Development of a HPLC Method for the Analysis of carotenoids in Vegetables and Fruits

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ABSTRACT

Preliminary studies were carried out to apply a HPLC method developed for the analysis of carotenoids in plant foods. The eight vegetables selected were prepared by two pre-treatment procedures, namely with and without prior saponification, and chromatographed by the reverse-phase HPLC method developed. Carotenoid peaks obtained were tentatively identified using eight reference standards similarly chromatographed. To assist in the confirmation of the pigments obtained, samples were also chromatographed by open-column chromatography employing a mixture of magnesia and diatomaceous earth. Fractions were eluted with an acetone-hexane mixture using a stepwise increase in the proportion of acetone in hexane, studied by UV-vis absorption spectrophotom etry, and re-chromatographed on HPLC. The major carotenoids α - and β -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 as well as prolonging the life of the HPLC columns. "Total carotenoid content" of the saponified vegetables could be calculated by taking the absorbance reading of the extracts at 450 nm. The saponification process did not result in a significant loss of β -carotene, although there appeared to be some loss of lutein, which, however, possesses no pro-vitamin A activity.

INTRODUCTION

In recent years, there has been particular emphasis on understanding the types and concentrations of various carotenoids in foods for two main reasons. Firstly, this is of importance in relation to the provitamin A activity of the carotenoids. It is thought that previously reported values of vitamin A activity in food composition tables may have been unreliable since methodologies used were not sufficiently discriminative to separate the various carotenoids of importance in human nutrition (Zakaria *et al.*, 1979; Beecher and Khachik, 1984; Underwood, 1984; Bureau and Bushway, 1986). Some of these carotenoids may occur in higher concentrations than β -carotene, the most potent precursor of vitamin A. 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 *et al.*, 1981; Olson, 1986; Temple and Basu, 1988).

Since the late 1970's, high-pressure liquid chromatography (HPLC) has become a widely used procedure for these purposes, mainly because of the ability of the technique to effect rapid separation, its non-destructiveness and, more importantly, the better resolution that can be achieved (Taylor, 1983). However, most of the reports in the literature deal with the analysis of a limited number of foods (Tee and Lim,1990) A systematic project was therefore undertaken to obtain more accurate data on vitamin A values of common foods, based on improved methodologies that enable the quantitative determination of retinol and the main carotenoids of nutritional importance. Various studies were first carried out to develop a suitable HPLC method, examining particularly solvent systems, peak detection and quantitation techniques, and studies into the physico-chemical characteristics of retinol and several carotenoids. The objective was to develop a simple HPLC method, 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.

MATERIALS AND METHODS

Solvents and Carotenoid Standards

Solvents used for sample preparation and pre-treatment and for open-column chromatography procedures, were all analytical-grade reagents. Solvents for high-pressure liquid chromatography were of HPLC grade. All solvents for use as the mobile phase in HPLC were filtered through a 0.45-mm regenerated cellulose membrane filter and degassed using an ultra-sonic bath.

Several carotenoid standards were obtained for use as references to aid in the identification of pigments from fruits and vegetables studied. α - and β -Carotenes and lycopene standards were purchased from Sigma Chemical Company.)-Carotene, cryptoxanthin, zeaxanthin, and lutein were gifts from F. Hoffmann La-Roche, Switzerland. Stock solutions of these carotenoids were prepared in hexane (except that lutein and zeaxanthin were prepared in ethanol) in concentrations of 100 g per ml and stored in amber bottles below -20°C. Working solu tions of 1 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 stand ard solutions were rapidly evaporated with the aid of nitrogen and the residues redissolved in the mobile phase. Alternatively, in order to reduce loss of carotenoids due to heating, working solutions suitable for HPLC could be prepared by diluting small volumes of the stock solution (e.g. 50 l) using the mobile phase. In such small volumes, the solvents in the stock solutions did not interfere in the chromatography process. 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) (Williams, 1984). The well-blended test material was extracted with 25-ml portions of hexane until the extract was colourless. The extracts were pooled and 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, referred to hereafter as the "test solution".

In the treatment procedure with saponification, a 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 chromatography method of the AOAC and the HPLC method developed in this study. UV-Vis absorption spectrophotometry was used for studying test solutions and chromatography eluants.

Open-column chromatography (AOAC method)

A suitable volume (e.g. 10 ml) of the test solution was pipetted into a glass column packed with a mixture of activated magnesia and diatomaceous earth, in the ratio of 1:1, for chromatography using the AOAC method (Williams, 1984). β -Carotene was eluted from the column with approximately 80 ml of 10% (v/v) acetone-in-hexane. The eluate was evaporated on a water-bath with the aid of a stream of nitrogen and made up to a suitable volume (e.g. 10 ml) with hexane. The absorbance of the solution was read in a spectrophotometer at 450 nm and the concentration of β -carotene calculated by comparison against a calibration curve prepared with the -carotene standard.

High-pressure liquid chromatography (HPLC) method

A Waters high-pressure liquid chromatograph equipped with a Model 440 fixed-wavelength detector was used. A 436-nm wavelength kit was fitted onto the detector and an attenuation of 0.02 absorbance units full scale (AUFS) was set. A stainless steel 30 cm x 3.9 mm I.D. 10-m Bondapak C_{18} column was used for the chromatographic separation. This was preceded by a Waters Guard-PAK pre-column module housing a disposable Guard-PAK pre-column insert packed with the same material as that in the analytical column. Sample injection volumes, dispensed with a Rheodyne 7125 injector, were usually 50 to 100 l. 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-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 similarly chromatographed. 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, employing elution solvent mixtures with a stepwise increase in the proportion of acetone in hexane and a corresponding decrease in the proportion of hexane. Starting with 10% acetone in hexane, the proportion of acetone in the 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 suitable volume to give a sufficiently large absorbance reading and scanned in the spectrophotometer, using a micro-cuvette if necessary. An aliquot of each fraction was also evaporated, redissolved in the mobile phase, and chromatographed by HPLC. In this way, 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. Especially for fractions with a single peak, these data obtained by this relatively simple technique were found to be useful in assisting in the confirmation of the carotenoids in the food samples.

RESULTS AND DISCUSSION

Absorption Spectra of Carotenoid Standards

The eight carotenoids used as reference standards have varying structures, including the acyclic conjugated polyenelycopene; carotenoids with *psi*, *beta* and *epsilon* end-groups; and an oxygenated carotenoids (Figure 1). Working solutions of the standards were prepared and scanned from 240 to 560 nm in a spectrophotometer. The UV-vis absorption spectra obtained (Figures 2a-2d) 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. Absorption spectroscopy has been the cornerstone for the characterization of carotenoids for more than fifty years. It is still the diagnostic tool most easily available to many laboratories.

Chromatographic Behaviour of Carotenoid Standards

High-pressure liquid chromatography

Working solutions of all carotenoid standards were subjected to open-column chromatography and HPLC to study the chromatographic behaviour of these carotenoids. After chromatography of individual carotenoids, mixtures of these standards, prepared by mixing 1 ml each of the working solutions, were also studied.

Figure 3 shows the HPLC profile of the carotenoid 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),) β -carotene (RT = 9.0 min), β -carotene (RT = 10.2 min), and -carotene (RT = 10.8 min) was obtained. α - and β -Carotenes, differing only in the position of the double-bond in one of the two end groups (Figure 1) were not completely separated. There was however no difficulty in accurate identification and quantitation of these two carotenoids. The inability of α - and β -carotenes to be completely resolved in HPLC systems can also be seen in reports of other investigators studying carotenoids in fruits and vegetables (e.g. Bushway and Wilson, 1982; Hsieh and Karel, 1983; Fisher and Rouseff, 1986; and Simon and Wolff, 1987). Zeaxanthin and lutein, the dihydroxy forms of β -carotene and β -carotene respectively (Figure 1), were also minimally separated, especially when the HPLC column has been used for some time. However, both carotenoids had slightly dif ferent absorption spectra (Figure 2d).

It can be seen from the chromatogram (Figure 3) that elution order of the carotenoids on the reverse-phase C_{18} column was as expected, i.e. the more polar compounds were eluted first, followed by the elution of the non-polar carotenoids. Thus, lutein and zeaxanthin, the dihydroxy pigments were eluted first. After the elution of these oxygenated carotenoids or xanthophylls, the hydroxy carotenoid, cryptoxanthin, was eluted, followed by the

straight-chain carotenoid lycopene. The non-polar carotenoid hydrocarbons,)-,α - and β -carotenes were eluted last from the column.

Open-column chromatography

In the original AOAC method, only β -carotene is eluted from the magnesia column using 10% acetone-in-hexane. In these studies, attempts were made to separate the carotenoid standards using the technique of stepwise increase in the propor tions of acetone-in-hexane. Each fraction was studied by UV-vis absorption spectrophotometry as well as re-chromatographed using HPLC to identify the peak(s) present.

The elution order from the magnesia column was established to be - carotene, -carotene,)-carotene, cryptoxanthin, lutein, and lycopene. In this normal-phase chromatography process, the non-polar carotenoid hydrocarbons were eluted first, followed by the more polar carotenoids. This order is the reverse of that observed using the reverse-phase HPLC method.

It was thus possible to achieve some degree of separation of carotenoids on the magnesia column using stepwise increase in the proportions of acetone-in-hexane. It is, however, an extremely tedious process and would require a long time. Furthermore, the separation was not complete, since each acetone-in-hexane eluate was shown by HPLC to consist of more than one carotenoid.

Characterization 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. As will be discussed later, it was noted that the saponification process did not significantly destroy carotenoids, particularly those with pro-vitamin A activity.

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. Examples of absorption spectra obtained for saponified and unsaponified samples are shown in Figure 4 for wolfberry leaves. The former spectrum is seen to be more characteristic of carotenoids. Chromatograms from HPLC of the unsaponified and saponified wolfberry leaves (Figures 5a and 5b) show that the former had additional peaks between five and nine minutes. Similarly for the leguminous vegetables, long bean and French bean, different absorption spectra and HPLC profiles were obtained for the unsaponified and saponified extracts (Figures 6 and 7a and 7b).

In contrast to the green vegetables, the hexane extracts of tomato and carrot prepared with and without saponification, gave rather similar absorp-

tion spectra (Figures 8 and 10) and HPLC profiles (Figures 9 and 11). As will be discussed in the following paragraphs, the close similarity between the with saponification and the without-saponication spectra was due to the fact that these two foods contained relatively less non-carotenoid pigments.

Based on the HPLC profiles, the area of each peak was calculated as a percentage of total peak area for the eight vegetables studied (Table 1). Each of the four groups of vegetables studied exhibited different patterns of peaks detected for the saponified and unsaponified samples.

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 46% of the total peak area in the saponified samples. The peak had a RT = 10.5 minutes, same as that of -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 correspond ing to the the β -carotene standard (Figure 2b). Thus, based on the similarity of the behaviour of the peak to -carotene in two separate chromatographic systems and its absorp tion spectrum, the peak was identified as β -caro tene.

Another peak that was observed to be 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 seen to be closer to that of lutein rather than zeaxanthin (Figure 2d). It was a significant peak in these vegetables, amounting to about 48% of the total peak area in the saponified samples. From the nutritional viewpoint, however, this peak is unimportant because both lutein and zeaxanthin are not 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 the peak.

The fruit vegetable studied, 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 9). The peak at 10.5 minutes was identified as -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 different 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 previously undetected peaks, eluting at 12.6 and 15.6 minutes were observed in the unsaponified samples of the beans, aside from peaks at 2.9, 3.7 and 10.5 minutes (Figure 7a). Both these late-eluting peaks were not eluted from the magnesia column, and were not detected in the saponified samples (Figure 7b). These were most likely 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. These two peaks, though not completely resolved, were sufficiently separated for quantitation (Figure 11). Retention times of the peaks (9.7 and 10.5 minutes) matched those of α - and β -caro tene. 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 chro matographing an extract of the saponified or unsaponified carrot extract.

In summary, β -carotene was found to be the ca rotenoid found in all the eight vegetables studied, although the concentration varied considerably. Lutein was another major pigment found in all the vegetables, except for tomato and car rot, in which the concentration was very much lower. Lycopene was detected only in tomato, whilst β -carotene was found only in carrot.

As has been discussed above, various non-carotenoid pigments were removed during saponification of the green vegetables, leavingβ -carotene and lutein as the major carote noids 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 β -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 sapon ified sample at 450 nm. This procedure would, however, not give an accurate quantitation for all vegetables because of the dif fering absorption maxima of 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 determining total carotenoid concentrations of fruits and vegetables.

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. An example is the case of -carotene and lutein. Both carotenoids gave similar absorption maxima (Figures 2b and 2d), but had widely differing retention times in the HPLC profile. On the other hand, although lutein and zeaxanthin were eluted very close together from the HPLC column, there were observable differences in absorption spectra of the two carotenoids (Figure 2d). Yet another example is the peak in unsaponified green leafy vegetables that was eluted with the same retention time as lycopene. However, because of its different chromatographic behaviour on the magnesia column, this peak was probably not lycopene.

Effect of saponification on carotenoid content

The concentration of major carotenoids determined by the HPLC method, with and without prior saponification of the sample is given in Tables 2a and 2b.

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 20% (the ratio of lutein concentration in the unsaponfied sample to that in the saponified sample is between 0.8 and 1.2). Larger 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 1.8 times that of 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. It is felt that 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 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,β -carotene concentration did not appear to be greatly affected by the sapon ification process. In all but two of the vegetables studied, the ratios ofβ -carotene concentration given by unsa ponified samples to the concentration in the saponified samples were all within the narrow range of 0.8 to 1.2. Similarly for -carotene in carrot, the concentration of this carotenoid was not drastically reduced by saponification.

CONCLUSION

Various studies carried out have shown that the HPLC method developed enabled the satisfactory separation and quantitation of several carotenoid standards of nutritional importance. Satisfactory results were also obtained when the method was applied to the determination of carotenoids in selected vegetables and fruits. Characterisation and identification studies carried out using a combination of procedures have shown that β -carotene were found in all the vegetables studied, although the concentration varied considerably. Lutein was another major carotenoid found in all the vegetables, except in carrot. Lycopene was detected only in tomato, whilst β -carotene was found only in carrot.

Results obtained show that prior saponification of the vegetable samples produced "cleaner" extracts, with less non-carotenoid pigments. The saponification process did not result in significant loss or destruction of β -carotene in the vegetables. There appeared to be some loss of lutein as a result of the saponification process, but this loss may not be of nutritional significance since the carotenoid 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.

The HPLC method established was next applied to the study of carotenoid composition of a variety of common local vegetables and fruits, for more accurate quantitation of vitamin A values of these foods. All the samples studied were simultaneously determined by the newly developed HPLC method and the open-column chromatography methods of the AOAC, to enable a systematic comparison of results obtained by the two methods. Results of the study will be presented in a subsequent report.

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Zakaria, M., Simpson, K., Brown, P.R. and Krstulovic, A. 1979. **Use of reversed-phase high-performance liquid chromatographic analysis for the determination of provitamin A carotenes in tomatoes.** J. Chromatogr. 176: 109-117.Table 1: Peaks in HPLC profiles of selected vegetables processed

Table 1. Peaks in HPLC profiles of selected vegetables processed with and without prior saponification of sample

	Area of peak, with indicated RT, as % of total peak area							
Vegetables	2.9	3.7	5.7	7.4	8.0	10.5	12.6	15.6
Drumstick leaves								
unsaponified	6.1	28.1	6.0	31.6	7.3	17.3	ND	ND
saponified	ND	42.8	ND	ND	ND	57.2	ND	ND
S. androgynus								
unsaponified	15.7	33.1	9.6	16.6	5.8	19.2	ND	ND
saponified	11.7	59.3	ND	ND	ND	28.9	ND	ND
Spinach								
unsaponified	6.5	20.6	8.2	31.5	16.9	16.3	ND	ND
saponified	ND	38.8	ND	ND	ND	61.2	ND	ND
Wolfberry leaves								
unsaponified	10.5	18.1	12.8	32.8	14.8	10.8	ND	ND
saponified	12.0	49.8	ND	ND	ND	37.4	ND	ND
Tomato								
unsaponified	ND	5.3	ND	69.7	ND	21.5	ND	ND
saponified	ND	7.0	ND	64.5	ND	25.4	ND	ND
French bean								
unsaponified	ND	30.0	ND	ND	ND	12.9	39.6	10.0
saponified	15.1	53.5	ND	ND	ND	31.4	ND	ND
Long bean								
unsaponified	16.2	18.4	10.5	ND	ND	19.2	26.5	9.1
saponified	18.9	32.4	ND	ND	ND	48.7	ND	ND
Carrot								
unsaponified	ND	3.1	ND	ND	ND	96.9[a]	ND	ND
saponified	ND	ND	ND	ND	ND	100	ND	ND

ND = peak not detected, or peak with area < 5% of total area

[a] consisted of two incompletely resolved peaks, with RT = 9.7 and 10.5 minutes

Table 2a. Lutein and lycopene content[1] of selected vegetables as determined by the HPLC method, with and without prior saponification of sample.

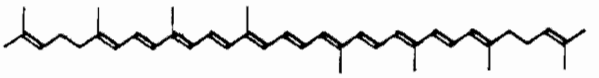
	Lutein			Lycopene		
	US	SS	US/SS	US	SS	US/SS
Drumstick leaves	12683	7128	1.8	0	0	NA
S. androgynus	37653	29913	1.3	0	0	NA
Spinach	3935	4175	1.0	0	0	NA
Wolfberry leaves	9642	7590	1.4	0	0	NA
Tomato	138	130	1.1	2069	723	2.9
French bean	616	460	1.3	0	0	NA
Long bean	227	300	0.8	0	0	NA
Carrot	0	0	NA	0	0	NA

[1] mean of duplicate analyses, expressed as μg/100 g edible portion of sample
US = unsaponified sample
SS = saponified sample
US/SS = ratio in unsaponified to saponified samples
NA = not applicable

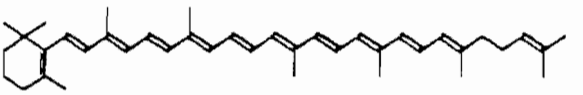
Table 2b. β- and α-Carotene content[1] of selected vegetables as determined by the HPLC method, with and without prior saponification of sample.

	Lutein			Lycopene		
	US	SS	US/SS	US	SS	US/SS
Drumstick leaves	8301	7536	1.1	0	0	NA
S. androgynus	16272	13351	1.2	0	0	NA
Spinach	2609	3177	0.8	0	0	NA
Wolfberry leaves	4947	5867	0.9	0	0	NA
Tomato	549	365	1.5	0	0	NA
French bean	247	236	1.1	0	0	NA
Long bean	275	412	0.7	0	0	NA
Carrot	6481	6769	1.0	3803	3410	1.1

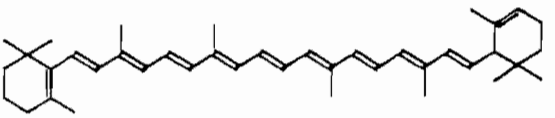
[1] mean of duplicate analyses, expressed as μg/100 g edible portion of sample
US = unsaponified sample
SS = saponified sample
US/SS = ratio in unsaponified to saponified samples
NA = not applicable



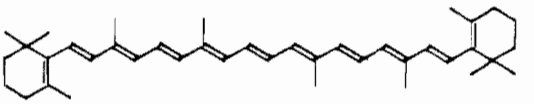
I· Lycopene (Ψ,Ψ –Carotene)



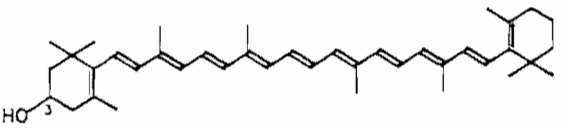
II· γ –Carotene (β,Ψ –Carotene)



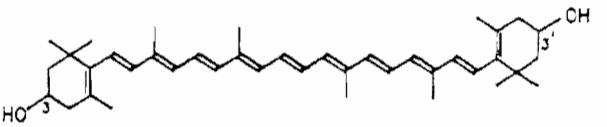
III· α –Carotene (β, ε –Carotene)



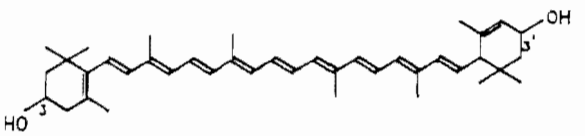
IV· β –Carotene (β,β –Carotene)



V· β–Cryptoxanthin, Cryptoxanthin, 3–Hydroxy– β –Carotene
(β,β –Caroten–3–ol)



VI· Zeaxanthin (β,β –Carotene–3,3'–diol)



VII· Lutein, "Xanthophyll", 3,3'–Dihydroxy– α –carotene
(β,ε –Carotene–3,3'–diol)

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

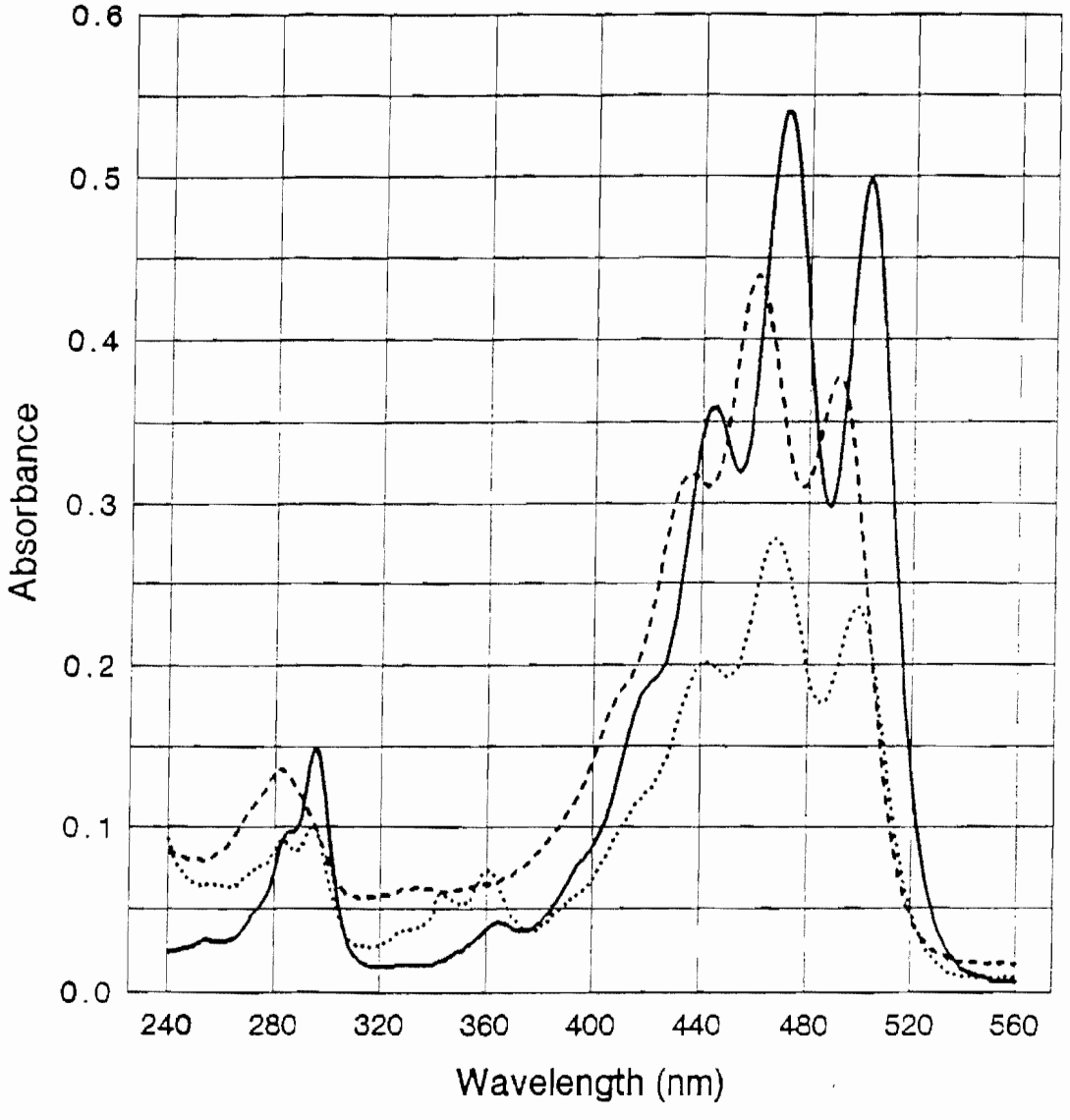


Figure 2a. UV-vis Absorption Spectra of Lycopene (———) and γ-Carotene (-----) in Hexane (2 μg/ml), and 35% acetone-in-hexane eluate from magnesia-diatomaceous earth column using hexane extract from tomato (.....).

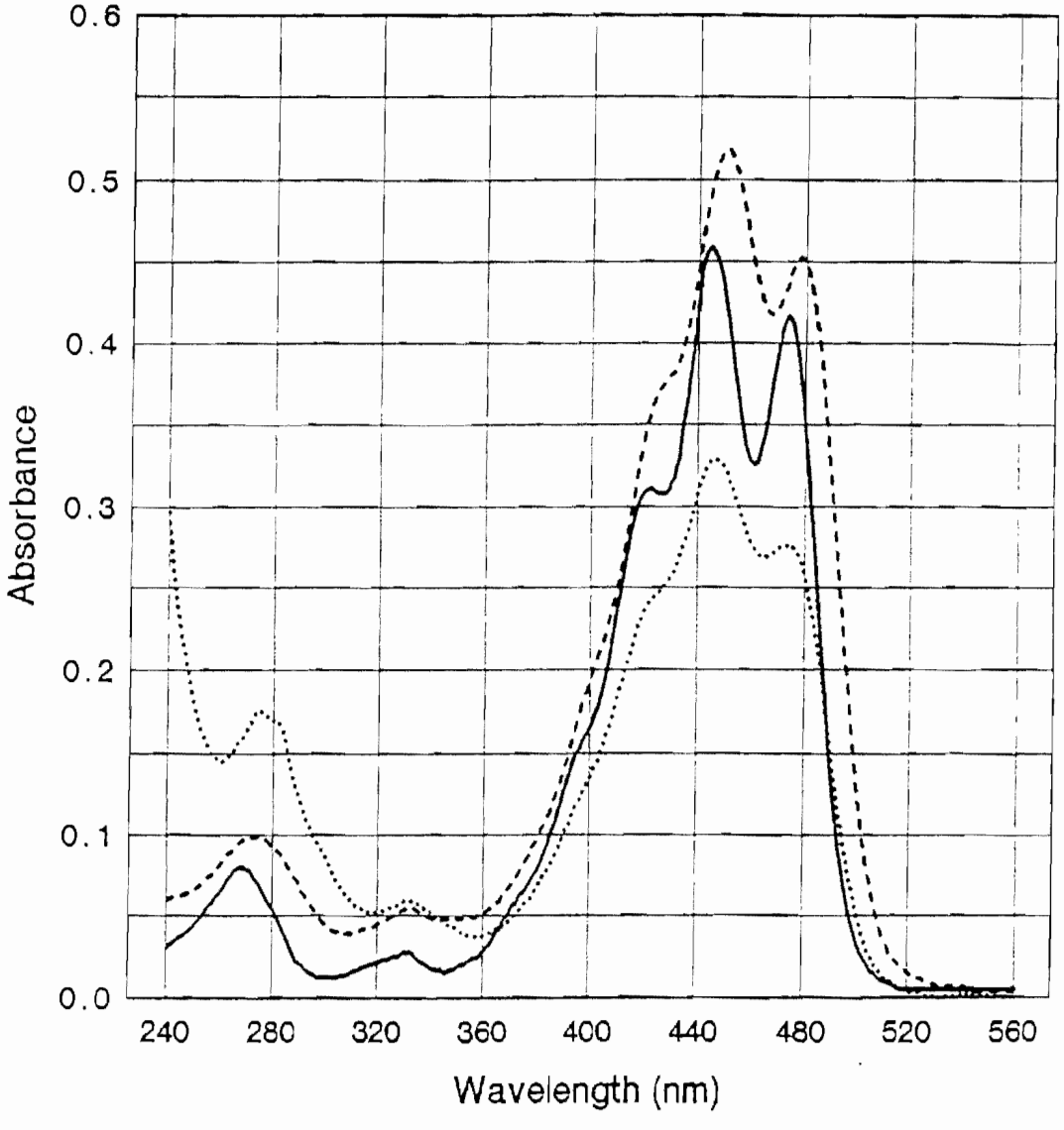


Figure 2b. UV-vis Absorption Spectra of α-Carotene (———) and β-Carotene (-----) in Hexane (2 μg/ml), and 10% acetone-in-hexane eluate from magnesia-diatomaceous earth column using hexane extract from all vegetables (.....).

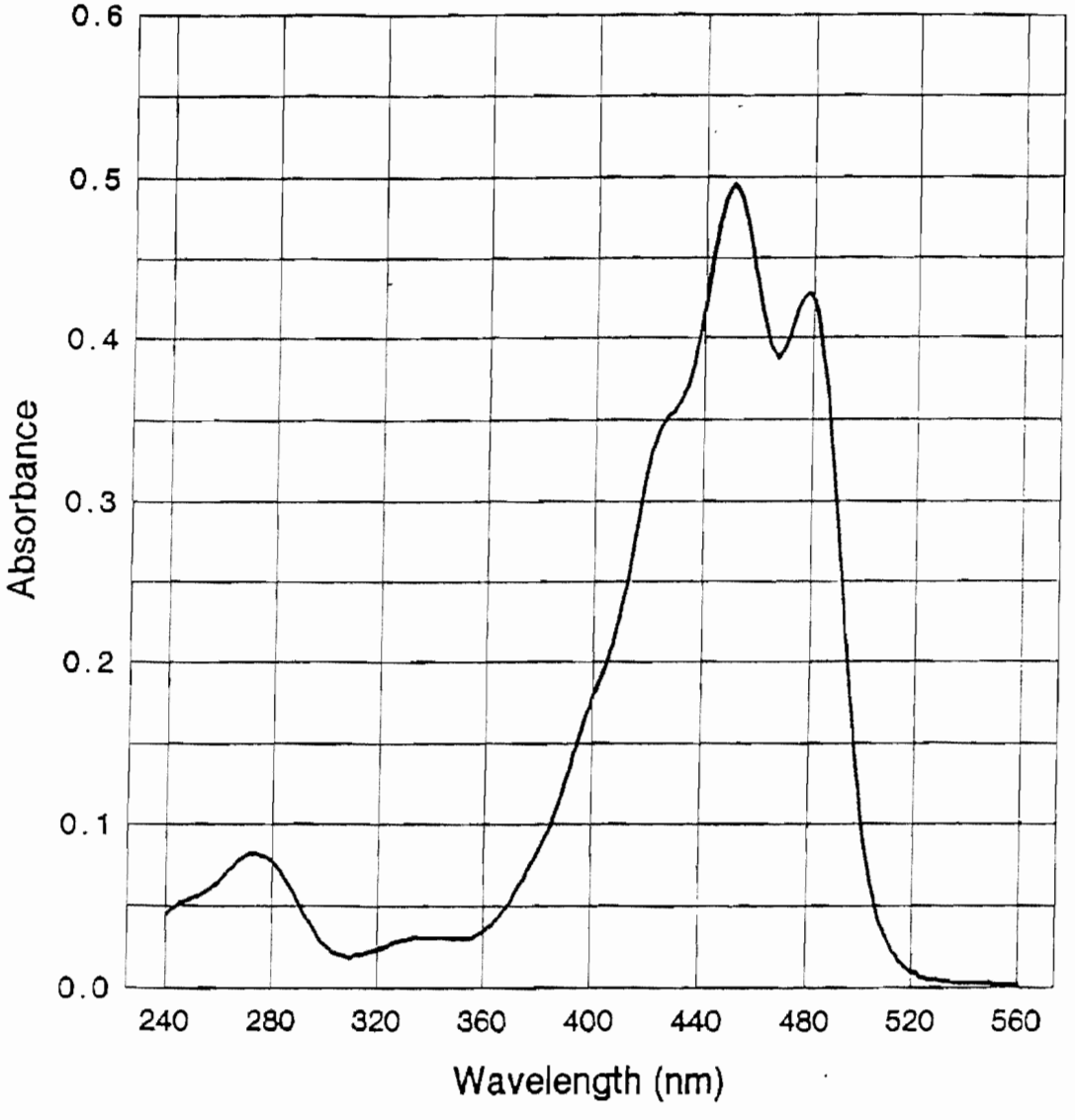


Figure 2c. UV-vis Absorption Spectrum of Cryptoxanthin in Hexane (2 μg/ml)

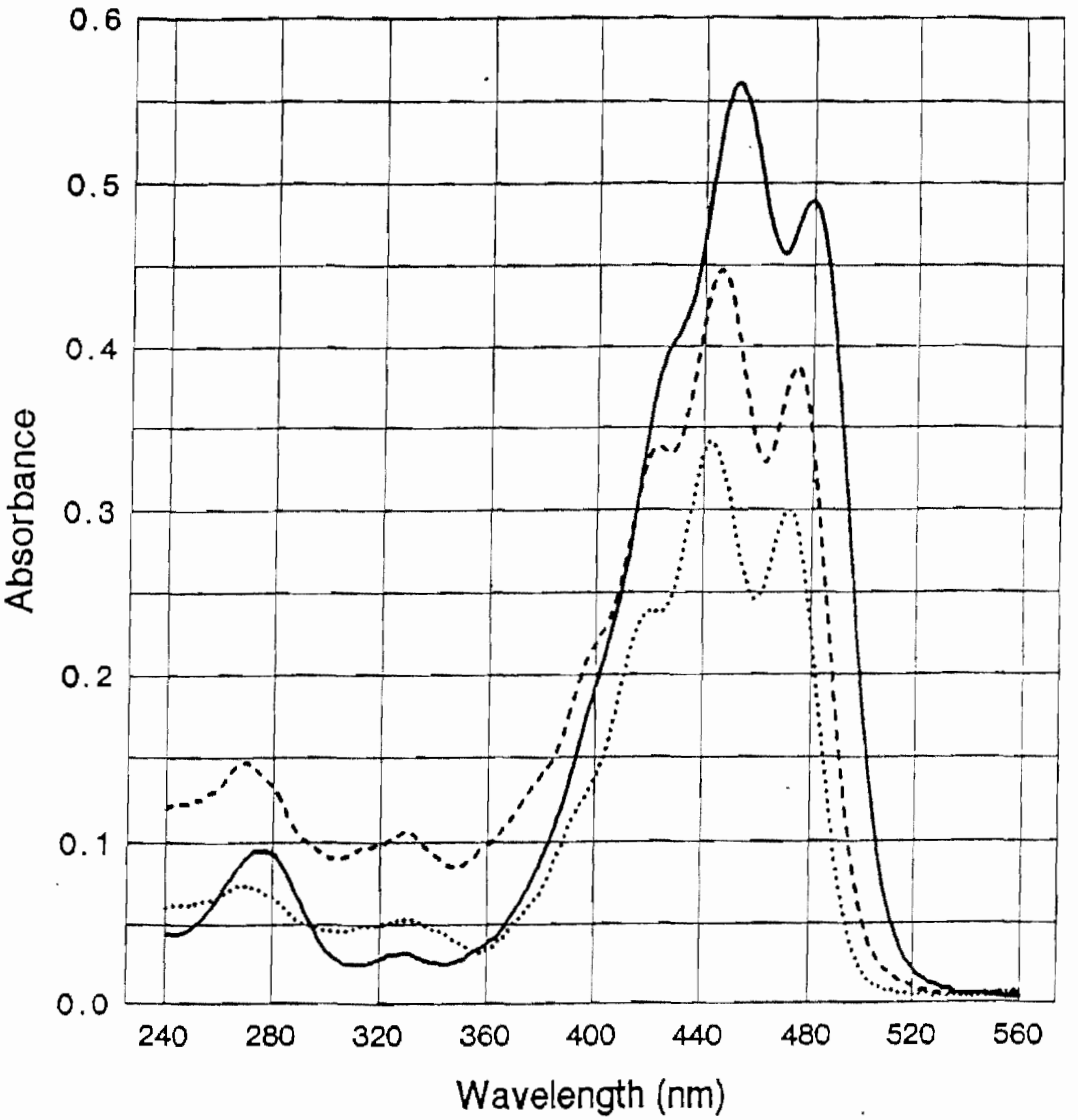


Figure 2d. UV-vis Absorption Spectra of Zeaxanthin (———) and Lutein (-----) in Ethanol (4 μg/ml), and 20% acetone-in-hexane eluate from magnesia-diatomaceous earth column using hexane extract from all vegetables (.....).

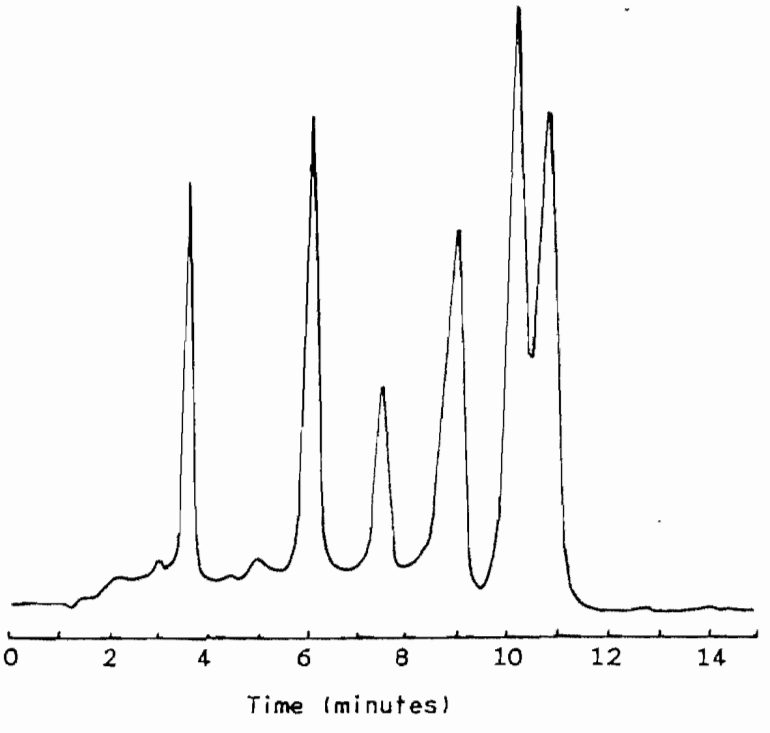


Figure 3. HPLC Profile of Carotenoid Standards. Detector at 436 nm, 0.02 AUFS. Other chromatography conditions as given in text. Concentrations of lutein, cryptoxanthin and lycopene were 0.5 μg/ml, and of α-, β- and γ-carotenes were 1.0 μg/ml. 100 μl used for injection.
1 = lutein; 2 = cryptoxanthin; 3 = lycopene; 4 = γ-carotene; 5 = α-carotene; 6 = β-carotene

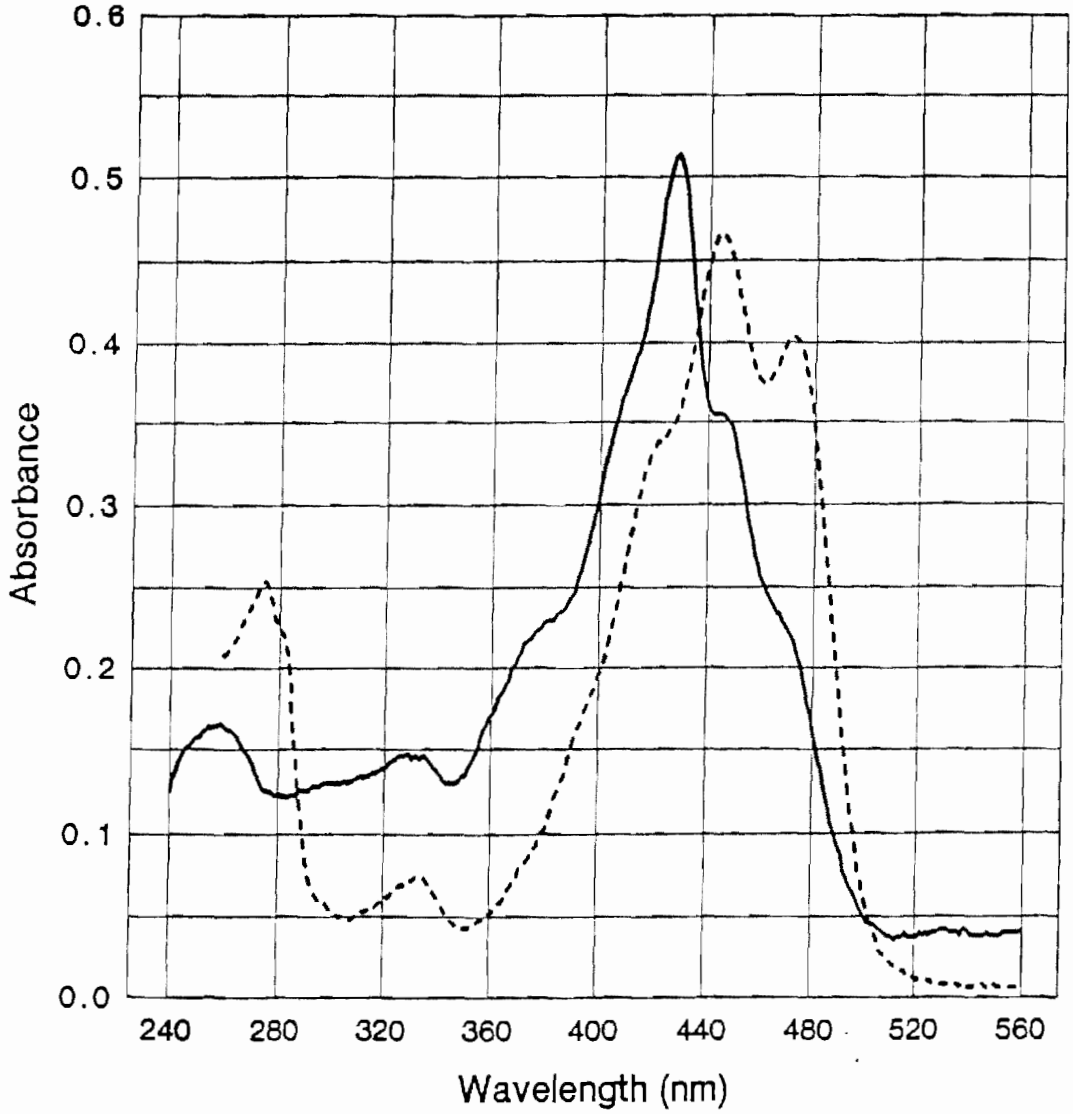


Figure 4. UV-vis absorption spectra of unsaponified (————) and saponified (--------) hexane extracts of wolfberry leaves.

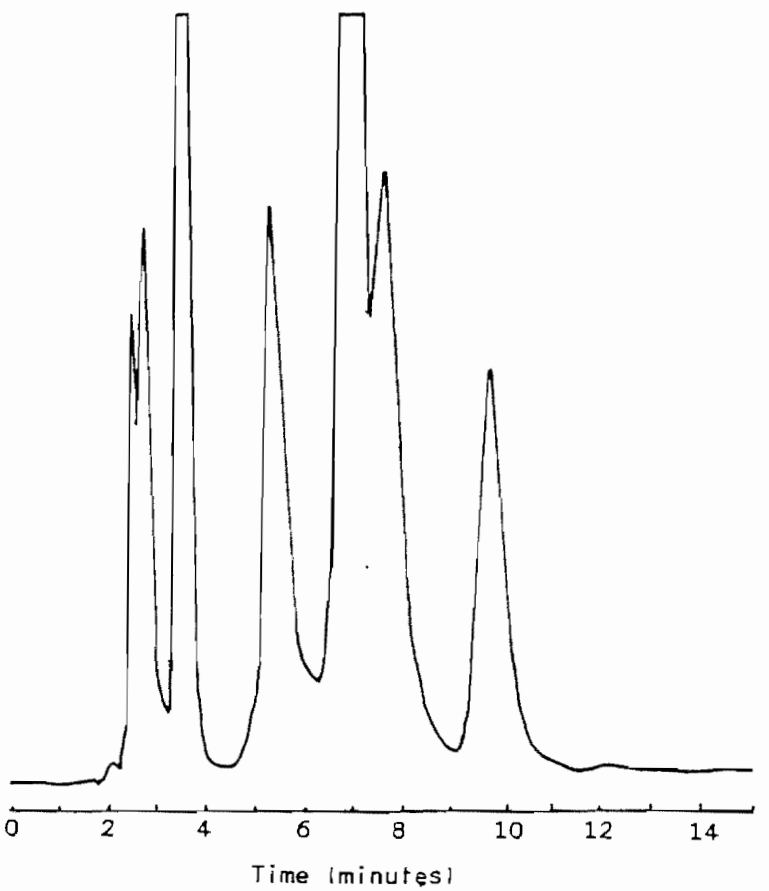


Figure 5a. HPLC chromatogram of unsaponified hexane extract of wolfberry leaves.

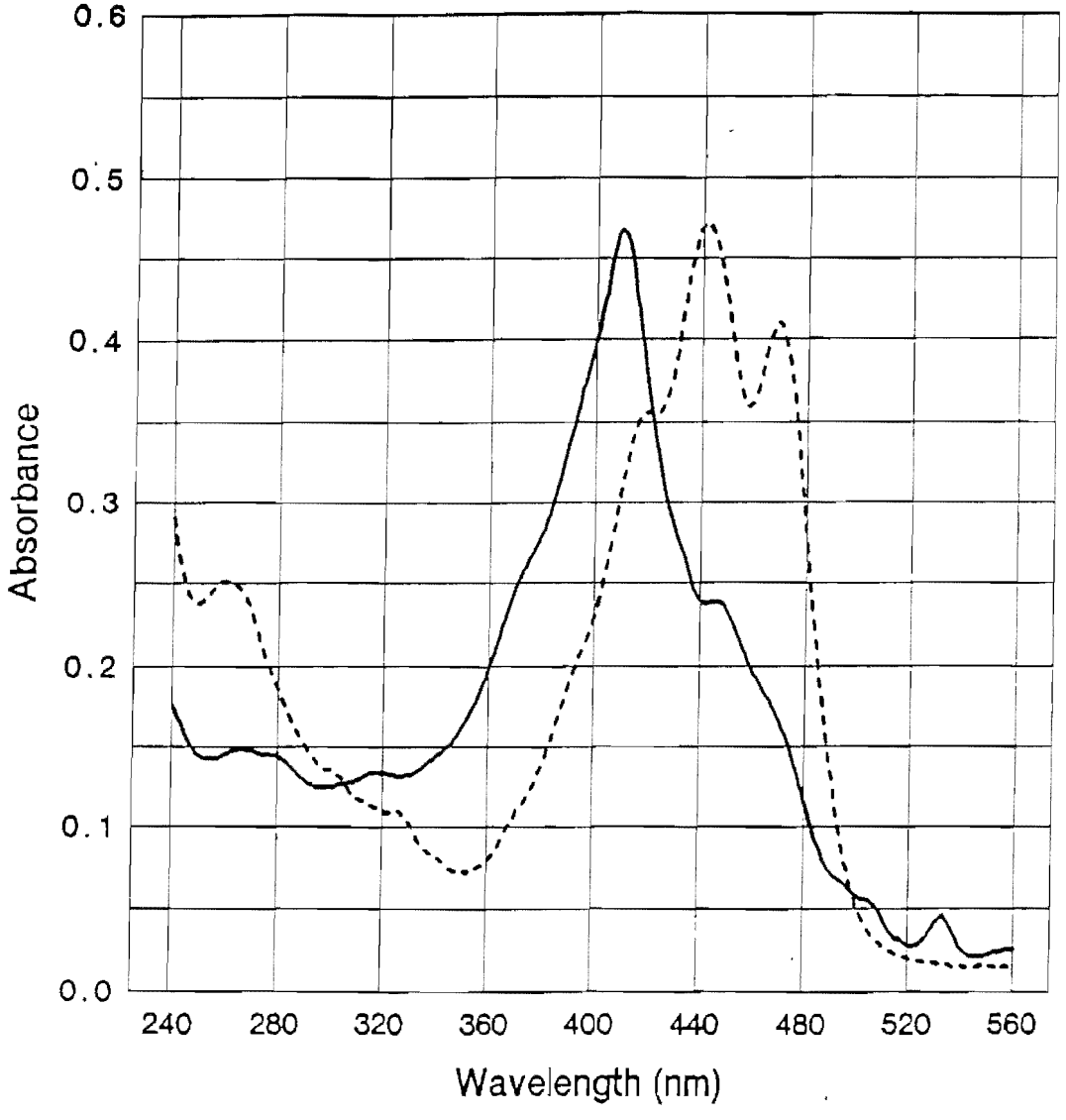


Figure 6. UV-vis absorption spectra of unsaponified (————) and saponified (--------) hexane extracts of long bean.

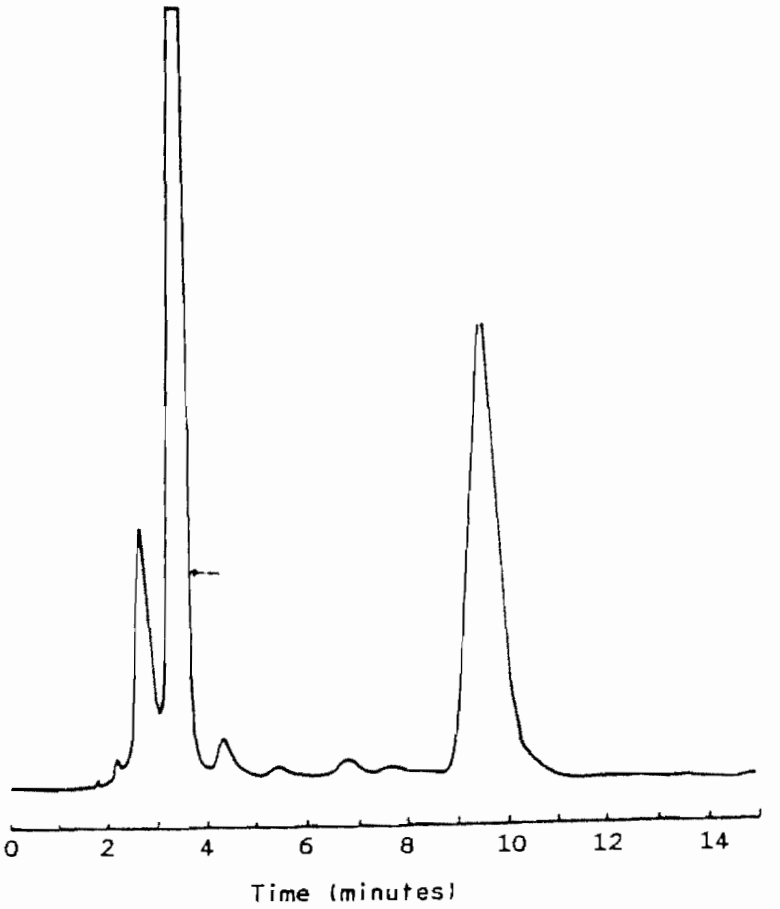


Figure 5b. HPLC chromatogram of saponified hexane extract of wolfberry leaves.

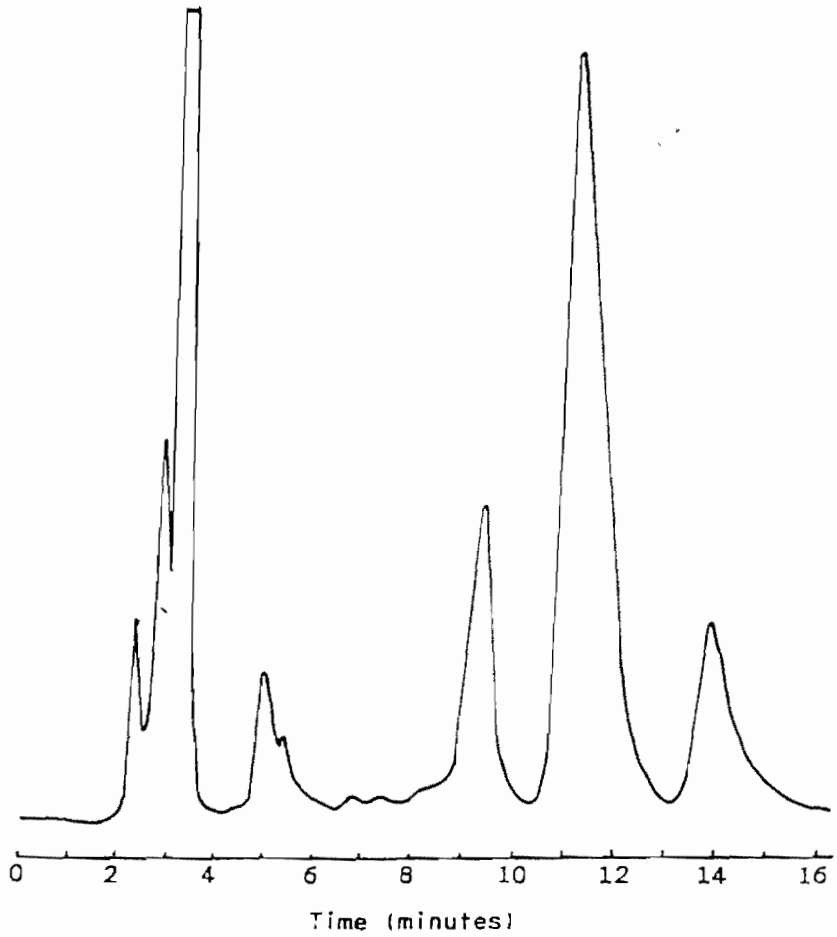


Figure 7a. HPLC chromatogram of unsaponified hexane extract of long bean.

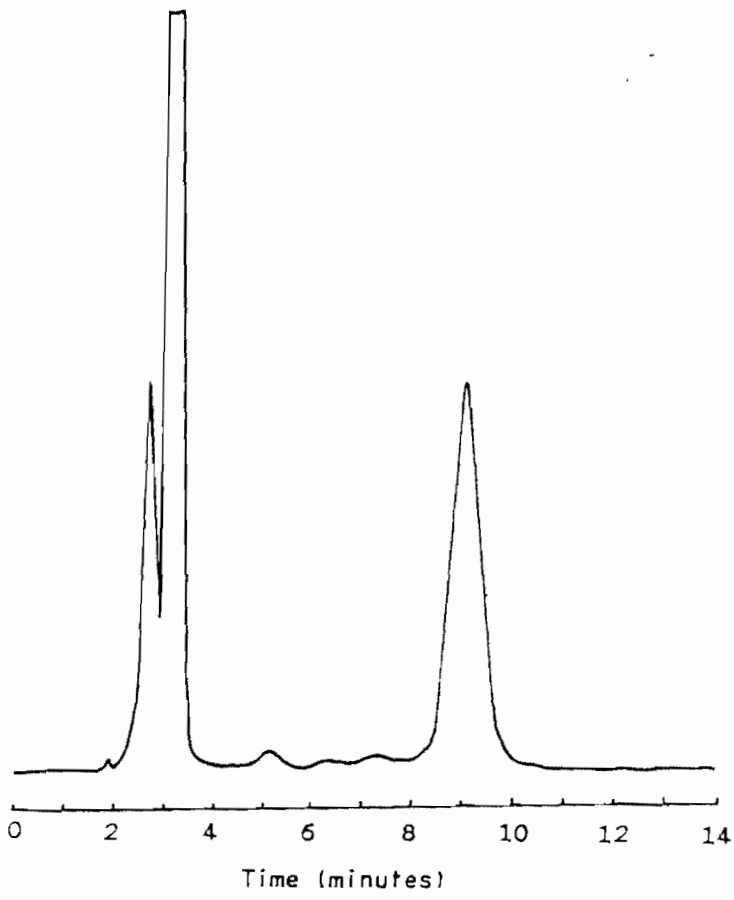


Figure 7b. HPLC chromatogram of saponified hexane extract of long bean.

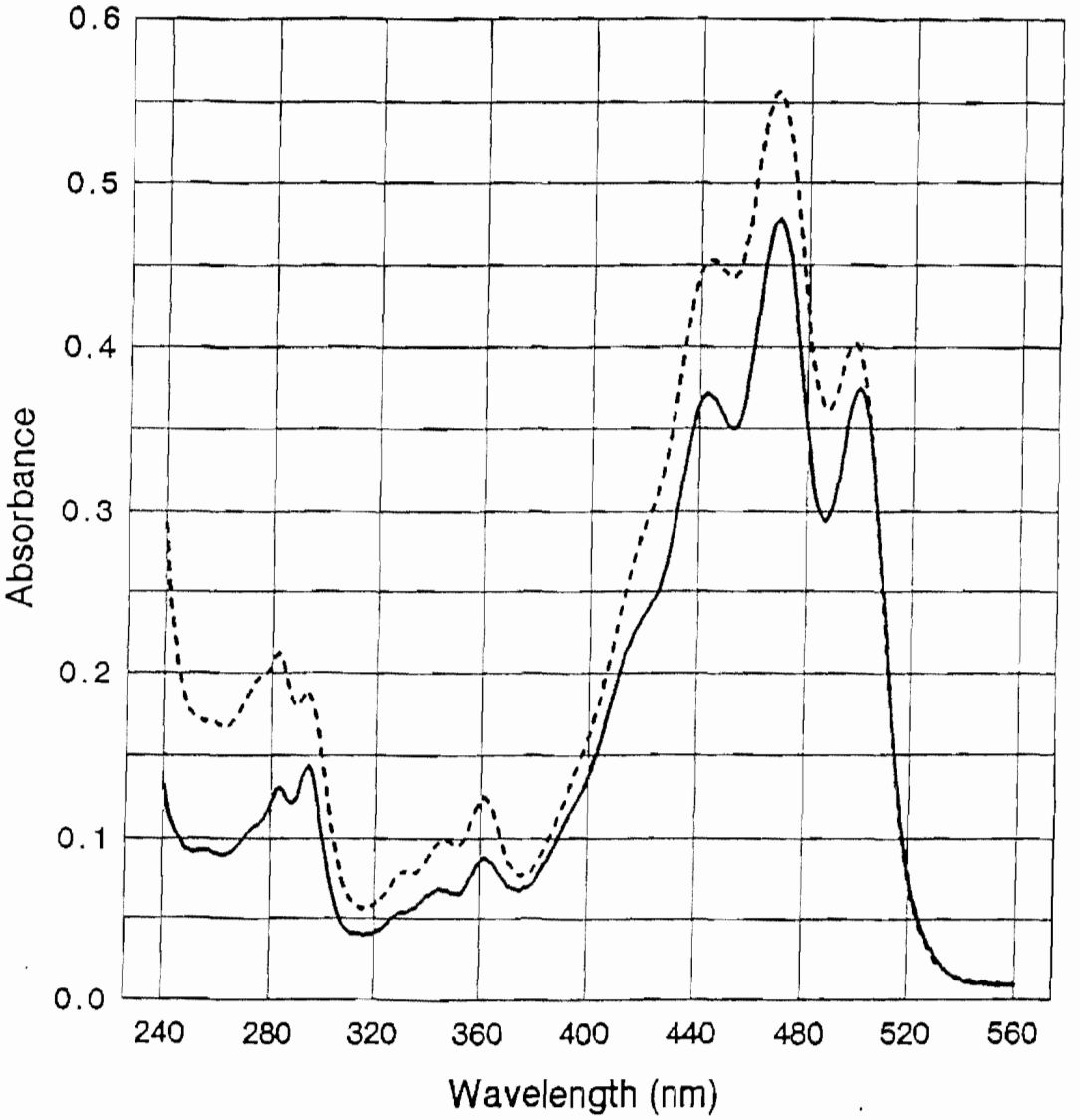


Figure 8. UV-vis absorption spectra of unsaponified (————) and saponified (--------) hexane extracts of tomato.

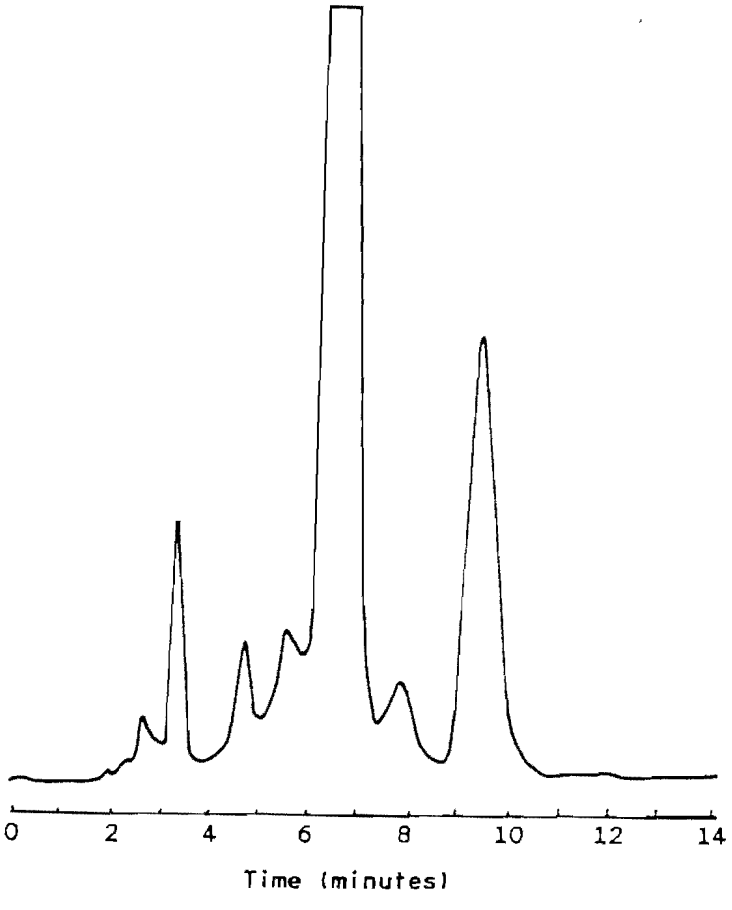


Figure 9. HPLC chromatogram of unsaponified hexane extract of tomato. Chromatogram for saponified extract is similar, except that peak at 7.4 minutes is reduced.

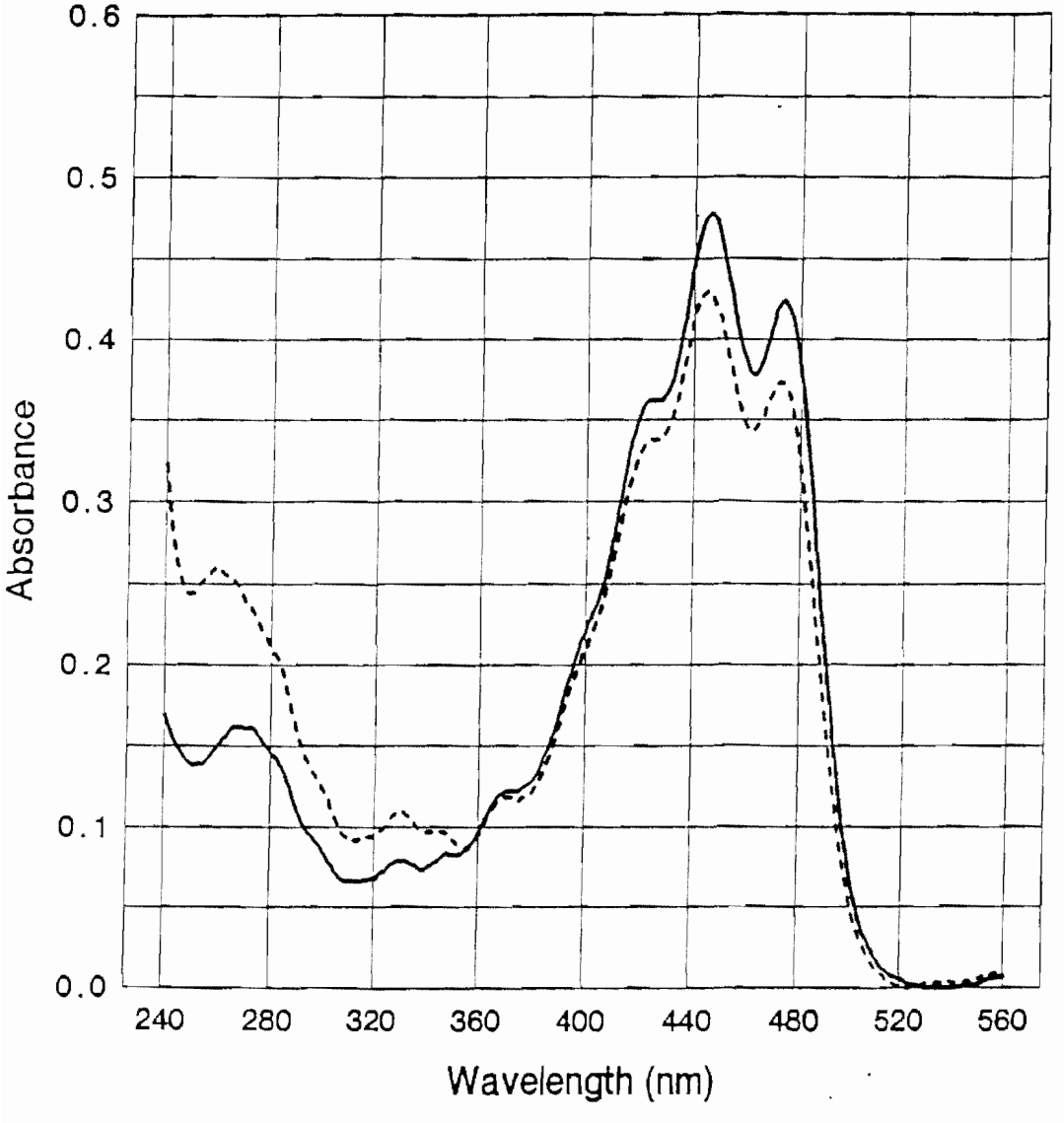


Figure 10. UV-vis absorption spectra of unsaponified (————) and saponified (-------) hexane extracts of carrot.

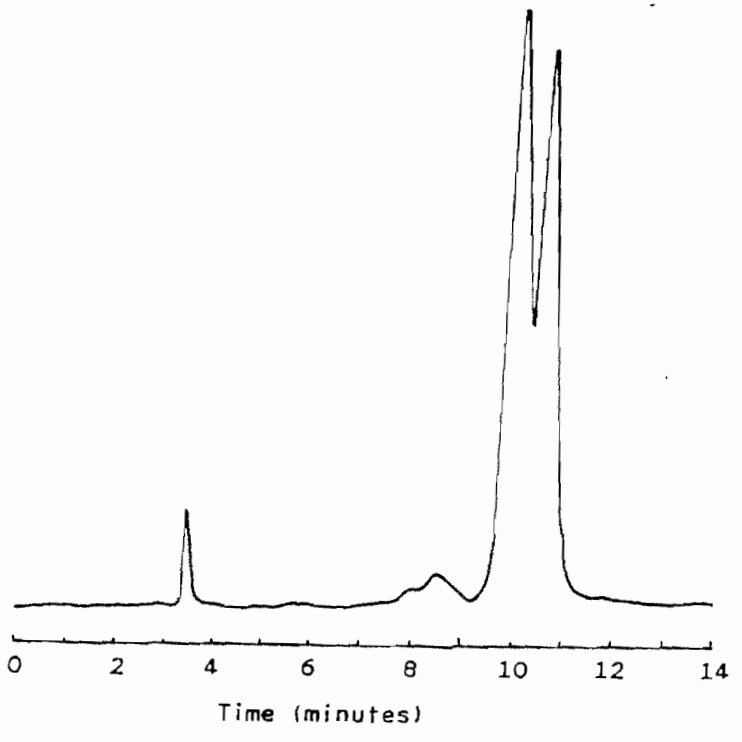


Figure 11. HPLC chromatogram of unsaponified hexane extract of carrot. Chromatogram for saponified extract is similar.

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