Vegetable Science (2013) 40 (1) : 109-110

Quantification of lutein and β carotene in underutilized green leafy vegetables using high performance liquid chromatography

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Received : November, 2012 / Accepted : April, 2013

Benefits imparted by β-carotene in orange and dark green fruits and vegetables in preventing and treating xerophthalmia are well known (Dowling and Wald, 1958). Plant foods, especially green leafy vegetables, provide other carotenoids with promise in eye health (Seddon, 2007). One of them, the xanthophyll lutein, has been identified as an important protective agent in several *in-vitro* assays, epidemiologic studies and intervention trials examining plant food consumption and prevention of age-related cataract and macular degeneration. In addition, carotenoids are attributed preventive properties against several forms of cancer. Green leafy vegetables are the major sources of lutein and β-carotene. Carotenoids play an important role in absorption of blue light and exert antioxidant activity preventing damages caused by light and free radicals. As leafy vegetables are widely available and can be cultivated at low cost, their consumption and conservation is being promoted for increased health benefits (Johns, 2007). The objective of present study is identification and quantification of lutein and β-carotene in six green leafy vegetables through reversed-phase HPLC column using UV detector.

Six commercially grown leafy vegetables viz., *Alternanthera sessilis* L., *Amaranthus tricolor* L., *Amaranthus cruentus* L., *Celosia argentea* L., *Hibiscus cannabinus* L., and *Rumex vesicarius* L. were selected from commercially produced farmers fields for present investigation. For each species, approximately 200 to 500 g of plants were collected. Plants were rinsed with distilled water, dried with absorbing paper. Before extraction, each sample was homogenized in a household blender. Carotenoid extraction was done as per Kimura and Rodriguez-Amaya (2002) and Rodriguez-Amaya (1999). Separation was performed on a Shimadzu model HPLC coupled with a tertiary pump LC-10AT, a SPD-10A UV-VIS detector and a column thermostat. The integration system was Class-VP version 7. The reversed-phase Phenomenex C_{18} 5µ (250 x 4.60 mm i.d.) column was kept at 25°C. The solvent composition was modified according to Kimura and Rodriguez-Amaya (2002) for a binary pump system: solvent A contained acetonitrile and 0.05% TEA and solvent B methanol: ethyl acetate (1:1). The selected flow rate was 1.0 ml/min. The initial proportion of solvent A and B was 95:5 increasing to 60:40 in 15 min following a concave gradient (curve 10) and the proportion was maintained until the end of the run (60 min). Re-equilibration took 15 min. Immediately before injection the sample was rediluted in 10 ml HPLC grade acetone, 1.5 ml were filtered through a 0.22m PTFE Millipore filter to a HPLC vial and 10 ml were injected in the system. Detection was performed at 450 nm.

Identification of carotenoids was carried out by comparison of the HPLC retention times with corresponding standards and co-chromatography with added standards. As major carotenoid patterns are highly constant in leafy vegetables (Britton, 1991) these procedures are sufficient to confirm the identification of the compounds for this validated analytical method (Rodriguez-Amaya, 2008). Standard curves were constructed for external quantification using lutein isolated by open-column chromatography from groundnut leaves (Kimura and Rodriguez-Amaya, 2002) and commercial β-carotene standard purchased from Sigma. Purity, verified with HPLC for the isolated lutein and the commercial β-carotene, was 93% and 97% respectively. Concentrations were corrected accordingly. The curves were constructed in triplicate at 3 and 4 different concentrations for β-carotene and lutein, respectively.

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The curves were linear, passed through the origin and their correlation coefficient were higher than 0.98.

According to the standard co-chromatography and retention times, lutein (β,α-carotene-3,3'-diol) and β-carotene (β,α-carotene) were identified. The elution patterns of all the six GLV were very similar. Lutein values ranged from 51 to 142 mg/g and β-Carotene contents were found to range from 41 to 103 mg/g (Table 1). Highest lutein and b-carotene values were recorded in *Hibiscus cannabinus L*. (142 and 103 mg/g) followed by *Amaranthus tricolor* L. (105 and 96 mg/g) respectively. Wills and Rangga (1996) and Kidmose *et al.* (2006) reported 29 and 23.10 mg/g for *A. tricolor*. Previous HPLC analyses yielded values for β-carotene contents of 57, 86, 11 and 12 mg/g for *A. sessilis, A. tricolor, A. viridis* and *C. argentea* (Bhaskarachary *et al.,* 1995). Rajyalakshmi *et al.* (2001) reported values of 55.7, 82.5, 74, 71.6, 60, 69.4, 52.1 g/g for β-carotene content in *A. nodiflora, A. sessilis, A. tricolor, A. viridis, C. argentea, D. muricata* and *H. cannabinus*, respectively.

Table 1: Botanical families, Scientific and Telugu names, lutein and β-carotene of the selected leafy vegetables species.

S. No.	Leafy Vegetable	Scientific name	Lutein (µg/g)	β-carotene (µg/g)
1	Sessile joy weed	*Alternanthera sessilis* L.	103	96
2	Joseph's-coat	*Amaranthus tricolor* L.	105	96
3	Red amaranth	*Amaranthus cruentus* L.,	95	78
4	Silver cock's comb	*Celosia argentea* L.,	83	72
5	Brown Indian hemp	*Hibiscus cannabinus* L.	142	103
6	Bladder dock	*Rumex vesicarius* L.	51	41

Based on our experimental results, green leafy vegetables are important sources of lutein and β-carotene that are beneficial to eye health. Highest lutein and β-carotene values were recorded in *Hibiscus cannabinus L*. followed by *Amaranthus tricolor* L.

Acknowledgements

Financial support of this work was provided by Acharya N G Ranga Agricultural University, Rajendranagar, Hyderabad under Head of Account "230 280 Aflatoxin lab and Quality Control Lab".

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