Short Communication

Contribution of selected wild and cultivated leafy vegetables from South India to lutein and β-carotene intake

Julie Bélanger PhD[1], Mungara Balakrishna MSc[2], Putta Latha PhD[2], Shoba Katumalla DNB[3], Timothy Johns PhD[1,4]

[1]*Department of Plant Science, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Quebec, Canada*
[2]*Regional Agricultural Research Station, Acharya N G Ranga Agricultural University, Tirupati, Andhra Pradesh, India*
[3]*Siloam Eye Centre, Unit of LV Prasad Eye Institute, Madanapalle, Chittoor District, Andhra Pradesh, India*
[4]*School of Dietetics and Human Nutrition, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Quebec, Canada*

Carotenoids, especially lutein and β-carotene, offer benefits to human health in general and to eye health in particular. However, more data on the contribution of plant foods to carotenoid intake is of importance for developing strategies for promoting eye health in regions where cataract is highly prevalent such as in South India. The most frequently consumed 5 uncultivated and 5 commercially grown South Andhra Pradesh leafy vegetables were selected based on interviews with 100 local women. The lutein and β-carotene contents of fresh and cooked samples were determined using reversed-phase high performance liquid chromatography. Lutein values ranged from 53 to 143 μg/g and 58 to 175 μg/g in fresh and cooked samples, respectively. β-carotene contents were found to range from 45 to 119 μg/g in fresh samples and from 40 to 159 μg/g in cooked samples. No significant difference was observed between the carotenoid contents of wild and commercially grown species. According to their reported frequency of consumption, the 10 species considered in this study contribute 40% of the daily recommended intake of β-carotene. This is the first report of lutein content in fresh samples of *Celosia argentea* L., *Rumex vesicarius* L., *Digera muricata* (L.) Mart., and *Amaranthus cruentus* L. as well as in cooked samples of all species included in this study.

Key Words: lutein, β-carotene, India, wild vegetables, cataract

INTRODUCTION

Benefits imparted by β-carotene in orange and dark green fruits and vegetables in preventing and treating xerophthalmia have long been established.[1] Plant foods, especially green leafy vegetables (GLV), provide other carotenoids with promise in eye health.[2,3] The xanthophyll lutein, found mostly in leafy vegetables, 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.[4] Antioxidant activity and absorbance of damaging blue and UV light constitute likely mechanisms of action.[5]

GLV are the major sources of lutein and, in developing countries where access to animal food is restricted, contribute substantially to fighting retinol deficiencies by being rich sources of the provitamin A β-carotene, notwithstanding bioavailability issues.[6] As leafy vegetables are widely available and easy to gather from the wild or in agro-ecosystems, or may be cultivated at low cost, their consumption and conservation is being promoted for increased health benefits.[7] However, epidemiologic studies on eye health and nutrition tend to overlook the contribution to diet of wild or less common species.[8]

This work is part of a multidisciplinary project to document the importance of GLV consumption in the prevention of cataract in women living in Madanapalle, Andhra Pradesh, involving an ethnobotanical survey and hospital-based case-control study. The objective of this analysis is to quantify carotenoids in common, local cultivated and wild GLV; and to estimate the contribution of these vegetables to daily lutein intake in local women. Because of its recognized importance for eye health, β-carotene content is also examined.

In accordance with local culinary habits in which leafy vegetables are mostly consumed cooked, carotenoid values

Corresponding Author: Dr Timothy Johns, School of Dietetics and Human Nutrition, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Quebec, Canada, H9X 3V9.
Tel: 514-398-7847; Fax: 514-398-7739
Email: tim.johns@mcgill.ca
Manuscript received 13 November 2009. Initial review completed 23 March 2010. Revision accepted 29 April 2010.

are reported for both fresh and cooked leafy vegetables. The effect of cooking on the retention of carotenoids is well documented and is not tested in this study.[9-11]

MATERIALS AND METHODS

Dietary intake

Ten local species of GLV were selected based on their frequency of consumption determined in ethnobotanical interviews and food frequency questionnaires conducted with 100 women randomly selected from 20 villages in the surroundings of the Madanapalle sub-district (Mandal) from September to December 2007. Villages were selected following an opportunistic sampling provided by the community outreach program of the Siloam Eye Center. Dietary intake was determined by administering a food frequency questionnaire among participants. Data are expressed as servings per person per week. With 78 24-hour recalls, one average portion was estimated to contains 20 g of fresh leaves, taking into account the variability between different recipes. Subject informed consent was sought before the interview and patient anonymity was respected. Ethics approval was obtained from the Human Subjects Ethics committee of McGill University and the Human Research Project Review Board of the L.V. Prasad Eye Institute, Hyderabad, Andhra Pradesh, to which the Siloam Eye Center is affiliated.

Plant material

The ten plant species with highest availability, frequency of consumption and unknown carotenoid contents are listed in Table 1. Five leafy vegetables among those are uncultivated, and the remaining are cultivated in small-scale farms. For each species, approximately 200 to 500 g of plant material was collected in three different locations (different gathering areas or cultivated in fields from different regions). Each sample was analyzed separately upon arrival in the laboratory. Plants were rinsed with distilled water, dried with absorbing paper and the composite sample of leaves without stems was divided into two portions, one to be analyzed fresh and the other after boiling for 5 minutes and draining the water. Before extraction each sample was homogenized in a household blender. Voucher specimens of each species were pressed and dried and identification was confirmed, with the collaboration of Dr. T. Pullaiah (Sri Krishnadevaraya University Herbarium, Anantapur).

Chemicals and standards

HPLC grade acetone, acetonitrile, methanol and ethyl acetate plus diethyl ether, petroleum ether, sodium sulfate and sodium chloride were purchased from Merck Ltd (Mumbai, India). Acetone and triethylamine (TEA) were purchased from S.D. Fine Chemicals Ltd (Mumbai, India) and SRL (Mumbai, India), respectively. Lutein and β-carotene standards were obtained from Sigma-Aldricht (Mississauga, Canada) and zeaxanthin was obtained from Extrasynthese (Lyon, France).

Carotenoid extraction

The procedure is described in Rodriguez-Amaya et al.[12-13] In brief, approximately 3 g of fresh or cooked homogenized sample was weighed and ground with mortar and pestle in Celite and acetone. Acetone was filtered through a sintered disk glass funnel mounted on a suction flask with the solid residue reground in acetone until complete discoloration of the material (usually 2-3 repetitions). The acetone fraction was then partitioned to 50 ml of diethyl ether and petroleum ether [1:1 v:v] and washed 5 times with distilled water, sometimes with addition of 2-3 g of sodium chloride if an emulsion formed. After removing all water, the remaining diethyl ether and petroleum ether fractions were dried over a sodium sulfate bed and evaporated with a rotary evaporator (Superfit Continental Pvt Ltd, Mumbai, India). The last 1-2 ml of solvent was evaporated under nitrogen gas and the extracted sample was stored at -20°C until HPLC analysis for a maximum

Table 1. Cultivation status, botanical families, scientific and Telugu names, yearly availability and dietary intake among Madanapalle women (n=100) of the selected leafy vegetables species

Cultivation status	Botanical family	Scientific name	Telugu name	English name	Availability[†]	Dietary intake (serv/pers/wk)[‡]
Uncultivated	Amaranthaceae	*Allmania nodiflora* (L.) R. Br.	Errabadaku	NA	Jul – Mar	1.28 (1.30)
		Alternanthera sessilis (L.) R. Br.	Ponnaganti aku	Sessile joy-weed	Aug-Jan	0.64 (0.89)
		Amaranthus viridis L.	Dantu aku	Slender amaranth	All year	0.77 (1.28)
		Celosia argentea L.	Gurugu aku	Silver cock's comb	Jul-Dec	1.58 (1.27)
		Digera muricata (L.) Mart.	Chenchali aku	False amaranth	All year	0.32 (0.61)
Cultivated		*Amaranthus cruentus* L.	Thota aku	Red amaranth	All year	0.41 (0.84)
		Amaranthus tricolor L.	Sirri aku	Joseph's-coat	All year	0.54 (0.78)
	Chenopodiaceae	*Chenopodium album* L.	Chakranta aku	Lambsquarter	All year	0.33 (0.68)
	Malvaceae	*Hibiscus cannabinus* L.	Gongura	Brown Indian hemp	All year	0.35 (0.67)
	Polygonaceae	*Rumex vesicarius* L.	Chukka aku	Bladder dock	All year	0.60 (0.79)

[†]According to Pullaiah et al.[49] and our observations.
[‡]Intake is expressed as mean servings/person/week with standard deviation.

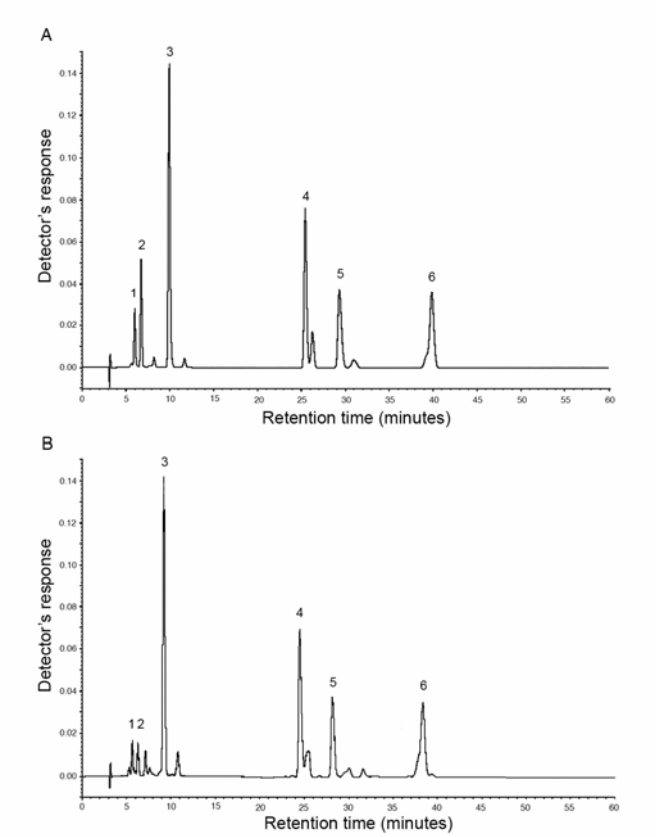

Figure 1. HPLC chromatograms of *Allmania nodiflora* (L.) R. Br. fresh (A) and cooked (B). Chromatographic conditions are described in the text. Peak identification: 1: neoxanthin, 2: violaxanthin, 3: lutein, 4: chlorophyll b, 5: chlorophyll a, 6: β-carotene.

duration of 1 week. The samples were not saponified as this treatment may alter the quantity of carotenoids and is not necessary in this case where the carotenoids of interest are separated from the chlorophylls.[13] All manipulations were conducted under dim laboratory light and glass material was covered with foil.

Analytical methodology

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 C18 5µ [250x4.60 mm i.d.] column was kept at 25°C. Solvent composition was modified according to Kimura and Rodriguez-Amaya for a tertiary pump system: solvent A contained acetonitrile and 0.05% TEA and solvent B methanol: ethyl acetate [1:1].[12] 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 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 0.22 µ PTFE Millipore filter to a HPLC vial and 10 µl were injected in the system. Detection was performed at 450 nm.

Identification of lutein and β-carotene 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 these procedures are sufficient to confirm the identification of the compounds for this validated analytical method.[14] Our method did not separate zeaxanthin from lutein, so values are reported together. However, it should be noted that GLV contain only trace amounts of zeaxanthin.[15] Values are reported for lutein and all-trans β-carotene.

Standard curves were constructed for external quantification using lutein isolated by open-column chromatography from groundnut leaves and commercial β-carotene standard purchased from Sigma.[12] Purity, verified with HPLC for the isolated lutein and the commercial β-carotene, was 93% and 97% respectively. The concentrations of the standards were determined spectrophotometrically, using the following absorption coefficient values $A^{1\%}_{1cm}$: β-carotene, 2592 in petroleum ether; and lutein, 2550 in ethanol. Concentrations were corrected accordingly. The curves were constructed in triplicate at 3 and 4 different concentrations for β-carotene and lutein, respectively. The curves were linear, passed through the origin and their correlation coefficient were higher than 0.98.

Statistical analysis

The means and standard deviation in µg/g were reported for fresh or cooked samples. One-way ANOVA analyses were used to test for difference between contents in non-cultivated and in cultivated GLV. Wilcoxon rank-sum test was used to compare consumption of cultivated and non-cultivated GLV.[16] Statistical analyses were conducted using R statistical software version 2.9.0.

RESULTS

Dietary intake

Celosia argentea and *Allmania nodiflora* were the two species most frequently consumed. In total the weekly average number of GLV servings per person was 6.81±3.91, comprised of 4.59±3.00 servings/person/week of uncultivated species and 2.22±2.14 of cultivated ones. There was a significant difference between the number of servings of cultivated and uncultivated GLV ($p < 0.01$) (Table 1).

Qualitative analysis

According to the standard co-chromatography and retention times, lutein (β,ε-carotene-3,3'-diol; t_R =9.6 min) and β-carotene (β,β-carotene; t_R =37.5 min) were identified. A typical GLV carotenoid chromatogram is presented in Figures 1A and 1B (fresh and cooked *A. nodiflora*). The elution patterns of all GLV were very similar, with the exception of *Hibiscus cannabinus* and *Rumex vesicarius*, which showed different patterns when fresh and cooked.

Fresh leafy vegetables

The concentrations obtained for *A. nodiflora, Alternanthera sessilis, Amaranthus cruentus, Amaranthus tricolor, Amaranthus viridis, C. argentea, Chenopodium album, Digera muricata, H. cannabinus* and *R. vesicarius* ranged from 53 to 143 μg/g for lutein and from 45 to 119 μg/g for β-carotene (Table 2).

Our results for *A. viridis* compare well with that of Kobori and Rodriguez-Amaya as well as Liu and col-

Table 2. Lutein and β-carotene contents (μg/g) of fresh and cooked leafy vegetables compared with values obtained from other studies reported in μg/g fresh weight

Leafy vegetables	Fresh (μg/g fresh weight)† (SD.)		Cooked (μg/g cooked weight)† (SD)	
	Lutein	β-carotene	Lutein	β-carotene
Uncultivated				
Allmania nodiflora				
This study	67 (14)	45 (10)	58 (15)	40 (10)
Rajyalakshmi et al.[9]	-	56	-	27§
Alternanthera sessilis				
This study	104 (25)	92 (26)	123 (46)	101 (31)
Bhaskarachary et al.[23]	-	57 (16)	-	-
Rajyalakshmi et al.[9]	-	83	-	36§
Amaranthus viridis				
This study	140 (15)	119 (10)	151 (63)	124 (47)
Kobori and Rodriguez-Amaya[17]	119 (21)	114 (22)	-	-
Tee and Lim[18]	42	32	-	-
Bhaskarachary et al.[23]	-	11 (4)	-	-
Rajyalakshmi et al.[9]	-	72	-	36§
Celosia argentea				
This study	81 (11)	69 (8)	109 (18)	96 (2)
Bhaskarachary et al.[23]	-	12 (2)	-	-
Rajyalakshmi et al.[9]	-	60	-	34§
Digera muricata				
This study	85 (17)	81 (19)	114 (18)	99 (16)
Rajyalakshmi et al.[9]	-	90	-	67§
Cultivated				
Amaranthus cruentus				
This study	92 (16)	76 (9)	143 (22)	115 (27)
Amaranthus tricolor				
This study	103 (18)	96 (16)	175 (77)	153 (61)
Tee and Lim[18]	20	51	-	-
Wills and Rangga[21]	29	20	-	-
Kidmose et al.[22]	23 (6)	18 (6)	-	-
Bhaskarachary et al.[23]	-	86 (30)	-	-
Liu et al.[11]	147‡ (7)	-	-	-
Rajyalakshmi et al.[9]	-	74	-	31§
Isabelle et al.[50]	23.55	-	36.68	-
Chenopodium album				
This study	107 (18)	93 (9)	175 (70)	159 (50)
Hibiscus cannabinus				
This study	143 (52)	107 (33)	82 (36)	121 (39)
Rajyalakshmi et al.[9]	-	83	-	42§
Rumex vesicarius				
This study	53 (1)	45 (1)	127 (14)	139 (31)
Bhaskarachary et al.[23]	-	26 (3)	-	-

†Values are reported as the mean of samples from three different locations with standard deviation. ‡Lutein only. §Fresh weight basis.

leagues who report 119 and 147 μg/g respectively.[11,17] For the same species, Tee and Lim report 42 μg/g after saponification and both Raju et al. and Lakshminarayana et al., from the same laboratory, reported 904 μg/g on a dry weight basis which does not allow comparison.[18-20] Kumar et al. reported 1850 μg/g for *C. album*, also on a dry weight basis.[21] Wills and Rangga and Kidmose et al. reported 29 and 23 μg/g for *A. tricolor* (syn. *A. mangostanus* and *A. gangeticus*).[22,23]

Previous HPLC analyses yielded values for β-carotene contents of 57, 86, 11 and 12 μg/g for *A. sessilis*, *A. tricolor, A. viridis* and *C. argentea*.[24] Our results also compare well with Kobori and Rodriguez-Amaya who reported 114 μg/g in *A. viridis*, and Rajyalakshmi et al. with values ranging from 56 to 90 μg/g (table 2).[17,9] Wills and Rangga found a lower value of 20 μg/g in *A. tricolor*.[22] A number of factors influence the carotenoid concentration among species, including timing of collection, seasonality, climate, growing conditions, geographic location, varieties (genetic variation) and cultivars, and may explain the variation among results.[10-13]

Cooked GLV

The means obtained for *A. nodiflora*, *A. sessilis*, *A. cruentus*, *A. tricolor*, *A. viridis*, *C. argentea*, *C. album*, *D. muricata*, *H. cannabinus* and *R. vesicarius* range from 58 to 175 μg/g for lutein and 40 to 159 μg/g of β-carotene in the cooked samples (Table 2).

Although no lutein values for cooked samples have been reported for the species of interest in this study, our results compare well with the USDA nutrient database for boiled kale (*Brassica oleracea* var. *acephala* DC.), spinach (*Spinacia oleracea* L.), turnip (*Brassica rapa* subsp. *rapa* L.), collards (*Brassica oleracea* var. *viridis* L.), mustard (*Brassica juncea* (L.) Czern.) and dandelion (*Taraxacum officinale* G.H. Weber ex Wiggers) greens, with reported lutein values of 182, 113, 84, 77, 60 and 47 μg/g (cooked weight basis).[25] During the cooking process, at least two mechanisms contribute to the decrease, increase or lack of modification in the concentration of carotenoids, namely the disruption of the food matrix and the consequent release of water and/or carotenoids and the degradation of the heat-labile carotenoids.[10]

Values ranging from 27 to 67 μg/g for β-carotene reported for cooked species by Rajyalakshmi et al. are different than ours because they are expressed on fresh weight instead of cooked weight basis.[9]

Contribution to total β-carotene and lutein intake

Daily intakes of β-carotene and lutein were calculated on the basis of 20 g fresh leaves portions and reported frequencies (Table 1). The selected species contribute to 1489 μg/day of β-carotene (980 μg from non-cultivated GLV) and 1788 μg/day of lutein (1186 μg from non-cultivated GLV). One-way ANOVA analyses of the results obtained between cultivated and non-cultivated GLV showed no significant differences.

DISCUSSION

Cataract is a condition affecting the transparency of the crystalline lens. It is the main cause of blindness in India and affects particularly women from rural areas.[26] In mammalian systems carotenoids originate exclusively from the diet. Lutein and zeaxanthin are the only carotenoids found in the human lens where their concentrations range between 15.1 to 44.1 ng/g of wet weight.[27-28] In both the lens and the macula lutea the two xanthophylls are responsible for blue light absorption and antioxidant protection which are the proposed mechanisms for their protective role against cataract.[5]

A number of studies in Western countries have demonstrated an inverse relationship between leafy vegetables consumption, especially lutein-rich species like spinach and kale,[15,25] and the risk of developing cataract.[29-34] However, no such studies have previously been conducted in India. To demonstrate the preventive effect of leafy vegetables consumption in countries where cataract is of high prevalence and occurs early in life, more data is needed on the carotenoid content of local foods. Increasing intake of leafy vegetables and associated carotenoids may constitute efficient strategies for promoting eye health and reducing the burden of cataract. In this study, we have determined the lutein and β-carotene concentrations of commonly consumed leafy vegetables of South Andhra Pradesh, thus providing useful data to further evaluate the contribution of wild and cultivated plant foods to cataract prevention.

Nutritional surveys tend to overlook the contribution of wild foods, GLV and varieties within species,[35-36] which may represent important sources of nutrients, including lutein and β-carotene. In our study, we found the carotenoid profile and contents of uncultivated leaves to be similar to the cultivated ones, confirming their important contribution to health and nutrition, and supporting their inclusion in nutritional and epidemiological surveys. Wild *A. viridis* and commercially available *H. cannabinus* had similar contents of lutein and β-carotene when fresh and cooked, and both compare well with globally available raw spinach (122 and 56 μg/g lutein and β-carotene, respectively).[25] In addition, within the selected species the uncultivated leaves were more frequently consumed on a weekly basis than the commercially grown ones, thus contributing further to carotenoid intake.

On a daily basis, the reported consumption of the 10 selected GLV species provides 1489 μg of β-carotene, contributing over 40% of the daily intake recommended by WHO/FAO.[37] To date, there is no recommendation for lutein intake. The reported intake attributable solely to the selected species in this study is 1788 μg/day. In the United States, various studies with women populations reported daily lutein intakes of 1832, 1300, 1860, 4404 and 1232 μg respectively.[38-42] Johnson-Down et al. estimated that Canadian women (18-65 years old) consumed 1382 μg/day of lutein.[43] Lutein intake in Europe was found to be 3250, 2500, 1590,1560 and 2010 μg/day in Spain, France, United Kingdom, Ireland and the Netherlands, respectively (male and female intakes not statistically different and reported together).[44] Recently, Hamulka et al. found a population of Polish women who consume 2160 μg/day of lutein.[45] In Asia, Zhang et al. estimated the lutein consumption of a Chinese women population to be 1810 μg/day.[46] To our knowledge, there is no published data on the lutein intake of Indian populations. However when compared with reported daily in-

takes in various countries, the 10 species of interest in this studies contribute an important portion of the lutein consumed by the women of Madanapalle.

Olmedilla et al. reported improved visual acuity and glare sensivity in patients with cataract with approximately 7 mg of lutein supplementation/day.[47] A portion of 100 g of either fresh *A. viridis* and *H. cannabinus* provides 14 mg of lutein. Moreover, 100 g of cooked *C. album* or *A. tricolor* will each contribute 18 mg of lutein with substantially increased bioavailability due to the disruption of the food matrix resulting from the cooking process.[11,48]

In conclusion, we have determined high contents of lutein and β-carotene in cultivated and wild leafy vegetable species commonly consumed by the women in the Madanapalle mandal. To our knowledge, this study is the first to report lutein values for fresh *C. argentea*, *R. vesicarius, D. muricata* and *A. cruentus* and for all cooked species, and β-carotene values for cooked *A. cruentus*, *C. album* and *R. vesicarius*. The reported concentrations can be used directly to estimate the contribution of either fresh or cooked species to lutein and β-carotene intake. One drawback of this work is the single time collection during the peak availability period. Based on these results, the selected cultivated and wild leafy vegetables are equally important sources of β-carotene and lutein. In a country such as India where cataract incidence is very high and occurs early in life, identifying strategies to help reduce or delay the burden of cataract is of primary importance. Increasing consumption and use of local GLV might be a valuable strategy. These data will be used in a population-based study to further evaluate the potential of leafy vegetables to contribute to cataract prevention in women from Madanapalle. This work providing evidence on the importance of local plant foods to health contributes to the implementation of Decision VIII/23A of the Convention on Biological Diversity (COP8, Curitiba, 2006), an initiative linking nutrition and food with global biodiversity conservation policy.[8]

ACKNOWLEDGEMENTS

Financial support of this work was provided by the International Development Research Center (IDRC Doctoral Research Award to JB), Fonds Québécois de la Recherche et des Nouvelles Technologies (FQRNT) (Bourse de Doctorat en Recherche B2 to JB) and by the Natural Sciences and Engineering Research Council (NSERC) (Discovery Grant to TJ). Special thanks to Dr. Palagiri Sudhakar, Dr. Delia Rodriguez-Amaya, Dr. M. Aruna, Dr. Jaqueline Bede, Dr. Alan Watson, Dr. K. R. Reddy, Mr. Anand, Girija Rani, K. Hymavathi, Mrs. S. Yesudas, Dr P. K. Nirmalan, the Siloam Eye Center staff, Dr. P. Owen and Dr. F. Fauteux.

AUTHOR DISCLOSURES

Julie Bélanger, Mungara Balakrishna, Putta Latha, Shoba Katumalla and Timothy Johns, no conflicts of interest.

REFERENCES

1. Dowling JE, Wald G. Vitamin-A deficiency and night blindness. Proc Natl Acad Sci U S A. 1958;44:648-61.
2. Seddon JM. Multivitamin-multimineral supplements and eye disease: Age-related macular degeneration and cataract. Am J Clin Nutr. 2007;85(Suppl 1):304-7.
3. Bartlett H, Eperjesi F. An ideal ocular nutritional supplement? Ophthalmic Physiol Optic. 2004;24:339-49.
4. Chiu CJ, Taylor A. Nutritional antioxidants and age-related cataract and maculopathy. Exp Eye Res. 2007;84:229-45.
5. Krinsky NI, Landrum JT, Bone RA. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr. 2003;23:171-201.
6. de Pee S, West CE, Permaesih D, Martuti S, Hautvast JGAJ. Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and beta-carotene in schoolchildren in Indonesia. Am J Clin Nutr. 1998;68:1058-67.
7. Johns T. Agrobiodiversity, diet and human health. In: Jarvis DI, Padoch C, Cooper HD, editors. Managing biodiversity in agricultural ecosystems. New York: Columbia University Press; 2007. pp. 492.
8. Bélanger J, Johns T. Biological diversity, dietary diversity and eye health in developing country populations: Establishing the evidence-base. EcoHealth. 2008;5:244-56.
9. Rajyalakshmi P, Venkata Lakshmi K, Padmavathi TVN, Suneetha V. Effect of processing on beta-carotene content in forest green leafy vegetables consumed by tribals of South India. Plant Foods Hum Nutr. 2003;58:1-10.
10. Calvo MM. Lutein: A valuable ingredient of fruit and vegetables. Crit Rev Food Sci Nutr. 2005;45:671-96.
11. Liu YT, Perera CO, Suresh V. Comparison of three chosen vegetables with others from South East Asia for their lutein and zeaxanthin content. Food Chem. 2007;101:1533-9.
12. Kimura M, Rodriguez-Amaya DB. A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoids. Food Chem. 2002;78:389-98.
13. Rodriguez-Amaya DB. A guide to carotenoid analysis in foods. Washington DC: International Life Sciences Institute Press; 1999.
14. Britton G. Carotenoids. Meth Plant Biochem. 1991;7:473-518.
15. Sommerburg O, Keunen JE, Bird AC, van Kuijk FJ. Fruits and vegetables that are sources for lutein and zeaxanthin: The macular pigment in human eyes. Br J Ophthalmol. 1998; 82:907-10.
16. Wilcoxon F. Individual comparisons by ranking methods. Biometrics Bull. 1945;1:80-3.
17. Kobori CN, Amaya DB. Uncultivated Brazilian green leaves are richer sources of carotenoids than are commercially produced leafy vegetables. Food Nutr Bull. 2008;29:320-8.
18. Tee ES, Lim CL. Carotenoid composition and content of Malaysian vegetables and fruits by the AOAC and HPLC methods. Food Chem. 1991;41:309-39.
19. Raju M, Varakumar S, Lakshminarayana R, Krishnakantha TP, Baskaran V. Carotenoid composition and vitamin A activity of medicinally important green leafy vegetables. Food Chem. 2007;101:1598-605.
20. Lakshminarayana R, Raju M, Krishnakantha TP, Baskaran V. Lutein and zeaxanthin in leafy greens and their bioavailability: Olive oil influences the absorption of dietary lutein and its accumulation in adult rats. J Agric Food Chem. 2007; 55:6395-400.
21. Kumar SR, Vallikannan B. Carotenoid composition and retinol equivalent in plants of nutritional and medicinal importance: Efficacy of beta-carotene from Chenopodium album in retinol-deficient rats. Food Chem. 2010;119:1584-90.
22. Wills RBH, Rangga A. Determination of carotenoids in Chinese vegetables. Food Chem. 1996;56:451-5.
23. Kidmose U, Yang RY, Thilsted SH, Christensen LP, Brandt K. Content of carotenoids in commonly consumed Asian vegetables and stability and extractability during frying. J Food Compos Anal. 2006;19:562-71.

24. Bhaskarachary K, Rao DSS, Deosthale YG, Reddy V. Carotene content of some common and less familiar foods of plant origin. Food Chem. 1995;54:189-93.
25. U.S. Departement of Agriculture. USDA national nutrient database for standard reference, release 18. Nutrient data laboratory homepage. 2005 [accessed 2006 Feb. 20]; Available from: http://www.nal.usda.gov/fnic/foodcomp/Data/SR18/sr18.html
26. Dandona L, Dandona R, Srinivas M, Giridhar P, Vilas K, Prasad MN, John RK, McCarty CA, Rao GN. Blindness in the Indian state of Andhra Pradesh. Invest Ophthalmol Vis Sci. 2001;42:908-16.
27. Yeum KJ, Shang FM, Schalch WM, Russell RM, Taylor A. Fat-soluble nutrient concentrations in different layers of human cataractous lens. Curr Eye Res. 1999;19:502-5.
28. Mares-Perlman JA, Millen AE, Ficek TL, Hankinson SE. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview. J Nutr. 2002;132:518-24.
29. Moeller SM, Jacques PF, Blumberg JB. The potential role of dietary xanthophylls in cataract and age-related macular degeneration. J Am Coll Nutr. 2000;19:522-7.
30. Moeller SM, Voland R, Tinker L, Blodi BA, Klein ML, Gehrs KM et al. Associations between age-related nuclear cataract and lutein and zeaxanthin in the diet and serum in the carotenoids in the age-related eye disease study (CAREDS), an ancillary study of the Women's Health Initiative. Arch Ophthalmol. 2008;126:354-64.
31. Hankinson SE, Stampfer MJ, Seddon JM, Colditz GA, Rosner B, Speizer FE, Willett WC. Nutrient intake and cataract-extraction in women - a prospective-study. BMJ. 1992; 305:335-9.
32. Mares-Perlman JA, Brady WE, Klein BE, Klein R, Haus GJ, Palta M, Ritter LL, Shoff SM. Diet and nuclear lens opacities. Am J Epidemiol. 1995;141:322-34.
33. Lyle BJ, Mares-Perlman JA, Klein BE, Klein R, Greger JL. Antioxidant intake and risk of incident age-related nuclear cataracts in the Beaver Dam Eye Study. Am J Epidemiol. 1999;149:801-9.
34. Brown L, Rimm EB, Seddon JM, Giovannucci EL, Chasan-Taber L, Spiegelman D, Willett WC, Hankinson SE. A prospective study of carotenoid intake and risk of cataract extraction in US men. Am J Clin Nutr. 1999;70:517-24.
35. Grivetti LE, Ogle BM. Value of traditional foods in meeting macro- and micronutrient needs: The wild plant connection. Nutr Res Rev. 2000;13:31-46.
36. Kennedy G, Islam O, Eyzaguirre P, Kennedy S. Field testing of plant genetic diversity indicators for nutrition surveys: Rice-based diet of rural Bangladesh as a model. J Food Compos Anal. 2005;18:255-68.
37. FAO/WHO. Vitamin and mineral requirements in human nutrition. 2nd edition. Geneva: World Health Organization and Food and Agriculture Organization; 2004.
38. Burke JD, Curran-Celentano J, Wenzel AJ. Diet and serum carotenoid concentrations affect macular pigment optical density in adults 45 years and older. J Nutr. 2005;135:1208-14.
39. Michaud DS, Giovannucci EL, Ascherio A, Rimm EB, Forman MR, Sampson L, Willett WC. Associations of plasma carotenoid concentrations and dietary intake of specific carotenoids in samples of two prospective cohort studies using a new carotenoid database. Cancer Epidemiol Biomarkers Prev. 1998;7:283-90.
40. Nebeling LC, Forman MR, Graubard BI, Snyder RA. Changes in carotenoid intake in the United States: The 1987 and 1992 National Health Interview Surveys. J Am Diet Assoc. 1997;97:991-6.
41. Chug-Ahuja JK, Holden JM, Forman MR, Mangels AR, Beecher GR, Lanza E. The development and application of a carotenoid database for fruits, vegetables, and selected multicomponent foods. J Am Diet Assoc. 1993;93:318-23.
42. Slattery ML, Benson J, Curtin K, Ma KN, Schaeffer D, Potter JD. Carotenoids and colon cancer. Am J Clin Nutr. 2000;71:575-82.
43. Johnson-Down L, Saudny-Unterberger H, Gray-Donald K. Food habits of Canadians: Lutein and lycopene intake in the Canadian population. J Am Diet Assoc. 2002;102:988-91.
44. O'Neill ME, Carroll Y, Corridan B, Olmedilla B, Granado F, Blanco I et al. A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. Br J Nutr. 2001;85:499-507.
45. Hamulka J, Wawrzyniak A, Gadomska M, Gronowska-Senger AB, Bawa S. The influence of selected demographic and lifestyle factors on lutein intakes by groups of Polish women. Int J Food Sci Nutr. 2009;29:1-7.
46. Zhang M, D'Arcy C, Holman J, Binns CW. Intake of specific carotenoids and the risk of epithelial ovarian cancer. Br J Nutr. 2007;98:187-93.
47. Olmedilla B, Granado F, Blanco I, Vaquero M. Lutein, but not alpha-tocopherol, supplementation improves visual function in patients with age-related cataracts: A 2-y double-blind, placebo-controlled pilot study. Nutrition. 2003;19: 21-4.
48. van het Hof KH, Tijburg LBM, Pietrzik K, Weststrate JA. Influence of feeding different vegetables on plasma levels of carotenoids, folate and vitamin C. Effect of disruption of the vegetable matrix. Br J Nutr. 1999;82:203-12.
49. Pullaiah T, Chennaiah E, Moulali DA, Babu PSP. Flora of Andhra Pradesh (vol. I-IV). Jodhpur: Scientific Publishers (India); 1998.
50. Isabelle M, Lee BL, Lim MT, Koh WP, Huang DJ, Ong CN. Antioxidant activity and profiles of common vegetables in Singapore. Food Chem. 2010;120:993-1003.

Short Communication

Contribution of selected wild and cultivated leafy vegetables from South India to lutein and β-carotene intake

Julie Bélanger PhD[1], Mungara Balakrishna MSc[2], Putta Latha PhD[2], Shoba Katumalla DNB[3], Timothy Johns PhD[1,4]

[1]*Department of Plant Science, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Quebec, Canada*
[2]*Regional Agricultural Research Station, Acharya N G Ranga Agricultural University, Tirupati, Andhra Pradesh, India*
[3]*Siloam Eye Centre, Unit of LV Prasad Eye Institute, Madanapalle, Chittoor District, Andhra Pradesh, India*
[4]*School of Dietetics and Human Nutrition, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Quebec, Canada*

印度南部野生及栽種的多葉蔬菜對葉黃素及β-胡蘿蔔素攝取的貢獻

類胡蘿蔔素，尤其是葉黃素及β-胡蘿蔔素，對人體健康有益，並特別有助於眼睛健康。然而，在白內障高盛行率的地區，例如印度南部，為了促進眼睛健康而發展的策略中，有關植物性食物對類胡蔔素攝取的貢獻之資料是非常重要的。在印度南安得拉邦(South Andhra Pradesh)地區，透過與 100 名當地婦女的面談，選出最常攝取的 5 種野生及 5 種商業栽種的多葉蔬菜。使用逆相高效液相層析儀定量新鮮及烹煮蔬菜中的葉黃素及β-胡蘿蔔素。在新鮮及烹煮後的樣本之葉黃素含量，範圍分別是 53-143 μg/g 及 58-175 μg/g。β-胡蘿蔔素含量在新鮮樣本是 45-119 μg/g，在烹煮樣本是 40-159 μg/g。比較野生與商業栽種的品種，胡蘿蔔素含量沒有顯著的差異。依據攝取頻率的報告，本研究選出的這 10 種蔬菜佔每日β-胡蘿蔔素建議攝取量的 40%。本文是第一個報告 *Celosia argentea* L.、*Rumex vesicarius* L.、*Digera muricata* (L.) Mart.、和 *Amaranthus cruentus* L.之新鮮樣本的葉黃素含量，也是首篇報告所有 10 種蔬菜的烹煮樣本之葉黃素含量。

關鍵字：葉黃素、β-胡蘿蔔素、印度、野生蔬菜、白內障