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Journal of Chromatography A, 923 (2001) 271–274

JOURNAL OF CHROMATOGRAPHY A

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Short communication

Preparative separation of isoflavone components in soybeans using high-speed counter-current chromatography

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Received 27 March 2001; received in revised form 31 May 2001; accepted 6 June 2001

Abstract

Four isoflavone components were purified from soybean extract by high-speed counter-current chromatography (HSCCC). Two types of multilayer coil separation columns were used: a small column made of standard 2.6-mm I.D. PTFE (polytetrafluoroethylene) tubing with a 260-ml capacity and a large column of convoluted PTFE tubing of 5.7-mm average I.D. with a 1200-ml capacity. Separation was performed with a two-phase solvent system composed of hexane–ethyl acetate–1-butanol–methanol–acetic acid–water (1:2:1:1:5:l, v/v) by eluting the lower aqueous phase at 2 ml/min (small column) and 5 ml/min (large column) at a revolution speed of 700 rpm. From 500 mg of crude sample the small column yielded 33 mg of daidzin, 41 mg of genistin, 27 mg of 6″-*O*-malonyldaidzin and 24 mg of 6″-*O*-malonylgenistin. The large convoluted column separated, from 3 g of crude sample, 203 mg of daidzin, 241 mg of genistin, 158 mg of 6″-*O*-malonyldaidzin and 135 mg of 6″-*O*-malonylgenistin all at over 90% purity. The convoluted tubing facilitated preparation of a large multilayer coil due to its high flexibility.

Keywords: Counter-current chromatography; Preparative chromatography; Soybean; Isoflavones

1. Introduction

Soybeans contain a high concentration of isoflavones (Fig. 1) as the glucosides of daidzin and genistin, of food consumed by humans. These isoflavones may have some important health-enhancing properties such as prevention of certain cancers [1], lowering the risk of cardiovascular disorders [2], and improvement of bone function [3]. Their estrogenic activities may play an important role in their health-enhancing properties. Genistin and daidzin account for the major portion of isoflavones in soybean foods and have been the focus of numerous studies. In the present study we separated isoflavones from soybean extract using high-speed counter-current chromatography (HSCCC) [4,5] to establish a preparative method of monomeric isoflavones.

2. Experimental

2.1. Apparatus

A J-type HSCCC instrument was used in the present study. It holds a separation column at a

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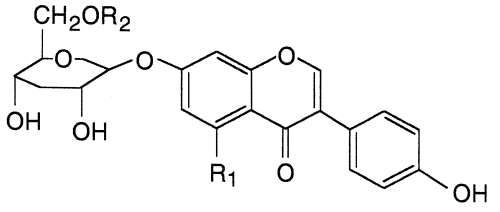
0021-9673/01/$ – see front matter
PII: S0021-9673(01)01031-7



	R_1	R_2
Daidzin:	H	H
Genistin:	OH	H
6"-O-malonyldaidzin:	H	$COCH_2COOH$
6"-O-malonylgenistin:	OH	$COCH_2COOH$

Fig. 1. Chemical structures of isoflavones in soybean.

distance of 10 cm from the central axis of the centrifuge. The column revolves around the central axis of the centrifuge and simultaneously rotates about its own axis at the same angular velocity in the same direction [4]. The column holder was 25-cm long×6-cm O.D. Two multilayer coils were as prepared each with a different type of PTFE (polytetrafluoroethylene) tubing (Zeus Industrial Products, Raritan, NJ, USA): one column was 50-m×2.6-mm I.D. and of 260-ml capacity, while the other was 48-m long convoluted tubing with 5.7-mm average I.D. and of 1200-ml capacity. The β values were 0.32–0.39 for the small column and 0.37–0.58 for the large column. The experiment was performed at a revolution speed of 700 rpm. The mobile phase was delivered using a Waters 510 HPLC pump (Millipore, Milford, MA, USA). An injection loop was used for sample loading and a UV–Vis detector (Model UV-752, Shanghai Instrument Factory, Shanghai, China) was used for monitoring the effluent.

2.2. Reagents

Organic solvents including hexane, ethyl acetate, acetic acid and *n*-butanol were of an analytical grade and purchased from Shanghai Chemical Company, Shanghai, China. Standard samples of daidzin genistin, 6″-*O*-malonyldaidzin and 6″-*O*-malonylgenistin were the gifts from Dr. Jiang Heyuan in Graduate School of Chinese Academy of Agricultural Sciences, Beijing, China. Crude isoflavone sample was purchased from Huabei Medicine Factory, Hebei, China.

2.3. HSCCC separation procedure

The HSCCC experiments were performed with a two-phase solvent system composed of hexane–ethyl acetate–*n*-butanol–methanol–acetic acid–water (1:2:1:1:5:1, v/v) where the upper organic phase was used as the stationary phase and the lower aqueous phase as the mobile phase. The sample solution was prepared by dissolving the crude isoflavones in a 1:1 mixture of each phase and loaded into the column by loop injection. For the 260-ml column, 500 mg of sample in a volume of 20 ml was injected and eluted with the aqueous phase at a flow-rate of 2.0 ml/min. For the 1200-ml column, 3 g of sample in a volume of 120 ml was injected and eluted with the aqueous phase at 5.0 ml/min. The effluent was monitored with a UV–Vis detector at 254 nm and collected with a fraction collector at 5-min intervals.

2.4. HPLC analysis of isoflavones

The HPLC analysis of the isoflavone fractions was carried out as follows [6]: a BDS column (5-μm, 250×4.6-mm I.D.) (Elite, Dalian, China) was used. The mobile phase was composed of methanol–acetic acid–water (30:3.5:66.5, v/v). The flow-rate of the mobile phase was 1.0 ml/min from 0 to 8 min and 1.5 ml/min from 8 to 25 min at column temperature of 50°C.

3. Results and discussion

A HPLC chromatogram of crude isoflavones is shown in Fig. 2 where four known isoflavones of daidzin (peak 3), genistin (peak 4), 6″-*O*-malonyldaidzin (peak 6) and 6″-*O*-malonylgenistin (peak 7) are resolved.

Fig. 3 shows the HSCCC separation of 500 mg of the crude sample with a 260-ml capacity column. Five peaks were resolved in 5.5 h. Peaks A–E (Fig. 3) obtained by HSCCC correspond to HPLC peaks

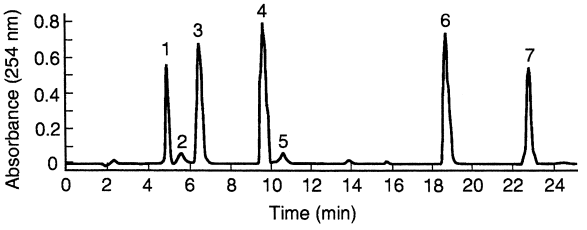


Fig. 2. HPLC analysis of crude isoflavones. Peaks: (3) daidzin; (4) genistin; (6) 6″-*O*-malonyldaidzin; (7) 6″-*O*-malonylgenistin. Experimental conditions: BDS column (5-μm, 250-mm×4.6-mm I.D.) from Elite, Dalian, China; mobile phase, methanol–acetic acid–water (30:3.5:66.5, v/v); flow-rate, 1.0 ml/min from 0 to 8 min and 1.55 ml/min from 8 to 25 min; column temperature, 50°C; detection, 254 nm.

of 1, 3, 4, 5 and 6 (Fig. 2), respectively, as shown by HPLC analysis of each peak fraction in Fig. 4. In both separations isoflavones were eluted in an increasing order of their hydrophobicity. The peak fractions of B–E were collected and lyophilized to yield 33 mg (89%) of daidzin, 41 mg (93%) of genistin, 27 mg (97%) of 6″-*O*-malonyldaidzin and 24 mg (93%) of 6″-*O*-malonylgenistin at purities indicated in the parentheses.

Fig. 5 illustrates HSCCC separation of 3 g of crude isoflavones with the 1200-ml capacity column. Although the large convoluted column produced a similar separation in a longer elution time of 9 h, it yielded 203 mg (93%) of daidzin, 241 mg (95%) of genistin, 156 mg (95%) of 6″-*O*-malonyldaidzin and 135 mg (92%) of 6″-*O*-malonylgenistin at comparable purity to those obtained from the smaller column.

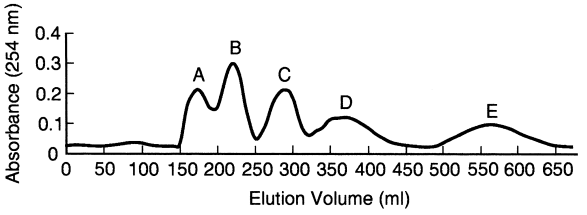


Fig. 3. HSCCC separation of 500 mg of crude isoflavones with 260-ml capacity column. Peaks: (B) daidzin; (C) genistin; (D) 6″-*O*-malonyldaidzin; (E) 6″-*O*-malonylgenistin. Experimental conditions: apparatus, HSCCC multilayer coil planet centrifuge with 10-cm revolution radius; column, standard wall 2.6-mm I.D. PTFE multilayer coil with a total capacity of 260 ml; sample, 500 mg of crude isoflavones; solvent system, hexane–ethyl acetate–*n*-butanol–methanol–acetic acid–water (1:2:1:1:5:1, v/v); mobile phase, lower aqueous phase: flow-rate, 2 ml/min; revolution, 700 rpm; detection, 254 nm; retention of stationary phase, 52%.

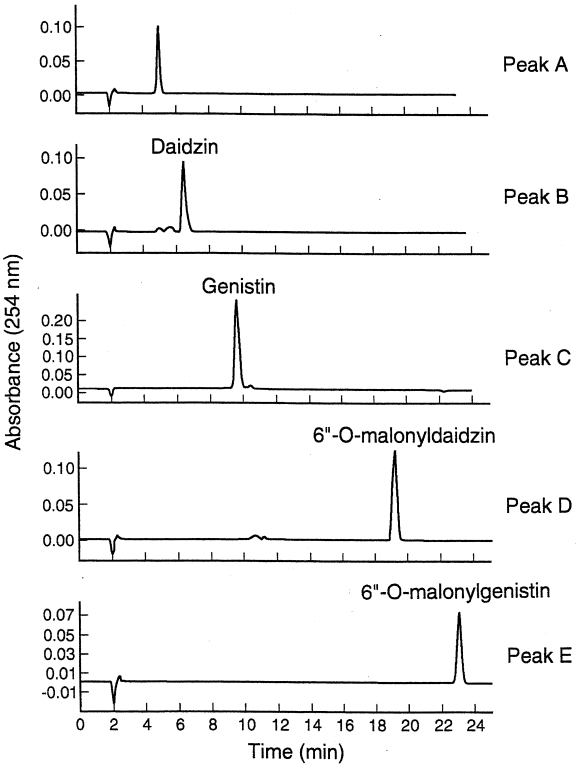


Fig. 4. HPLC analysis of HSCCC peak fractions obtained from the 260-ml capacity column. Experimental conditions are described in the caption to Fig. 2.

Previously, the convoluted tube was used for a low-speed rotary CCC machine [7] to improve retention of stationary phase while its high flexibility

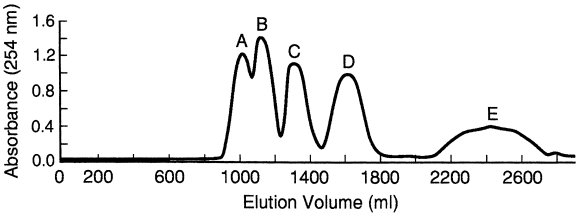


Fig. 5. HSCCC separation of 3 g of crude isoflavones obtained from the 1200-ml capacity convoluted column. Peaks: (B) daidzin; (C) genistin; (D) 6″-*O*-malonyldaidzin; (E) 6″-*O*-malonylgenistin. Experimental conditions: column, convoluted multilayer coil with a 1200-ml capacity; sample, 3 g of crude isoflavones; flow-rate, 5 ml/min; retention of stationary phase, 55%. Other conditions are described in the caption to Fig. 3.

facilitated fabrication of large multilayer coils without deformation of the tubing. The present study demonstrated that the convoluted multilayer coil is also useful for purification of isoflavone standards in HSCCC.

References

[1] S. Barnes, M. Messina, J. Natl. Cancer Inst. 83 (1991) 541.
[2] J.M. Anderson, B.M. Johnstone, M.E. Cook-Newell, New Engl. J. Med. 333 (1995) 276.
[3] H.A. Bahram, L. Alekel, B.W. Hollis, D. Amin, M. Stacewicz-Sapuntzakis, P. Guo, S.C. Kukreja, J. Nutr. 126 (1996) 161.
[4] Y. Ito, CRC Crit. Rev. Anal. Chem. 17 (1986) 65.
[5] Y. Ito, W.D. Conway, in: High-Speed Countercurrent Chromatography, Wiley, New York, 1996.
[6] H. Jiang, F. Lu, J. Tai, H. Dong, Chin. J. Food Sci. 21 (2000) 56.
[7] Q. Du, P. Wu, Y. Ito, Anal. Chem. 72 (2000) 3363.