

# Analysis of Carotenoids in Vegetables by HPLC

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## ABSTRACT

Eight vegetables were pre-treated with and without saponification, and chromatographed by reversed-phase HPLC. Carotenoid peaks were identified using seven reference standards. Samples extracts were also chromatographed using a mixed magnesia and diatomaceous earth column. Fractions were eluted using stepwise increases in the proportion of acetone in hexane, studied by UV-VIS absorption spectrophotometry, and re-chromatographed by HPLC. The major carotenoids,  $\alpha$ - and  $\beta$ -carotenes, lutein and lycopene were thus identified. Saponification removed various non-carotenoid pigments in green vegetables, resulting in HPLC profiles which were easier to quantitate, while prolonging the life of the HPLC column. The process did not result in a drastic loss of  $\alpha$ - and  $\beta$ -carotene, although there appeared to be some loss of lutein, which possesses no pro-vitamin A activity. "Total carotenoid content" of the saponified vegetables could be obtained from the absorbance reading of the extracts at 450 nm.

## INTRODUCTION

Rapid advances have taken place in methodologies for more accurate quantitation of the carotenoids in foods for two main reasons. Firstly, it was thought that previously reported values of vitamin A activity in food may have been unreliable since methodologies were not sufficiently discriminative to separate the various carotenoids of importance in human nutrition (Zakaria, Simpson, Brown and Krstulovic, 1979; Beecher and Khachik, 1984; Underwood, 1984; Bureau and Bushway, 1986). Secondly, carotenoids, including those without vitamin A activity, are now thought to play important roles beyond their classical functions in nutrition and vision (Tee, 1988). With their highly conjugated double bonds, carotenoids may act as free radical traps or antioxidants, and therefore play important roles in cancer causation and prevention (Peto, Doll, Buckley and Sporn, 1981; Olson, 1986; Temple and Basu, 1988).

Since the late 1970's, high-pressure liquid chromatography (HPLC) has become a widely used procedure for the determination of carotenoids, mainly because the technique effects rapid separation, is

non-destructive and, more importantly, achieves better resolution (Taylor, 1983). However, most of the reports in the literature deal with the analysis of a limited number of foods (Tee and Lim, 1991). Some studies deal with the characterisation of a large number of carotenoids, but the procedures employed were complicated and unsuitable for routine analysis. The application of HPLC to the analysis of carotenoids in foods is still being developed and improved because of the complicated nature of these procedures.

A systematic project was therefore undertaken to develop improved methodologies for the quantitative determination of retinol and the main carotenoids of nutritional importance. The HPLC method developed should be suitable for routine analysis of a wide variety of foods of both plant and animal origins. The method should also be applicable to the analysis of blood samples for the assessment of vitamin A status of communities. Such a system would then obviate the use of different and complicated chromatographic conditions for different food samples, as well as blood.

This paper describes initial work carried out on the use of the developed HPLC method for the analysis of carotenoids in several types of vegetables.

## MATERIALS AND METHODS

### Solvents and Carotenoid Standards

Solvents for liquid chromatography were of HPLC grade. All solvents for use as the mobile phase in HPLC were filtered through a 0.45  $\mu$ m regenerated cellulose membrane filter and degassed using an ultrasonic bath.

$\alpha$  and  $\beta$ -Carotenes and lycopene standards were purchased from Sigma Chemical Company.  $\gamma$ -Carotene, cryptoxanthin, zeaxanthin, and lutein were gifts from F. Hoffmann La-Roche, Switzerland. Stock solutions of these carotenoids were prepared in hexane, except lutein and zeaxanthin which were prepared in ethanol, in concentrations of 100  $\mu$ g per mL and stored in amber bottles below  $-20^{\circ}\text{C}$ . Working solutions of 1  $\mu$ g per mL of the standards were prepared daily. The appropriate extinction coefficients published in the literature (De Ritter, 1981) were used to calculate the exact concentration of each of the carotenoids. For HPLC, the solvents in the standard solutions were rapidly evaporated

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with the aid of nitrogen and the residues redissolved in the mobile phase. The preparation of all standard carotenoids was carried out without undue delay, in a room with subdued light and with all windows tinted with a light-protective film. All sample treatment and analytical procedures were also carried out in this room.

### Sample Pre-treatment Procedures

The studies were carried out on selected vegetables from four different groups, namely four green leafy vegetables (drumstick leaves, *S. androgynus* or *cekor manis*, spinach, and wolfberry leaves), a fruit vegetable (tomato), two leguminous vegetables (French bean and long bean) and a root vegetable (carrot). Edible portions of the foods were comminuted in a blender and 2-10 g immediately weighed for analysis.

Two pre-treatment procedures were studied, namely with and without prior saponification of the samples. For the latter procedure, the pre-treatment steps were essentially those of the Association of Official Analytical Chemists (AOAC) (Deutsch, 1984). The well-blended test material was extracted with hexane, and the pooled extract reduced to a small volume by heating over a water-bath with the aid of a stream of oxygen-free nitrogen. The resulting solution was made up immediately to a suitable volume (e.g. 25 mL) with hexane (test solution).

In the treatment procedure with saponification, volume of 100% (w/v) potassium hydroxide equal to the weight of the test material and 40 mL of ethanol were added to the sample. The mixture was saponified on an electric heating mantle for 30 minutes, cooled and extracted with hexane. The hexane extracts were washed till free of alkali and treated as above for preparation of the test solution.

### Analytical Procedures

Carotenoids in the test solutions prepared were studied by a combination of several procedures. All samples were studied by two chromatographic procedures, namely the open-column chromatographic method of the AOAC and the HPLC method developed by the authors. UV-VIS absorption spectrophotometry was used for studying test solutions and chromatographic eluants.

### Open-column Chromatography (AOAC method)

In the original AOAC method (Deutsch, 1984) only  $\beta$ -carotene is eluted from the magnesia-diatomaceous earth column using 10% acetone in hexane. In these studies, the method was modified to separate the carotenoid standards using the technique of stepwise increase in the proportions of acetone-in-hexane,

described in the section on identification and characterisation of carotenoids.

### High-pressure Liquid Chromatography (HPLC) method

A Waters HPLC equipped with a Model 440 fixed-wavelength detector fitted with a 436-nm wavelength kit at an attenuation of 0.02 absorbance units full scale (AUFS). A Waters Guard-Pak pre-column and a stainless steel 30 cm x 3.9 mm I.D. 10- $\mu$ m  $\mu$ Bondapak C<sub>18</sub> column were used for the chromatographic separation. Sample injection volumes (50 to 100  $\mu$ L), were dispensed with a Rheodyne 7125 injector. A Waters 6000A solvent delivery system was used to deliver the mobile phase (acetonitrile-methanol-ethyl acetate, 88:10:2, v/v) at the rate of 2.0 mL/min. Peak areas were quantitated with a Waters 730 Data Module.

Hexane in the test solution was first evaporated off on a water-bath with the aid of nitrogen gas. The residue was immediately redissolved in a suitable volume of the mobile phase. After passing through a 0.45- $\mu$ m regenerated cellulose membrane filter, suitable volumes were chromatographed using the conditions described above. Quantitation of the carotenoids was carried out by comparing with reference standards. Peak areas of samples and standards used for calculation were based on mean values obtained from at least three injections.

### Identification and Characterisation of Carotenoids

Carotenoid peaks in food samples were tentatively identified by chromatography of reference standards using the same HPLC conditions. To assist in the identification and confirmation of the pigments isolated from the samples, the HPLC and open-column chromatography techniques were used in combination with UV-VIS spectrophotometry. Test solutions prepared from food samples were chromatographed on the magnesia-diatomaceous earth column, and eluted with 80 mL 10% acetone in hexane, the proportion of acetone in the successive 80 mL eluting solvent was increased progressively in 5% increments (i.e. 15%, 20%, 25%, 30% and 35% acetone in hexane). The fraction obtained with each elution solvent mixture was evaporated and made up to a sufficient volume to give an adequate absorbance reading and scanned in the spectrophotometer, using a micro-cuvette as necessary. An aliquot of each fraction was evaporated, redissolved in the mobile phase, and chromatographed by HPLC. The peaks present in each fraction could be studied by HPLC. Retention times and UV-VIS absorption spectra obtained were compared with those given by authentic carotenoid standards. The data obtained by this technique were useful in the confirmation of the carotenoids in the food samples.

## RESULTS AND DISCUSSION

### Absorption Spectra of Carotenoid Standards

The seven carotenoids used as reference standards have varying structures, including the acyclic conjugated polyenelycopene; carotenoids with *psi*, *beta* and *epsilon* end-groups; and oxygenated carotenoids (Figure 1). UV-VIS absorption spectra of the standards (Figures 2a-2c) were in agreement with absorption maxima published in the literature (e.g. De Ritter, 1981). The spectra were used in assisting the identification of carotenoids in the vegetables studied.

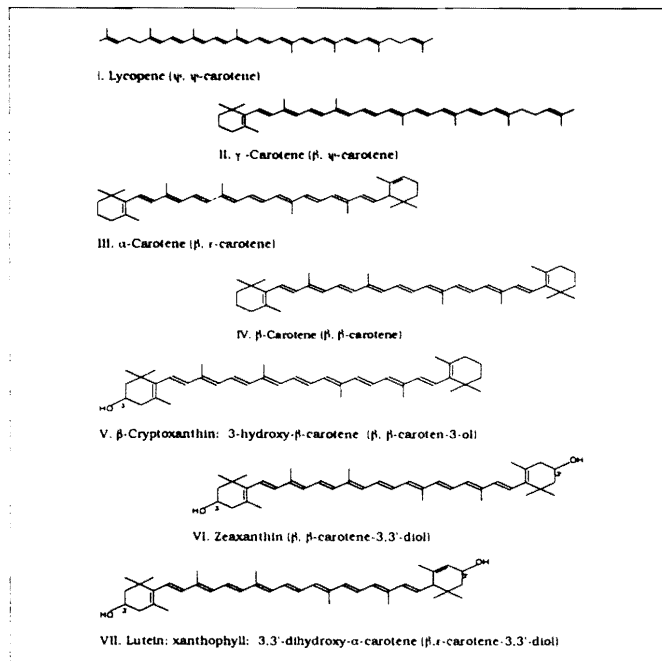


Figure 1. Structures of Carotenoid Standards (Systematic names of carotenoids given in parentheses)

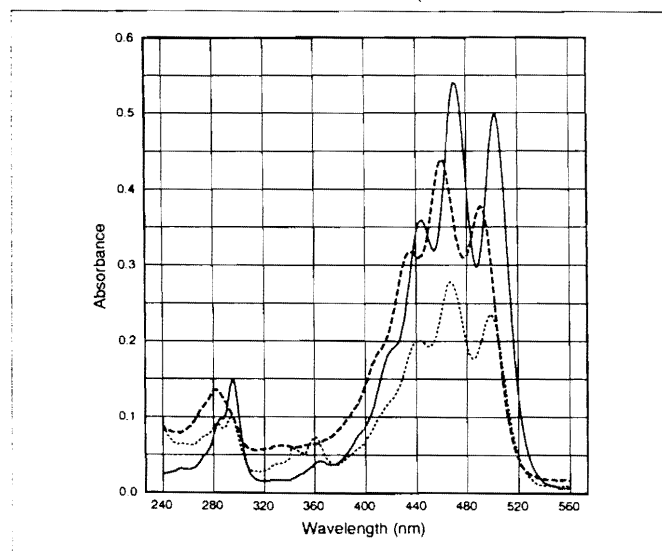


Figure 2a. UV-VIS Absorption Spectra of Lycopene (—) and  $\gamma$ -Carotene (---) in Hexane (2  $\mu\text{g}/\text{mL}$ ), and 35% acetone-in-hexane eluate from magnesia-diatomaceous earth column using hexane extract from tomato (.....).

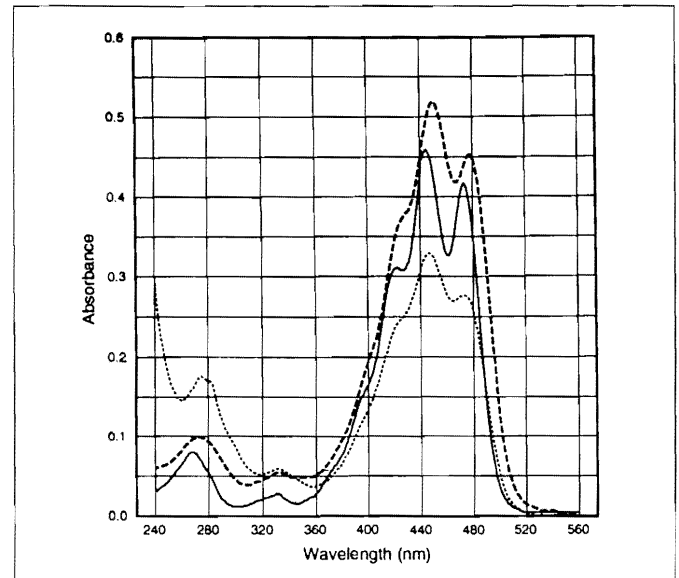


Figure 2b. UV-VIS Absorption Spectra of  $\alpha$ -Carotene (—) and  $\beta$ -Carotene (---) in Hexane (2  $\mu\text{g}/\text{mL}$ ), and 10% acetone-in-hexane eluate from magnesia-diatomaceous earth column using hexane extract from all vegetables (.....).

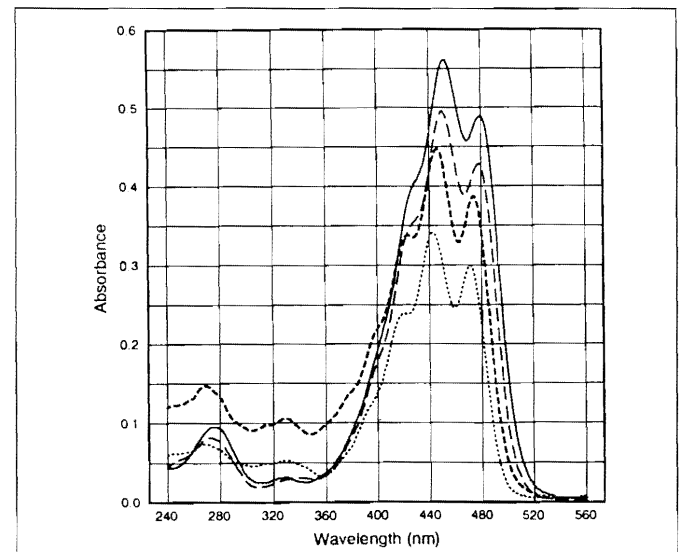


Figure 2c. UV-VIS Absorption Spectra of Cryptoxanthin (---) in hexane (2  $\mu\text{g}/\text{mL}$ ), Zeaxanthin (—) and Lutein (.....) in Ethanol (4  $\mu\text{g}/\text{mL}$ ), and 20% acetone-in-hexane eluate from magnesia-diatomaceous earth column using hexane extract from all vegetables (.....).

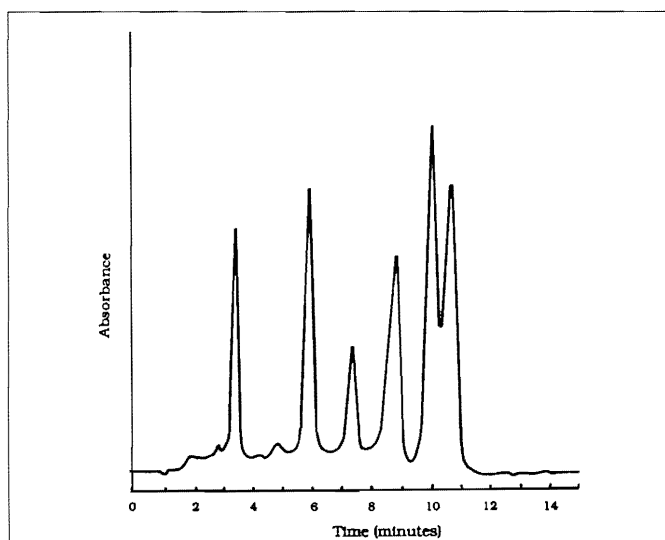
### Chromatographic Behaviour of Carotenoid Standards

#### High-pressure Liquid Chromatography

Figure 3 shows the HPLC profile of the carotenoid standard mixture. Using the chromatography conditions developed, satisfactory separation for lutein (retention time, RT = 3.7 min), cryptoxanthin (RT = 6.0 min), lycopene (RT = 7.5 min),  $\gamma$ -carotene (RT = 9.0 min),  $\alpha$ -carotene (RT = 10.0 min), and  $\beta$ -carotene (RT = 10.5 min) was obtained.  $\alpha$ - and  $\beta$ -Carotenes, differing only in the position of the double-bond in one of the two end groups (Figure 1) were not com-

pletely separated. There was no difficulty in accurate identification and quantitation of these two carotenoids. The incomplete separation of  $\alpha$ - and  $\beta$ -carotenes by HPLC systems has also been described by other investigators studying carotenoids in fruits and vegetables (Bushway and Wilson, 1982; Hsieh and Karel, 1983; Fisher and Rouseff, 1986; and Simon and Wolff, 1987). Zeaxanthin and lutein, the dihydroxy forms of  $\beta$ -carotene and  $\alpha$ -carotene respectively (Figure 1), were also poorly separated, especially when the HPLC column has been used for some time. However, these carotenoids had slightly different absorption spectra (Figure 2c).

It can be seen from the chromatogram (Figure 3) that elution order of the carotenoids on the reversed-phase  $C_{18}$  was as expected, i.e. the more polar compounds were eluted first, followed by the elution of the non-polar carotenoids. Lutein and zeaxanthin, the dihydroxy pigments, were eluted first, followed by the hydroxy carotenoid, cryptoxanthin, then the straight-chain carotenoid lycopene. The non-polar carotenoid hydrocarbons,  $\gamma$ -,  $\alpha$ - and  $\beta$ -carotenes were eluted last.



**Figure 3.** HPLC Profile of Carotenoid Standards, at 436 nm, 0.02 AUFS, 100  $\mu$ L. Other chromatography conditions described in text.  
1 = lutein (0.5  $\mu$ g/mL); 2 = cryptoxanthin (0.5  $\mu$ g/mL); 3 = lycopene (0.5  $\mu$ g/mL); 4 =  $\gamma$ -carotene (1.0  $\mu$ g/mL); 5 =  $\alpha$ -carotene (1.0  $\mu$ g/mL); 6 =  $\beta$ -carotene (1.0  $\mu$ g/mL).

### Open-column Chromatography

Using stepwise increase in the proportions of acetone in hexane as eluants, the elution order of the carotenoids from the magnesia-diatomaceous earth column was established to be  $\alpha$ -carotene,  $\beta$ -carotene,  $\gamma$ -carotene, cryptoxanthin, lutein, and lycopene. This normal-phase chromatography process eluted the non-polar carotenoid hydrocarbons first, followed by the more polar carotenoids.

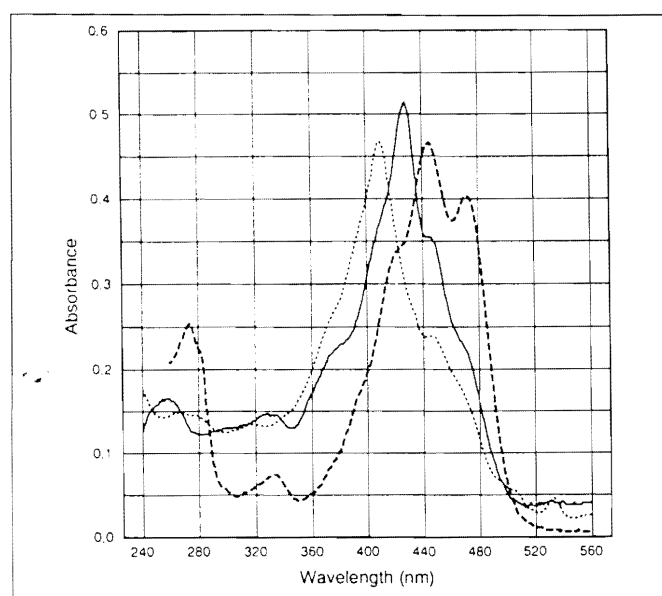
This order is the reverse of that observed using the reversed-phase HPLC method.

It was thus possible to achieve some degree of separation of carotenoids using the modified AOAC method. It is, however, an extremely tedious process and would require a long time. Furthermore, the separation was not complete, since each acetone-hexane eluate was found by HPLC to consist of more than one carotenoid.

### Characterisation and Identification of Carotenoids

Visual inspection of the saponified and unsaponified green vegetables showed obvious differences as the latter samples were generally greenish in colour, whereas the saponified samples were more orange-yellow. This colour difference indicates that saponification removed most of the green pigments (which are not carotenoids). Saponification would be advantageous, especially for HPLC, since there would be less interfering substances to contaminate and block the column.

UV-VIS absorption spectra and chromatograms from HPLC confirmed that pigments contained in the hexane extracts of green vegetables processed with and without saponification were rather different. The spectrum from the saponified sample of wolfberry leaves is more characteristic of carotenoids (Figure 4). HPLC chromatograms of the unsaponified wolfberry leaves (Figure 5) had additional peaks between five and nine minutes compared with the saponified sample. Different absorption spectra and HPLC profiles were obtained for the unsaponified and saponified extracts of the leguminous vegetables, long bean and French bean (Figures 4 and 6).



**Figure 4.** UV-VIS absorption spectra of hexane extracts of unsaponified wolfberry leaves (—) and long bean (.....), and hexane extracts of saponified samples of both vegetables (-----).

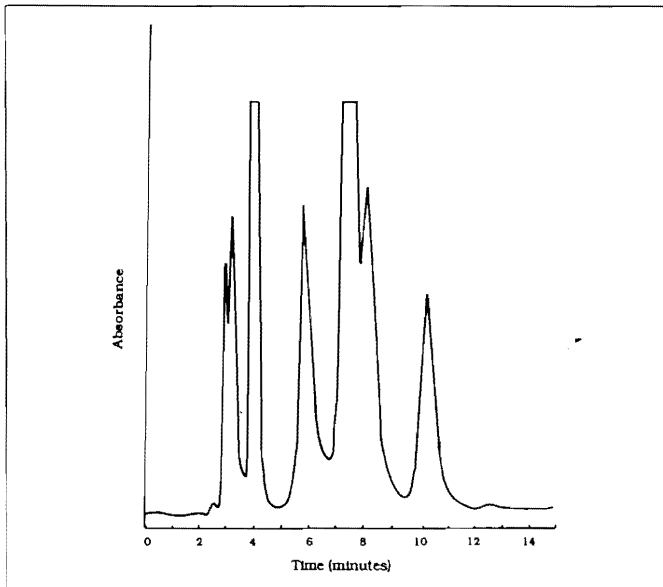


Figure 5a. HPLC chromatogram of hexane extract of unsaponified wolfberry leaves.

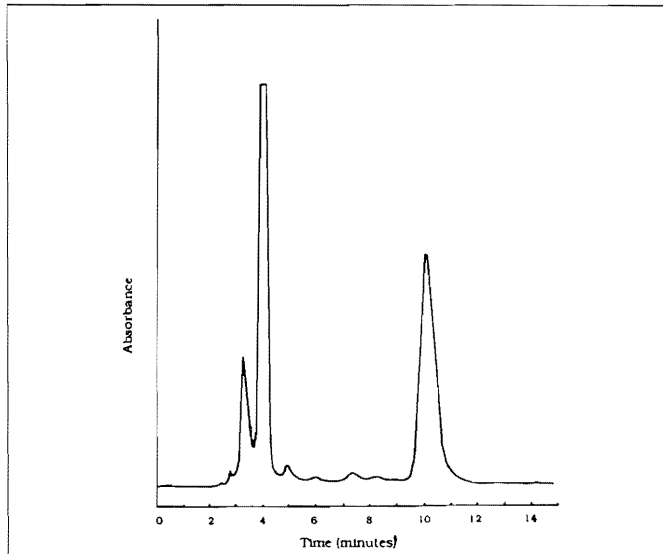


Figure 5b. HPLC chromatogram of hexane extract of saponified wolfberry leaves.

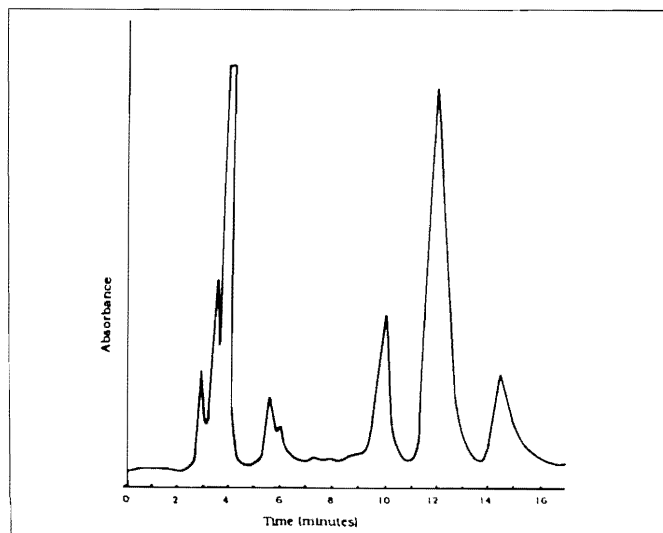


Figure 6a. HPLC chromatogram of hexane extract of unsaponified long bean.

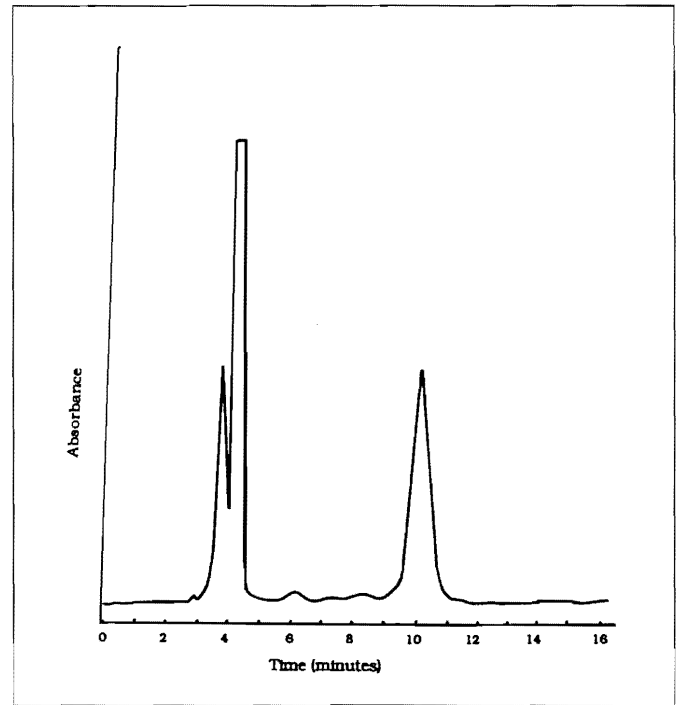


Figure 6b. HPLC chromatogram of hexane extract of saponified long bean.

By contrast, the hexane extracts of tomato and carrot prepared with and without saponification gave rather similar absorption spectra (Figure 7) and HPLC profiles (Figures 8 and 9), because these two foods contained relatively few non-carotenoid pigments.

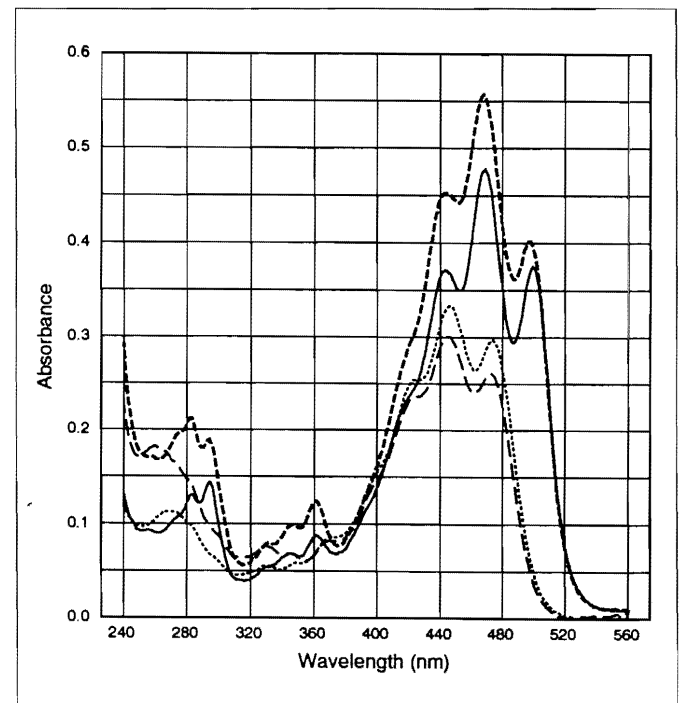
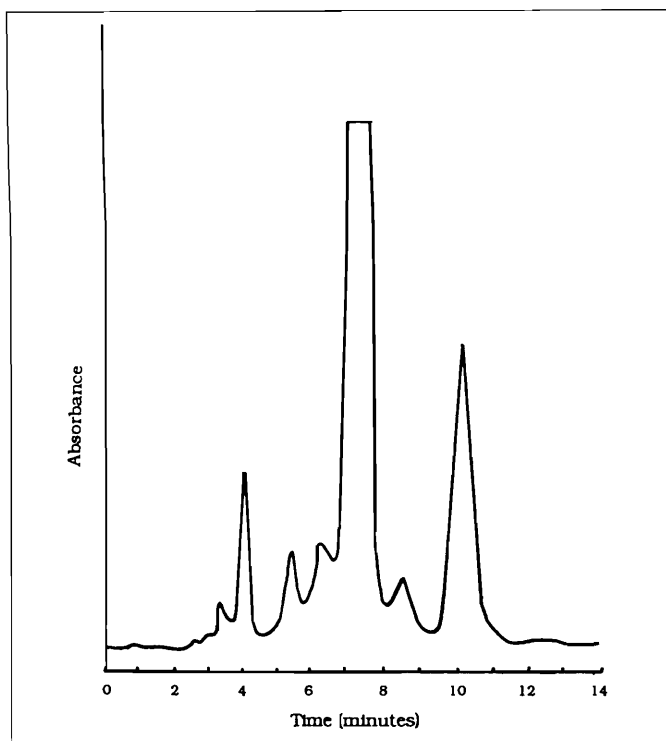
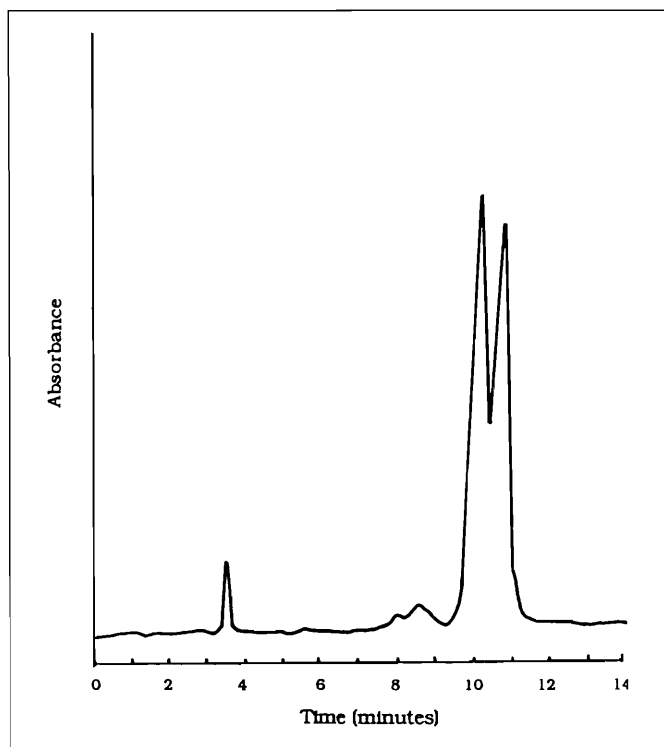


Figure 7. UV-VIS absorption spectra of hexane extracts of unsaponified (---) and saponified (—) tomato, and hexane extracts of unsaponified (.....) and saponified (-.-.-) carrot.



**Figure 8.** HPLC chromatogram of hexane extract of unsaponified tomato. Chromatogram for saponified sample is similar, except that peak at 7.4 minutes is reduced.



**Figure 9.** HPLC chromatogram of hexane extract of unsaponified carrot. Chromatogram for saponified sample is similar.

HPLC profiles, open-column chromatography and UV-VIS absorption spectra of the unsaponified and saponified samples were studied to identify and confirm the carotenoids present in the vegetables studied.

For the four unsaponified green leafy vegetables studied, the major peaks in the HPLC profile were those with RT = 3.7, 7.4 and 10.5 minutes (Figure 5a). Three minor peaks were eluted at 2.9, 5.7 and 8 minutes. After subjecting these vegetables to saponification, only two major peaks remained in the chromatogram, with RT at 3.7 and 10.5 minutes (Figure 5b). The peak common to all the four green leafy vegetables studied, and present in saponified as well as unsaponified samples, was a major peak averaging about half of the total peak area in the saponified samples, and RT of 10.5 minutes corresponding to that of  $\beta$ -carotene. For each of the four leafy vegetable samples, the peak eluted from the magnesia-diatomaceous earth column with 10% acetone-in-hexane, had an UV-VIS absorption spectrum corresponding to the  $\beta$ -carotene standard (Figure 2b). Thus, based on the similarity of the behaviour of the peak to  $\beta$ -carotene in two separate chromatographic systems and its absorption spectrum, the peak was identified as  $\beta$ -carotene.

Another peak that was present in all the four vegetables treated by the two procedures had a HPLC retention time similar to that for lutein or zeaxanthin (RT = 3.7 minutes). In all cases, the peak was eluted from the magnesia column using a 20% acetone-in-hexane mixture. The absorption spectrum obtained for this fraction was closer to that of lutein rather than zeaxanthin (Figure 2c). It was a significant peak in these vegetables, amounting to about 50% of the total peak area in the saponified samples. However, neither lutein nor zeaxanthin are precursors of vitamin A.

The peak with RT of 7.4 minutes obtained for the unsaponified green leafy vegetables was most likely not that of lycopene, a carotenoid with the same retention time. The fraction was thought to be a non-carotenoid pigment because (a) the peak could not be eluted from the magnesia column, and (b) after saponification, the peak was not detected. For the same reasons, the peaks with RT = 5.7 and 8 minutes were probably non-carotenoid pigments.

The peak eluted at 2.9 minutes using HPLC was detected in all the four vegetables. It was eluted from the magnesia column using 15% acetone in hexane. In most cases, HPLC showed that this fraction also contained the lutein peak. UV-VIS spectra therefore were not useful in assisting the identification of this relatively small peak.

Tomato gave a rather different pattern of pigments from that of the green leafy vegetables. Only three peaks were obtained for the unsaponified and saponified samples, with RT = 3.7, 7.4 and 10.5 minutes (Figure 8). The peak at 10.5 minutes was identified as  $\beta$ -carotene, and that at 3.7 minutes as lutein.

The peak with RT of 7.4 minutes found in the

unsaponified tomato sample was eluted from the magnesia column using 35% acetone-in-hexane. The UV-VIS absorption spectrum obtained matched that of lycopene standard (Figure 2a). The peak was also obtained in the saponified sample (in contrast to the peak with the same retention time in green leafy vegetables, but shown to be a non-carotenoid).

The leguminous vegetables studied, French bean and long bean, presented yet another pattern of pigments from those of the previous two types of vegetables. In this group, peaks with RT between 5 and 9 minutes were not detected. In contrast, two peaks, eluting at 12.2 and 14.5 minutes were observed in the unsaponified samples of the beans, in addition to peaks at 2.9, 3.7 and 10.5 minutes (Figure 6a). Both these late-eluting peaks were not eluted from the magnesia column, and were not detected in the saponified samples (Figure 6b). These were likely to be non-carotenoid pigments.

The carrot sample studied also presented a different pattern of pigments. The HPLC profiles of the saponified and unsaponified samples were similar and were dominated by two large peaks, and a much smaller lutein peak at 3.7 minutes. These two large peaks, though not completely resolved, were sufficiently separated for quantitation (Figure 9). Retention times of the peaks (10.0 and 10.5 minutes) matched those of  $\alpha$  and  $\beta$ -carotene. The two peaks

were eluted together in the same fraction from the magnesia column. Upon subjecting this fraction to HPLC, the two incompletely resolved peaks were obtained, just as chromatographing an extract of the saponified or unsaponified carrot extract.

As has been discussed above, various non-carotenoid pigments were removed during saponification of the green vegetables, leaving  $\beta$ -carotene and lutein as the major carotenoid remaining in the hexane extracts. The absorption maximum for the hexane extracts of all the vegetables studied, except tomato, was at about 450 nm, the absorption maxima for  $\beta$ -carotene and lutein. An approximation of total carotenoid content of the vegetables could thus be calculated by taking the absorbance reading of the hexane extract of the saponified sample at 450 nm.

### Effect of Saponification on Carotenoid Content

The concentration of major carotenoids determined by the HPLC method, with and without saponification of the sample is given in Table 1.

Lutein was detected in seven of the vegetables studied. In three of these vegetables, the concentrations in the saponified and unsaponified samples varied within the narrow range of  $\pm 20\%$  (the ratio of lutein concentration in the unsaponified sample to that in the saponified sample is between 0.8 and 1.2). Larger

**Table 1.** Lutein, lycopene,  $\beta$ - and  $\alpha$ -carotene content<sup>1</sup> of selected vegetables as determined by the HPLC method, with and without saponification of sample.

	Lutein			Lycopene			$\beta$ -Carotene			$\alpha$ -Carotene		
	US	SS	US/SS	US	SS	US/SS	US	SS	US/SS	US	SS	US/SS
Drumstick leaves	12700	7130	1.8	0	0	NA	8300	7540	1.1	0	0	NA
S. androgynus	37700	29900	1.3	0	0	NA	16300	13400	1.2	0	0	NA
Spinach	3940	4180	1.0	0	0	NA	2610	3180	0.8	0	0	NA
Wolfberry leaves	9640	7590	1.4	0	0	NA	4950	5870	0.9	0	0	NA
Tomato	138	130	1.1	2070	723	2.9	549	365	1.5	0	0	NA
French bean	616	460	1.3	0	0	NA	247	236	1.1	0	0	NA
Long bean	227	300	0.8	0	0	NA	275	412	0.7	0	0	NA
Carrot	0	0	NA	0	0	NA	6480	6770	1.0	3800	3410	1.1

<sup>1</sup>mean of duplicate analyses, expressed as  $\mu\text{g}/100$  g edible portion of sample

US = unsaponified sample

SS = saponified sample

US/SS = ratio in unsaponified to saponified samples

NA = not applicable

variations were observed for the remaining four vegetables, and the unsaponified samples gave higher lutein concentrations. Thus, for most of the vegetables, there was a loss of lutein after the samples were saponified, but in none of the vegetables was the loss more than 43% of that in the unsaponified sample.

In tomato, the only vegetable with lycopene detected, there appeared to be almost a three times reduction in the concentration of this carotenoid after the sample was saponified. The peak at 7.4 minutes in the HPLC profile of the unsaponified sample could be made up of lycopene and another non-carotenoid pigment eluted at the same retention time. This non-carotenoid pigment could be the same compound that was present in the green leafy vegetables and eluted at 7.4 minutes in the HPLC system. Saponification has probably removed the non-carotenoid pigment and only lycopene was eluted, thereby reducing the area of the 7.4 minute peak in the saponified sample. The loss in lycopene concentration in the saponified sample may thus be more apparent than real.

For most of the vegetables studied,  $\beta$ -carotene concentration did not appear to be greatly affected by the saponification process. In all but two of the vegetables studied, the ratios of  $\beta$ -carotene concentration given by unsaponified samples to the concentration in the saponified samples were within the narrow range 0.8 to 1.2. Similarly for  $\alpha$ -carotene in carrot, the concentration of this carotenoid was not drastically reduced by saponification.

## CONCLUSION

The HPLC method developed enabled the satisfactory separation and quantitation of several carotenoid standards of nutritional importance. Satisfactory results were obtained when the method was applied to the determination of carotenoids in selected vegetables. Characterisation and identification studies carried out using a combination of procedures have shown that  $\beta$ -carotene was found in all the vegetables studied, although the concentration varied considerably. Lutein was a major carotenoid found in all the vegetables except carrot. Lycopene was detected only in tomato, whilst  $\alpha$ -carotene was found only in carrot. It can be noted that the combined use of HPLC and open-column chromatography and UV-VIS absorption spectra was necessary for the identification of carotenoids.

Saponification of the vegetable samples produced "cleaner" extracts, containing less non-carotenoid pigments. The saponification process did not result in significant loss or destruction of  $\beta$ -carotene in the vegetables. There appeared to be some loss of lutein

as a result of the saponification process, a carotenoid that does not possess pro-vitamin A activity.

"Total carotenoid content" of the vegetables could be calculated by taking the absorbance reading of the saponified extracts at 450 nm. This procedure would not give an accurate quantitation for all vegetables because of the differing absorption maxima of the various carotenoids. For example, owing to the influence of lycopene, the hexane extract from tomato gave an absorption maximum at 470 nm. This procedure is nevertheless proposed as a convenient method for estimating total carotenoid concentrations of vegetables.

The HPLC method established will be applied to the study of carotenoid composition and content of a variety of common local vegetables and fruits, for more accurate quantitation of vitamin A values of these foods. All the samples to be studied will be concurrently determined by this HPLC method and the open-column chromatography methods of the AOAC, for further systematic comparison of results obtained by the two methods.

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## REFERENCES

- Beecher, G.R. and Khachik, F. Evaluation of vitamin A and carotenoid data in food composition tables. *J. Nat. Cancer Inst.* 73: 1397-1404; 1984.
- Bureau, J.L. and Bushway, R.J. HPLC determination of carotenoids in fruits and vegetables in the United States. *J. Food Sci.* 51: 128-130; 1986.
- Bushway, R.J. and Wilson, A.M. Determination of  $\alpha$ - and  $\beta$ -carotene in fruit and vegetables by high performance liquid chromatography. *Can. Inst. Food Sci. Technol. J.* 15: 165-169; 1982.
- De Ritter, E. Absorption maxima and E (1%/1cm) values for carotenoids. Bauernfeind, J.C. (Ed.). *Carotenoids as colorants and vitamin A precursors.* New York: Academic Press, 1981. 883-923.
- Deutsch, M.J. Vitamins and other nutrients. Williams, S. (Ed.). *Official methods of analysis of the AOAC.*



- 14th ed. Virginia: Association of Official Analytical Chemists, 1984. 834-835.
- Fisher, J.F. and Rouseff, R.L. Solid-phase extraction and HPLC determination of  $\beta$ -cryptoxanthin and  $\alpha$ - and  $\beta$ -carotene in orange juice. *J. Agri. Food Chem.* 34: 985-989; 1986.
- Hsieh, Y-P C. and Karel, M. Rapid extraction and determination of  $\alpha$ - and  $\beta$ -carotenes in foods. *J. Chromatogr.* 259: 515-518; 1983.
- Olson, J.A. Carotenoids, vitamin A and cancer. *J. Nutr.* 116: 1127-1130; 1986.
- Peto, R., Doll, R., Buckley, J.D. and Sporn, M.B. Can dietary beta-carotene materially reduce human cancer rates? *Nature* 290: 201-208; 1981.
- Simon, P.W. and Wolff, X.Y. Carotenes in typical and dark orange carrots. *J. Agric. Food Chem.* 35: 1017-1022; 1987.
- Taylor. R.F. Chromatography of carotenoids and retinoids. *Adv. Chromatogr.* 22: 157-213; 1983.
- Tee, E.S. Carotenoids and retinoids in human nutrition. Lim, C.L. and Chong, Y.H. (Eds.). Kuala Lumpur: Institute for Medical Research, 1988.
- Tee, E.S. and Lim, C.L. The analysis of carotenoids and retinoids: a review. *Food Chem.* 41:147-193; 1991.
- Temple, N.J. and Basu, T.K. Does beta-carotene prevent cancer? A critical appraisal. *Nutr. Res.* 8: 685-701; 1988.
- Underwood, B.A. Vitamin A in animal and human nutrition. Sporn, M.B., Roberts, A.B. and Goodman, D.S. (Eds.). the retinoids. Volume 1. Orlando: Academic Press, 1984. 281-392.
- Zakaria, M., Simpson, K., Brown, P.R. and Krstulovic, A. Use of reversed-phase high-performance liquid chromatographic analysis for determination of provitamin A carotenes in tomatos. *J. Chromatog.* 176: 109-117; 1979.