased from the peel of citrus fruits harvested in January (Fig. 1). Crude carotenoids obtained from flesh of the citrus fruits exhibited a similar pattern for β-cryptoxanthin content, but only a small amount of other pigments was detected (Fig. 2). Comparing with the β-cryptoxanthin contents between peel and flesh, the highest concentration of β-cryptoxanthin was found in the peel of citrus fruits. Also, other investigators found that β-cryptoxanthin content of the peel of citrus fruit was higher than that of flesh (20,21). Furthermore, the content of β-cryptoxanthin in both peel and flesh increased as the citrus fruits fully matured. These results demonstrate that the harvesting of Shiranuhi mandarin fruits during February is ideal for optimizing the β-cryptoxanthin content.

Analysis of β-cryptoxanthin by HPLC

β-Cryptoxanthin content was quantitatively analyzed by HPLC using a standard calibration curve. Generally, the yellow pigment of carotenoids absorbs maximally at 450 nm. β-Cryptoxanthin can be easily determined using a spectrophotometer in the visible range (22). The HPLC pattern of crude carotenoids from peel and flesh are shown in Fig. 3 and Fig. 4, respectively. As shown in Fig. 3, the crude carotenoids from peel were fractionated into various peaks including β-cryptoxanthin. The reten-

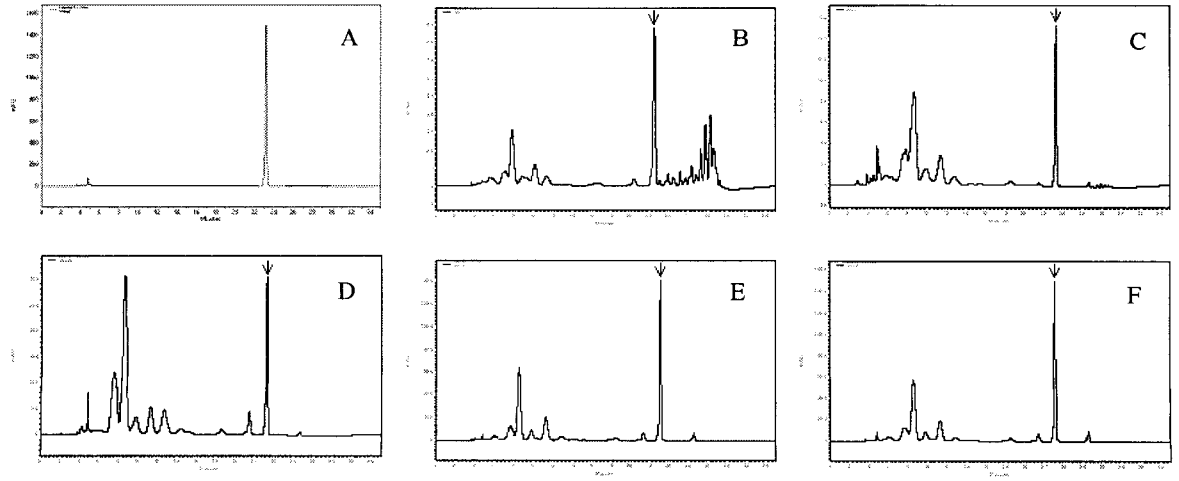

Fig. 3. β-Cryptoxanthin content from peel of Shiranuhi mandarin fruits. A: standard of β-cryptoxanthin, B: Oct., C: Nov., D: Dec, E: Jan., F: Feb.

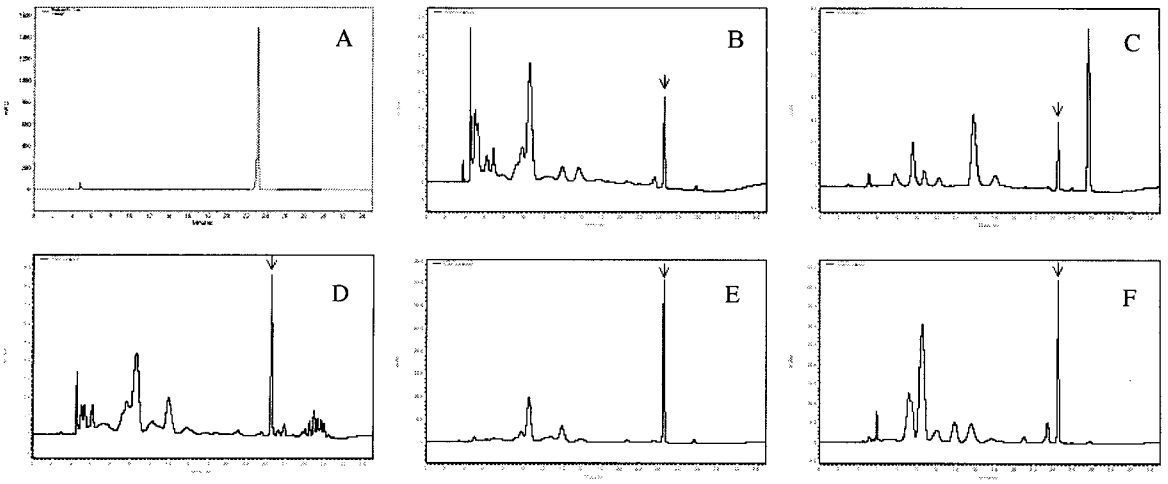

Fig. 4. β-Cryptoxanthin content from flesh of Shiranuhi mandarin fruits. A: standard of β-cryptoxanthin, B: Oct., C: Nov., D: Dec, E: Jan., F: Feb.

tion time of β-cryptoxanthin was about 23 min. Crude carotenoids from peel contained various carotenoid compounds with lower retention times of 4~13 min. The β-cryptoxanthin content from peel was increased, resulting in higher yields as the harvesting season of citrus fruits postponed from October to February in the next year (Fig. 3). The crude carotenoids from flesh showed a similar pattern compared to that of peel of citrus fruits. As shown in Fig. 4, β-cryptoxanthin content gradually increased as the harvesting season of citrus fruits were delayed. Consequently, β-cryptoxanthin content was greatly changed by both the harvesting season of citrus fruits as well as by the part of citrus fruit. As shown in Fig. 5, as the harvesting season was delayed, β-cryptoxanthin contents of peel were greatly increased from 0.15 mg% (October), 0.28 mg% (November), 0.38 mg% (December), 1.23 mg% (January), and 1.71 mg% (February). In the flesh of citrus fruits, β-cryptoxanthin contents were 0.06 mg% (October), 0.08 mg% (November), 0.19 mg% (December), 0.26 mg% (January), and 0.65 mg% (February) (Fig. 5).

β-cryptoxanthin contents of peel and flesh were 0.38 mg% and 0.19 mg%, from citrus fruit harvested in December, respectively. In addition, β-cryptoxanthin content was greatly increased in citrus fruits harvesting in February, reaching 1.71 mg% (peel) and 0.65 mg% (flesh) (Fig. 5). Considering the higher content of β-cryptoxanthin in the peel of Shiranuhi mandarin fruits, it is necessary to harvest it late in the season in order to fully utilize the peel as functional ingredient effectively. Generally, the β-cryptoxanthin contents varied according to various citrus cultivars. The amount of β-cryptoxanthin ranged from 0.3 to 2.1 mg% in the peel of domestic Citrus cultivars (21). It has been reported that the β-cryptoxanthintin content was 2.18 mg% and 0.66

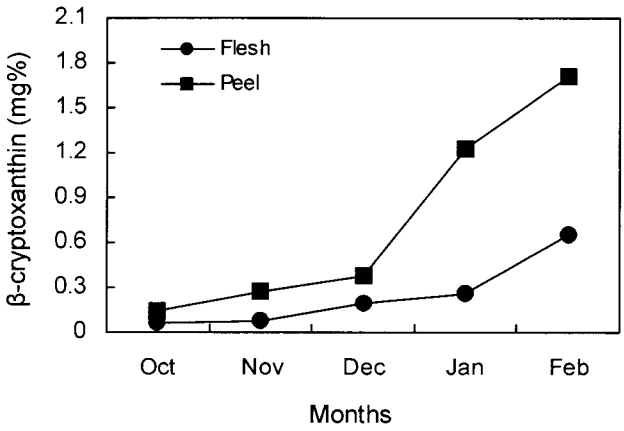

Fig. 5. Comparison of β-cryptoxanthin content from flesh and peel of Shiranuhi mandarin fruits according to harvesting season.

mg% from peel and flesh of Satsuma, respectively (22). Miyagawa wase showed 5.26 mg% in peel and 0.78 mg% in flesh (4). However, the β-cryptoxanthin contents in lemon and grapefruit were considerably lower than those found in other Citrus cultivars, containing less than 0.1 mg% in both peel and flesh (21). Based on the comparison of β-cryptoxanthin, Shiranuhi mandarin fruit contained reasonably higher β-cryptoxanthin both peel and flesh. It has been known that carotenoids have various biological properties (23) and play specific roles in mamalian tissues (22). It is recently known that β-cryptoxanthin in *Citrus* is synthesized by enzymatic conversion of β-carotene, catalyzing by β-carotene hydroxylase. A gene coding β-carotene hydroxylase has been isolated from *Citrus*, which has higher content of β-cryptoxanthin (8). In addition to the superior characteristics of Shiranuhi mandarin fruit, β-cryptoxanthin content could be increased by the novel technology of genetic manipulation developed in plant molecular biology. For

this approach the basic evaluation of β-cryptoxanthin content from Shiranuhi mandarin fruit will be the important information for improving the nutritional value of the citrus cultivar in the future.

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