Journal of Chromatography B, 867 (2008) 282–286

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Preparative isolation and purification of theaflavins and catechins by high-speed countercurrent chromatography

Kunbo Wang[a,b], Zhonghua Liu[a,b,*], Jian-an Huang[a,b], Xinrong Dong[a], Lubing Song[a,b], Yu Pan[a,b], Fang liu[a,b]

[a] Key Laboratory of Tea Science of Ministry of Education, Hunan Agricultural University, Furong District, Changsha, Hunan 410128, People's Republic of China
[b] Department of Tea Science of Horticulture and Landscape College, Hunan Agricultural University, Changsha, Hunan 410128, People's Republic of China

ARTICLE INFO

Article history:
Received 4 January 2008
Accepted 3 April 2008
Available online 9 April 2008

Keywords:
High-speed countercurrent chromatography
Theaflavins
Black tea
Catechins
Green tea

ABSTRACT

High-speed countercurrent chromatography (HSCCC) has been applied for the separation of theaflavins and catechins. The HSCCC run was carried out with a two-phase solvent system composed of hexane–ethyl acetate–methanol–water–acetic acid (1:5:1:5:0.25, v/v) by eluting the lower aqueous phase at 2 ml/min at 700 rpm. The results indicated that pure theaflavin, theaflavins-3-gallate, theaflavins-3′-gallate and theaflavin-3,3′-digallate could be obtained from crude theaflavins sample and black tea. The structures of the isolated compounds were positively confirmed by ^{1}H NMR and ^{13}C NMR, MS analysis, HPLC data and TLC data. Meanwhile, catechins including epigallocatechin gallate, gallocatechin gallate, epicatechin gallate and epigallocatechin were isolated from the aqueous extract of green tea by using the same solvent system. This study developed a modified method combined with enrichment theaflavins method by using HSCCC for separation of four individual theaflavins, especially for better separation of theaflavins monogallates.

1. Introduction

Black tea accounts for about 80% of tea consumed worldwide. Theaflavins (TFs) are a group of polyphenol pigments found in black tea formed by oxidative coupling of an appropriate pair of catechins at the fermentation stage of black tea manufacture [1]. Although, theaflavins constitute about 2% of the dry weight, they significantly contribute to the bright color and brisk taste of tea brews [1]. In addition to the four major theaflavins (theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate and theaflavin-3,3′-digallate) identified many years ago, several novel theaflavin compounds have recently been isolated and characterized from black tea [2]. Recently, theaflavins have received much attention because of their potential benefits for human health, including anti-oxidation [3,4], anti-virus [5,6], antimutagenicity [7,8], anti-inflammatory [9,10] and cancer chemopreventive action [11,12].

However, the preparative separation of TFs is of significant technical challenge because of their low-level amount in black tea. Although, NMR and MS data of TFs have been published in detail [13], their pure standards are still not commercially available. In the past, the isolation of TFs had been based on chromatography on Sephadex LH-20 and preparative high-performance liquid chromatography (HPLC). However, these procedures are rather time-consuming. Therefore, a rapid separation by high-speed countercurrent chromatography (HSCCC) was developed. HSCCC is a unique liquid–liquid partition chromatography using a liquid stationary phase without solid support, no irreversible adsorption, low risk of sample denaturation, total sample recovery, large load capacity, and low cost [14]. HSCCC has been applied for the separation of four theaflavins (TFs), while the separation of two theaflavin monogallates is incomplete [15–19].

The major catechins in fresh tea leaves and green tea are (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG) and (−)-epicatechin (EC) [20]. Green tea catechins have anti-oxidant activity [3,12]. They may also have anticarcinogenic, anti-inflammatory, anti-atherogenic, thermogenic and antimicrobial activities [12,21]. Due to their possible contributions to the health benefits of tea, catechins have also attracted considerable attention. Isolation of catechins has been based on chromatography on (semi-) preparative HPLC and sephadex LH-20 [22,23]. The procedures are time-consuming and tedious. Thereafter, it is ideal for the separation of catechins by HSCCC [24,25].

The objective of this study was to develop a modified method combined with enrichment theaflavins method by using HSCCC

* Corresponding author at: Key Laboratory of Tea Science of Ministry of Education, Hunan Agricultural University, Furong District, Changsha, Hunan 410128, People's Republic of China. Tel.: +86 731 4635306; fax: +86 731 4635306.
E-mail address: zhonghualiu163@163.com (Z. Liu).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.04.005

for separation of four individual theaflavins, especially for better separation of theaflavins monogallates. In previous study, we have devised a simple method for synthesizing theaflavins from tea catechins mixture using enzymatic oxidation methods [26]. An in vitro model fermentation system, containing tea catechins and crude pear polyphenol oxidase from pear fruits has been used to determine the effect of tea catechins of different proportions and physical and chemical conditions on the formation of theaflavins [26]. Meanwhile, the separation of catechins (EGCG, ECG, EGC and GCG) was also investigated by using the same solvent system.

2. Experimental

2.1. Reagents and materials

Epigallocatechin gallate (EGCG, ≥98%), epigallocatechin (EGC, ≥98%), gallocatechin gallate (GCG, ≥98%), epicatechin gallate (ECG, ≥98%), caffeine (≥99%) and theaflavins standard mixture (≥80% theaflavins) were purchased from Sigma Chemicals, USA. Hexane, methyl isobutyl ketone, ethyl propionate, ethyl acetate, methanol, acetone, and chloroform were of analytical grade and were purchased from Changsha Chemical Company (Changsha, China). Polyamide thin layer chromatography (TLC) plates were purchased from TZSHSL (Taizhou, China).

2.2. Preparation of TFs and tea extracts

TFs were prepared by enzymatic oxidation of tea catechins using crude polyphenol oxidase from pear fruits. The procedures to produce TFs were similar to that described by Wang et al. [26], except that the reaction was carried out at defined pH 5.5 at 30 °C in the incubation and terminated after 30 min. Tea extracts were prepared by extracting 5 g of powdered tea by 100 ml of boiling water for 10 min. After filtration, the water extract was further extracted 2 times with 100 ml ethyl acetate in the separation funnel. The ethyl acetate layer of black tea was mixed with 2.5% (w/v) $NaHCO_3$ (100 ml) and shaken for 30 s. The aqueous layer was discarded. The ethyl acetate phase was collected and evaporated to dryness.

2.3. HSCCC separation procedure

The HSCCC (GS-10A, Beijing Institute of New Technology Application, China) experiments were performed with a two-phase solvent system composed of hexane–ethyl acetate–methanol–water–acetic acid (1:5:1:5:0.25, v/v). The upper phase was used as the stationary phase, and the lower aqueous phase as the mobile. The experiment was performed at a revolution speed of 700 rpm. Usually, in each separation, the coiled column was first filled with the stationary phase. Then, the mobile phase was pumped into the column at a flow-rate of 2.0 ml/min. After the mobile phase front emerged and the system established a hydrodynamic equilibrium, the sample solution was loaded through an injection valve (with 10 ml loop). The sample solution was prepared by dissolving 30 mg of crude theaflavin sample or tea extract (20 mg green tea extract, 40 mg black tea extract) in mobile phase (5 ml) and loaded into the column by loop injection. The effluent was monitored with the UV–VIS detector (Model UV-8823B, Beijing, China) at 280 nm and collected using a fraction collector at 2.5 min intervals (5 ml per tube). The fractions were analyzed by HPLC-DAD. The fractions containing purified theaflavins were collected and dried separately.

2.4. HPLC analysis with diode array detection (HPLC-DAD) and measurement of partition coefficient

A Shimadzu SCL-10ATVP system equipped with a model LC-10ATVP pump, SPD-M20A diode array detector, and a LC-solution data system was used. Photodiode array spectra (range 200–600 nm) were obtained. The analysis of TFs was performed on Hypersil C_{18} column (5 μm, 150 mm × 4.6 mm, Shimadzu, Japan) [27]. The mobile phases were composed of solvent A, 2% acetic acid in high pure water and solvent B, acetonitrile:ethyl acetate (7:1, v/v). The elution was programmed as follows: initial, 18% B; gradient to 26% B in 30 min; at 30.01 min, back to initial condition 18% B and isocratic for 10 min; flow-rate, 0.9 ml/min. The partition coefficients (*K*-values) were determined by HPLC as follows: about 5.0 mg of crude sample was added to a test tube, to which 5 ml of each phase of the two-phase solvent system was added. The test tube was shaken vigorously for several minutes. The partition coefficients of all components in sample were obtained according to the peak areas.

2.5. TLC characterization

Theaflavins were submitted to the characterization by thin layer chromatography (Polyamide TLC plates; mobile phase, chloroform–methanol (2:3, v/v, two-fold development)). TLC was sprayed with Iron(III) chloride–ethanol reagent (5:100, w/v). Chromatograms were evaluated under visible light to detect the presence of theaflavins.

2.6. LC/MS analysis and NMR spectroscopy

LC/MS spectra were measured with Agilent 1100 LC/MSD SL (Agilent Inc., USA) equipped with an atmospheric pressure chemical ionization (ApCI) interface [28]. LC/MS was performed on a Zarbax C8 column, 200 mm × 4.6 mm i.d. (Agilent Inc., USA), flow-rate was 0.9 ml/min, and eluents were mixtures (75:25, v/v) of methanol and water containing 4 g L^{-1} ammonium formate. The effluent from the LC column was delivered to the ion source (150 °C) through a heated nebulizer probe (400 °C) using nitrogen as drying gas (5 l min^{-1}, 350 °C) and nebulizer pressure was set to 60 psi. The mass spectrometer was scanned from *m*/*z* 50 to 1000 in full scan mode. Nuclear magnetic resonance (NMR) spectrometer used here was Varian Unity INOVA 300 NMR. Chemical shifts (δ) are reported in ppm relative to the residual solvent signals (δH 3.35 and δC 49.0 ppm) and coupling constants (*J*) in Hz.

3. Results and discussion

3.1. Screening of solvent systems

For the separation of TFs using HSCCC, ten solvent systems have been examined to optimize the partition coefficients (*K*) of four main TFs by HPLC analysis. The sample used for this purpose was a crude mixture of four TFs, as illustrated in Fig. 1. The results listed in Table 1 showed most of the TFs *K* values lied closely in the range of 0.5–2, and generally, the *K* values of TF and TFDG are clearly different from that of TF3G and TF3′G under most of solvent systems. Among those solvent systems, No. 4 and 5 were the best for the separation of TF, TF3G, TF3′G and TFDG. So the system composed of hexane–ethyl acetate–methanol–water–acetic acid was employed. When using neutral solvent systems, it is considerable to modify the *K* value of the negatively charged analytes, e.g., carboxylic acids, by adding acids such as TFA and acetic acid to the solvent system [29]. Due to protonation, these molecules become more hydrophobic and favor partition to the organic phase. Since these acidic analytes

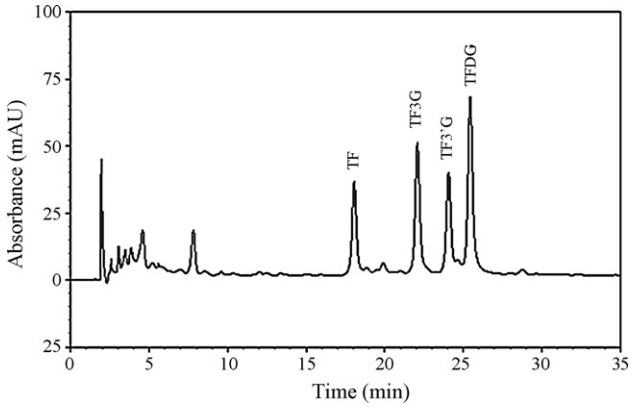

Fig. 1. HPLC chromatogram of the crude TFs mixture. TF: theaflavin, TF3G: theaflavins-3-gallate, TF3′G: theaflavins-3′-gallate, TFDG: theaflavins-3,3′-digallate.

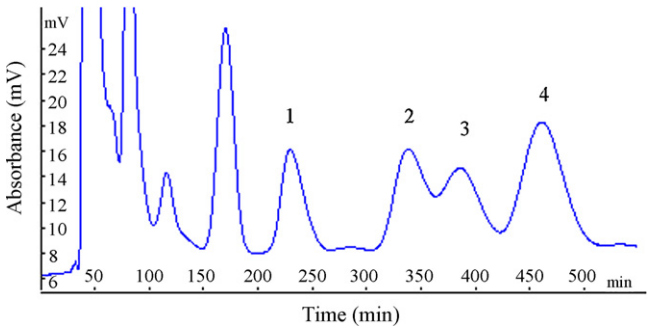

Fig. 2. Preparative separation of four TFs by HSCCC. Solvent system: hexane–ethyl acetate–methanol–water–acetic acid (1:5:1:5:0.25, v/v); mobile phase: lower aqueous phase; flow-rate: 2 ml/min; column volume: 243.0 ml; sample size: 30 mg; retention of stationary phase: 66.3%.

have two molecular forms, protonated (COOH) and deprotonated (COO—), each having a different *K* value, they form a broader peak when partly ionized. Therefore, the use of TFA or acetic acid to the solvent system is recommended.

The results obtained with the above TFs mixture, showed that slight differences between volume ratios of each composition can make a great difference in the separation (HSCCC chromatograms not given). This was consistent with that predicted from the partition data in Table 1. The results of HSCCC tests indicated that when (1:5:1:5:0.125, v/v) was used as the solvent system, the separation time was too long. When hexane–ethyl acetate–methanol–water–acetic acid (1:5:1:5:0.5, v/v) was used, the three compounds (TF3G, TF3′G and TFDG) could not get satisfactory separation. When hexane–ethyl acetate–methanol–water–acetic acid (1:5:1:5:0.25, v/v) was used as the solvent system, good separation results and acceptable separation time could be obtained.

3.2. Preparative separation of TFs

Fig. 2 shows the separation of 30 mg of the theaflavin sample by HSCCC. Four TFs peaks were resolved in about 500 min. Peak 1 represents theaflavin, the other three peaks (2, 3, 4) are theaflavin-3-gallate, theaflavin-3′-gallate and theaflavin-3,3′-gallate, respectively. The peak fractions separated by HSCCC were analyzed by HPLC (HPLC analysis data not shown). Pure compounds were obtained after recrystallization of these fractions with aqueous methanol, while minor impurities were present in the isolated fractions.

3.3. The structure identification

The structural identification of peak fractions of HSCCC was performed according to ^{1}H NMR data, ^{1}C NMR data, MS data, HPLC data and TLC data.

Peak 1 (The effluent was collected from 220 to 245 min): ^{1}H NMR (300 MHz, CD_3OD): δ_H 7.99 1H s, 7.87 1H s, 7.38 1H s, 6.06 1H d, J = 2.4 Hz, 6.03 1H d, J = 2.4 Hz, 6.01 1H d, J = 2.4 Hz, 6.00 1H d, J = 2.4 Hz, 5.66 1H brs, 4.93 1H brs, 4.49 1H m, 4.36 1H m, 2.95–3.06 2H m, 2.82–2.90 2H m; ^{13}C NM R (75 MHz, CD_3OD): δ_C 185.8, 158.2, 158.1, 157.9, 157.7, 157.4, 156.7, 155.5, 151.1, 146.5, 134.6, 131.6, 129.0, 126.5, 124.0, 122.2, 118.4, 100.2, 99.8, 96.8 × 2, 96.1, 95.7, 81.3, 77.3, 66.6, 65.7, 30.0, 29.4 ppm; negative APCI-MS m/z 563 $[M–H]^-$. These data were identical with the literature [28,30], in combination with its retention time, absorbance spectrum in HPLC-DAD analysis (data not given), Rf value in TLC analysis (Fig. 3) and by cochromatography with authentic standard, and peak 1 was identified as theaflavin.

Peak 2 (The effluent was collected from 320 to 345 min): ^{1}H NMR (300 MHz, CD_3OD): δ_H 7.91 1H s, 7.90 1H s, 7.39 1H s, 6.89 2H s, 6.02–6.11 4H m, 5.90 1H m, 5.85 1H brs, 4.97 1H s, 4.38 1H m, 2.87–3.18 4H m; ^{13}C NM R (75 MHz, CD_3OD): δ_C 185.8,167.3,158.1, 158.0 × 2, 157.9, 157.1, 156.7, 155.6, 151.2, 146.4, 146.3 × 2, 139.8, 135.1, 130.5, 129.0, 126.0, 123.2, 122.2, 121.1, 118.5, 111.1 × 2, 99.9, 99.6, 96.9, 96.8, 96.0, 95.7, 81.4, 75.9, 68.3, 66.6, 29.3, 27.2 ppm; negative APCI-MS m/z 715 $[M–H]^-$. These data were identical with the literature [28,30], in combination with its retention time,

Table 1
Solvent selection for the separation of TFs by HSCCC

Solvent system	Volume									
No.	1	2	3	4	5	6	7	8	9	10
Hexane	1	1	1	1	1	1	1	2	2	1.5
Methyl isobutyl ketone								5	5	5
Ethyl propionate			5							
Ethyl acetate	5	4		5	5	5	5			
Methanol	1	1	1	1	1	1	1			
Acetone								2	2	1.5
Water	5	5	5	5	5	5	5	5	5	5
Acetic acid			0.5	0.25	0.5	1	1			
n-Butanol							0.25	0.25		
Partition coefficient (K)[a]										
TF	1.89	1.10	0.17	1.74	1.93	0.49	1.76	0.11	0.14	0.55
TF3G	3.05	1.39	0.51	2.44	2.86	1.59	2.37	0.86	0.70	1.69
TF3′G	4.07	1.78	0.62	3.43	3.43	1.85	2.86	1.05	1.19	2.18
TFDG	5.13	1.94	0.95	3.73	3.88	1.91	3.03	1.26	1.04	2.54

[a] K is expressed as the solute concentration in the upper phase divided by that in the lower phase.

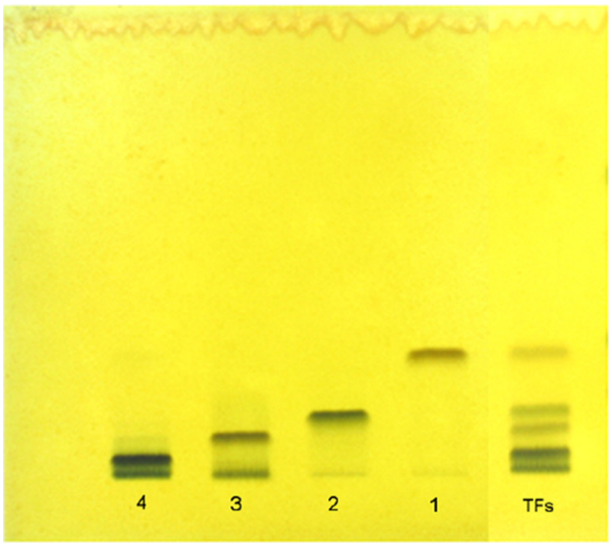

Fig. 3. Separation of theaflavins on polyamide TLC plates. (1) TF, (2) TF3G, (3) TF3′G, (4) TFDG. TFs: theaflavins standard mixture.

absorbance spectrum in HPLC-DAD analysis (data not given), Rf value in TLC analysis (Fig. 3) and by cochromatography with authentic standard, and peak 2 was identified as theaflavin-3-gallate.

Peak 3 (The effluent was collected from 380 to 405 min): ^{1}H NMR (300 MHz,CD_3OD): δ_H 7.95 1H s, 7.82 1H s, 7.41 1H s, 6.84 2H s, 6.03–6.07 4H m, 5.82 1H s, 5.58 1H s, 5.15 1H s, 4.22 1H s, 2.85–3.16 4H m; ^{13}C NMR (75 MHz, CD_3OD): δ_C 185.8, 167.3, 158.2, 158.1, 157.9, 157.7, 157.4, 156.5, 155.5, 151.2, 146.6, 146.3 × 2, 139.9, 133.6, 131.5, 128.8, 125.8, 124.0, 122.1, 121.1, 117.6, 110.1 × 2, 100.2, 99.3, 97.0, 96.9, 96.2, 95.8, 79.9, 77.0, 69.0, 65.8, 30.1, 27.1 ppm; negative APCI-MS m/z 715 $[M–H]^-$. These data were identical with the literature [28,30], in combination with its retention time, absorbance spectrum in HPLC-DAD analysis (data not given), Rf value in TLC analysis (Fig. 3) and by cochromatography with authentic standard, and peak 3 was identified as theaflavin-3′-gallate.

Peak 4 (The effluent was collected from 440 to 485 min). ^{1}H NMR (300 MHz, CD_3OD): δ_H 7.83 1H s, 7.80 1H s, 7.51 1H s, 6.93 2H s, 6.84 2H s, 6.04–6.12 4H m, 5.90 1H s, 5.81 1H m, 5.71 1H m, 5.24 1H s, 2.93–3.28 4H m; ^{13}C NMR (75 MHz, CD_3OD): δ_C 185.9, 167.5, 167.1, 158.1, 157.9 × 2, 157.2, 156.6, 155.6, 151.3, 146.3 × 2, 146.2 × 2, 134.3, 130.5 × 2, 129.0 × 2, 125.8, 122.9, 122.3, 121.1, 121.0, 117.5 × 2, 110.2 × 2, 110.1 × 2, 99.8, 99.3, 97.0 × 2, 96.1, 95.8, 80.3, 75.7, 69.5, 68.2, 27.3, 26.9.0 ppm; negative APCI-MS m/z 867 $[M–H]^-$. These data were identical with the literature [28,30], in combination with its retention time, absorbance spectrum in HPLC-DAD analysis (data not given), Rf value in TLC analysis (Fig. 3) and by cochromatography with authentic standard, and peak 4 was identified as theaflavin-3,3′-digallate.

3.4. Separation of tea extracts

For the separation of different kinds of tea products and high-molecular-mass pigments in fermented tea, HSCCC separations were carried out in the normal-phase partition mode using the two-phase solvent system composed of hexane–ethyl acetate–methanol–water–acetic acid (1:5:1:5:0.25, v/v). Fig. 4A and B shows chromatograms of the extracts of green tea and black tea. In this HSCCC separation, esterified catechins (EGCG, ECG and GCG) were detected and separated (HPLC analysis data not shown) in green tea extract. The purity of EGCG, ECG and GCG was 96.3%, 97.6% and 98.0%, respectively, as determined by HPLC. Data of EGCG,

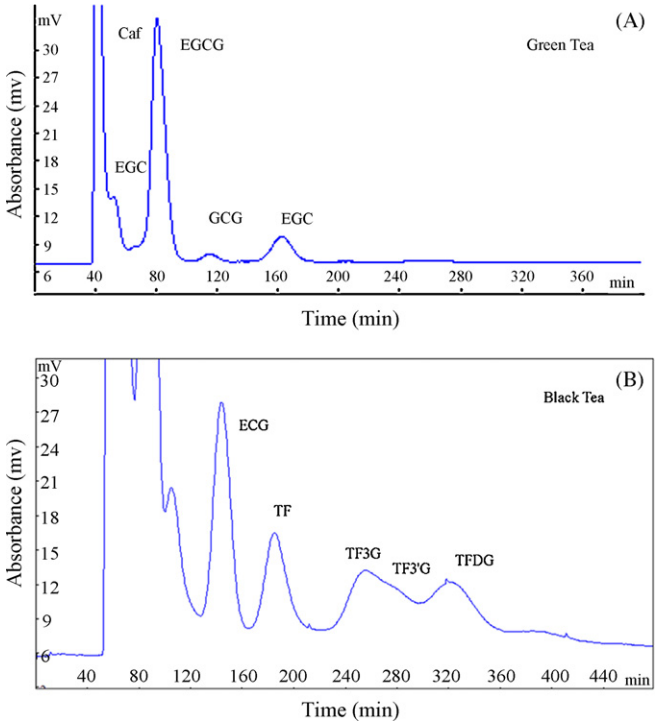

Fig. 4. (A and B) HSCCC profiles of green tea and black extracts. Chromatographic conditions were described in caption of Fig. 2. Caf: caffeine, GCG: gallocatechin gallate.

GCG and ECG are in good agreement with catechin standards (data not given). The apparent shoulder on the first peak was determined to be EGC. Minor impurities were present in the isolated fractions. In those cases, crystallization from an aqueous solution resulted in pure catechins. Decaffeination of the tea by extraction with chloroform prior to HSCCC separation enables the isolation of pure EGC with the purity of 91.7%. In addition to these peaks, theaflavins (TF, TF3G, TF3′G and TFDG) were also separated from the extract of fermented black tea whereas these peaks were not detected in the extract of non-fermented green tea. It is noteworthy that a successful separation of catechins by HSCCC employing the solvent system hexane–ethyl acetate–methanol–water–acetic acid (1:5:1:5:0.25, v/v) is performed and allows isolation of catechins from green tea extract and theaflavins from black tea extract.

4. Conclusion

It was demonstrated that HSCCC has a great potential for the preparative isolation of polyphenols from tea. In combination with a suitable extraction and cleanup procedure prior to HSCCC separation, pure reference compounds are obtained on a preparative scale. The results of our study clearly demonstrated that HSCCC could provide highly efficient preparative separation of theaflavins from enzymatic oxidation products of green tea catechins. Meanwhile, the HSCCC isolation of catechins (EGCG, ECG, EGC and GCG) from the aqueous extract of green tea has proved to be successful based on the solvent system mentioned above.

Acknowledgements

This study was supported by a grant from the Ministry of Science and Technology of People's Republic of China (2006DFA33220). We thank Pro. Du FL, Dr. Gong YS and Fu DH for helpful discussions and Chen JH, Huang H and Li YH for some work in

this experiment. We thank Pro. Zhang TY for reviewing the manuscript.

References

[1] D.A. Balentine, Manufacturing and Chemistry of Tea, American Chemical Society, Washington, DC, 1992, pp. 102.
[2] J.R. Lewis, A.L. Davis, Y. Cai, A.P. Davies, J.P.G. Wilkins, M. Pennington, Phytochemistry 49 (1998) 2511.
[3] L.K. Leung, Y. Su, R. Chen, Z. Zhang, Y. Huang, Z.Y. Chen, J. Nutr. 131 (2001) 2248.
[4] N.J. Miller, C. Castelluccio, L. Tijburg, C. Rice-Evans, FEBS Lett. 392 (1996) 40.
[5] S.W. Liu, H. Lu, Q. Zhao, Y.X. He, et al., BBA 1723 (2005) 270.
[6] C.N. Chen, P.C. Lin, K.K. Huang, W.C. Chen, H.P. Hsieh1, et al., eCAM 2 (2005) 209.
[7] Z. Apostolides, D.A. Balentine, M.E. Harbowy, Y. Hara, J.H. Weisurger, Mutat. Res. 359 (1996) 159.
[8] M. Shiraki, Y. Hara, T. Osawa, H. Kumon, T. Nakayama, S. Kawakishi, Mutat. Res. 323 (1994) 29.
[9] Y.L. Lin, S.H. Tsai, S.Y. Lin-Shiau, C.T. Ho, J.K. Lin, Eur. J. Pharmacol. 367 (1999) 379.
[10] J.B. Lu, C.T. Ho, G. Ghai, K.Y. Chen, et al., Cancer Res. 60 (2000) 6455.
[11] Y.Y. Tu, A.B. Tang, N. Watanabe, ABBS 36 (2004) 508.
[12] Y.S. Zhen, Z.M. Chen, S.J. Cheng, et al., Tea Bioactivity and Therapeutic Potential, Taylor & Francis, CRC Press Inc., 2002.
[13] A.L. Davies, Y. Cai, A.P. Davies, Magn. Reson. Chem. 33 (1995) 549.
[14] X.L. Cao, High-Speed Counter-Current Chromatography Technique and Application, Chemical Industry Press, Beijing, 2005.
[15] Q.Z. Du, H.Y. Jiang, Y. Ito, J. Liq. Chrom. Rel. Technol. 24 (2001) 2363.
[16] C.J. Yang, D.X. Li, X.C. Wan, J. Chromatogr. B 861 (2008) 140.
[17] X.L. Cao, J.R. Lewis, Y. Ito, J. Liq. Chrom. Rel. Technol. 27 (2004) 1893.
[18] A. Degenhardt, U.H. Engelhardt, A.S. Wendt, P. Winterhalter, J. Agric. Food Chem. 48 (2000) 5200.
[19] A. Yanagida, A. Shoji, Y. Shibusawa, H. Shindo, M. Tagashira, M. Ikeda, Y. Ito, J. Chromatogr. A 1112 (2006) 195.
[20] H.F. Wang, G.J. Provan, K. Helliwell, Trends Food Sci. Technol. 11 (2000) 152.
[21] Y. Hara, Green Tea: Health Benefits and Applications, CRC Press, New York, 2001.
[22] R. Amarowicz, F. Shahidi, Food Res. Intern. 29 (1996) 71.
[23] A.L. Davis, Y. Cai, A.P. Davies, J.R. Lewis, Magn. Reson. Chem. 34 (1996) 887.
[24] A. Degenhardt, U.H. Engelhardt, C. Lakenbrink, P. Winterhalter, J. Agric. Food Chem. 48 (2000) 3425.
[25] T.Y. Zhang, X.L. Cao, X. Han, J. Liq. Chrom. Rel. Technol. 26 (2003) 1565.
[26] K.B. Wang, Z.H. Liu, S.J. Zhao, D.H. Fu, J.A. Huang, J. Tea Sci. (Chin.) 27 (2007) 192.
[27] K.B. Wang, Z.H. Liu, J.A. Huang, Y.S. Gong, Chin. J. Chromatogr. 22 (2004) 151.
[28] S.M. Sang, J.D. Lambert, S.Y. Tian, J. Hong, Z. Hou, et al., Bioorg. Med. Chem. 12 (2004) 459.
[29] Y. Ito, J. Chromatogr. A 1065 (2005) 145.
[30] S. Scharbert, M. Jezussek, T. Hofmann, Eur. Food Res. Technol. 218 (2004) 442.