Note

Separation of the Components in Black Tea Infusion by Chromatography on Toyopearl®

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Received September 10, 1981

The methods commonly used for separation of the components in an infusion of black tea are chromatographic using silica gel, cellulose and Sephadex as the adsorbents. Among them, column chromatography on Sephadex LH-20, reported by Crispin *et al.*,¹⁾ is a most useful method. Indeed, this technique can be applied to the separation of black tea pigments^{2~4)} and an evaluation of the quality of tea.^{5~8)}

Toyopearl is a hydrophilic porous polymer packing material for gel filtration. It is produced from the polymerization of hydrophilic vinyl monomers. In this study, the gel was applied to separation of the phenolic components in black tea infusion. As a principal result, it showed a much higher resolving power than that of Sephadex LH-20.⁷⁾ The methods and results are described below.

Twinings Queen Mary tea was brewed for 3 min with distilled water (60 ml) at 95°C. The extract was filtered through a filter paper and rapidly freeze-dried.

For the gel filtration chromatography, Toyopearl HW-40F gels (Toyo Soda Mfg. Co., Ltd.) were equilibrated in a glass column (0.9 × 25 cm) with aqueous acetone (15% by vol.). The freeze-dried sample (60 mg) was dissolved in the initial solvent (0.4 ml) and placed on the column. The column was eluted with a stepwise gradient of aqueous acetone at a flow rate of 1 ml per 13 min using a peristaltic pump. The eluate was collected in 1 ml (45 drops) fractions and their optical densities at 375 nm were measured. The fractions corresponding to the peak number were collected, freeze-dried and examined by two-dimensional paper chromatography.

The elution pattern for the hot aqueous infusion of black tea is shown in Fig. 1. The components in the infusion were separated into at least thirty zones. The results of paper chromatographic analyses of each zone are listed in Table I. The phenolic components in black tea infusion have been classified mainly into catechins, flavonol glycosides, phenolic acids and depsides, theaflavins, nondialyzable phenolic substances, and other phenolic substances (bisflavanols, dialyzable thearubigins plus other fermentation products).⁶⁾ The most important pigments, theaflavins, were eluted in the peaks 27, 29, and 30. These correspond to theaflavin, theaflavin mono- and digallate, respectively. They were eluted in order of increasing molecular size, contrary to the principle of molecular sieving. On this chromatography, the crystalline theaflavin digallate could be obtained readily. Brown colored substances eluted in the peaks 1, 2, and 3 may be high

FIG. 1. Elution Pattern of Tea Infusion on Toyopearl HW-40F.

TABLE I. PAPER CHROMATOGRAPHIC IDENTIFICATION OF THE SUBSTANCES SEPARATED BY TOYOPEARL COLUMN CHROMATOGRAPHY

Peak and fraction number	<i>R_f</i> values × 100, ^a fluorescence under UV light ^b and color reaction ^c
1	10~12 47/43, V(+); 51/54, B(-); Brown colored substances
2	14~17 42/64, V(+), gallic acid; 64/57, B(+), chlorogenic acid; 77/76, V(-), caffeine; Brown colored substances
3	18~20 41/64, V(+), gallic acid; 61/48, YB(-); 79/80, V(-), caffeine; Brown colored substances
4	21~26 26/90, B(-); 28/36, D(±); 37/66, (+); 41/45, D(+), orange colored substance; 56/34, (+); 60/56, B(-); 67/39, (+); 74/12, V(+)
5	27~33 29/38, D(±); 78/70, V(-); Polymerized material
6	35~46 54/39, D(+), quertrin; 61/46, D(±), kaempferin; Polymerized material
7	47~55 29/48, (+); 33/71, B(-); 38/64, (+); Polymerized material
8	56~60 37/44, D(+), rutin; 48/90, B(-); Polymerized material
9	61~68 09/45, V(±); 37/44, D(+), rutin; 39/64, (+); 43/53, D(±), nicotiflorin; 42/33, V(+); 59/31, V(+)
10	69~75 23/37, V(+); 41/34, V(+); 69/28, (+), theogallin
11	76~82 27/45, V(+); 68/31, V(+), theogallin
12	83~85 09/44, D(+), thearubigin; 27/37, V(+), (-)-epigallocatechin; 29/89, B(-); 56/31, (+)
13	86~89 05/37, D(+), thearubigin; 13/49, D(+); 19/60, D(+), isoquercitrin; 37/91, B(-); 39/50, V(+), (-)-epicatechin
14	90~94 16/58, D(+), isoquercitrin; 22/73, D(±), astragalin; 36/53, V(+), (-)-epicatechin
15	96~100 41/29, V(+); 47/66, V(+), (+)-catechin
16	101~105 03/35, V(±), tricetinidin; 35/23, V(+); 40/62, (+)
17	106~110 03/35, V(±), tricetinidin; Polymerized material
18	111~118 03/34, V(±), tricetinidin; Polymerized material
19	119~125 16/60, V(+); 47/35, V(+); Polymerized material
20	126~130 09/36, V(+); 41/36, V(+)
21	131~135 09/41, V(+); 24/63, V(+); 38/32, (+)
22	136~147 06/43, V(+); 21/57, V(+), (-)-epigallocatechin gallate; 31/28, V(+)
23	148~153 28/75, V(+), (-)-epicatechin gallate
24	154~158 30/78, V(+), (-)-epicatechin gallate; 35/50, V(+)
25	159~168 15/32, D(+), orange colored substance; 31/41, V(+)
26	169~175 Orange colored substances
27	180~190 05/37, D(+), theaflavin; 13/76, V(+)
28	195~203 Orange colored substances
29	205~219 16/60, D(+), theaflavin monogallate
30	223~238 19/58, D(+), theaflavin digallate

^a Solvent system, 2% AcOH/BuOH-AcOH-H₂O (4:1:5).

^b V, violet; B, blue; YB, yellowish blue; D, dark spot.

^c 0.5% iron alum and 0.5% potassium ferricyanide: +, positive; ±, slight; -, negative.

polymerized non-dialyzable components. The other colored substances, thearubigins, were separated into two regions. The polymerized materials eluted from the peaks 4 to 8 may be butanol soluble thearubigins. The thearubigins soluble in ethyl acetate were recognized in the peaks 12 and 13.

Five kinds of catechins were separated from each other on this column. The catechins in the free state were eluted from the column before those galloyl esters, contrary to the principle of molecular sieving. However, the rate of elution between (-)-epigallocatechin and (-)-epicatechin was in the order expected on a molecular weight basis. This phenomenon was also observed between their gallates. As Toyopearl has a weak adsorptive activity for aromatic compounds, separation of the phenolics cannot

be necessarily achieved on the basis of molecular size.

In the previous methods, flavonol glycosides were not separated from each other. However, they were separated into at least five peaks on this chromatography in the range from 25 to 30% of aqueous acetone. The rate of elution depended on the number of the sugar, the order of elution being tri-, di-, and monoglycoside. Among the flavonol glycosides having the same sugar composition but different aglycone, the order of elution depended on the number of the hydroxyl group in the B-ring. Thus, the flavonol glycosides were eluted in order of decreasing molecular size.

Tricetinidin was eluted in the peaks 16 to 18 as a broad band, and theogallin was detected from peak 11. In addition several phenolic constituents were recognized on

the paper chromatogram as distinct spots. However, they could not be identified.

As described above, Toyopearl gels had a high resolving power to the phenolic compounds in black tea infusion. So this technique should be useful to evaluate the characteristics of tea liquors. At the same time, this gel could be applied to the separation of natural products, especially glycosides.

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