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Preparation of the monomers of gingerols and 6-shogaol by flash high speed counter-current chromatography

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ABSTRACT

The flash high speed counter-current chromatographic (FHSCCC) separation of gingerols and 6-shogaol was performed on a HSCCC instrument equipped with a 1200-ml column (5 mm tubing i.d.) at a flow rate of 25 ml/min. The performance met the FHSCCC feature that the flow rate of mobile phase (ml) is equal to or greater than the square of the diameter of the column tubing (mm). The separation employed the upper phase of stationary phase of the *n*-hexane–ethyl acetate–methanol–water (3:2:2:3, v/v) as the stationary phase. A stepwise elution was performed by eluting with the lower phase of *n*-hexane–ethyl acetate–methanol–water (3:2:2:3, v/v) for first 90 min and the lower phase of the *n*-hexane–ethyl acetate–methanol–water (3:2:6:5, v/v) for the second 90 min. In each separation 5 g of the ethyl acetate extract of rhizomes of ginger was loaded, yielding 1.96 g of 6-gingerol (98.3%), 0.33 g of 8-gingerol (97.8%), 0.64 g of 6-shogaol (98.8%) and 0.57 g of 10-gingerol (98.2%). The separation can be expected to scale up to industrial separation.

1. Introduction

The rhizomes of ginger, *Zingiber officinale* Roscoe, are used by consumers for relief of nausea [1] or inflammation associated with arthritis [2]. Recently, ginger has received extensive attention as a botanical dietary supplement in the USA and Europe because of its anti-oxidative, anti-inflammatory, and anti-tumor activities [3,4]. The gingerols, a series of homologues differentiated by the length of their unbranched alkyl chains, were identified as the major pungent components in the ginger oleoresin from fresh rhizome, of which 6-gingerol is the most abundant [1]. It has been reported that topical application of 6-gingerol onto the shaven backs of female ICR mice prior to each topical dose of 12-O-tetradecanoylphorbol-3-acetate (TPA) significantly inhibited 7,12-dimethylbenz [R] anthracene (DMBA)-induced skin tumor incidence and tumor burden [5]. The same researchers also found that 6-gingerol inhibited TPA-induced cyclooxygenase-2 (COX-2) expression in mouse skin *in vivo* by blocking the p38 MAP kinase-NF-κB signaling pathway [6]. 6-Gingerol was found to decrease the number of lung metastases in mice implanted with B16F10 melanoma cells [7]. It also was reported 6-gingerol effectively suppressed *in vivo* tumor growth in HCT-116 cancer cell-bearing nude mice [8]. 6-Shogaol is an anhydride of 6-gingerol. Recent studies have shown that it can significantly inhibit the growth of HL-60 human leukemia cells and human colon COLO-205 cells [9], A-549 human lung cancer cells, SK-OV-3 human ovarian cancer cells, SKMEL-2 human skin cancer cells, and HCT-15 human colon cancer cells [10], and induce apoptosis through modulation of mitochondrial functions regulated by reactive oxygen species [11]. Therefore, it is important to establish a quick preparative method providing gram-scale quantities of 6-gingerol and 6-shogaol for further studies of their biological activities. Separation by silica gel liquid chromatography could give gram-scale quantities of the monomers of gingerols including 6-gingerol and 6-shogaol, but the processing was tedious and solvent-consumed [12]. Countercurrent chromatography was used for the isolation of gingerols from ginger extracts [13,14], but the separation did not involve 6-shogaol. Therefore, a fast separation of gingerols and 6-shogaol to prepare the monomers of gingerols including 6-gingerol and 6-shogaol is still expected.

Flash high speed counter-current chromatography (FHSCCC) is defined as a separation process in which the flow rate of the mobile phase (ml/min) is equal to or greater than the square root of the square of the diameter of the column tubing (mm), i.e. $F_c/d^2 \geq 1$, here d and F_c are the inner diameter of the column tubing and the applied flow rate of mobile phase, respectively, suggested by Du [15]. Thereby, a FHSCCC separation will be finished in a time less than 4 h for target compound with a partition coefficient of 3 for all size columns with a tubing length of 130 m. Therefore, successful FHSCCC is possibly scaled-up for industrial HSCCC separation since the flow rate can be scaled up to the level of industrial separation.

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The present study describes the FHSCCC method for the preparation of the monomers of gingerols and 6-shogaol, which was rapid, simple and solvent-saved. The method could pilot the industrial preparation of 6-gingerol and 6-shogaol.

2. Experimental

2.1. Materials

All solvents for extraction and separation were of analytical grade, purchased from Hangzhou Huadong Chemicals Inc., China. The ginger rhizomes, belonging to *Z. officinale* Roscoe, were purchased at a local vegetable store in Hangzhou, China.

2.2. Extraction

The ginger rhizomes (50 kg) at a similar state of maturity were selected for extraction of gingerols and shogaols. The damp dry rhizomes were crushed, and then dried at 50–70 °C and powdered at room temperature. The powder material was extracted with 50 l of 95% ethanol at 50 °C for 3 h twice. The combined extracts were evaporated to a syrup, dissolved in 1500 ml water, and was partitioned with 2000 ml of ethyl acetate three times. The ethyl acetate solution was evaporated to dryness resulting in 457 g of crude extract which was subjected to FHSCCC separations.

2.3. FHSCCC Separation

2.3.1. Selection of solvent system

Selection of solvent system started from tests of partition coefficient of 6-gingerol and 6-shogaol in a series of solvent systems composed of *n*-hexane–ethyl acetate–methanol–water. The selected system was checked for the availability of FHSCCC on an analytical HSCCC instrument (tubing bore: 0.8 mm), i.e. whether or not the flow rate of the separation could reach more than 0.64 ml/min which met the condition of $F_c/d^2 \geq 1$. The system with good retention of stationary phase and excellent resolution for the gingerols and 6-shogaol was selected for preparative FHSCCC separation with gram-scale.

2.3.2. Apparatus

The analytical and preparative HSCCC columns used in the present study were constructed at the Institute of Food and Biological Engineering, Zhejiang Gongshang University (Hangzhou, China). The analytical apparatus was equipped with a 4-layer coil column of 15.5 ml capacity which made by wounding 30 m of 0.8 mm bore polytetrafluoroethylene (PTFE) tubing on a 7.0 cm O.D. holder. The extra coil volume was 0.5 ml and β ranges 0.71–0.79 with a rotor radius (R = 5 cm). The preparative one equipped with a 6-layer coil column of 1200 ml capacity which was made by wounding 61 m of 5.0 mm bore PTFE tubing on a 17.0 cm O.D. holder. The β ranges 0.59–0.78 with a rotor radius (R = 14 cm). A Waters 510 pump (Waters, USA), a Rheodyne 7725 injection valve and an Elite UV-220 detector (Elite, Dalian, China) were used for analytical separations. A K-1800 Wellchrom pump (Knauer, Germany), a 100 ml sample loop made of 3 mm i.d. PTFE tubing, an Elite UV-200 detector (Elite, Dalian, China) and a B-684 collector (Büchi, Switzerland) were used for preparative separation.

2.3.3. Separation procedure

The separation of gingerols and 6-shogaol was performed by a stepwise elution mode with the lower phase of *n*-hexane–ethyl acetate–methanol–water (3:2:2:3, v/v) for the first stage and eluted with the lower phase of the *n*-hexane–ethyl acetate–methanol–water (3:2:6:5, v/v) for the second stage. The stationary phase in the whole separation was the upper phase of the *n*-hexane–ethyl acetate–methanol–water (3:2:2:3, v/v). For each separation, the HSCCC column was entirely filled by the stationary phase first. Subsequently, the sample solution (5%, w/v) which was prepared by dissolving the ethyl acetate extract in the initial mobile phase, was loaded to the sample loop. Then, the apparatus was rotated at a given rotation speed and the sample solution was injected into the HSCCC system through the sample loop by the push of the mobile phase. The rotation speed for the analytical and preparative separation was 1500 rpm and 900 rpm respectively. The mode for HSCCC separation was "head to tail". The effluent was monitored at 254 nm.

2.4. HPLC analysis

HPLC separation was performed with a Shimaduz LC-10A HPLC system on an YMC-Pack ODS-AQ, S-3 μm, 150 × 4.6 mm column (YMC Separation technology, Kyoto, Japan) at 30 °C. Gradient elution was carried out with 40% to 90% aqueous methanol from 0 to 65 min at a flow rate of 0.8 ml/min. The chromatograms were monitored by a Shimadzu SPD10A UV detector at 254 nm.

2.5. Identification of gingerols and 6-shogaol

Electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR) were used for identification of 6-gingerol and 6-shogaol. All ESI-MS experiments were performed on a Bruker Esquire LC–MS ion trap multiple mass spectrometer (Bremen, Germany) in positive ionization mode with analysis of ions up to m/z 2200. Drying gas was nitrogen (gas flow 7.0 L/min, 330 °C), and nebulizer pressure was set to 34.5 kPa. ESI-MS parameters (positive mode): capillary, −4500 V; end plate, −4000 V; cap exit, +90 V; cap exit offset, +60 V; skim 1, +30 V; skim 2, +10 V. NMR spectra were recorded on a Bruker Avance 500 NMR instrument (Karlsruhe, Germany) with 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR measurements in [^{2}H] chloroform ($CDCl_3$), respectively.

3. Results and discussion

3.1. Selection of solvent system

The ethyl acetate extract of ginger rhizomes provided a mixture of compounds with a wide range of polarities. 6-Gingerol, 8-gingerol, 6-shogaol and 10-gingerol, which were primarily identified by LC–MS, were the most abundant and corresponded to peaks 1, 2, 3 and 4, in the HPLC chromatogram (Fig. 1), respectively. Separation of the extract by HSCCC requires solvent systems with lower polarity. In consideration of the high flow rate of FHSCCC, a hydrophobic organic phase composed of *n*-hexane–ethyl acetate (3:2, v/v) in a series of solvent systems composed of *n*-hexane–ethyl acetate–methanol–water was tested for estimation of partition coefficients of 6-gingerol and 6-shogaol. The $K_{U/L}$ val-

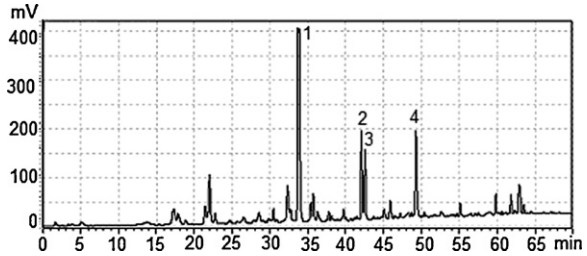

Fig. 1. Chromatogram of HPLC analysis of ethyl acetate extract of ginger rhizomes. Column: YMC-Pack ODS-AQ (S-3 μm, 150 mm × 4.6 mm); gradient elution: 40% to 90% from 0 to 65 min; flow rate: 0.8 ml/min; detection wavelength: 254 nm. **1**,6-gingerol; **2**,6-shogaol; **3**,8-gingerol; **4**,10-gingerol.

Table 1
The partition coefficient ($K_{U/L}$) of 6-gingerol and 6-shogaol for different *n*-hexane–ethyl acetate–methanol–water phase systems when upper phase was defined as stationary phase.

n-Hexane–ethyl acetate–methanol–water (v/v)	6-Gingerol	6-Shogaol
3:2:2:4	2.5	17.1
3:2:2:3	1.6	9.7
3:2:3:3	0.7	5.2
3:2:6:5	0.2	1.3
3:2:4:3	0.1	0.8
3:2:2:1	0.03	0.3

ues (the concentration in upper phase/the concentration in lower phase) were listed in Table 1. As the ratio of *n*-hexane:ethyl acetate:methanol:water was 3:2:2:3, the $K_{U/L}$ values of 6-gingerol and 6-shogaol were1.6 and 9.7, while they were 0.2 and 1.3 when the ratio of the solvents was 3:2:6:5, respectively. Thus, when the upper phase of solvent system composed of *n*-hexane:ethyl acetate:methanol:water (3:2:2:3) was used as the stationary phase, 6-gingerol and 6-shogaol could be eluted out at a desired time, by a consequently stepwise elution which was performed by eluting with the lower phase of *n*-hexane–ethyl acetate–methanol–water (3:2:2:3) first, and then eluting with the lower phase of *n*-hexane–ethyl acetate–methanol–water (3:2:6:5), when the upper phase of *n*-hexane–ethyl acetate–methanol–water (3:2:2:3) as stationary phase of the whole separation.

To check the availability of the solvent system for FHSCCC separation, preliminary separation was performed on an analytical HSCCC at flow rate of 0.8 ml/min since the inner diameter of the tubing was 0.8 mm. The separation completed in 70 min and gave a retention rate of stationary phase of 63% after the end of separation (Fig. 2), which indicated that the separation was a very successful FHSCCC separation. HPLC analysis (Fig. 3) showed the fractions corresponding peaks I, II, III and IV in the FHSCCC chromatogram reached purity more than 97%. The spectra of ESI-MS data indicated compounds 1, 2, 3 and 4 corresponding peaks I, III, II and IV in the FHSCCC chromatogram were possibly 6-gingerol (*m*/*z*: 317 [M+Na$^+$], 611 [2M+Na$^+$]) and 6-shogaol (*m*/*z*: 299 [M+Na$^+$], 575 [2M+Na$^+$]), 8-gingerol (*m*/*z*: 345 [M+Na$^+$], 667 [2M+Na$^+$]) and 10-gingerol (*m*/*z*: 373 [M+Na$^+$], 723 [2M+Na$^+$]) [16].

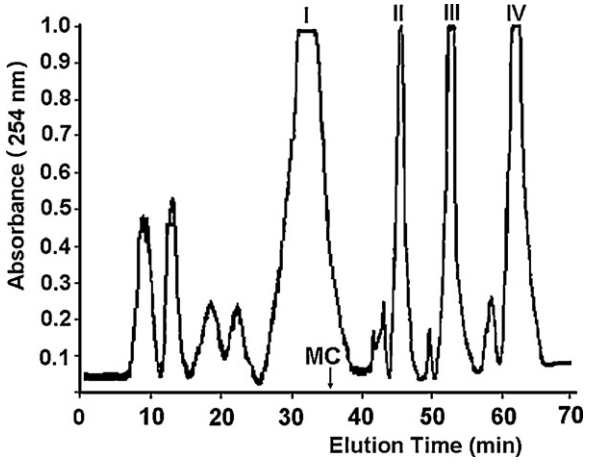

Fig. 2. Chromatogram of analytical FHSCCC separation of 20 mg of the ethyl acetate extract. Stationary phase: the upper phase of the *n*-hexane–ethyl acetate–methanol–water (3:2:2:3, v/v); stepwise elution: lower phase of *n*-hexane–ethyl acetate–methanol–water (3:2:2:3, v/v) for the first 35 min and lower phase of the *n*-hexane–ethyl acetate–methanol–water (3:2:6:5, v/v) for the second 35 min; flow rate: 0.8 ml/min; rotation speed: 1500 rpm; elution mode: "head to tail". I, 6-gingerol; II, 8-gingerol; III, 6-shogaol; IV, 10-gingerol; MC, mobile phase change.

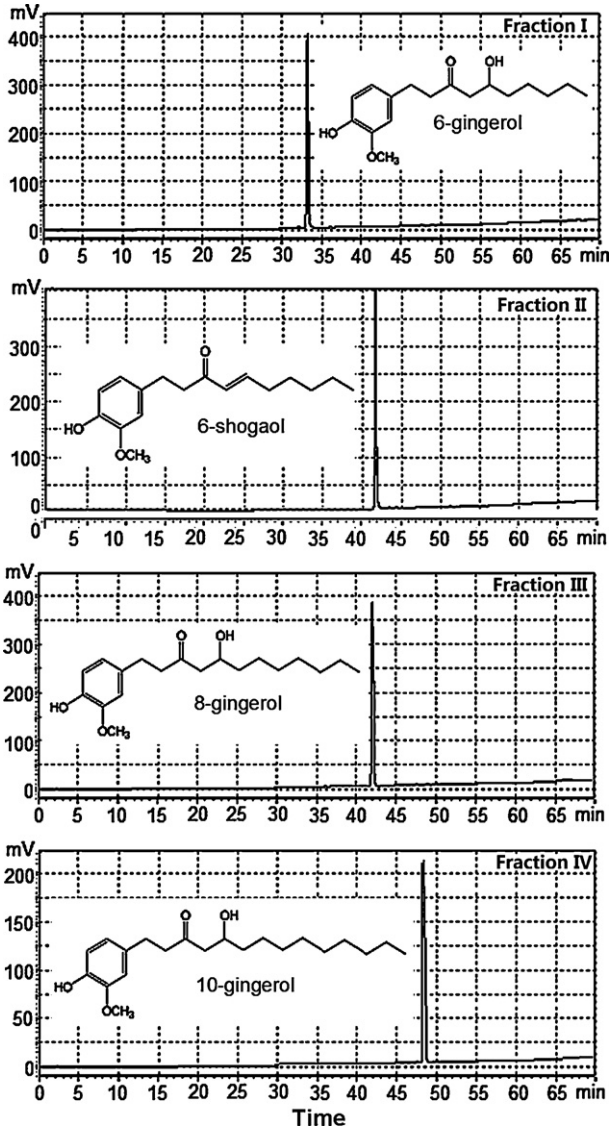

Fig. 3. Chromatogram of HPLC analysis of fractions I, II, III and IV from the analytical FHSCCC separation. HPLC conditions: the same as those in Fig. 1.

3.2. Preparative FHSCCC separation

Preparative separation of gingerols and 6-shogaol by FHSCCC was scaled up according to the above analytical separation. The present study employed a HSCCC instrument with a column made of 5 mm bore PTFE tubing. Thereby a flow rate of 25 ml/min was applied. The separation was performed using the lower phase of *n*-hexane–ethyl acetate–methanol–water (3:2:2:3, v/v) as mobile phase for the first 90 min and the lower phase of *n*-hexane–ethyl acetate–methanol–water (3:2:6:5, v/v) for the second 90 min while the upper phase of *n*-hexane–ethyl acetate–methanol–water (3:2:2:3, v/v) was used as stationary phase in the whole separation. The sample amount of the injection for separation was 100 ml of initial mobile phase containing 5 g of the ethyl acetate extract. The retention rate of stationary phase was 55% after the end of separation. The separation chromatogram was exhibited in Fig. 4. Evaporation of the organic solvents of fractions I, II, III and IV under reduced pressure, and subsequent lyophilization yielded 1.96 g of 6-gingerol (98.3%), 0.33 g of 8-gingerol (97.8%), 0.64 g of 6-shogaol (98.8%) and 0.57 g of 10-gingerol (98.2%).

The four substances were further identified by ^{1}H and ^{13}C NMR spectra as below:

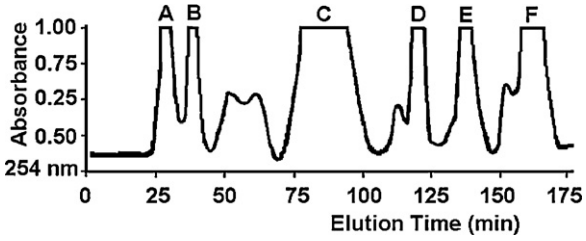

Fig. 4. Chromatogram of preparative FHSCCC separation of 5 g of the ethyl acetate extract. Stationary phase: the upper phase of the *n*-hexane–ethyl acetate–methanol–water (3:2:2:3, v/v); stepwise elution: lower phase of *n*-hexane–ethyl acetate–methanol–water (3:2:2:3, v/v) for the first 90 min and lower phase of the *n*-hexane–ethyl acetate–methanol–water (3:2:6:5, v/v) for the second 90 min; flow rate: 25 ml/min; rotation speed: 900 rpm; elution mode: "head to tail". I, 6-gingerol; II, 8-gingerol; III, 6-shogaol; IV, 10-gingerol.

Compound **1** (6-gingerol), ^{1}H NMR (δ in $CDCl_3$): 6.80 (1H,d, J = 8.0 Hz, H-6′), 6.66 (1H, d, J = 1.75 Hz, 138 H-2′), 6.63 (1H, d, J = 9.2 Hz, H-5′), 4.04 (1H, m, H-5), 3.83 (3H, s, 3′-OMe), 2.82 (2H, m, 139 H-1), 2.73 (2H, m, H-2), 2.56 (2H, m, H-4), 1.23–1.48 (8H, m, H-6, H-7, H-8, H-9), 0.87 (3H, t, J = 6.85, H-10); ^{13}C NMR (δ in $CDCl_3$): 36.4 (C-1), 31.7 (C-2), 211.4 (C-3), 49.3 (C-4), 67.7 (C-5), 45.3 (C-6), 25.1 (C-7), 29.2 (C-8), 22.5 (C-9), 14.0 (C-10), 132.6 (C-1′), 111.1 (C-2′), 146.6 (C-3′), 144.0 (C-4′), 114.5 (C-5′), 120.7 143 (C-6′), 55.8 (3′-OMe).

Compound **2** (6-shogaol), ^{1}H NMR (δ in $CDCl_3$): 6.07 (1H, d, J = 14.5 Hz, H-4), 6.81 (1H, d, J = 13.5 Hz, H-5), 6.83 (1H, d, J = 6.9 Hz, H-5′), 6.66 (1H, dd, J = 1.8, 6.2 Hz, H-6′), 6.70 (1H, d, J = 1.8 Hz, H-2′), 3.82 (3H, s, 3′-OMe), 2.83 (4H, m, H-1, H-2) 2.17 (2H, m, H-6), 1.42 (2H, m, H-7) 1.29 (4H, m, H-8, H-9), 0.88 (3H, t, J = 7.1 Hz, H-10); ^{13}C NMR (δ in $CDCl_3$): 32.4 (C-1), 41.8 (C-2), 199.9 (C-3), 130.2 (C-4), 147.9 (C-5), 31.3 (C-6), 27.7 (C-7), 29.9 (C-8), 22.4 (C-9), 13.9 (C-10), 133.1 (C-1′), 111.2 (C-2′), 146.5 (C-3′), 143.9 (C-4′), 114.4 (C-5′), 120.7 (C-6′), 55.8 (3′-OMe).

Compound **3** (8-gingerol), ^{1}H NMR (δ in $CDCl_3$): 6.80 (1H, d, J = 8.0 Hz, H-6′), 6.66 (1H, d, J = 1.75 Hz, H-2′), 6.63 (1H, d, J = 9.2 Hz, H-5′), 4.04 (1H, m, H-5), 3.83 (3H, s, 3′-OMe), 2.82 (2H, m, H-1), 2.73 (2H, m, H-2), 2.56 (2H, m, H-4), 1.23–1.48 (8H, m, H-6, H-7, H-8, H-9), 0.87 (3H, t, J = 6.85, H-10); ^{13}C NMR (δ in $CDCl_3$): 36.4 (C-1), 31.7 (C-2), 211.4 (C-3), 49.3 (C-4), 67.7 (C-5), 45.3 (C-6), 25.1 (C-7), 29.2 (C-8), 22.5 (C-9), 14.0 (C-10), 132.6 (C-1′), 111.1 (C-2′), 146.6 (C-3′), 144.0 (C-4′), 114.5 (C-5′), 120.7 (C-6′), 55.8 (3′-OMe).

Compound **4** (10-gingerol), ^{1}H NMR (δ in $CDCl_3$): 6.81 (1H,d, J = 8.0 Hz, H-6′), 6.66 (1H, s, H-2′), 6.64 (1H, d, J = 8.1 Hz, H-5′), 4.03 (1H, dd, J = 3.8, 7.8 Hz, H-5), 3.84 (3H, s, 3′-OMe), 2.83 (2H, t, J = 7.5 Hz, H-1), 2.73 (2H, t, J = 7.4 Hz, H-2), 2.57 (2H, m, H-4), 1.26–1.48 (12H, m, H-6, H-7, H-8, H-9, H-10, H-11), 0.88 (3H, t, J = 6.7, H-12); ^{13}C NMR (δ in $CDCl_3$): 36.5 (C-1), 31.7 (C-2), 211.4 (C-3), 49.3 (C-4), 67.7 (C-5), 45.4 (C-6), 25.4 (C-7), 29.2 (C-8), 29.2 (C-9), 29.4 (C-10), 22.6 (C-11), 14.0 (C-12), 132.6 (C-1′), 111.0 (C-2′), 146.5 (C-3′), 144.0 (C-4′), 114.5 (C-5′), 120.7 (C-6′), 55.8 (3′-OMe)

A typical preparation of gingerols and 6-shogaol by traditional liquid column chromatography (LC) has to follow several steps [12]. First, the ginger extract was chromatographed on a Sephadex LH-20 column with 95% ethanol as an eluant to remove the nonphenolic compounds and to generate the gingerol- and shogaol-enriched fraction which was then loaded into a Diaion HP-20 column, eluted first with water to remove the water-soluble compounds and then with 40% aqueous ethanol to obtain fraction A, followed by 95% aqueous ethanol to obtain fraction B. Fraction A was subjected to a normal phase silica-gel column with a stepwise gradient of hexane/ethyl acetate to give pure 6-gingerol, 8-gingerol, and 10-gingerol. Fraction B was also subjected to a normal phase silica-gel column with a stepwise gradient of hexane/ethyl acetate to generate a fraction of 6-shogaol-enriched fraction which was subjected to a C-18 reverse-phase column eluted with a stepwise gradient of methanol/water to yield 6-shogaol. Obviously, the separation by LC was very tedious. The present FHSCCC separation only needs one step separation to yield pure monomers of gingerols including 6-gingerol and 6-shogaol, which will be more attractive than the traditional LC method. Moreover, industrial preparation of the monomers of gingerols and 6-shogaol by FHSCCC is possible since CCC technologies have been scaled up to semi-industrial level [17,18].

4. Conclusions

Rapid purification to obtain the monomers of gingerols including 6-gingerol and 6-shogaol on a gram scale was achieved by FHSCCC. The larger quantities of 6-gingerol and 6-shogaol obtained by this method can support further investigation into the biological activities of the compounds. The more important point is that the above FHCCC method may scale-up to industrial separation.

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References

[1] V.S. Govindarajan, Crit. Rev. Food Sci. Nutr. 17 (1982) 1.
[2] B.H. Ali, G. Blunden, M.O. Tanira, A. Nemmar, Food Chem. Toxicol. 46 (2008) 409.
[3] Y. Shukla, M. Singh, Food Chem. Toxicol. 45 (2007) 683.
[4] Y. Surh, Food Chem. Toxicol. 40 (2002) 1091.
[5] K.K. Park, K.S. Chun, J.M. Lee, S.S. Lee, Y. Surh, Cancer Lett. 129 (1998) 139.
[6] S.O. Kim, J.K. Kundu, Y.K. Shin, J.H. Park, M.H. Cho, T.Y. Kim, Y. Surh, Oncogene 24 (2005) 2558.
[7] E.C. Kim, J.K. Min, T.Y. Kim, S.J. Lee, H.O. Yang, S. Han, Y.M. Kim, Y.G. Kwon, Biochem. Biophys. Res. Commun. 335 (2005) 300.
[8] C.H. Jeong, A.M. Bode, A. Pugliese, Y.Y. Cho, H.G. Kim, J.H. Shim, Y.J. Jeon, H. Li, H. Jiang, Z. Dong, Cancer Res. 69 (2009) 5584.
[9] M.H. Pan, M.C. Hsieh, P.C. Hsu, S.Y. Ho, C.S. Lai, H. Wu, S. Sang, C.T. Ho, Mol. Nutr. Food Res. 52 (2008) 1467.
[10] J.S. Kim, S.I. Lee, H.W. Park, J.H. Yang, T.Y. Shin, Y.C. Kim, N.I. Baek, S.H. Kim, S.U. Choi, B.M. Kwon, K.H. Leem, M.Y. Jung, D.K. Kim, Arch. Pharm. Res. 31 (2008) 415.
[11] M.H. Pan, M.C. Hsieh, J.M. Kuo, C.S. Lai, H. Wu, S. Sang, C.T. Ho, Mol. Nutr. Food Res. 52 (2008) 527.
[12] S. Sang, J. Hong, H. Wu, J. Liu, C.S. Yang, M.-H. Pan, V. Badmaev, C.-T. Ho, J. Agric. Food Chem. 57 (2009) 10645.
[13] J.E. Farthing, M.J. O'neill, J. Liquid Chromatogr. Rel. Technol. 13 (1990) 941.
[14] X. Wang, Z. Zheng, X. Guo, J. Yuan, C. Zheng, Food Chem. 125 (2011) 1476.
[15] Q. Du, BOOK of ABSTRACTS, 6th International Symposium on Countercurrent Chromatography, Villeurbanne, France, July 28–30, 2010.
[16] N. Ohtake, Y. Nakai, M. Yamamoto, I. Sakakibara, S. Takeda, S. Amagaya, M. Aburada, J. Chromatogr. B 812 (2004) 135.
[17] A. Ian, Sutherland, J. Chromatogr. A 1151 (2007) 6.
[18] Q. Du, G. Jerz, Y. He, L. Li, Y. Xu, Q. Zhang, Q. Zheng, P. Winterhalter, Y. Ito, J. Chromatogr. A 1074 (2005) 43.