Solid-Phase Microextraction Coupled with Liquid Chromatography for Determination of β-Carotene in Food

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Abstract

β-Carotene in vegetables and nutritional products is analyzed using solid-phase microextraction (SPME) coupled with liquid chromatography (LC) to improve the speed of analysis and to reduce the consumption of organic solvents. The relative standard deviations (RSDs) of this analytical method for β-carotene determinations in vegetables and nutritional products are approximately 10% and 5%, respectively. The amount of β-carotene was found to vary from 0.35 ± 0.05 ppm to 76.5 ± 6.9 ppm for several vegetables in Taiwan. This method was linear over the range of 0.4–40 ppm with correlation coefficients higher than 0.997. The experimentally determined level of β-carotene in nutritional products varied from 3.8 ± 0.2 ppm to 24.6 ± 1.1 ppm following SPME–LC. The recoveries of β-carotene for these measurements following SPME were all higher than 97% ± 2% (*n* = 3). The detection limits of β-carotene for this method were from 0.027 to 0.054 ppm. Conventional solvent extractions take approximately 4–6 h for extraction and reconcentration but SPME takes approximately 1 h. From several tens to hundreds of milliliters, organic solvents can be saved using SPME. SPME provides better analyses on β-carotene than conventional solvent extraction for nutritional products in terms of speed, precision, simplicity, and solvent consumption.

Introduction

Carotenoids are very important nutrients for our bodies (1–12). The major sources of carotenoids for humans in developed countries are vegetables and fruits. There have been many reports on the intake of carotenoids-rich food inversely related to the risk of cancers and cardiovascular diseases (2–4). The main attractive factors of carotenoids for health benefits are the provitamin A and antioxidant capabilities. There are many nutritious products related with carotenoids advertised for potential health benefits. Carotenes are carotenoids composed of hydrocarbons. β-Carotene is the most predominant and active carotenoid in plant food for vitamin A.

There is considerable research being done on β-carotene because of its importance to life (5–12). Carotenes have also been reported to be associated with harmful events, such as at high doses for heavy smokers (13–14). Therefore, quantitative determination of β-carotene in food is important for the evaluation of the nutritional values, quality of fresh and processed products, and health benefits to humans. Extraction of β-carotene from vegetable samples generally takes several hours and uses several tens to hundreds of milliliters of organic solvents (7–8). The solvents commonly used for extraction are acetone, methanol, ethanol, hexane, tetrahydrofuran, dichloromethane, petroleum ether, and their mixtures. Alternative methods are needed to improve the extraction.

Solid-phase microextraction (SPME) has become a very useful method for sample pretreatment in recent years (15–18). There are more applications using the SPME coupled with gas chromatography (GC) and GC–mass spectrometry (MS) than those coupled with liquid chromatography (LC) and LC–MS in the literature because of the considerations of speed, simplicity, and sensitivity. The interface of SPME–GC is much simpler than that of SPME–LC. The interface of SPME–LC consists of a six-port injection valve and a desorption chamber that replaces the injection loop in the high-performance liquid chromatograph (HPLC). SPME coupled with GC and GC–MS is very popular and routinely used for many analytical applications. LC is still the analytical technique of choice for those nonvolatile and thermolabile samples. Further study of SPME–LC on those nonvolatile and thermolabile samples deserves further attention. SPME coupled with LC is used for β-carotene analysis in vegetables and nutritional products to improve the speed of analysis and to reduce the consumption of organic solvents.

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Experimental

Chemicals

The acetone, methanol, acetonitrile, and tetrahydrofuran (THF) were HPLC grade from Tedia (Fairfield, OH). The ethyl-β-apo-8'-carotenoate was from Fluka Chemie (Buchs, Switzerland). The β-carotene (99%) standard was from Tokyo Kasei (Tokyo, Japan). β-Carotenes were stored in dark brown glass containers wrapped with aluminum foil and handled under a yellow light to minimize exposure of these compounds to the light throughout experiments. Stock solutions of β-carotene standards were used as prepared or stored in methanol after N_2 degas at 4°C within 5 days of preparation.

Samples and preparation procedures

Nutritional products for a baby, pregnant woman, and senior citizen were manufactured from abroad. The vegetables used were carrot, spinach, and green cabbage. The juices used were mixed vegetable juices. The vegetables and juices were purchased from local markets of Taichung in Taiwan. Vegetables were cut into small pieces and sampled after cleaning. A 30-g sample of vegetables slices was thoroughly blended with 100 mL of methanol in a food processor. The vegetable muds were filtered with suction and washed three times with 5-mL aliquots of methanol. A 5-mL amount of filtrate was diluted to 10 mL with methanol after adding 0.6 mL of 100 ppm ethyl-β-apo-8'-carotenoate (internal standard). Vegetable juices were filtered before use. A nutrient tablet was ground into powder, sonicated with 100 mL methanol for 5 min, and filtered before use. A 5-mL amount of filtrate was diluted to 10 mL with methanol after adding internal standard, as treated with vegetable samples.

SPME procedures

Manual SPME holders were used with 50 µm carbowax (CW)–templated resin (TPR), 60 µm poly(dimethylsiloxane) (PDMS)–divinyl benzene (DVB), and 85 µm poly(acrylate) (PA) fiber assembly (Supelco, Bellefonte, PA). The fibers were conditioned as recommended by the manufacturer before use. All fibers were cleaned and conditioned with mobile phase until a stable baseline was reached before each new SPME experiment. A 6.0-mL sample was loaded into an 8.0-mL vial with a Teflon septum cap for sampling under room temperature at 24°C ± 1°C. The SPME fiber was put into a vial under the sample solutions with stirring at 500 rpm during each extraction, except for the study of stirring optimization. The fiber absorption time was 30 min, except for its optimal study. Immediately after the SPME extraction, the SPME fiber was inserted into the desorption chamber for 4 min, mobile phase was eluted at a flow rate of 0.1 mL/min for 2 min, and the flow rate was then increased to 1.0 mL/min.

The parameters optimized were fiber type, absorption time, desorption time, solution pH, solution temperature, organic solvent, and salt effect. Three different fibers coated with 50 µm CW–TPR, 60 µm PDMS–DVB, and 85 µm PA were tested using spiked 10- and 500-ppm β-carotene standards with 30 min of absorption time, 5 min of static desorption time, and 500 rpm of stirring rate for fiber type optimization, respectively. The PDMS–DVB fiber was used for the rest of the experiments after fiber optimization. The absorption time optimization was studied using 10 ppm of β-carotene standards with 500 rpm of stirring rate and 5 min of desorption time at different (1, 5, 10, 30, 60, and 90 min) absorption times. The 30 min of absorption time and 500-rpm stirring rate were used for the rest of the experiments after absorption time optimization. The desorption time optimization was studied from 0.5 to 5 min with 0.5-min increments using saturated β-carotene standards. Experimental parameters including 10 ppm of β-carotene standards, 30 min of absorption time, and a 500-rpm stirring rate were used to study solution pH optimization (pH = 3, 5, 7, and 9), solution temperature optimization (4°C, 25°C, and 35°C), effect of organic solvent addition [1%, 5%, 10%, and 20% (v/v) of acetonitrile], effect of salt addition [2%, 4%, 8%, and 16% (w/v) of NaCl], and storage stability (1, 3, 5, 7, and 11 days at 4°C). The recoveries were calculated from the detected β-carotene standards divided by the spiked β-carotene standards.

Calibration and standard addition methods were used for β-carotene analysis in food. Standards of fixed concentrations of different volumes (0.3, 0.6, 0.9, 1.2, and 1.5 mL) were added to five flasks containing 5 mL of sample solutions in each flask, respectively. Each flask was diluted to 10 mL with methanol and mixed well after adding 0.6 mL of 100-ppm internal standard. The slopes and intercepts of the linear equations for calibration measurements with peak area ratios of β-carotene standard to internal standard were obtained for calculation of β-carotene concentrations in the sample. Fibers were cleaned three times with 0.5 mL of the desorption solvent (CH_3OH–THF, 75:25, v/v) in the desorption chamber for 1 min and reconditioned before the new experiments.

LC analyses

A Vydac 201TP54 (Hesperia, CA) column (250 × 4.6 mm, 5 µm) was used. All LC analyses were carried out on a PU-1580 pump (Jasco, Tokyo, Japan) with a UV–vis detector (UV-975, Jasco). The wavelength for the detection was 450 nm. The LC flow rate was set at 1 mL/min except for SPME desorption periods. The mobile phase was a mixture of methanol–acetonitrile–tetrahydrofuran (57:42:1, v/v) and was degassed with N_2 gas before use. Data acquisition and processing were carried out using a chromatography data system from Scientific Information Service, (Taipei, Taiwan).

The quantity of β-carotene was calculated from the 8-point calibration curve covering the range from 1.0, 2.0, 4.0, 6.0, 8.0, 10, 20, to 40 mg/L, each divided by the 6 mg/L of internal standard. The limit of detection was calculated at a signal-to-noise ratio of 3:1 based on the calibration curves of the standard solutions. International unit (IU), retinol equivalent (RE), and the amount of β-carotene were calculated interchangeably as follows: 1 RE = 10 IU = 6 µg β-carotene.

Results and Discussion

Optimization of SPME experimental conditions

Three different SPME fibers coated with 50 µm CW–TPR, 60 µm PDMS–DVB, and 85 µm PA were tested for extraction with

spiked 10 and 200 ppm of β-carotene standards. The LC peak response areas on 10 and 200 ppm of standards from the 60-µm PDMS–DVB fibers were at least 20% higher than the other coated fibers, thus they were used for the rest of the experiments. The optimal fiber extraction time was determined to be 30 min based on LC peak response areas of standards with stirring rate at 500 rpm (as shown in Figure 1). The optimal fiber extraction time was 45 min for no stirring solution. The optimal stirring rate was determined to be 500 rpm based on LC peak response area with fiber extraction time fixed at 30 min. Fiber extraction with a stirring rate at 500 rpm for 30 min was used for the rest of the experiments. The optimal fiber desorption time was determined to be 4 min based on the equilibrium approach of LC peak responses using the residual test of fiber for saturated β-carotene standards.

The effect of pH (3–9) on the sample solutions was investigated. Figure 2 demonstrates the peak area of β-carotene obtained when the pH of sample solutions varied from 3 to 9. The best peak area of β-carotene was obtained when sample solutions were at pH 5 and 7. The solution pH was held at 7 for the rest of the experiments. The effect of solution temperature on β-carotene determination was also studied using SPME–LC. The relative peak areas of 10-ppm β-carotene standards increased two and three times as temperature increased from 4°C to 25°C and from 4°C to 35°C, respectively. The extracted β-carotene increased as solution temperature increased. It was reported that β-carotene might have degradation with temperatures higher than 35°C (1). The extraction temperature was fixed at 24°C ± 1°C for the rest of the experiments.

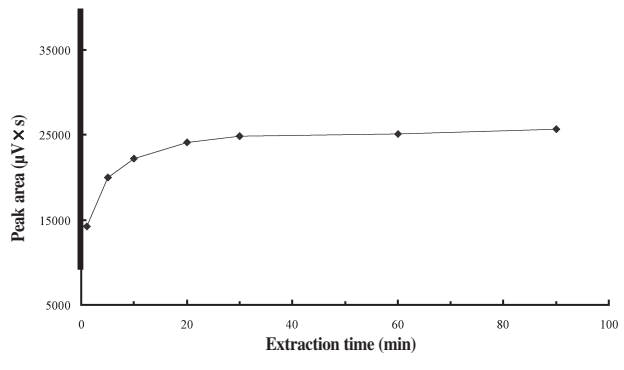

Figure 1. Plot of fiber exposition time versus detector response for 10 ppm β-carotene standard using SPME–LC. The wavelength for the detection was 450 nm.

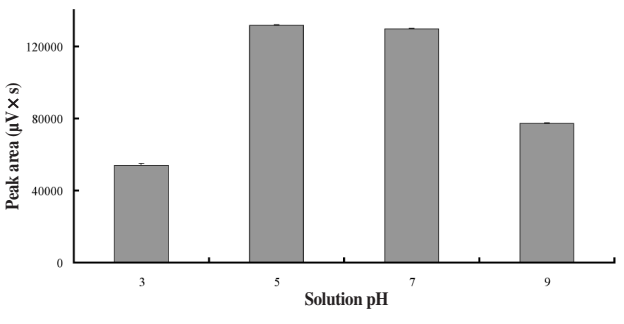

Figure 2. Solution pH effect of 10 ppm β-carotene standard using SPME–LC. The wavelength for the detection was 450 nm and the stirring rate was 500 rpm.

The effect of organic solvent addition on the sample solutions was also studied. The organic solvent studied was acetonitrile. The preconcentrated β-carotenes obtained from SPME–LC varied within 5% after adding 1%, 5%, 10%, and 20% (v/v) of acetonitrile. No organic solvent was added in this SPME study because the added organic solvents did not show a clear increase in LC response of β-carotene. The effect of salt addition on β-carotene solutions was also studied with sodium chloride solution from 2% to 16% with increments of two. The peak areas of detector response from SPME–LC changed within 6% variations after adding different concentrations of sodium chloride solution. No salt was added for this SPME–LC study.

The stability of β-carotene was important for quantitative analysis. β-Carotene was reported to be relatively sensitive toward light, heat, and oxygen (1). β-Carotene was handled carefully on these sensitive factors in all experiments to avoid or minimize degradation. The stability of β-carotene solutions was studied for different storage times at 4°C (as shown in Figure 3). The relative percentage of β-carotene concentration decreased approximately 5% after a 5-day storage based on the SPME–LC analysis. Therefore, all β-carotene solutions were used as freshly prepared as possible and discarded after a 5-day storage.

Determination of β-carotene from vegetables

Several domestic vegetables were studied for β-carotene using SPME–LC. The vegetables used for the study were carrot, spinach, and green cabbage. Carrot and spinach were used as representative samples of root and leafy vegetables. Various amounts of β-carotene were detected in these vegetables grown in Taiwan (as shown in Table I). The SPME–LC analyses were generally consistent within each type of vegetables at 10% variation ranges. The correlation coefficients of calibration from SPME were all larger than or equal to 0.997. The amount of β-carotene in carrots was found to be approximately 76.5 ± 6.9 ppm. The amount of β-carotene in spinach was found to be approximately 37.2 ± 3.9 ppm. Green cabbage was found to contain around 0.35 ± 0.05 ppm of β-carotene. The amount of β-carotene found in these vegetables differed from the literature by 10%. Recoveries of β-carotene from the SPME–LC in all vegetables were all higher than 95%. The detection limits of β-carotene using the SPME–LC were from 0.027 to 0.54 ppm.

A typical SPME–LC chromatogram of spinach vegetables for

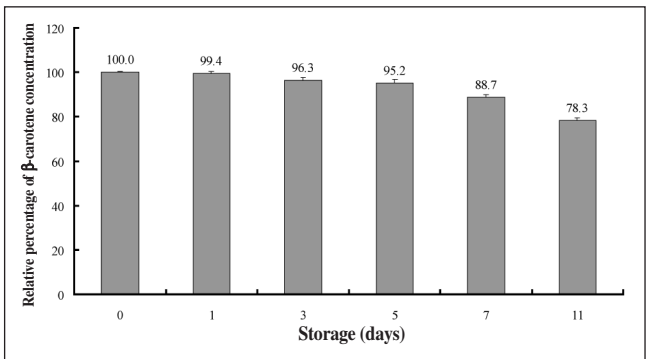

Figure 3. Stability of β-carotene solution for storage at 4°C using SPME–LC. The concentration of β-carotene standard in methanol was 10 ppm. The wavelength for the detection was 450 nm, and the stirring rate was 500 rpm.

β-carotene determination is shown in Figure 4. This is commonly observed from vegetable extracts. The precision of SPME–LC for β-carotene determination from vegetables was comparable to the precision (~ 8%) of traditional solvent extraction (7). The main advantages of SPME–LC versus traditional solvent extraction for β-carotene determination were saving several tens to hundreds of milliliters of organic solvents and reducing the extraction time by three fourths. Traditional solvent extraction took approximately 4–6 h, but SPME took approximately 1 h. The amount of β-carotene in vegetable juices was also studied (as shown in Table I). The precision of β-carotene measurement using the SPME–LC from vegetable juice was approximately two times higher than those from vegetables.

Table I. Summary of Experimentally Determined β-Carotene in Vegetables and Nutritional Products

Samples	β-Carotene (ppm) mean ± SD (RSD %)	Limit of detection (ppm)	Recovery (%) (*n* = 3)	Literature (ppm)
Cabbage green	0.45 ± 0.04 (8.9)	0.041	97 ± 2	0.51*
Spinach	37.2 ± 3.9 (10.5)	0.050	97 ± 1	34.0*
Carrot	76.5 ± 6.9 (9.0)	0.035	98 ± 2	85.2*
Vegetable juice	4.0 ± 0.2 (5.0)	0.027	98 ± 1	3.6†
Nutrients for Pregnant woman	3.83 ± 0.19 (4.6)	0.048	98 ± 2	4.5†
Nutrients for baby	9.45 ± 0.38 (4.1)	0.054	97 ± 2	10.42†
Nutrients for senior citizen	24.6 ± 1.1 (4.5)	0.045	98 ± 2	27†

* See literature (7).
† Manufacturer specification.

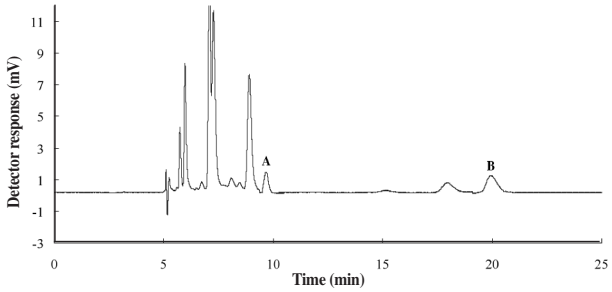

Figure 4. A typical SPME–LC chromatogram of spinach vegetables. The carrier flow rate was 0.1 mL/min, elution for 2 min, then raised to 1.0 mL/min. Ethyl-β-apo-8'-carotenoate (internal standard) (A) and β-carotene (B).

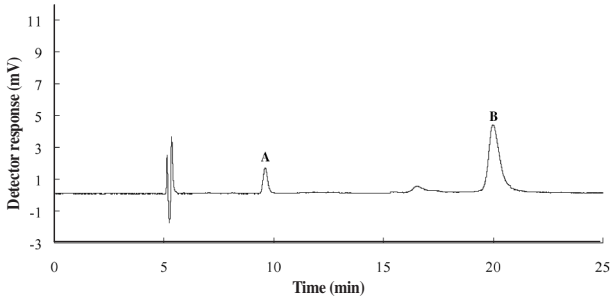

Figure 5. A typical SPME–LC chromatogram from nutritional products for a baby. The carrier flow rate was 0.1 mL/min, elution for 2 min, then raised to 1.0 mL/min. Ethyl-β-apo-8'-carotenoate (internal standard) (A) and β-carotene (B).

Determination of β-carotene from nutritional products

The optimal extraction conditions of vegetables were used for nutritional product study. Three samples of each nutritional product were examined for β-carotene using SPME–LC (as shown in Table I). The precisions of SPME were improved approximately 4% than those of solvent extractions. The amount of β-carotenes found in nutritional products differed by 8.9–14.8% from manufacturer specification. A typical SPME–LC chromatogram from nutritional products for a baby is shown in Figure 5. This is commonly observed from nutritional extracts. The advantages of SPME for β-carotene analysis in nutritional products versus traditional solvent extraction techniques are as follows: SPME has at least three times shorter sample pretreatment time and two times better precision. The recovery and detection limit of SPME–LC were comparable to those of solvent extractions.

Conclusion

The SPME–LC method shows great promise for simple and fast β-carotene analysis in vegetables and nutritional products with this preliminary study. SPME–LC can reduce organic solvent consumption and environmental pollution. SPME provides better analyses of β-carotene in nutritional products than conventional solvent extraction pretreatments in term of simplicity, speed, precision, and solvent consumption.

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References

1. G. Britton, S. Liaaen-Jensen, H. Pfander, Eds. *Carotenoids-Handbook*. Birhauser, Verlag, Springer, New York, NY, 2004.
2. N.I. Krinsky and S.T. Mayne, Eds. *Carotenoids in Health and Disease*. Marcel Dekker, New York, NY, 2004.
3. L. Packer, U. Obermueller-Jevic, K. Kramer, and H. Sies, Eds. *Carotenoids and Retinoids: Molecular Aspects and Health Issues*. AOCS Press, Champaign, IL, 2005.
4. S.B. Kritchevsky. (Beta-carotene), carotenoids, and the prevention of coronary heart disease. *J. Nutr.* **129:** 5–8 (1999).
5. B.T. Burri, T.R. Neidlinger, A.O. Lo, C. Kwan, and M.R. Wong. Supercritical fluid extraction and reversed-phase liquid chromatography methods for vitamin A and β-carotene heterogeneous distribution of vitamin A in the liver. *J. Chromatogr. A* **762:** 201–206 (1997).
6. P. Astorg. Food carotenoids and cancer prevention: An overview of current research. *Trends Food Sci. Technol.* **8:** 406–13 (1997).
7. D.J. Hart and K.J. Scott. Development and evaluation of an HPLC method for the analysis of carotenoids in food, and the measurement of carotenoid content of vegetables and fruits commonly consumed in UK. *Food Chem.* **54:** 101–11 (1995).
8. F. Khachik and G.R. Beecher. Separation, identification, and quan-

tification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. *J. Agric. Food. Chem.* **34:** L603–606 (1986).
9. B. Olmedilla, F. Granado, E. Rojas-Hidalgo, and I.A. Blanco. A rapid separation of ten carotenoids, three retinoids, alpha-tocopherol and δ-α-tocopherol acetate by HPLC and its application to serum and vegetable samples. *J. Liq. Chromatogr.* **13:** 1455–83 (1990).
10. G.T. Handleman, B. Shen, and N.I. Krinsky. High resolution analysis of carotenoids in human plasma by high performance liquid chromatography. *Methods Enzymol.* **213:** 336–46 (1992).
11. W.G. Rushin, G.L. Catignani, and S.J. Scwartz. Determination of β-carotene and its cis isomers in serum. *Clin. Chem.* **36:** 1986–89 (1990).
12. F. Khachik, G.R. Beecher, M.B. Goli, and W.R. Lusby. Separation, identification, and quantification of carotenoids in fruits, vegetables and human plasma by high performance liquid chromatography. *Pure Appl. Chem.* **63:** 71–80 (1991).
13. G.S. Omenn, G. Goodman, M. Thornquist, J. Grizzle, L. Rosenstock, and S. Barnhart. The beta-carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. *Cancer Res.* **54:** 2038s–43s (1994).
14. The alpha-tocopherol, beta-carotene cancer prevention study group. The effect of vitamin E and beta-carotene on the incident of lung cancer and other cancers in male smokers. *New Engl. J. Med.* **330:** 1080–1035 (1994).
15. H. Kataoka, H.L. Lord, and J. Pawliszyn. Applications of solid-phase microextraction in food analysis. *J. Chromatogr. A* **880:** 35–62 (2000).
16. Z. Zhang, M. Yang, and J. Pawliszyn. Solid phase microextraction. *Anal. Chem.* **66:** 844A–53A (1994).
17. J. Pawliszyn. *Applications of Solid Phase Microextraction.* Royal Society of Chemistry, Cambridge, U.K., 1999.
18. J. Pawliszyn. *Solid Phase Microextraction-Theory and Practice.* Wiley-VCH, New York, NY, 1997.

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