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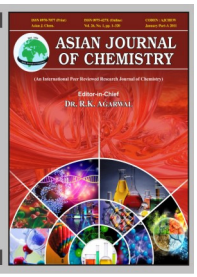

Carotenoid Contents in Different Millets Cultivars Collected from China and Korea

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Millet (*Panicum miliaceum* L.) cultivars from China and Korea were analyzed using HPLC to determine the levels of 5 different carotenoids: lutein, zeaxanthin, β-carotene, 9-*cis*-β-carotene and 13-*cis*-β-carotene. There was a significant difference in carotenoid content among cultivars from the 2 locations, with the Chinese cultivars generally having a higher content than the Korean cultivar. Lutein was present at the highest concentration in all of the cultivars, but was 34, 33 and 16 % higher in Joongback (China) than in Joongjukyumi (China), Hwangguem (Korea) and Joongjuk (China), respectively. Joongback also contained 58, 55 and 37 % higher concentrations of zeaxanthin than Joongjukyumi, Hwangguem and Joongjuk, respectively and a 33 % higher concentration of β-carotene than Hwangguem (Korea). The other two carotenoids (9-*cis*-β-carotene and 13-*cis*-β-carotene) were present at similar levels in all 4 cultivars. The results suggest that the millet cultivar Joongback from China could potentially be used as a source of carotenoids in the human diet.

Keywords: Carotenoid, Cultivars, Millet, HPLC.

INTRODUCTION

Panicum is one of the largest genera of grasses, comprising more than 400 species. Within this genus, *Panicum miliaceum* (proso millet) and *Panicum sumatrense* (little millet) are of economic importance. Proso millet, which is also known as common millet, hog millet or white millet is one of the world's oldest cultivated crops. This cereal plant is cultivated for its grain, mostly in Asia and North America, but may also be used for forage. Proso millet differs from other millets, such as pearl, foxtail, finger or barnyard millets. This annual grass is found in central and eastern Asia, covering a wide area from the Caspian Sea, east to Xinjiang and Mongolia and is also found in the mountains of the former U.S.S.R. and India at altitudes of up to 1,200 and 3,500 m, respectively[1]. The plant can grow to heights of 50-100 cm and its leaves grow up to 30 cm long. The stems are stout and erect and both the stems and leaves are covered with small hairs. The seed heads grow in bunches and can be divided into 3 types, based on the shape of the panicle: spreading, loose and one-sided and erect. The seeds are very small and oval in shape. Seed colour depends on species and can be white, cream, yellow, orange, red, or black through to brown in pearl millet. Millet has a higher protein value than corn, as well as high fiber levels due to the attached hulls.[1] Millet can be successfully grown in a wide range of environmental conditions, being better adapted than most crops to hot, dry regions and is particularly valuable in semiarid regions because of its short growing season. It can tolerate drought and intense heat, or avoids these conditions by growing to maturity very quickly. Millet plays an important role in the economies of many developing countries in the world, where it is consumed directly as human food. In contrast, in the Western world, millet is grown primarily for birdseed, livestock feed, hay, or as an emergency catch crop[2].

Traditionally, Korea's major crops were rice, barley, sorghum and millet, but cultivating area of these crops gradually decreased due to grow other economic crops. However, recently minor crops such as barley, sorghum and millet have attracted attention due to their high energy, protein, vitamin and mineral contents, which are believed to help prevent and treat diseases associated with aging[3-6].

Carotenoids are isoprenoid pigments that are widely distributed in nature[7]. In plants, carotenoids play an important role in photosynthesis (where they contribute to the light-

harvesting process), photomorphogenesis and photoprotection. They are also the precursors of an important plant hormone, abscisic acid, which is involved in regulating the plant stress response[8,9]. Carotenoids contain abundant pigments and are accumulated in the plastids of leaves, flowers and fruits[10].

Carotenoids also have anticarcinogenic and antioxidant activities. For example, the provitamin A carotenoid β-carotene is a nutritional factor that is important for chronic disease prevention and it has been shown that carotenoids can lower the levels of bad cholesterol and triglycerides in the bloodstream[11]. Consequently, in recent years, several carotenoids have been used as colourants, nutritional supplements and nutraceuticals for food, cosmetic and pharmaceutical purposes[12]. In the present study, we analyzed the carotenoid content of 4 different millet cultivars collected from Korea and China.

EXPERIMENTAL

Four millet cultivars were used in this study: 3 from China and 1 from Korea. Millet seeds were germinated in a greenhouse and the seedlings were then transferred to the experimental farm at Chungnam National University (Daejeon, Korea). All of the cultivars were harvested after 4 months, at their ripening stage. They were then manually hulled and ground to obtain a fine powder using a cyclone mixer mill (HMF-590; Hanil, Seoul, Korea) and a mortar and pestle. The milled powders were immediately frozen in liquid nitrogen and stored at -80 ºC until required for use in the carotenoid analysis.

Extraction and HPLC analysis of carotenoids: Carotenoids were extracted from millet samples (0.1 g) with 3 mL of ethanol containing 0.1 % ascorbic acid (w/v). This mixture was vortexed for 20 s and incubated in a water bath at 85 ºC for 5 min. Subsequently, 120 µL of potassium hydroxide (80 % w/v) was added to saponify any potentially interfering oils and the mixture was again vortexed and incubated at 85 ºC for 10 min. The samples were then placed on ice and 1.5 mL of cold deionized water and 0.05 mL of β-apo-8-carotenal (12.5 µg mL^{-1}; an internal standard) were added. The carotenoids were subsequently extracted twice with 1.5 mL of hexane and centrifuged at 1200 × g following each extraction in order to separate the layers. Finally, the extracts were freeze dried under a stream of nitrogen gas and resuspended in 50:50 (v/v) dichloromethane/methanol.

For HPLC analysis, the carotenoids were separated using an Agilent 1100 HPLC system using a C_{30} YMC column (250 mm × 4.6 mm, 3 µm; Waters Corporation, Milford, MA) and detected using a photodiode array (PDA) detector at 450 nm. Solvent A consisted of methanol/water (92:8 v/v) with 10 mM ammonium acetate, whereas solvent B comprised 100 % methyl *tert*-butyl ether (MTBE). The flow rate was maintained at 1 mL min^{-1} and samples were eluted with the following gradient: 0 min, 83 % A/17 % B; 23 min, 70 % A/30 % B; 29 min, 59 % A/41 % B; 35 min, 30 % A/70 % B; 40 min, 30 % A/70 % B; 44 min, 83 % A/17 % B and 55 min, 83 % A/17 % B.

Statistical analysis: For HPLC statistical analysis, the data were analyzed by using the computer software Statistical Analysis System (SAS version 9.2). All data are given as the mean and standard deviation of triplicate experiments. Treatment mean comparisons were performed with the least significant difference (LSD).

RESULTS AND DISCUSSION

Carotenoid contents of different cultivars of millet collected from China and Korea: Five different carotenoids were detected in the 4 millet cultivars collected from China and Korea: lutein, zeaxanthin, β-carotene, 9-*cis*-β-carotene and 13-*cis*-β-carotene. There was significant variation in carotenoid contents among the millet cultivars (Fig. 1), with the Chinese cultivars generally having a higher content than the Korean cultivar. Among the different cultivars, Joongback from China was found to contain the highest concentration of carotenoids. Lutein was found to occur at the highest concentration in all 4 cultivars, but was 34, 33 and 16 % higher in Joongback than in Joongjukyumi (China), Hwangguem (Korea) and Joongjuk (China), respectively. There was much greater variation in the content of zeaxanthin among cultivars than in lutein, but once again, Joongback contained the highest amount, with concentrations that were 58, 55 and 37 % higher than those found in Joongjukyumi, Hwangguem and Joongjuk, respectively. The cultivar Joongback also contained 33 % more β-carotene than Hwangguem. The other two carotenoids (9-*cis*-β-carotene and 13-*cis*-β-carotene) were present at similar levels in all 4 cultivars.

This study showed that the levels of carotenoids varied widely among 4 different cultivars of millet from 2 locations. In particular, the Joongback cultivar from China contained the highest concentration of all 5 types of carotenoid sampled. These findings are consistent with previous findings, which have shown that the carotenoid contents of some vegetables varies between cultivars and geographical locations. For example, Park *et al.*[13] showed that carotenoid contents varied in the skins and flesh of 2 types of kohlrabi. Habicht *et al.*[14] reported that the levels of saponin, linoleic acid and linolenic acid varied between cultivars of bitter melon, with white bitter gourd varieties containing significantly lower concentrations of saponin (0.25 %) than green varieties (0.67 %).

Kim *et al.*[15] reported that different coloured rice grains contained different levels of carotenoids, with black cultivars containing mean levels of 0.482 mg/100 g, red cultivars containing 0.052 mg/100 g and white cultivars containing 0.021 mg/100 g. The same authors also mentioned that the level of the vitamin A precursor β-carotene was significantly higher in black rice than in red and white rice. Frei and Becker[16] reported that black rice samples from the Philippines contained β-carotene concentrations of up to 0.013 mg/100 g. In contrast, the β-carotene content of black varieties in the present study ranged from 0.026-0.048 mg/100 g. Kim *et al.*[15] also found that rice cultivars showed distinct differences in lutein content depending upon grain colour, with the highest average content being found in black cultivars, which had values of up to 0.643 mg/100 g. Our findings are in agreement with those of Kim *et al.*[15] and Frei and Becker[16], who reported that carotenoid levels varied among cultivars of rice.

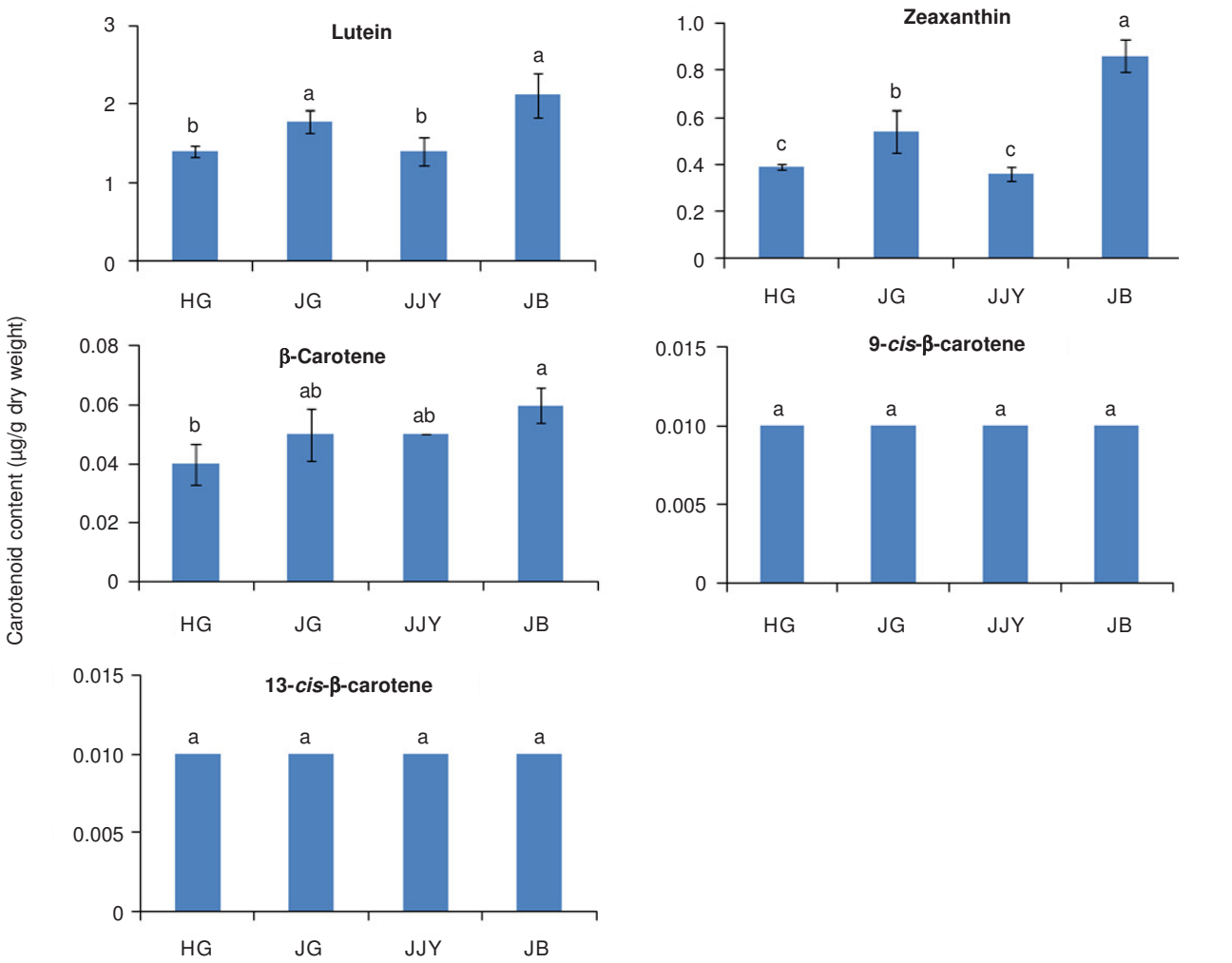

Fig. 1. Carotenoid content of different cultivars of millet. HG, hwangguem; JG, joongjuk; JJY, joongjukyumi; JB, joongback. The values and error bars represent the mean and standard deviation of three independent measurements, respectively. Different letters represent LSD (p = 0.05)

Because carotenoids are precursors to vitamin A, millet could be used as a food supplement to meet human carotenoid requirements. It may be concluded from the present study that the millet cultivar Joongback from China is a particularly good source of carotenoids.

ACKNOWLEDGEMENTS

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