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Note

Rapid extraction and determination of α- and β-carotenes in foods

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Approximately half of human dietary vitamin A intake is derived from red, yellow and green fruits and vegetables containing α-, β- and γ-carotenes. Thin-layer and column chromatography have been used to separate biologically potent carotenoids, but these separations lacked sensitivity and reproducibility and required long periods of elution and recalibration. Recently, high-performance liquid chromatography (HPLC) has provided sensitivity and reproducibility in separating various carotenoids[1–3] but sample preparations before HPLC analysis are normally time consuming and complicated. We report here a simple, modified extraction procedure for the determination of β-carotene in fruits and vegetables using HPLC.

EXPERIMENTAL

Apparatus

A Waters Model 6000A solvent delivery system (Waters Assoc., Milford, MA, U.S.A.) equipped with a Valco simple injection valve (Model CV-6UHPa-N60) was used. The column effluents were monitored by a UV–visible detector (Waters Model No. 440) operated at 436 nm for α- and β-carotenes. The chromatographic peaks were recorded on an Omniscribe recorder.

Column. Stainless-steel (30 cm × 3.9 mm I.D.) μBondapak C_{18} (10-μm particle size) (Waters Assoc.). A protective pre-column C_{18}/Corasil; Waters Assoc.) was attached to the front of the C_{18} chromatographic column to protect it from interfering substances. Food samples often require frequent changes of pre-column packing material or frequent flushing of the pre-column with methanol. Need for replacement of the pre-column was indicated by an increase in column pressure.

Reagents and materials

α- and β-carotenes (Sigma, St. Louis, MO, U.S.A.) were used without further purification. HPLC-grade solvents [methylene chloride, acetonitrile and chloroform (Fisher Scientific, Pittsburgh, PA, U.S.A.)] were filtered through a 0.5-μm filter.

Fruits and vegetables were purchased from local supermarkets in large

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amounts and stored in a refrigerator at 4°C. Six replicate determinations were carried out at one time for each type of vegetable or fruit.

Mobile phase

The mobile phase used was a slight modification of that described previously[1]. Chloroform–acetonitrile (8:92) was used as the mobile phase for the C_{18} reversed-phase column. The flow-rate was maintained at 1.0 ml/min.

Sample extraction

Fruits or vegetables were homogenized in a blender or food processor. Samples (2–5 g) were immediately weighed into 50-ml centrifuge tubes and extracted with 25 ml of acetone–light petroleum (b.p. 35–60°C) (50:50) by vigorous shaking or vortexing. The two layers were allowed to separate and the top layer was transferred into a 500-ml round-bottomed flask using a pasteur pipette. The bottom layer was re-extracted several times with acetone–light petroleum until the top layer became colorless. The number of re-extractions of the bottom layer varied with the type of fruit and vegetable. It was essential that all carotenoid materials be extracted and that the top layer became completely colorless. The top layers collected in the 500-ml round-bottomed flask were evaporated to dryness at 30°C. The vacuum was broken with nitrogen and the flask was immediately stoppered. The residue was dissolved in light petroleum and filtered through a Fluoropore filter (Millipore, Bedford, MA, U.S.A.). The sample was diluted with elution buffer (chloroform–acetonitrile, 8:92), before injection into the HPLC column.

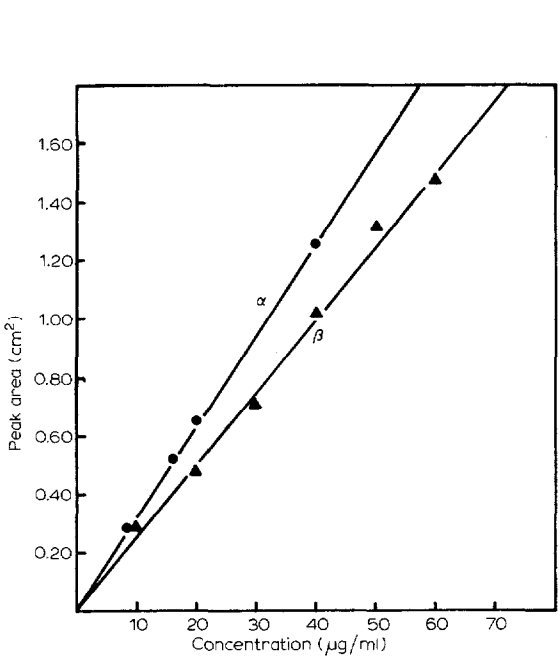
RESULTS AND DISCUSSION

As HPLC is a very sensitive detection method, only a small amount of sample was needed for analysis (1 g or less). We simplified the extraction method by using 50-ml centrifuge tubes in which samples can be extracted without additional transferring of the contents. Further, the headspace in the centrifuge tube is very small, which limits the volume of oxygen present and thus minimizes oxidation of carotenes during extraction. The cap must be tightly sealed and pressure released periodically while shaking or vortexing the tube.

Fig. 1 shows the calibration graph for α- and β-carotene standards. The peak area is directly proportional to the concentration in the range examined. The chromatographic separation of α- and β-carotenes in carrots is shown in Fig. 2.

The quantification of α- and β-carotenes in five batches of carrots is shown in Table I. The standard deviations for α- and β-carotene are 3.0 and 4.2%, respectively. The concentrations of α- and β-carotene in various fruits and vegetables are shown in Table II. Carrots and butternut squash contained both α and β-carotenes, whereas tomato and sweet potatoes only have β-carotene. The α- and β-carotene levels of many of the fresh fruits and vegetables are dependent on the season, variety and storage time and cannot be directly compared with other published values. However, most foods were within the range of published values.

Dried vegetables such as sweet potato powder, which contains only β-carotene and a few interfering substances, could be extracted with hexane and measured spectrophotometrically at 440 nm. No difference in absorbance was observed between



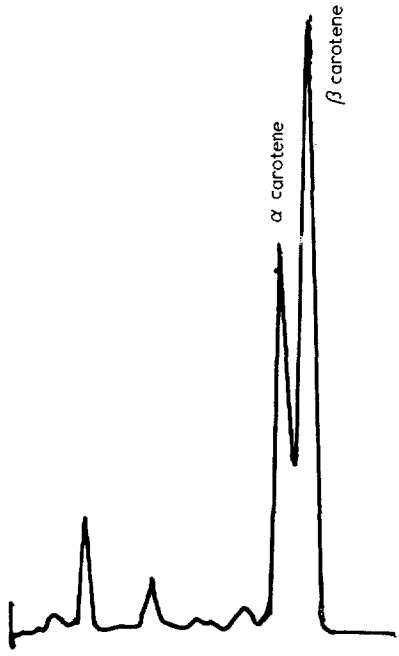


Fig. 1. Calibration graphs for α- and β-carotene.

Fig. 2. Chromatographic separation of α- and β-carotene in carrots.

the two methods of extraction. α-Carotene was not found in cantaloupe or in dried apricots. Recovery studies were made on β-carotene added to tomatoes and to dried apricots. The recoveries were 91 and 99%, respectively.

In conclusion, a rapid procedure has been developed for the extraction of α- and β-carotenes from fruits and vegetables. α- and β-carotenes can be determined in several fruits and vegetables using a reversed-phase column with chloroform–acetonitrile as the mobile phase. The recovery of β-carotene in various food products ranged between 91.0 and 99%.

TABLE I

QUANTITATION OF α- AND β-CAROTENE IN CARROTS

No. of batch of carrots	*α-Carotene (μg/g)*	*β-Carotene (μg/g)*
1	201.5	432.3
2	201.5	438.9
3	212.8	415.6
4	212.8	399.0
5	212.8	402.3
Mean	208.3	417.6
Standard deviation	6.2	17.7
Standard deviation (%)	3.0	4.2

TABLE II

QUANTITATION OF α- AND β-CAROTENE IN FRUITS AND VEGETABLES

Material	*α-Carotene* (g/g)*	*β-Carotene* (g/g)*	*Published value (g/g)*
Carrots	207.2 ± 6.5	421.4 ± 17.9	146.5 (α) 324.3 (β)
Tomatoes	–	9.1 ± 0.1	5.4–6.6
Butternut squash	19.5 ± 1.06	25.5 ± 4.2	29.7 (total α + β)
Sweet potatoes		61.8 ± 2.3	46.2–48.6
Cantaloupe	–	52.8 ± 3.8	20.4
Dried apricots	–	55.1 ± 0.8	44.5–65.4

* Average for five samples. Results ± standard deviation.

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