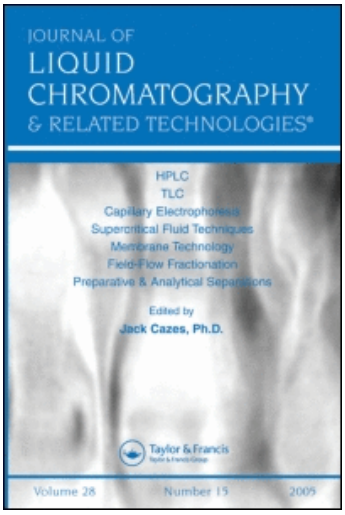

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SEPARATION OF THEAFLAVINS OF BLACK TEA. HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY VS. SEPHADEX LH-20 GEL COLUMN CHROMATOGRAPHY

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ABSTRACT

Performance of two chromatographic techniques, high-speed countercurrent chromatography (HSCCC) and conventional Sephadex LH-20 gel column chromatography (GLC), was compared in the separation of theaflavins from the extract of black tea leaves. The HSCCC run was carried out with a two-phase solvent system composed of hexane/ethyl acetate/methanol/water (1:3:1:6, v/v) by eluting the lower aqueous phase at 2 mL/min at 800 rpm where 200 mg of the sample was resolved into three peaks corresponding to theaflavin, theaflavin-3,3'-gallate, and a mixture of theaflavin-

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3-gallate and theaflavin-3'-gallate in 4.1 hrs. Although, Sephadex column chromatography yielded a similar separation for 100 mg of the same sample, it required a much longer elution time of 21 hrs.

INTRODUCTION

Theaflavins (Fig. 1) are one of the most important components effecting the quality of black tea(1). Recent studies indicated that theaflavins possess anti-atherosclerosis and anti-mutagenesis activities(2). In the past, various monomers of theaflavins were prepared from the extract of black tea leaves using the conventional Sephadex LH-20 column chromatographic method(3,4). The method, however, requires a long separation time due to a limited flow rate through the Sephadex column. Recently, we succeeded in the separation of catechins from green tea extract using high-speed countercurrent chromatography (HSCCC) and large preparative-scale low-speed countercurrent chromatography (LSCCC)(5,6).

In the present study, we separated theaflavins from the black tea leaves extract using HSCCC and conventional Sephadex LH-20 column chromatography to compare the performance of these two methods.

EXPERIMENTAL

Apparatus

An HSCCC instrument used in the present study was a Model GS-10A manufactured by Beijing Institute of New Technology Application, Beijing, China. It was equipped with a multilayer coil of 1.6 mm ID PTFE (polytetrafluoroethylene) tubing with a total capacity of about 230 mL. The experiment was performed at a revolution speed of 800 rpm. The mobile phase was delivered using a Waters 510 HPLC pump. An injection loop was used for sample loading and a UV-VIS detector (Model UV-752, Shanghai, China) was used for monitoring the eluent.

Reagents

Organic solvents including hexane, ethyl acetate, methanol, acetone, isoamyl alcohol, ethanol, acetic acid, and butanol were of an analytical grade and purchased from Shanghai Chemical Company, Shanghai, China. Crude theaflavin sample was a gift from Dr. Ya Cai, Unilever Research Laboratory, Unilever, Colworth House, U.K.

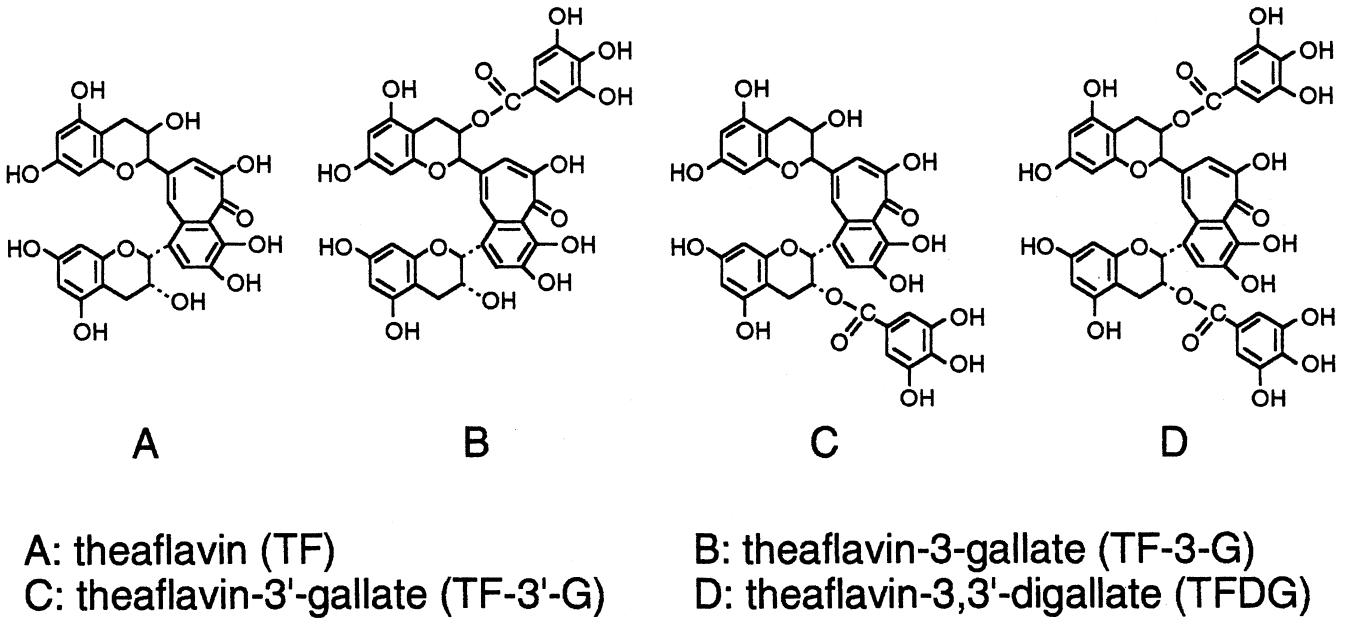

Figure 1. Structures of theaflavins in black tea.

HSCCC Separation Procedure

The HSCCC experiments were performed with a two-phase solvent system composed of hexane-ethyl acetate-methanol-water (1:3:1:6, v/v) The upper phase was used as the stationary phase, and the lower aqueous phase as the mobile. The flow rate of the mobile phase was 2.5 mL/min. The sample solution was prepared by dissolving 250 mg of crude theaflavin sample in a 1:1 mixture of each phase and loaded into the column by loop injection. The effluent was monitored with the UV-VIS detector at 380 nm. and collected using a fraction collector.

Sephadex LH-20 Gel Chromatography

A glass column with a capacity of 237 mL (32cm long and 3.2 cm I.D.) was filled with a slurry of Sephadex LH-20 (Pharmacia, Stockholm, Sweden) in 35% aqueous acetone and equilibrated with the same solvent, and 100 mg of crude theaflavin sample in 20 mL water was added into the column before eluting with 35% acetone at an average flow rate of 0.9 mL/min. The effluent was monitored with the UV-VIS detector.

HPLC Analysis of Theaflavins(7)

The HPLC analysis of the theaflavin fractions was carried out as follows: A BDS column (5 μm, 250 mm x 4.6 mm) from Elite (Dalian, China) was used. The mobile phase was eluted in a linear gradient between Solvents A (acetoni-

Table 1. Partition Coefficients of Four Monomers of Theaflavins in Six Solvent Systems

Solvent System	TF	TF-3-G	TF-3'-G	TFDG
1	0.62	1.6	1.7	2.9
2	0.83	4.0	4.1	5.3
3	0.42	1.1	1.2	1.2
4	2.8	3.9	4.1	4.2
5	1.0	1.9	1.9	3.7
6	2.2	2.7	2.6	3.1

1. Methanol/water/ethyl acetate/hexane 1:1:3:1.
2. Isoamyl alcohol/methanol/acetic acid/water 4:5:2:5.
3. Isoamyl alcohol/ethanol/acetic acid/water 2:2:1:5:1.
4. Isoamyl alcohol/ethanol/acetic acid/water 3:2:1:5.
5. Isoamyl alcohol/ethyl acetate/water/hexane 2:3:10:3.
6. Butanol/ethyl acetate/acetic acid/water/methanol 4:1:1:5:1.

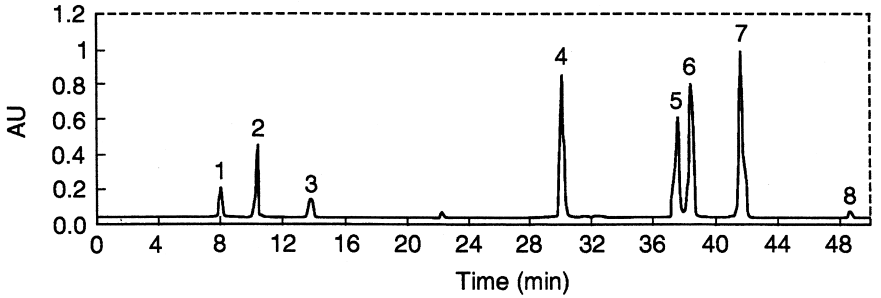

Figure 2. HPLC analysis of crude theaflavin sample. Peak 4: theaflavin; Peak 5: theaflavin-3-gallate; Peak 6: theaflavin-3'-gallate; Peak 7: theaflavin-3,3'-digallate. Experimental conditions: BDS column (5 μm, 250 mm long, 4.6 mm ID); mobile phase: solvent A (acetonitrile) and solvent B (2% aqueous acetic acid) using a linear gradient from 8% A and 92% B to 31% A and 69% B for 50 min; flow rate: 1.4 mL/min.

trile) and B (2%, v/v, aqueous acetic acid), starting at 8% A and 92 % B and ending at 31% B and 69% A, from 0 to 50 min at a flow rate of 1.4 mL/min.

RESULTS AND DISCUSSION

Table 1 shows partition coefficients of four main theaflavin components determined by HPLC analysis. The results indicated that all of these six solvent

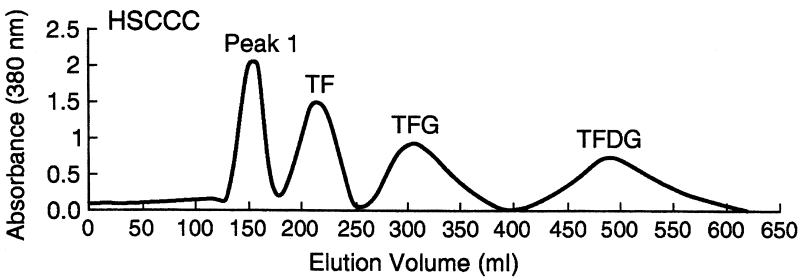

Figure 3. HSCCC separation of crude theaflavin sample. TF:theaflavin; TFG: theaflavin-3-gallate + theaflavin-3'-gallate; TFDG: theaflavin-3,3'-digallate. Experimental conditions: Apparatus: Type J coil planet centrifuge with 10 cm revolution radius equipped with a multilayer coil of 1.6 mm ID and 230 mL capacity; sample: crude theaflavin mixture 250 mg; solvent system: hexane/ethyl acetate/methanol/water (1L3:1:6); mobile phase: lower aqueous phase; flow rate: 2.5 mL/min: detection: 380 nm; revolution: 800 rpm.

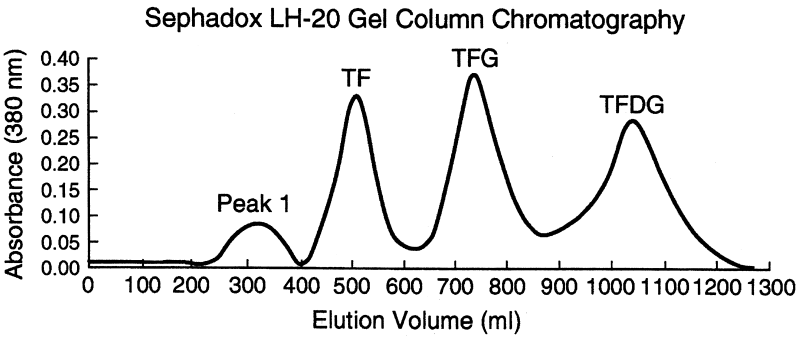

Figure 4. Sephadex-LH20 gel chromatography of crude theaflavin sample, TF:theaflavin; TFG: theaflavin-3-gallate + theaflavin-3'-gallate; TFDG: theaflavin-3,3'-digallate. Experimental conditions: Column: glass column with a 238 mL capacity, 32 cm long and 3.0 cm ID; sample: 100 mg of crude theaflavins; mobile phase: 35% acetone; flow rate: 0.9 mL/min; detection: 380 nm.

systems could not resolve TF-3-G and TF-3'-G. Among those solvent systems, 1 is the best for the separation of TF, TFG (TF-3-G + TF-3'-G), and TFDG.

HPLC analysis of the crude theaflavin sample is shown in Fig. 1 where eight peaks are as numbered. Figure 2 shows the separation of 250 mg of the theaflavin sample by HSCCC. Four peaks were resolved in about 4 hours. The first peak represents unknown impurities, while the other three peaks are theaflavin (Peak 2), a mixture of theaflavin-3-gallate and theaflavin-3'-gallate (Peak 3), and theaflavin-3,3'-gallate.

The separation of 100 mg of the same sample using the conventional Sephadex-20 gel column is shown in Fig. 3. It produced a similar separation for four peaks at a flow rate of 0.9 mL/min in 21 hours. The above results indicate that the separation efficiency of HSCCC is 12 times that of GLC: 61 mg/hr of crude theaflavins for HSCCC and 4.8 mg/hr for GLC (Table 2).

Overall results of the above experiments clearly show that HSCCC can separate, more efficiently, a large amount of crude theaflavin sample in a shorter elu-

Table 2. Separation of Theaflavins by HSCCC and GLC

Method	Flow Rate (mL/min)	Sample Size (mg)	Separation Time (hr)	Efficiency (mg/hr)
HSCCC	2.5	250	4.1	61
GLC	0.9	100	21	4.8

tion time. We expect that HSCCC will play an important role in the research on bioactive components in a black tea extract.

REFERENCES

1. Owuor, P.O.; Reeves, S.G.; Wanyoko, J.K. J. Sci. Food Agri. **1986**, *37*, 507.
2. Santosh, K.K.; Hasan,, M. Proc.'95 International Tea-Quality Human Health Symposium, Shanghai, China, 1995; pp 7-15.
3. Lea, A.G.H.; Crispin, D.J. J. Chromatogr. **1971**, *54*, 133.
4. Collier, P.K.; Bryce, T.; Marrows, R.; Thomas, P.E. Tetrahedron **1973**, *29*, 125.
5. Du, Q.Z.; Li, M.-J.; Cheng, Q.K. Proc. '95 International Tea-Quality Human Health Symposium, Shanghai, China, 1995; pp. 188-189.
6. Du, Q.-Z.; Wu, P.D.; Ito, Y. Anal. Chem. **2000**, *72*, 3353.
7. Bailey, R.G.; Nursten, H.E.; McDowell, I. J. Sci. Food Agri. **1990**, *52*, 509.

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