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Short communication

Application of analytical and preparative high-speed counter-current chromatography for separation of lycopene from crude extract of tomato paste

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Abstract

Lycopene was isolated from 100 mg of crude extract of tomato paste containing about 9% of lycopene. Analytical high-speed counter-current chromatography (HSCCC) was first used for the systematic selection of the two-phase solvent system. Then preparative HSCCC separation was performed with a nonaqueous solvent system composed of *n*-hexane–dichloromethane–acetonitrile at an optimum volume ratio of 10:3.5:6.5. This yielded 8.6 mg of lycopene at over 98.5% purity as determined by HPLC analysis.

Keywords: Tomato paste; Counter-current chromatography; Lycopene

1. Introduction

It has been shown that oxidative damage, either to DNA or membranes, might play a role in the development of prostate and other cancers [1,2]. Increased consumption of vegetables and fruits may be associated with a reduced risk of cancer, and research on possible anticarcinogenic compounds was often focused on carotenoids [3]. Many epidemiological and ontological studies suggest that carotenoid-rich vegetables and fruits help to maintain high levels of serum carotenoids and reduces the incidence of several types of cancer and cardiovascular diseases [4].

Recent research indicated that the anticarcinogenic effects of fruits and vegetables may be ascribed not only to beta-carotene, but also to other carotenoids such as lutein and lycopene [3]. Levy [5] reported that lycopene was far more efficient than either α- or β-carotene for inhibiting endometrial, mammary and lung human cancer cells. Lycopene significantly reduced the occurrence of spontaneous mammary tumors in mice fed with a lycopene-enriched diet [6]. In a large prospective cohort study, Giovannucci found that consumption of lycopene was inversely related to prostate malignancy. Apart from the cohort analysis by Giovannucci, several other epidemiological studies also suggest that lycopene could have a protective effect against prostate cancer as well as

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several cancers of the digestive tract [7–9]. Dietary consumption of the carotenoid lycopene (mostly from tomato products) has been associated with a lower risk of prostate cancer. Therefore increased consumption of tomato products and other lycopene-containing foods could reduce the occurrence or progression of prostate cancer.

High-speed counter-current chromatography (HSCCC), being a support-free liquid–liquid partition chromatographic technique, eliminates irreversible adsorption of the sample on to the solid support [10], and has been widely used for the preparative separation of natural products [11–15]. Isolation and purification of lycopene by column chromatography were previously reported [16,17]. The aim of the present study is to introduce an efficient HSCCC-method for purifying lycopene. Purification of lycopene by HSCCC has advantages over that by column chromatography in the following two aspects: The first is the maximum capacity in HSCCC which is much larger than that in HPLC with an excellent sample recovery. The second is that HSCCC permits introduction of a crude sample directly into the column.

The present paper describes the successful preparative separation and purification of lycopene, which is unstable in air and under light, from the crude extract of tomato paste by HSCCC.

2. Experimental

2.1. Apparatus

The analytical HSCCC instrument employed in the present study is a Model GS 20 analytical high-speed counter-current chromatograph designed and constructed in Beijing Institute of New Technology Application (Beijing, China). The apparatus holds a pair of column holders symmetrically on the rotary frame at a distance of 5 cm from the central axis of the centrifuge. The multilayer coil separation column was prepared by winding a 50 m long, 0.85 mm I.D. PTFE (polytetrafluoroethylene) tube directly on to the holder hub forming multiple coiled layers with a total capacity of 40 ml. The β-value varied from 0.4 at the internal terminal to 0.7 at the external terminal ($\beta = r/R$ where r is the distance from the coil to the holder shaft, and R, the revolution radius or the distance between the holder axis and central axis of the centrifuge). Although the revolution speed of the apparatus could be regulated with a speed controller in a range between 0 and 2000 rpm, an optimum speed of 1800 rpm was used in the present studies.

Preparative HSCCC was performed using a Model GS10A2 multilayer coil planet centrifuge (Beijing Institute of New Technology Application, Beijing, China) equipped with a PTFE multilayer coil of 110 m length and 1.6 mm I.D. with a total capacity of 230 ml. The β-value of the preparative column ranges from 0.5 to 0.8.

The solvent was pumped into the column with a Model NS-1007 constant-flow pump (Beijing Institute of New Technology Application, Beijing, China). Continuous monitoring of the effluent was achieved with a Model 8823A-UV Monitor (Beijing Institute of New Technology Application, Beijing, China) at 254 nm. A manual sample injection valve with a 1.0-ml loop (for the analytical HSCCC) or a 20-ml loop (for the preparative HSCCC) (Tianjin High-New Science & Technology Company, Tianjin, China) was used to introduce the sample into the column, respectively. A portable recorder (Yokogawa Model 3057, Sichuan Instrument Factory, Chongqing, China) was used to draw the chromatogram.

The high-performance liquid chromatography (HPLC) equipment used was a Shimadzu LC-10AVP system including two LC-10ATVP solvent delivery units, an SPD-M10AVP UV–Vis photodiode array detector, a Model 7726 injection valve with a 20-μl loop, an SCL-10AVP system controller, a CTO-10ASVP column oven, a DGU-12A degasser and a Class-VP-LC workstation (Shimadzu, Kyoto, Japan).

2.2. Reagents

All organic solvents used for HSCCC were of analytical grade and purchased from Beijing Chemical Factory, Beijing, China. Acetonitrile used for HPLC analysis was of chromatographic grade and purchased from Tianjin Huaxi Special Reagent Factory, Tianjin, China. Lycopene (90% standard) was purchased from Sigma (St Louis, MO).

2.3. Preparation of sample

A 2 g amount of crude extract of tomato paste (supplied from Kelong Company, China) was percolated five times each with 50 ml of chloroform/methanol (2:1) at room temperature, and then concentrated to dryness under a stream of nitrogen [18–20]. The residue (crude lycopene) consisted of 9.0% lycopene, which is quantitatively determined by HPLC.

2.4. Preparation of two-phase solvent system and sample solutions

The present study used a nonaqueous two-phase solvent system composed of *n*-hexane, dichloromethane and acetonitrile at a volume ratio of 10:3.5:6.5. The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature, and the two phases were separated shortly before use.

The sample solutions were prepared by dissolving the crude lycopene in the lower phase at suitable concentrations according to the analytical or the preparative purpose.

2.5. Separation procedure

Analytical HSCCC was performed with a Model GS 20 HSCCC instrument as follows: the multilayer coiled column was first entirely filled with the upper phase. The lower phase was then pumped into the head end of the inlet column at a flow-rate of 1.0 ml/min, while the apparatus was run at a revolution speed of 1800 rpm. After hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting at the tail outlet, the sample solution (15 mg in 1 ml of lower phase) was injected through the sample port. The effluent from the tail end of the column was continuously monitored with a UV detector at 254 nm. Each peak fraction was collected according to the chromatogram. The retention of the stationary phase relative to the total column capacity was computed from the volume of the stationary phase collected from the column after the separation was completed. Preparative HSCCC was similarly performed with a Model GS10A2 HSCCC instrument as follows: the multilayer coiled column was first entirely filled with the upper phase as stationary phase. Then the lower phase was pumped into the head end of the inlet column at a flow-rate of 2 ml/min, while the apparatus was rotated at 800 rpm. After hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting at the tail outlet, the sample solution (100 mg in 20 ml of lower phase) was injected through the injection valve. The effluent from the outlet of the column was continuously monitored with a UV detector at 254 nm. Each peak fraction was collected according to the chromatogram.

2.6. HPLC analyses and identification of CCC peak fractions

The crude extract of tomato paste, lycopene (standard) and each peak fraction from HSCCC were analyzed by HPLC. The analyses were performed with a Supelcosil ODS column (250×4.6 mm I.D.) at a column temperature of 30°C. The mobile phase composed of acetonitrile/methanol/chloroform (47:47:6, v/v/v) was isocratically eluted at a flow-rate of 1.0 ml/min and the effluent monitored at 472 and 254 nm by a PAD detector.

The retention time of each HSCCC peak fraction (lycopene) was compared with that of the pure

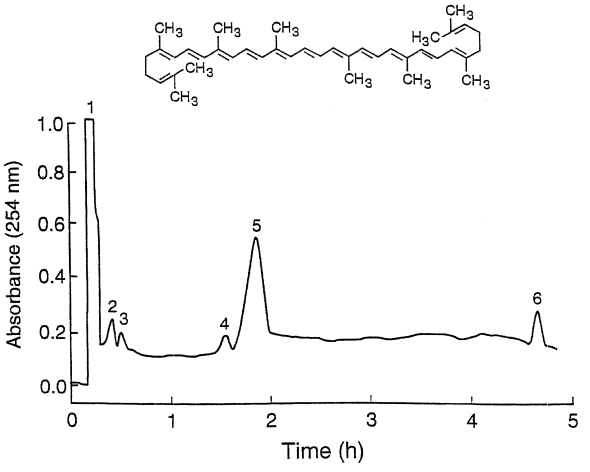

Fig. 1. Chromatogram of the crude lycopene by analytical HSCCC. Solvent system: *n*-hexane–dichloromethane–acetonitrile (10:3.5:6.5/v:v:v); stationary phase: upper phase; mobile phase: lower phase; flow-rate: 1.0 ml/min; revolution speed: 1800 rpm; sample: 15 mg dissolved in 1 ml lower phase; retention of the stationary phase: about 50%. Peak 5: lycopene.

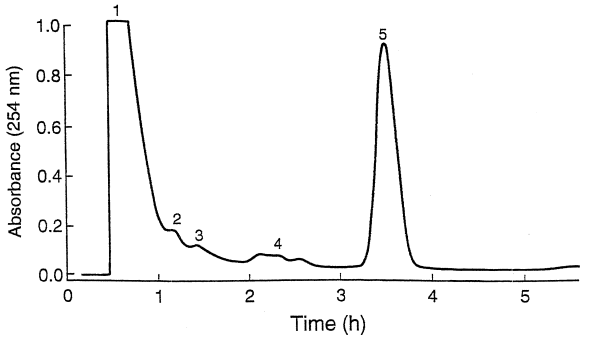

Fig. 2. Chromatogram of the crude lycopene by preparative HSCCC. Solvent system: *n*-hexane–dichloromethane–acetonitrile (10:3.5:6.5/v:v:v); stationary phase: upper phase; mobile phase: lower phase; flow-rate: 2.0 ml/min; revolution speed: 800 rpm; sample: 100 mg dissolved in 20 ml lower phase; retention of the stationary phase: 62.5%. Peak 5: lycopene.

standard and the final identification was performed by MS, ^{1}H-NMR and ^{13}C-NMR spectra.

3. Results and discussion

The crude extract of tomato paste was analyzed by HPLC (Fig. 3). The result indicated that the crude sample contained several compounds among which the lycopene represents 9.0% of the total.

In order to achieve an efficient resolution of the target compound, performance of the nonaqueous two-phase solvent system composed of *n*-hexane–dichloromethane–acetonitrile at various volume ratios was examined by analytical HSCCC. The results indicated that the volume ratio of 10:3.5:6.5 could isolate lycopene well (Fig. 1).

Fig. 2 shows the chromatogram obtained from 100 mg of the crude lycopene by preparative HSCCC. The fraction from peak 5 of this separation yielded 8.3 mg of lycopene at over 98% purity measured from HPLC peak areas and at 99.9% based on the external standard curve as determined by HPLC (Fig. 3). The structural identification of lycopene was carried out by MS, ^{1}H-NMR and ^{13}CNMR spectra as follows: ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 1.62 (3H, s, 17-CH_3), 1.69 (3H, s, 16-CH_3), 1.82 (3H, s, 18-CH_3), 1.97 (6H, s, 19, 20-CH_3), 2.11 (4H, m, 3, 4-CH_2), 5.11 (1H, m, 2-H), 5.94 (1H, d, 6-H), 6.17 (1H, d, 10-H), 6.25 (2H, d, 8-H, 14-H), 6.34 (1H, d, 12-H), 6.49 (1H, dd, 7-H), 6.62, 6.63 (2H, m, 11-H, 15-H). ^{13}C-NMR (500 MHz, $CDCl_3$) δ ppm: 139.51 (2C, C-5, C-5′), 137.35 (2C, C-12, C-12′), 136.55

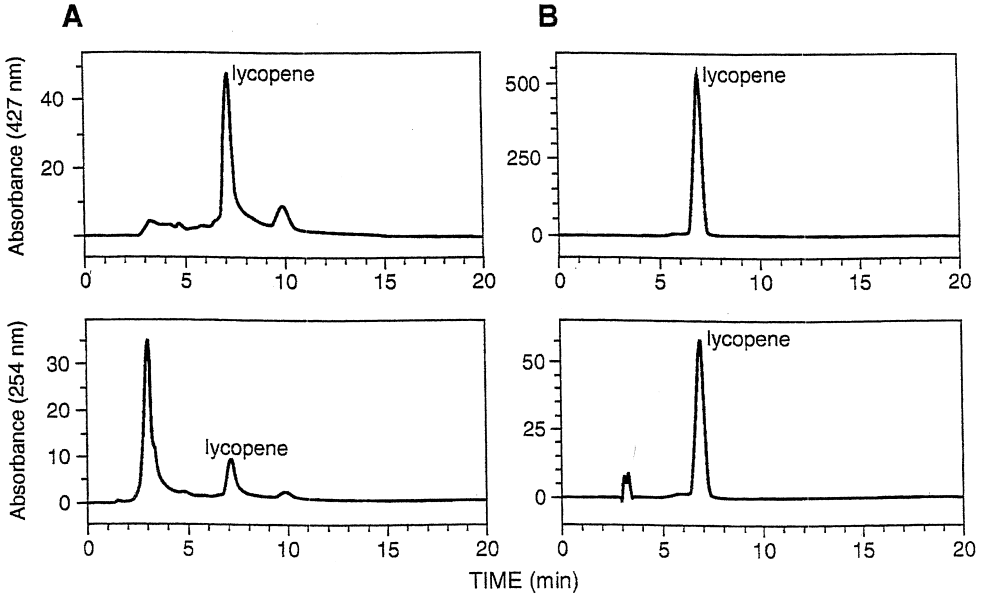

Fig. 3. The result of HPLC analysis of the crude lycopene and Peak 5 (lycopene) shown in Fig. 2. (A) The crude sample; (B) Peak 5; Column: Supelcosil ODS column (250 mm×4.6 mm I.D.); Column temperature: 30°C; Mobile phase: methanol/acetonitrile/chloroform (47:47:6, v/v/v); Flow-rate: 1.0 ml/min.

(2C, C-13, C-13′), 136.18 (2C, C-9, C-9′), 135.40 (2C, C-8, C-8′), 132.64 (2C, C-14, C-14′), 131.75 (2C, C-1, C-1′), 131.55 (2C, C-10, C-10′), 130.07 (2C, C-15, C-15′), 125.70 (2C, C-6, C-6′), 125.16 (2C, C-11, C-11′), 124.77 (2C, C-7, C-7′), 123.94 (2C, C-2, C-2′), 40.23 (2C, C-4, C-4′), 26.67 (2C, C-3, C-3′), 25.69 (2C, C-16, C-16′), 17.70 (2C, C-17, C-17′), 16.95 (2C, C-18, C-18′), 12.89 (2C, C-19, C-19′), 12.79 (2C, C-20, C-20′).

The results of the present study clearly demonstrated that HSCCC is very useful in the preparative separation of lycopene from the crude extract of tomato paste.

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