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Reversed phase HPLC analysis of α- and β-carotene from selected raw and cooked vegetables

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Abstract. Traditional AOAC colorimetric procedures for carotenoid analysis are known to lack specificity and accuracy. Newer HPLC methods provide the investigator with a more precise tool for carotenoid quantification in foods and tissues. In the present studies, reverse phase HPLC was utilized to evaluate the α- and β-carotene content in raw and cooked leaves of lettuce, spinach and winged bean as well as in the carrot root. The vegetables were boiled or steamed and the true retention of α- and β-carotene in the cooked products was determined. Boiling for 30 minutes resulted in a 53 and 40% loss of β-carotene from lettuce and carrots, respectively. Full retention or even an increase in β-carotene content in boiled winged bean leaves and spinach was noted. Steaming resulted in very good retention of α- and β- carotene in all vegetables (83–139% retention). Thus, although cooking procedures (especially boiling) may result in oxidative loss of carotenoids in some vegetables, heat treatment increases the chemical extractability of α- and β-carotene in others. The presence of carotenoproteins in some vegetables may effect the heat stability of extractability of α- and β- carotene.

Introduction

Carotenoids are associated with chlorophylls in many plants, especially the green leafy vegetables (Bauernfeind, 1972; Goodwin, 1980). Biologically, carotenoids are important for their metabolism as provitamin A, with α- and β-carotene being the most commonly found in plant tissues and having the highest activity (β-carotene 100% and α-carotene 53% biopotency; Bauernfeind, 1972). The oxygenated carotenoids (xanthophylls), which generally lack vitamin A activity, can be found in human plasma but their exact function there, if any, is not well defined.

The evaluation of the major biologically active provitamin A-rich carotenoids (α- and β-carotene) requires efficient separation of these pigments from other plant pigments such as chlorophylls, xanthophylls and lycopene. The traditional methods of carotene determination measured only total

carotenes; and varying biopotency of various provitamin A carotenoids were ignored (Panalaks and Murray, 1970; Bauernfeind, 1972). Even the Association of Official Analytical Chemists (AOAC, 1984) procedure utilizes the spectrophotometric estimation of total carotenoids, without specifically quantitating individual carotenoids. The reported Food and Agricultural Organization (FAO, 1972, 1982) values of carotenoids for edible plants list only the figures for β-carotene equivalents, estimated by spectrophotometric methods. These values have been reported to be less accurate and arbitrary (Sweeny and Marsh, 1970; Bauernfeind, 1972, 1983; Reeder and Park, 1975; Bushway and Wilson, 1982; Bureau and Bushway, 1986).

Though the National Academy of Sciences of USA (NAS, 1981) cities a range of 5,240–20,000 International Units/100 g of edible portion as vitamin A content in the leaves of winged bean (*Psophocarpus tetragonolobulin*) there still exists a paucity of verified data in this regard. Furthermore, there are no published reports in the winged bean literature indicating the effects of common cooking techniques upon the carotene content of winged bean leaves (Khan, 1982; Sri Kantha and Erdman, 1984). Winged beans grow very well in the high humid tropics and thus, might provide an excellent source of vitamin A for some tropical countries where vitamin A deficiency is often a problem. Therefore, in this study, the α- and β-carotene content of raw and cooked winged bean leaves were estimated using reversed phase (rp) HPLC and compared with the values of other provitamin A-rich vegetables such as carrots, spinach and lettuce.

Materials and methods

Samples

Winged bean leaves were obtained during two consecutive summers from plants grown in a home garden in Urbana, IL. Other vegetables were purchased fresh from the local supermarket. Raw vegetables were cleaned and the edible part (green leaves of lettuce, deribbed spinach and young tender winged bean leaves) was divided into three portions. Efforts were made to distribute the samples as homogenously as possible. The first portion was used for estimation of carotenes in raw state. The second and third portions received either water blanching or steam blanching treatments prior to carotene analysis.

Apparatus

A Tracor 950 chromatographic pump and 970A variable wavelength detector were used for carotenoid analysis. The column was a Supelcosil LC-18

(Supelco Inc., Bellefonte, PA) stainless steel column (4.6 × 25 cm) packed with ODS (octadecyldimethylsilyl) C18 of 5 μ particle size and a Hewlett Packard Model 3390A recording integrator was utilized.

Methods

For the water blanching treatment, 10 g of raw, chopped, fresh vegetable was blanched for 30 minutes in 100 ml of boiling water. The water was boiling prior to addition of vegetables and was kept at 95°C during blanching. The steam blanching treatment consisted of steaming 10 g of the raw, chopped, fresh vegetable in a household vegetable steamer for 3 minutes at 100°C. Excess water on the cooked vegetable from both treatments was removed by blotting with absorbant paper.

The methodology for α- and β-carotene analysis consisted of three consecutive steps. The first 2 steps were followed as described in AOAC (1984) for fresh plant materials. The third step was performed by rp-HPLC analysis (Broich et al., 1983). In the initial step, the pigments (chlorophylls and carotenes) from the vegetables were extracted by homogenizing at least twice with an acetone:hexane mixture (40:60) and concentrated on a rotary evaporator at reduced pressure (Bucher Instruments, Fort Lee, NJ). The carotenes were separated from chlorophyll and the more polar carotenoids during step 2 by employing column chromatography (deactivated alumina, prepared as described by Olson, 1961). The eluent from the alumina column was monitored for carotenoids and the results exhibited one distinct yellow band which eluted early in the chromotogram. Spectral analysis of this yellow band using a Beckman DU 40 spectrophotometer, determined it to be predominantly carotenes. The third phase was an HPLC separation using an ODS C18 column and a mobile phase of CH_3OH:CH_3CN:$CHCl_3$ (47:47:6)(Broich et al., 1983). The flow rate was set at 2 ml/min and all readings were taken at 461 nm.

The identification of α- and β-carotene was made using retention time comparison with commercially available standards (Sigma Chemical Co., St. Louis, MO). Purity of standards on a day to day basis was determined to be at best 85%. Thus, appropriate corrections based on spectrophotometric determination of purity using published extinction coefficients (Davis, 1976) were made in the calculation of α- and β-carotene content of the analyzed samples. Extinction coefficients used for β-carotene was 2396 in chloroform at 465 nm; and for α-carotene, 2800 in light petroleum ether at 444 nm. Percent true retentions of α- and β-carotene in cooked samples were calculated according to Murphy et al. (1975):

$$\% \text{ TR} = \frac{\text{nutrient content per (g) of cooked food} \times \text{(g) of food after cooking}}{\text{nutrient content per (g) of raw food} \times \text{(g) of food before cooking}}.$$

Total analysis time was approximately 60 minutes (extraction 10 min; washing of solvents 10 min; concentration of pigments by flash evaporator 10 min; column separation of carotenoids 10 min; and rp-HPLC determination 20 min). Under the conditions employed, mean retention time recorded for 1 ng/1 μl of standard α-carotene and β-carotene were 15.31 min (cv 1.1%) and 16.95 min (cv 2.2%), respectively. The peak areas recorded for α-carotene and β-carotene were 300×10^4 (cv 2.5%) and 289×10^4 (cv 11.2%), respectively.

Results

Alpha- and β-carotene levels determined for the vegetables analyzed in this study are compared with past research work and reported FAO (1972; 1982) values in Table 1. Each of the compared research studies (Sweeney and Marsh, 1971; Takagi, 1985; and Bureau and Bushway, 1986) used an HPLC method for analysis of α- and β-carotene. The FAO values are based on a spectrophotometric method and are expressed in β-carotene equivalents.

The amount of α- and β-carotene found in plant foods is influenced by growing season, variety, and storage time prior to analysis (Goodwin, 1980; Hsieh and Karel, 1983). Therefore, it is difficult to compare the levels reported previously with those α- and β-carotene levels in the present study. The comparisons drawn in Table 1 show this study's analysis of α- and β- carotene in vegetables to be in the same range as past research findings. The wide range of reported α- and β- carotene content in the carrots can be explained by varietal differences. Buishand (1978) found α- and β- carotene to be the major pigment in orange varieties of carrots, while lycopene and β-carotene predominated in red varieties. Comparison of α- and β-carotene analysis in winged bean leaves versus other vegetables indicates wing bean leaves to be a significant source of provitamin A.

The present HPLC solvent system was developed by Broich et al. (1983) for carotenoid analysis of human plasma (Curran-Celentano, 1985). This solvent system also exhibits good application for carotenoid analysis of vegetables. For this study an alumina precolumn was utilized to remove chlorophyll and more polar carotenoid pigments. Khachik et al. (1986) utilized an alternative approach for a more complete pigment analysis. They bypassed use of a pre-

Table 1. Comparison α- and β-carotene values of vegetables analyzed by HPLC techniques (expressed as mg/100 g fresh weight basis)

Sample	FAO values (1972; 1982)[a]	Sweeney and Marsh (1971)		Takagi (1985)	Bureau and Bushway (1986)		Present study[b]	
	Total Carotene	α-car.	β-car.	carotene	α-car.	β-car.	α-car.	β-car.
Winged bean leaves	3.2	–[c]	–	–	–	–	n.d.[d]	3.1
Lettuce	1.3	–	–	2.9	0.004	0.3	n.d.	2.3
Carrots	3.9	2.4–5.5	3.4–11.0	3.6	3.8	7.6	5.2	8.1
Spinach	5.7	–	2.7–3.2	–	n.d.	4.3	n.d.	1.5

[a] Winged bean values are from FAO (1972) and the values of other vegetables from FAO (1982).
[b] Mean of two determinations.
[c] Not determined.
[d] n.d. = none detected; limit of detection for α-carotene was 1 μg/100 g product.

Table 2. Content and retention of α- and β-carotene before and after heat processing in four vegetables

Sample[c]	Carotene content (mg/100 g fresh weight)						The retention of α- & β-car. (%)			
	Raw		Water-blanched[a]		Steam-blanched[b]		Boiled		Steamed	
	α-car.	β-car.	α-car.	β-car.	α-car.	β-car.	α-car.	β-car.	α-car.	β-car.
Winged bean	n.d.	3.1	n.d.	2.4	n.d.	2.7	–	119	–	83
Lettuce	n.d.	2.3	n.d.	1.1	n.d.	2.3	–	47	–	104
Carrots	5.2	8.1	3.7	5.3	5.8	8.2	64	60	139	99
Spinach	n.d.	1.5	n.d.	1.7	n.d.	3.2	–	112	–	132

[a] 10 g sample cooked in 100 ml of boiling water for 30 min.
[b] 10 g sample steamed in household steamer for 30 min. at 100°C.
[c] Mean of two determinations.
n.d. = none detected; limit of detection for α- and β-carotene was determined to be 1 μg/100 g product.

column and used gradient HPLC to efficiently separate chlorophylls and xanthophylls from less polar carotenes.

The effects of heat processing on the α- and β-carotene content of vegetables are reported in Table 2. Alpha-carotene was only detected in carrot samples. Water blancing resulted in a 53 and 40% loss of β-carotene from lettuce and carrots, respectively. Loss of α-carotene in the boiled carrot samples was similar to β-carotene (36% loss).

The water blanch treatment for winged bean leaves and spinach resulted in full retention or even an increase in the analytical value of β-carotene in the extracts. Beta-carotene content remained high for steamed winged bean, lettuce and carrot samples (83, 104 and 99% respectively). An increase in detectable α-carotene in carrots and β-carotene in spinach (139 and 132%, respectively) was noted with steaming.

Conflicting results are found in past studies as to whether extractable/analytical carotene levels increase or decrease with heat treatments. Sweeney and Marsh (1971) reported a 15–20% decrease in carotene content of green vegetables cooked for 30 minutes. However, other researchers (Panalks and Murray, 1970; Gomez, 1981; Bushway and Wilson, 1982) reported carotene increases ranging from 24 to 88% in carrots and cooked leaves of cassava, cowpea, kale and amaranthus. 200–400% increases in carotenes during heat processing of leafy vegetables have also been recorded in the literature (Bender, 1978).

Incomplete description of the methods reported for cooking in the previous studies makes it difficult to arrive at a general consensus of the changes occurring to α- and β-carotene content during heat processing. However, a theory can be suggested to explain the stability and apparent analytical increase of carotenes occurring during steam blanching. It is possible that carotenes from some vegetable sources are resistant to complete extraction while in the raw state. Reports by Anderson et al. (1978), Krinsky (1979), Braumann et al. (1982) and Grimme et al. (1984) have shown many carotenoids present in plants to be protein bound. In the bound form, the carotenes have important functions in photosynthetic processes. Some heat treatments such as steaming may help to release these bound carotenoids and cause them to be more readily extracted. The bioavailability of protein-bound carotenes is yet to be determined.

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References

1. Anderson JM, Waldron JC, Thorne SW (1978) Chlorophyll-protein complexes of spinach and barley thylakoids. FEBS Letters 92: 227–233
2. AOAC (1984) Official methods of analysis, 13th edn. Washington DC: Association of Official Analytical Chemists
3. Bauernfeind JC (1972) Carotenoid vitamin A precursors and analogs in foods and feeds. J Agric Food Chem 20: 456–473
4. Bauernfeind JC (1983) Vitamin A: Technology and applications. World Rev Nutr and Dietet 41: 110–199.
5. Bender AE (1978) Food Processing and Nutrition. London: Academic Press. p. 13
6. Braumann T, Weber G, Grimme LH (1982) Carotenoid and chlorophyll composition of light harvesting and reaction centre proteins of the thylakoid membrane. Photobiochem and Photobiophys 4: 1–8
7. Broich CR, Gerber LE, Erdman JW Jr (1983) Determination of lycopene, alpha- and beta-carotene and retinyl esters in human serum by reversed-phase high performance liquid chromatography. Lipids 18: 253–257
8. Buishand JG (1978) Investigation on the inheritance of root color and carotenoid content in carrot, *Daucus carota* L. Diss Abstr Internat B 39: 2656B–2657B
9. Bureau JL, Bushway RJ (1986) HPLC determination of carotenoids in fruits and vegetables in the United States. J Food Sci 51: 128–130
10. Bushway RJ, Wilson AM (1982) Determination of α- and β-carotene in fruit and vegetables by high performance liquid chromatography. Canad Inst Food Sci Technol J 15: 165–169
11. Curran-Celentano J, Erdman JW Jr., Nelson RA, Grater SJE (1985) Alterations in vitamin A and thyroid hormone status in anorexia nervosa and associated disorders. Am J Clin Nutr 42: 1183–1191
12. Davis BH (1976) In: Goodwin TW (ed.) Chemistry and Biochemistry of Plant Pigments, Vol. 2. New York: Academic Press
13. FAO (1972) Food Composition Table for Use in East Asia. Rome: FAO
14. FAO (1982) Food Composition Tables for the Near East. Rome: FAO
15. Gomez MI (1981) Carotene content of some green leafy vegetables of Kenya and effects of dehydration and storage on carotene retention. J Plant Foods 3: 231–244
16. Grimme LH, Brown JS (1984) Functions of chlorophylls and carotenoids in thylakoid membranes. Adv in Photosyn Res 2: 141–144
17. Goodwin TW (ed.) (1980) The Biochemistry of Carotenoids, vol. 1, 2nd end. London: Chapman and Hall
18. Hsieh YPC, Karel M (1983) Rapid extraction and determination of α- and β-carotenes in foods. J Chromatogr 259: 515–518
19. Khan TN (1982) Winged Bean Production in the Tropics. Rome: FAO
20. Khachik F, Beecher GR, Whittacker NF (1986) Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. J Agric Food Chem 34: 603–616
21. Krinksy NI (1979) Carotenoid protection against oxidation. Pure and Appl Chem 51: 649–660
22. Murphy EW, Criner PE, Gray BC (1975) Comparisons of methods for calculating retentions of nutrients in cooked foods. J Agric Food Chem 23: 1153–1157
23. NAS (1981) The Winged Bean – A High Protein Crop for the Tropics, 2nd edn. Washington, DC: National Academy of Sciences

24. Olson JA (1961) Conversion of radioactive β-carotene into vitamin A by the rat intestine in vivo. J Biol Chem 236: 349–356
25. Panalaks T, Murray TK (1970) The effect of processing on the content of carotene isomers in vegetables and peaches. Canad Inst Food Technol J 3: 145–151
26. Reeder SK, Park GL (1975) A specific method for the determination of pro-vitamin A carotenoids in orange juice. J Assoc Off Anal Chem 58: 595–598
27. Sri Kantha S, Erdman JW Jr (1984) The winged bean as an oil and protein source. J Amer Oil Chem Soc 61: 515–525
28. Sweeney JP, Marsh AC (1970) Separation of carotene stereoisomers in vegetables. J Assoc Off Anal Chem 53: 937–940
29. Takagi S (1985) Determination of green leaf carotenoids by HPLC. Agric Biol Chem 49: 1211–1213
30. Zakaria M, Simpson K, Brown P, Krstulovic A (1979) Use of reverse phase HPLC analysis for the determination of provitamin A carotenes in tomatoes. 176: 109–117