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Determination of carotenoids in Chinese vegetables

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Seven leafy Chinese vegetables, gau choi (*Allium tuberosum*), een choi (*Amaranthus tricolor*), baak choi (*Brassica chinensis*), choi sum (*Brassica parachinensis*), wong baak (*Brassica pekinensis*), ong choi (*Ipomoea aquatica*) and sai yeung choi (*Rorippa nasturtium aquaticum*) were analysed for carotenoids by reversed-phase HPLC and gradient elution. Sixteen carotenoids were identified as being present at levels of more than 2% of total carotenoids. For all leafy vegetables, lutein was the major carotenoid with β-carotene present at substantial levels. Violaxanthin, neoxanthin, antheraxanthin and mutatoxanthin were also important contributors in specific produce. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Carotenoids are pigmented constituents present in most fruit and vegetables. Nutritional interest was initially on the provitamin A carotenoids, particularly in vegetables, as they provide the major source of dietary vitamin A in most countries. Expanded interest in plant carotenoids was stimulated in the 1980s by epidemiological and laboratory studies indicating they may have anti-carcinogenic (Shekelle *et al.*, 1981), anti-ulcer (Javor *et al.*, 1983) or anti-aging (Cutler, 1984) properties. While the initial focus was with β-carotene, many of the fruit and vegetables suggested as being protective were poor sources of β-carotene but good sources of the oxygenated xanthophylls which have little vitamin A activity (Beecher & Khachik, 1984).

Most available data on carotenoids in fruit and vegetables were obtained to assess provitamin A status and are therefore β-carotene levels obtained using open column chromatography (AOAC, 1984). The advent of HPLC has accelerated the quality of carotenoid separation that can be achieved with a wide array of normal and reversed-phase systems using isocratic and gradient solvent systems developed (Simpson & Tsou, 1986; Khachik *et al.*, 1986; Wills *et al.*, 1988; Olmedilla *et al.*, 1992; Barua *et al.*, 1993), and a better understanding of maintaining carotenoid integrity during analysis (Scott, 1992).

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Vegetables traditionally consumed by Chinese populations around the world are now popular with non-Chinese people not only in Asia but also in Western countries. Most studies on the carotenoid constituents of Chinese vegetables have tended to concentrate on β-carotene with high levels reported for many vegetables (Wills *et al.*, 1984; Tee & Lim, 1991). A better understanding of other carotenoids in Chinese vegetables appears warranted as many have traditionally been considered to confer specific health benefits to consumers. In addition, many leafy Chinese vegetables are of the *Brassica* genus, Western representatives of which have been associated with anti-carcinogenicity (Graham *et al.*, 1978). This paper reports on the major carotenoid profile of seven Chinese vegetables using the reversed-phase gradient elution HPLC method developed by Wills *et al.* (1988).

MATERIALS AND METHODS

Seven Chinese leafy vegetables were purchased from the wholesale market in Sydney, Australia, as commercially mature produce during the summer months (January–March). Each produce was obtained from three market outlets with about 500 g or one piece of larger produce comprising a purchase lot. On arrival at the laboratory, the lower stems of all produce which are normally discarded by consumers were removed, except for ong choi and sai yeung choi which were considered to comprise 100% edible material. The edible portions of all batches

Table 1. Nomenclature of Chinese vegetables analysed

Botanical name	Cantonese name	English name
Leafy vegetable		
Allium tuberosum	Gau choi	Chinese chives
Amaranthus tricolor	Een choi	Chinese spinach
Brassica chinensis	Baak choi	Chinese white cabbage
Brassica parachinensis	Choi sum	Chinese flowering cabbage
Brassica pekinensis	Wong baak	Chinese cabbage
Ipomoea aquatica	Ong choi	Water spinach
Rorippa nasturtium aquaticum	Sai yeung choi	Water cress

of each produce were mixed in a domestic blender and sub-samples (100 g) taken for carotenoid analysis. Analysis was conducted on three sub-samples taken from the homogenized mixture. The common Western, Cantonese and botanical names of the vegetables analysed are given in Table 1.

The method of extraction and HPLC analysis was that described by Wills *et al.* (1988). The extraction method, which was a modification of that described by Zakaria *et al.* (1979), involved the blending of produce in acetone, shaking with diethyl ether and water, and collecting the organic layer which was washed with water, dried over anhydrous sodium sulphate, and reduced in volume under vacuum. The extract was saponified to remove chlorophylls and hydrolyse carotenoid esters using a method similar to that used by Curl (1953) as it was shown by Nurdin (1989) to quantitatively hydrolyse a range of carotenoid types. Saponification was performed at room temperature with methanolic potassium hydroxide and, after washing with water, the organic fraction was dried over sodium sulphate and evaporated under vacuum at room temperature. The carotenoid residue was dissolved in chloroform and after making up to volume was filtered and immediately analysed by HPLC.

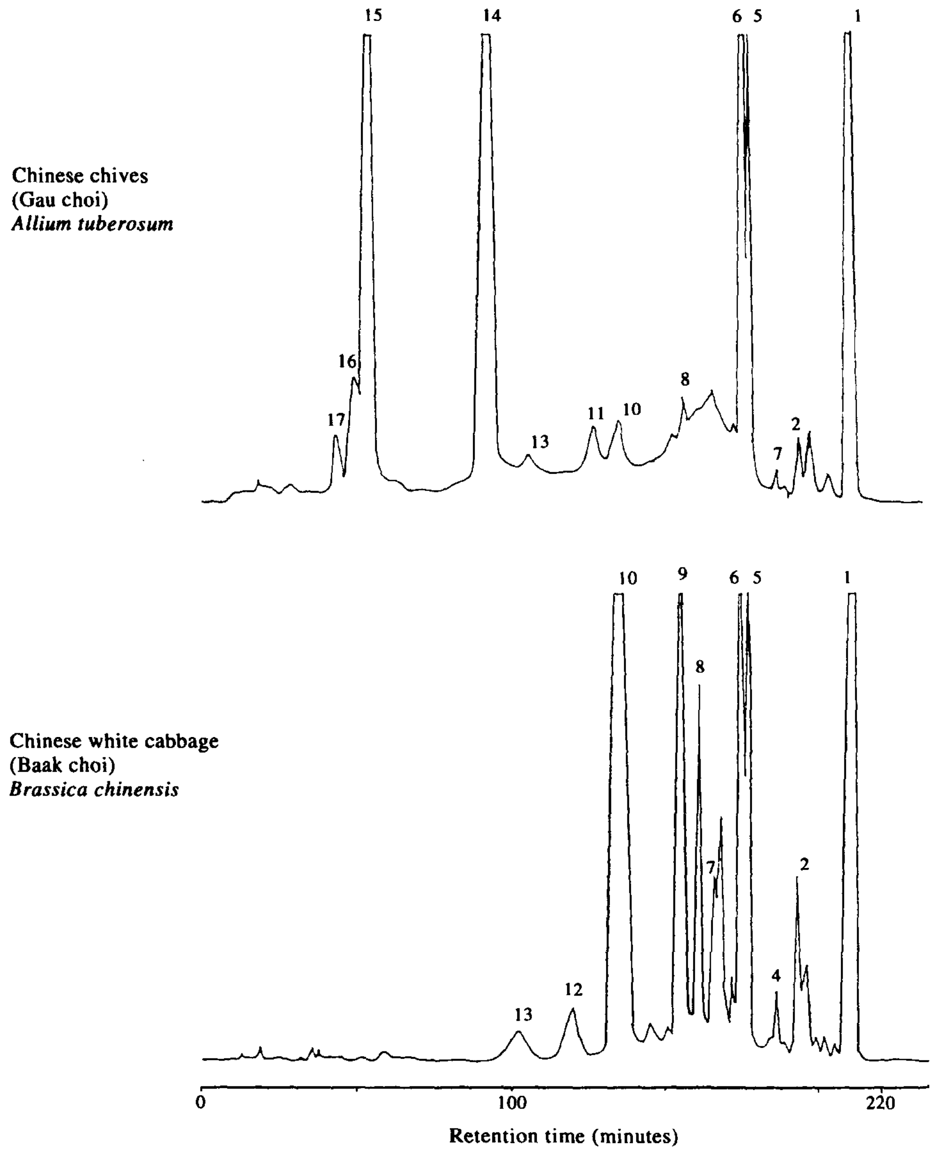

Fig. 1. Chromatograms of two Chinese vegetables (identification of peaks is given in Table 2).

HPLC analysis was carried out on two-reversed phase columns (Novapak C18, 5 μm; Waters, Milford, Massahusetts, USA) connected in series. The columns were attached to a Waters U6K injector and 41-MPa pumps. The mobile phase was a gradient of acetonitrile–methanol–water, 50:25:15 v/v/v (Solvent A) and acetonitrile–methanol 75:25 v/v (Solvent B). A Waters 660 Solvent Programmer was used to maintain a flow rate of 3 ml/min. Solvent A entered the columns for 60 min, the No. 10 programme then increased the proportion of Solvent B over the next 120 min, and Solvent B was maintained for the remaining 70 min of analysis. The column eluant was monitored by a Waters 490 Programmable Multiwavelength Detector at 440 nm.

Peak identification was based on information derived by Nurdin (1989), as the columns he used were also utilized in this study. Identification by Nurdin (1989) was based on comparison of HPLC retention times with chemical standards, separation of carotenoids into structural groups by open-column and thin-layer chromatography, and collection of all eluted peaks for epoxide testing, and visible, NMR and mass spectral examinations of the collected or acetylated peaks, with the responses compared to standards or literature data. Determination of the proportion of individual carotenoids present was based on peak area. The amount of β-carotene was calculated from chromatographic responses to standard solutions. The levels of other carotenoids present in leafy vegetables was based on peak area assuming a similar factor as for β-carotene.

RESULTS AND DISCUSSION

Two examples of the chromatograms obtained from separation of the seven Chinese vegetables by reversed-phase HPLC with gradient elution over a 250-min analytical run are shown in Fig. 1. A total of 16 different peaks were noted in the seven vegetables. Identification of these peaks, as determined by Nurdin (1989), is noted in Table 2 along with the proportion of each compound in the total carotenoids. The peaks are numbered in reverse order of elution and are thus ranked in order of increasing polarity from hydrocarbons to polyhydroxylated xanthophylls.

A range of carotenoids was observed in the leafy vegetables, but for each vegetable about 80% of the total carotenoids were represented by only four compounds. The only carotenoids present in all leafy vegetables at measurable levels were lutein and β-carotene. Lutein was the major carotenoid in all leafy vegetables, being 20–36% of total carotenoids while β-carotene was present at 14–23%. The next most persistent carotenoids were violaxanthin, neoxanthin and zeaxanthin. Violaxanthin was present at 16–23% in all leafy vegetables except sai yeung choi where it was present at 4% and was absent (< 2%) in baak choi. A similar trend was found for neoxanthin but at slightly lower levels than violaxanthin except for sai yeung choi where it comprised 17% of total carotenoids. While zeaxanthin was present in all leafy vegetables it only represented 5–11% of carotenoids. The only other carotenoids present at > 10% were antheraxanthin and mutatoxanthin in baak choi.

Table 2. Identification and quantification of major carotenoid constituents of Chinese vegetables

Peak	retention (min)	Carotenoid	Gau choi	Een choi	Baak choi	Choi sum	Wong baak	Ong	Sai yeung
Percentage of total carotenoids[a]									
1	209	β-Carotene	16	18	21	16	20	14	23
2	193	ε-Carotene				2		2	
3	190	Lycopene							
4	185	β-Cryptoxanthin				2			
5	170	Zeaxanthin	7	6	6	5	11	5	10
6	167	Lutein	23	22	25	20	36	24	33
7	155	Lutein epoxide			2				
8	151	Lutein diepoxide			4				
9	145	Antheraxanthin		2	17			3	
10	126	Mutatoxanthin	5		18				
11	121	Flavoxanthin	5	4		4		4	
12	112	Auroxanthin		4	3	4		3	3
13	92	Luteoxanthin		5	2	8	2	5	4
14	80	Violaxanthin	21	20		19	16	23	4
15	45	Neoxanthin	16	14		18	12	15	17
16	40	Neoxanthin a	2				2		
17	36	Neoxanthin b	2	2		2			
Amount of carotenoid present (mg/100 g)[b]									
		β-Carotene		2.0	1.7	2.3	2.2	0.4	1.5
		Zeaxanthin	0.9	0.6	0.7	0.7	0.2	0.5	0.7
		Lutein		2.9	2.1	2.7	2.7	0.6	2.6
		Violaxanthin	2.5	1.9		2.6	0.3	2.5	0.3
		Neoxanthin	2.0	1.3		2.4	0.2	1.6	1.2
		Total	**12.6**	**9.6**	**11.0**	**13.5**	**1.8**	**10.7**	**7.2**

[a]Carotenoids present at > 2% of total carotenoids.
[b]Limit of detection 0.2 mg/100 g.

Table 3. Literature values of the carotenoid composition of Chinese vegetables and some Western *Brassica* vegetables

Carotenoid	% of total carotenoids					
	Wong baak[a]	Gau choi[a]	Ong choi[b]	Gai choi[c]	Broccoli[d]	Cabbage[d]
β-Carotene	76	76	31	20	10	9
Neolutein					7	3
Lutein	24	24	27	33	42	31
Lutein epoxide			9		22	20
Neochrome						6
Antheraxanthin				19		
Violaxanthin			18	15	7	11
Neoxanthin			15	6	11	19
Total (mg/100 g)	4.0	4.6	33.5	2.2	4.2	0.9

[a]Tee & Lim (1991).
[b]Chen & Chen (1992).
[c]Takagi (1985).
[d]Khachik *et al.* (1986).

Table 2 also converts the compositional profile to absolute amounts for the major carotenoids. This shows that the highest carotenoid levels were in choi sum and gau choi at about 13 mg/100 g. Baak choi, ong choi and een choi had levels at about 10 mg/100 g, sai yeung choi contained about 7 mg/100 g, while wong baak contained the lowest level at < 2 mg/100 g. Considering the individual carotenoids, the levels of β-carotene and lutein in wong baak (0.4 and 0.6 mg/100 g, respectively) were thus much lower than in the other leafy vegetables (1.5–2.3 and 2.1–2.9 mg/100 g, respectively), with gau choi, baak choi and choi sum tending to have higher levels than een choi, ong choi and sai yeung choi. Violaxanthin levels were highest in gau choi, een choi, choi sum and ong choi (1.9–2.6 mg/100 g) but were ≤0.3 mg/100 g in baak choi, wong baak and sai yeung choi. Neoxanthin was highest in gau choi and choi sum (2.0–2.4 mg/100 g) with een choi, ong choi and sai yeung choi having 1.2–1.6 mg/100 g, and baak choi and wong baak at ≤0.2 mg/100 g.

The data show that the compositional profiles of gau choi, een choi, choi sum and wong baak were not greatly dissimilar and could, therefore, be expected to have similar effects on human physiology. However, the absolute level of all carotenoids was much lower in wong baak and would, therefore, not have the same quantitative impact as the other leafy vegetables. Similarly, een choi had slightly lower amounts of all carotenoids. Baak choi differed, being the only vegetable with antheraxanthin and mutatoxanthin present at high proportions in lieu of violaxanthin and neoxanthin. Sai yeung choi had a similar composition and amount of individual carotenoids as een choi except for a lower amount of violaxanthin.

There is little information in the literature to compare with the current findings but relevant data have been summarized in Table 3. Tee & Lim (1991) in a large study on vegetables which included gau choi and wong baak reported only β-carotene and lutein, with the former comprising 75% of the carotenoids. Two more comprehensive studies by Chen & Chen (1992) on ong choi and by Takagi (1985) on gai choi (*Brassica juncea*), which was not examined in this study, showed a not dissimilar compositional profile to the leafy vegetables analysed in this study. The major differences between the two analyses for ong choi were a higher proportion of β-carotene and the presence of lutein epoxide by Chen & Chen (1992). Data by Khachik *et al.* (1986) for the Western *Brassica* vegetables broccoli and cabbage differ from the Chinese *Brassica* vegetables with a lower proportion of β-carotene but higher proportions of lutein epoxide.

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