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Comparative carotenoid compositions during maturation and their antioxidative capacities of three citrus varieties

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ABSTRACT

This study investigated total carotenoid content, comparative carotenoid composition, vitamin C content, and total antioxidant capacity of three citrus varieties which are Yuza (*Citrus junos* Sieb ex Tabaka), Kjool (*Citrus unshiu* Marcow), and Dangyooja (*Citrus grandis* Osbeck). Seven carotenoids were identified, with β-cryptoxanthin, astaxanthin, and zeaxanthin being predominant in citrus varieties. Ripening increased the total carotenoid in three citrus varieties. Individual carotenoid of canthaxanthin, astaxanthin, and α-carotene in citrus varieties decreased with maturation, whereas the others increased with ripening. Yuza exhibited the highest total antioxidant capacity in 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays, with VCEAC values of 582.9 mg/100 g and 451.5 mg/100 g, respectively. The relative VCEAC values were vitamin C (1.00) > lycopene (0.375), α-carotene (0.304), β-carotene (0.289), β-cryptoxanthin (0.242), and zeaxanthin (0.099). These results indicate that Yuza contains higher amounts of total carotenoids, individual carotenoids, and vitamin C than other Korean citrus varieties.

1. Introduction

Several species of the Genus *Citrus* are cultivated worldwide, and consumed millions tons per year (USDA, 2015). *Citrus* L. is consumed as either a fresh fruit (e.g., tangerine, orange, grapefruits, pummelo) or processed juices and beverages (e.g., lime, lemon, Yuza) (Yoo, Hwang, Park, & Moon, 2009). Citrus fruits production in Korea was estimated at 588,000 tons in 2011 (Forestry MoAa, 2012). According to the Korea Ministry of Agriculture and Fisheries, citrus production accounted for 20% of the total fruit production (Yoo et al., 2009). Tanaka (1969) has classified 36 mandarin species, which are commonly known as tangerine. It has been estimated that there are more than 200 cultivars of *Citrus unshin* (Ye et al., 2011). Kjool (*Citrus unshiu*), Yuza (*Citrus junos* Sieb ex Tanaka) and Dangyooja (*Citrus grandis* Osbeck) are citrus fruits mainly cultivated in the southern part of Korea. Kjool is generally used as juice or a fresh fruit (Kim, Kim, & Koh, 2001). Yuza is commonly used in preparing tea as a herbal medicine, especially for the common cold (Kim & Shin, 2013; Yoo, Lee, Park, Lee, & Hwang, 2004). Recently, studies have been reported for the physiological activity of Dangyooja and its antidiabetic activity (Kim, Shin, & Jang, 2009).

Numerous dietary components of these fruit, which have been found to exhibit potential nutraceutical effects, contain antioxidants such as vitamins, phenolics, (Block, Patterson, & Subar, 1992; Yoo et al., 2004), and carotenoids (Kim, Kim, & Koh, 2001). Carotenoids, which are found in yellow and green citrus fruit, are gaining importance in food products because of their health-related benefits (rich in provitamin A and anticarcinogenic effect) (Rao & Rao, 2007). In citrus fruit, the antioxidant activity is primarily caused by the presence of hydrophilic components (Cano, Alcaraz, Acosta, & Arnao, 2002). Therefore, to estimate the antioxidant activity of citrus fruit, the overall concentration and composition of the different antioxidants present therein needs to be considered.

Antioxidant activity of citrus fruit, including lemons, limes, and oranges, has been reported to be caused by vitamin C and phenolic substances in it (Silalahi, 2002; Tsai, Chang, & Chang, 2007). However, the antioxidant activity of Korean citrus cultivars with different carotenoid compositions during maturation has not been reported hitherto. Especially, individual carotenoids composition during ripening in Korean citrus varieties is very limited. Therefore, we aimed to evaluate the composition and distribution of carotenoids during maturation, evaluate their antioxidant activity, and

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quantify the proportion of vitamin C in three different citrus cultivars grown in Korea.

2. Materials and methods

2.1. Samples

Yuza (*C. junos* Sieb ex Tabaka), Kjool (*C. unshiu* Marcow), and Dangyooja (*C. grandis* Osbeck) were harvested at the Agricultural Research Center in Jeolla Province, Korea. Three citrus were harvested with the degree of maturation obtained from the fruit color: unripe fruits picked on October 30 were green, ripe fruits picked on November 15 were yellow, and ripe fruits picked on December 1 were deep yellow with three stages of maturation. After the citrus varieties were collected, they were peeled, sliced, and frozen at −80 °C. The resulting citrus samples were pulverized (FM-700 W food mixer, Han Il, Korea) and stored at −20 °C for analysis.

2.2. Chemicals

Canthaxanthin, astaxanthin, lycopene, zeaxanthin, lutein, neoxanthin, beta-cryptoxanthin, and beta-carotene standards were obtained from Extrasynthese (Genay, France). 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), vitamin C, and Folin–Ciocalteu phenol were purchased from Sigma (St. Louis, MO, USA). 2,2′-azobis-(2-amidino-propane) dihydrochloride (AAPH) was purchased from Wako Chemicals (Richmond, VA, USA). All other chemicals used in this study were obtained from Fisher (Springfield, NJ, USA).

2.3. Ascorbic acid analysis

The ascorbic acid of the citrus varieties was determined using the method described by Yoo et al. (2009). Five gram of sample was mixed with 2% (v/v) acetic acid and ascorbic acid was extracted by shaking for 3 min. HPLC (HP 1090 series II, Hewlett–Packard) was equipped with a C_{18} column (particle size of 5 μm, and an internal diameter of 25 cm × 3.0 nm) (Shiseido, Tokyo, Japan) and a UV diode-array detector. The mobile phase was 2% (v/v) acetic acid/acetonitrile at a flow rate of 0.5 mL/min; wavelength and oven temperature were set to 265 nm and 40 °C. The content of ascorbic acid was expressed in milligrams per 100 g of fresh weight or dry matter.

2.4. Identification of carotenoids using HPLC

A slightly modified version of the method by Prasad et al. (2011) was used for identification of carotenoids. Five gram of dried citrus peel powder was extracted with 50 mL acetone, and kept at 4 °C overnight. For the analysis of unsaponified pigment composition, the extracts were filtered using a 0.45 μm PTFE syringe filter (Millipore Co., Bedford, USA) and injected into the HPLC (HP 1100 series II; Hewlett–Packard, MO, U.S.A.). The saponified pigment composition was analyzed by mixing 5-mL extracts with 5 mL acetone, 10 mL MeOH (methanol) and 2 mL of 30% KOH/MeOH; the resulting mixture was kept at 30 °C overnight. Then 10 mL distilled water and 10 mL diethylether were added to the mixture and it was allowed to settle after shaking. Subsequently, 10 mL of 10% (w/v) NaCl solution was added, and the ether phase was extracted for carotenoids analysis. Anhydrous Na_2SO_4 (2% w/v) was added to the NaCl solution phase to recover the residual ether, which was then combined with the previous ether extract bringing the total volume to 20 mL. The individual carotenoid compositions were analyzed by injecting 20 μL of the pigment solution into a HPLC system with a μ-Bondapak C_{18} column (3.9 mm × 300 mm, 10 μm; Waters, Millipore Co., Bedford, USA) connected to a guard column and mobile phase was acetonitrile/methanol (95:5, v/v) at a flow rate of 1.0 mL/min; wavelength and oven temperature were set to 450 nm and 25 °C.

2.5. Determination of anti-oxidant activity

The 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS) assay was employed to determine antioxidant activity. A method developed by Yoo et al. (2009) was used to evaluate the ABTS radical scavenging activity with a slight modification. Five gram of citrus powder and 20 mL of absolute methanol were homogenized for 15 min at 3000×*g* using a homogenizer (Ultra Turrax T25, IKA Works, Wilmington, NC, USA). After the solution was kept still for 6 h in the dark, the solution was then centrifuged in a refrigerated superspeed centrifuge (Sorvall RC-5B, Biomedical Products Department, Du Pont, Wilmington, DE, USA) at 6000×*g* for 10 min at 4 °C. The supernatant was collected and used as sample solution. ABTS (2.5 mM) in phosphate-buffered saline solution (100 mM potassium phosphate buffer) was mixed with AAPH (1.0 mM). The mixture was heated in a water bath (68 °C) for 13 min. and its absorbance was adjusted to 0.650 ± 0.020 at 734 nm. Subsequently, 20 μL of sample solutions were mixed with 980 μL of ABTS radical solution. After the mixture was incubated in a 37 °C water bath for 10 min. in darkness, the absorbance was measured again at 734 nm. The ABTS radical scavenging activity is expressed in milligrams per serving of vitamin C equivalent antioxidant capacity (VCEAC).

A slightly modified version of the method by Brand-Williams, Cuvelier, and Berset (1995) was used for determining 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Aliquots of 0.3 mL of the sample solutions with different concentrations were mixed with 2.7 mL of the DPPH radical solution (in 80% aqueous methanol). After shaking, the mixture was incubated at 23 °C in the dark for 30 min. Thereafter, the absorbance change of the mixture was determined using a spectrophotometer at 517 nm (Shimadzu, Kyoto, Japan).

2.6. Quantification of total antioxidant capacity

A method by Yoo et al. (2004) was used to quantify the antioxidant capacity of samples with a slight modification. The area under the kinetic curve was calculated by integration and the total antioxidant capacity (TAC) was calculated by the following equation:

$$\mathrm{TAC} = 100 - \left(\int \mathrm{SA} \Big/ \int \mathrm{CA} \times 100 \right) \quad (1)$$

$\int$SA and $\int$CA are the integrated areas under the curve of sample and control, respectively.

2.7. Antioxidant capacity

The antioxidant capacity was measured and the VCEAC values were calculated, using ABTS and DPPH radical scavenging assays, by Kim, Lee, Lee, and Lee (2002) method. The vitamin C standard curves were determined using the ABTS and DPPH radical scavenging assay. The VCEAC values of the samples were determined using vitamin C standard curves. The EC_{50} values of the samples were calculated from the dose–response curves.

2.8. Statistical analysis

Data are expressed as mean ± standard deviation (SD) of three measurements. The significance was calculated by one-way