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RESEARCH NOTE

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Changes in β-Cryptoxanthin Contents of *Citrus unshiu* Markovich Fruits Ripened in Greenhouse versus Open Field Cultivation

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Abstract β-Cryptoxanthin contents were determined from *Citrus unshiu* Markovich fruits grown in a greenhouse and open field of Jeju Island, off the southern coast of Korea. In a greenhouse and open field, the β-cryptoxanthin content in the peel was greatly increased by harvesting citrus fruits in the late season from August through November. However, β-cryptoxanthin content in the flesh was gradually increased and was superior to that of the citrus fruits grown in a greenhouse. β-Cryptoxanthin was efficiently purified from the flesh of citrus fruits harvested in the late harvesting season in November. The β-cryptoxanthin contents in the peel and flesh of citrus fruits harvested from a greenhouse in November were 0.89 mg% and 0.35 mg%, respectively, and in that obtained from an open field were 1.12 mg% and 0.35 mg%, respectively.

Key worlds: β-cryptoxanthin, *Citrus unshiu* Markovich, peel, flesh, HPLC

Introduction

Citrus are the most widely cultivated fruit trees in the world (1), and citrus fruits have been used as valuable ingredients for oriental medicine and functional foods (2). *Citrus unshiu* Markovich fruits are representative Citrus for greenhouse cultivation in Jeju Island, off the southern coast of Korea, and begin their pigmentation from August and fully ripen in the middle of November. It was reported that citrus fruits contain various bioactive compounds such as flavonoids and carotenoids (3).

Carotenoids are isoprenoid molecules that are widespread as natural pigments in nature. In addition to their obvious contribution to food quality as natural pigments, they have been shown to play vital physiological roles (4). Over recent years there has been considerable interest in dietary carotenoids with respect to their potential in alleviating chronic diseases in humans (5). Carotenoids present in fruits and vegetables are widely believed to protect human health, prevent some cancers (6, 7) and function as quenchers of single oxygen, as antioxidants (8). Various natural carotenoids, being important biological precursors of vitamin A, have been associated with a reduced risk of cardiovascular and other chronic diseases (9). Some epidemiological studies have confirmed an inverse relation between carotenoid consumption and the development of some cancers (10, 11).

More than 600 carotenoids have been identified in foods, but most nutrition research has focused on α-carotene, β-carotene, lycopene, lutein, and β-cryptoxanthin (12). In particular, β-cryptoxanthin is present in human serum and tissue and plays a protective role against human disease (13, 14). β-Cryptoxanthin has demonstrated anti-breast cancer activity and an inhibitory effect on osteoclast-like cell formation in mouse marrow cultures *in vitro* (15, 16). It was reported that β-cryptoxanthin from Satsuma Mandarin (*Citrus unshiu* Marc.) had inhibitory effects on colon carcinoma of rats (13).

In particular, β-cryptoxanthin has received research attention as one of the target carotenoids because of its great biological activity for human health. To evaluate the nutritive and biological value of food carotenoids, the absolute concentrations need to be determined (4). There has been particular emphasis on obtaining more accurate data on the concentrations of various carotenoids in foods for various health and nutrition activities (17). In addition, accurate characterization of the association between carotenoid intake and various chronic diseases has been required (12). Generally, the accumulation of β-carotenoid in citrus fruits could be varied by the cultivar type and environment, including climate and cultivation condition. Therefore, it is necessary to distinguish the compositional difference between citrus fruits cultivated in a greenhouse and those cultivated in an open field. Such a comparison will provide valuable information for obtaining citrus fruits fortified with a higher β-cryptoxanthin content.

The purpose of this study was to evaluate the contents of β-cryptoxanthin in citrus fruits harvested from a greenhouse or open field during the ripening season.

Material and Methods

Reagents and material Citrus fruits (*Citrus unshiu* Markovich) were cultivated in a greenhouse and open field of the National Institute of Subtropical Agriculture in Jeju Island and were harvested from August to November 2003. The peel and flesh from the citrus fruits

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were separated and sliced. Each part of the citrus fruit was stored at -70°C in a 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). Other chemicals used were HPLC or analytical grade. A 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 mm) was obtained from Waters Co. (Waters Chromato-graphy, Milford, MA, USA).

Chromatography of carotenoids The carotenoid extraction was performed using the method reported by Ko et al. (18). The carotenoids extracted from the peel or flesh of the citrus fruit was analyzed using silica gel TLC plates. For the separation of pigment compounds, hexane/ acetone (3/1, v/v) was used as the mobile solvent. The components were separated by holding for 30 min in darkness at room temperature. β-Cryptoxanthin as a standard carotenoid was used for determining the R_f value. The quantitative separation of carotenoids was also performed by HPLC (19). The concentrations for the carotenoid standard ranged from 0.1-0.5 μg/mL. The linearity of the calibration between β-cryptoxanthin concentration and absorbance was determined. Duplicate samples were injected for each extract for HPLC analysis.

Results and Discussion

TLC analysis of carotenoids in citrus fruits Crude carotenoids were extracted from the peel and flesh of citrus fruits grown in a greenhouse and open field of Jeju Island. The composition of the crude carotenoids prepared by solvent extraction was analyzed by TLC method. As shown in Figure 1, crude carotenoids extracted from the peel were composed of several carotenoid compounds, including β-cryptoxanthin with R_f value of 0.4. It was reported that β-cryptoxanthin from citrus fruits produced in Jeju Island had R_f value of 0.39 (18). It was expected that β-carotene was separated with R_f of 0.97. The β-cryptoxanthin content in the flesh and peel was increased as the harvest season for the citrus fruits was delayed from August (coloring season) to November (harvesting season). It was concluded that carotenoids containing β-cryptoxanthin were successfully extracted from both the peel and flesh of citrus fruits.

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 19 min. The β-cryptoxanthin in the flesh was gradually increased and purified effectively as the harvesting season of citrus fruits was delayed from August to November. However, β-cryptoxanthin in the crude carotenoids was less purified compared to those of the flesh from citrus fruits, including various carotenoid components (data not shown). Based on this previous HPLC experiment, it was considered that crude carotenoids from the peel contained xanthophyll-like compounds with a retention time of 4-9 min, along with β-carotene and lutein with R_f of 24 min and 8.5 min, respectively. As shown in Fig. 3, β-cryptoxanthin content was greatly changed by the harvesting season and cultivation method of the citrus fruits. In citrus fruits harvested from an open field, β-cryptoxanthin contents of the flesh and peel were increased from 0.06 mg% and 0.05 mg% (August) to 0.35 mg% and 1.12 mg% (November), respectively.

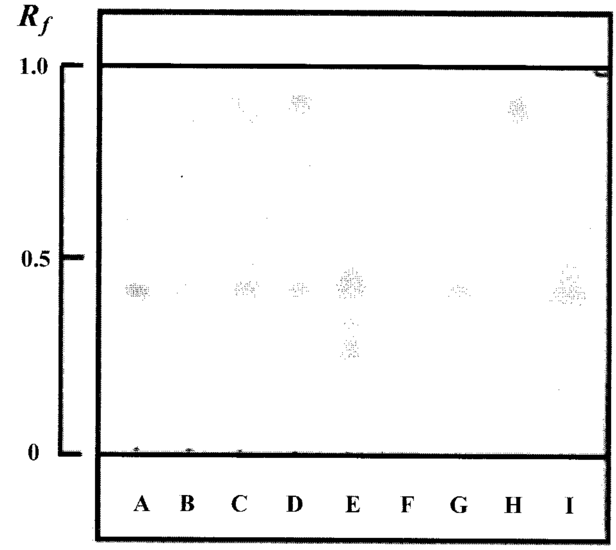

Fig. 1. TLC chromatograms of a standard of β-cryptoxanthin and carotenoids from the flesh of citrus fruits. A: standard of β-cryptoxanthin; B-E: citrus fruits from a greenhouse; F-I: citrus fruits from an open field; B, F: Aug., C, G: Sep., D, H: Oct., E, I: Nov.

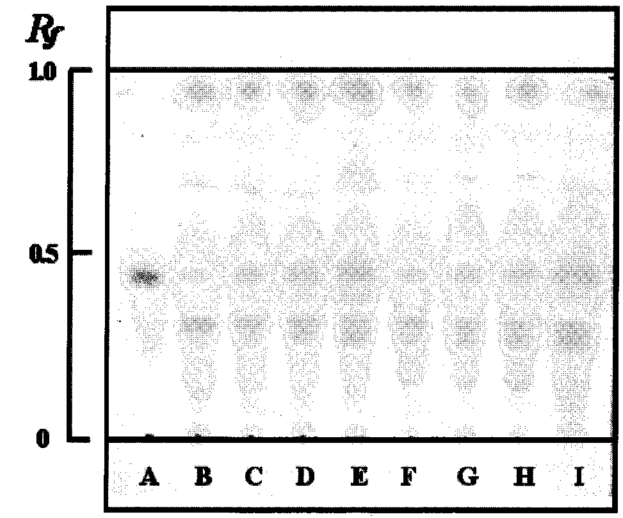

Fig. 2. TLC chromatograms of a standard of β-cryptoxanthin and carotenoids from the peel of citrus fruits. A: standard of β-cryptoxanthin; B-E: citrus fruits from a greenhouse; F-I: citrus fruits from an open field; B, F: Aug., C, G: Sep., D, H: Oct., E, I: Nov.

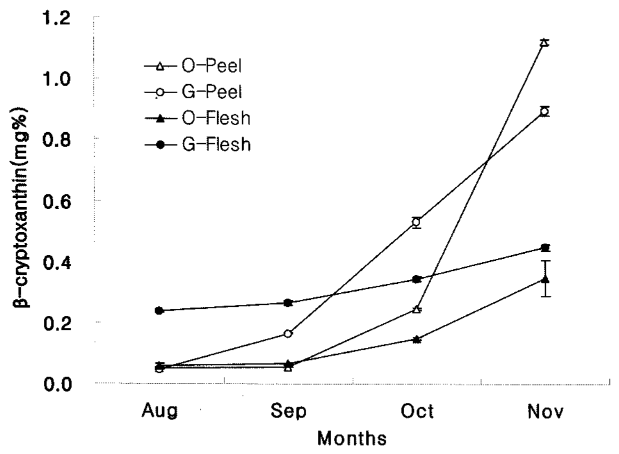

Fig. 3. Comparison of β-cryptoxanthin from the peel and flesh of citrus fruits according to the harvesting season. Abbreviation: O-open field, G- greenhouse.

In citrus fruits grown in a greenhouse, β-cryptoxanthin contents of the peel were 0.05 mg% (August), 0.17mg% (September), 0.53 mg% (October), and 0.89 mg% (November), and β-cryptoxanthin contents of the flesh were gradually increased with 0.24 mg% (August), 0.27 mg% (September), 0.34 mg% (October) and 0.45 mg% (November). Previously, it was recognized that β-cryptoxanthin content in the peel of citrus fruit was higher than that in the flesh (17, 18). It was concluded that the β-cryptoxanthin contents in both peel and flesh increased as the citrus fruits fully matured. β-Cryptoxanthin contents from the peel and flesh of citrus fruits cultivated in November in an open field were 1.12 mg% and 0.35 mg%, respectively, and in a greenhouse were 0.89 mg% and 0.45 mg%, respectively.

In both a greenhouse and open field, β-cryptoxanthin content was greatly increased in citrus fruits harvested in November (Fig. 3), which suggests that the harvesting of *Citrus unshiu* Markovich fruits in November is quite reasonable for increased content of β-cryptoxanthin. In particular, β-cryptoxanthin of the flesh from citrus fruits grown in a greenhouse was higher than that of an open field during the ripening season, being indicating 0.45 mg% (greenhouse) and 0.35 mg% (open field) (Ed- there is no respective comparison here) (Fig. 3). However, in the peel of citrus fruits, β-cryptoxanthin was gradually increased until September, and was higher than that of an open field. β-cryptoxanthin of the peel reached 1.12 mg% (open field) and 0.89 mg% (greenhouse) in November as the harvesting season. Considering the higher content of β-cryptoxanthin in the peel of citrus fruits, it is necessary to utilize the peel effectively as a functional ingredient. Generally, the β-cryptoxanthin contents varied according to various citrus cultivars. 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 exhibited 5.26 mg% in the peel and 0.78 mg% in the flesh (2). However, the β-cryptoxanthin contents in lemon and grapefruit were considerably lower than those found in other citrus cultivars, containing below 0.1 mg% in both peel and flesh (19). Based on the comparison of β-cryptoxanthin content, *Citrus unshiu* Markovich fruit harvested from a greenhouse in November contained reasonably higher β-cryptoxanthin in both peel (0.89 mg%) and flesh (0.35 mg%), which confirms that *Citrus unshiu* Markovich fruits are suitable Citrus for greenhouse cultivation in Jeju Island. The accumulation of β-cryptoxanthin could be affected by various environmental conditions as well as by genetic manipulation. It is expected that improve-ments of culture environment and cultivar will increase the levels of β-cryptoxanthin in *Citrus unshiu* Markovich fruits. For this approach the basic evaluation of β-cryptoxanthin content from *Citrus unshiu* Markovich fruit will provide important information to improve the citrus cultivar in the future.

References

1. Abkenar AA, Isshiki S, Tashiro Y. Phylogenetic relationships in the true citrus fruit trees revealed by PCR-RFLP analysis of cpDNA. Scientia Horticulturae 102: 233-242 (2004)
2. Whang HJ, Yoon KR. Carotenoid pigment of citrus fruits cultivated in Korean. J. Food Sci Technol. 27: 950-957 (1995)
3. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am. J. Med. 113: 71S-88S (2002)
4. Wilberg VC, Rodriguez-Amaya DB. HPLC quantitation of major carotenoids of fresh and processed guava, mango and papaya. Lebensm -Wiss U-Technol. 28: 474-480 (1995)
5. Fraser PD, Bramley PM. The biosynthesis and nutritional uses of carotenoids. Prog. Lipid Res. 43: 228-265 (2004)
6. Nishino H, Tokuda H, Murakoshi M, Satomi Y, Masuda M, Onozuka M., Yamaguchi S, Takayasu J, Tsuruta J, Okuda M, Khachik F, Narisawa T, Takasuka, N, Yano, M. Cancer prevention by natural carotenoids. Biofactors 13: 89-94 (2000)
7. Riso P, Porrini M. Determination of carotenoids in vegetable foods and plasma. Internat J. Vit. Nutr. Res. 67: 47-54 (1997)
8. Setiawan B, Sulaeman A, Giraud DW, Driskell JA. Carotenoid content of selected Indonesian fruits. J. Food Comp. Anal. 14: 169-176 (2001)
9. Su Q, Rowley KG, Balazs NDH. Carotenoids: separation methods applicable to biological samples. J. Chromatogr. B 781: 393-418 (2002)
10. Slattery ML, Benson J, Curtin K, Ma KN, Schaefer D, Potter JD. Carotenoids and colon cancer. Am. J. Clin. Nutr. 71:575-582 (2000)
11. Cooper DA, Eldridge AL, Peters JC. Dietary carotenoids and certain cancers, heart disease, and age-related macular degeneration: A review of recent research. Nutr. Rev. 57: 201-214 (1999)
12. Holden JM, Eldridge AL, Beecher GR, Buzzard IM, Bhagwat S, Davis CS, Douglass LW, Gebhardt S, Haytowitz D, Schakel S. Carotenoid content of U.S. Foods: An update of the database. J. Food Comp. Anal. 12: 169-196 (1999)
13. Sumida T, Azuma Y, Ogawa H, Tanaka T. Inhibitory effects of β-cryptoxanthin rich powder prepared Satsuma Mandarin (*Citrus Unshiu* Marc.) juice on azoxymethane-induced aberrant crypt foci of rats. Nippon Shokuhin Kagaku Kogaku Kaishi. 46:473-479 (1999)
14. Krinsky NI, Russett MD, Handelman GJ. Structural and geometrical isomers of carotenoids in human plasma. J. Nutr. 120:1654-1662 (1990)
15. Dorgan JF, Swanson CA, Potischman N, Miller R, Schussler N, Stephenson HE. Relation of serum carotenoids, retinol, α-tocopherol, and selenium with breast cancer risk: results from a prospective study in Colombia, Missouri U.S.A. Cancer Causes Control 9: 89-97 (1998)
16. Uchiyama S, Yamaguchi M. Inhibitory effect of β-cryptoxanthin on osteoclast-like cell formation in mouse marrow cultures. Biochem. Pharmacol. 67: 1297-1305 (2004)
17. Tee ES, Lim CL. The analysis of carotenoids and retinoids: A review. Food Chem. 41: 147-193 (1991)

18. Ko KC, Kim, CS, Lee NH, Lee SP, Moon DK. Determination of β-cryptoxanthin in peel and flesh of citrus fruits produced in Cheju Island. Food Sci. Biotechnol. 9: 288-291 (2000)

19. Nam TS, Lee, SP, Kim CS. Determination of β-cryptoxanthin in peel and flesh of domestic and foreign citrus fruits. Food Sci. Biotechnol. 11: 628-633 (2002)