Determination of catechins in matcha green tea by micellar electrokinetic chromatography

David J. Weiss*, Christopher R. Anderton

Department of Chemistry, University of Colorado at Colorado Springs, Colorado Springs, CO 80918, USA

Abstract

Catechins in green tea are known to have many beneficial health properties. Recently, it has been suggested that matcha has greater potential health benefits than other green teas. Matcha is a special powdered green tea used in the Japanese tea ceremony. However, there has been no investigation to quantitate the catechin intake from matcha compared to common green teas. We have developed a rapid method of analysis of five catechins and caffeine in matcha using micellar electrokinetic chromatography. Results are presented for water and methanol extractions of matcha compared with water extraction of a popular green tea. Using a mg catechin/g of dry leaf comparison, results indicate that the concentration of epigallocatechin gallate (EGCG) available from drinking matcha is 137 times greater than the amount of EGCG available from China Green Tips green tea, and at least three times higher than the largest literature value for other green teas.
© 2003 Elsevier B.V. All rights reserved.

Keywords: Tea; Food analysis; Catechins; Epigallocatechin gallate

1. Introduction

Tea is perhaps the most popular drink in the world besides water [1–3]. Recently, there has been increased interest in understanding the relationship between our health and diet. With this in mind, a number of studies have been performed in order to investigate the relationship between green tea consumption and health. For example, recent evidence suggests that there is an inverse relationship between tea consumption and ischemic heart disease [4]. In addition, green tea polyphenolic compounds are known to act as strong antioxidants [5–10].

One of the most intriguing properties of green tea has been its proposed chemo-preventative effects [9–15]. For example, Imai and co-authors studied 8552 Japanese men and women and found a negative relationship between green tea consumption and cancer incidence [13]. Tea drinking has also been found to decrease the concentration of biomarkers for oxidative stress after smoking [12]. One possible explanation for this effect is that polyphenolic compounds show good inhibition of proteolytic enzymes such as urokinase which cancers need to invade cells and form metastases [11]. More recently, Fujiki and co-authors demonstrated the inhibition of tumor necrosis factor-α (TNF-α) by epigallocatechin gallate (EGCG) found in green tea. This cytokine is

---

*Corresponding author. 1420 Austin Bluffs Parkway, SB-1, Colorado Springs, CO 80918, USA. Tel.: +1-719-262-3565; fax: +1-719-262-3047.

E-mail address: dweiss@uccs.edu (D.J. Weiss).

required for tumor development [14] and the inhibition of TNF-α is believed to be one of the most important activities of EGCG in green tea.

EGCG is the catechin best known for its chemoprotective properties and is the main bioactive component of green tea after caffeine [1,11,14,15]. However, in green teas the other predominant flavonoids are (−)-epigallocatechin (EGC), (+)-catechin (C), (−)-epicatechin (EC), and (−)-epicatechin gallate (ECG). Fig. 1 shows the structures of the major bioactive compounds in green tea. The polyphenolic compounds shown here are also believed to possess similar properties to EGCG. Tea is a popular beverage in the US and can be found in grocery stores and coffee houses such as Starbucks Coffee®. Considering the nutraceutical properties of green tea, health specialists [16] have suggested that people drink green tea for its therapeutic value.

Weil et al. [16], have suggested that instead of drinking more common green teas, people should drink the green tea matcha which is used in the Japanese tea ceremony. Matcha is grown in 90% shade, whereas green teas are usually grown in bright sunlight [17]. As a result, the amount of catechins available from matcha are expected to be different than catechin levels from other green teas as sunlight affects the composition and concentrations of catechins present in the tea leaves [9]. In addition, matcha leaves are finely ground into a powder using a stone mill [17]. In other green teas the leaves are rolled and packed into bags. The teas are prepared differently as well. With more widely
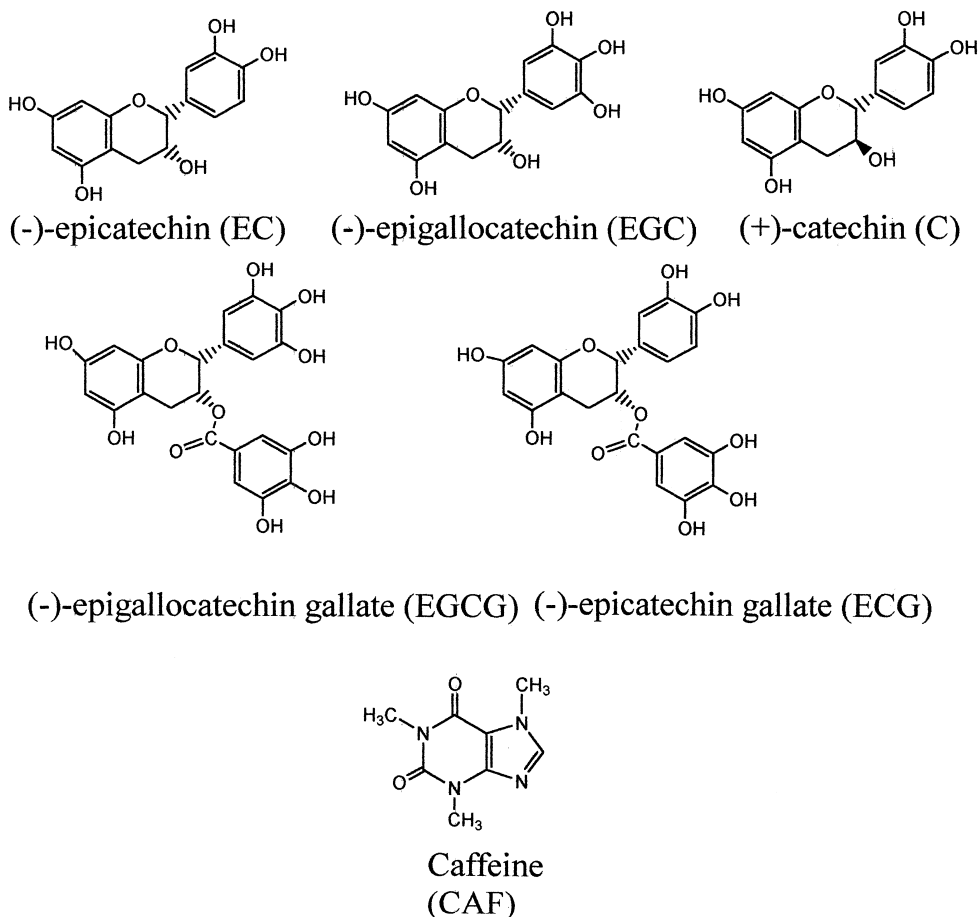

Fig. 1. Structures of catechins and caffeine present in matcha and other green teas.

consumed green teas, the leaves are extracted with water and the extract is consumed. For matcha, water is added to the fine powdered leaves and the leaves themselves are consumed.

Goto et al. [18] used the acetonitrile–water extraction method of Suematsu et al. [19] for the analysis of matcha by gradient LC and identified EC, EGC, ECG, and EGCG present in the tea. This report indicated that as a percentage of the dry leaf, there were greater quantities of catechins in the leaves of another green tea, sencha, than in matcha if the leaves were extracted in the same manner. However, if the health and wellness experts Weil and his co-workers [16] are correct, there must be a greater amount of EGCG consumed as a result of drinking matcha than other green teas since the powdered leaves themselves are ingested. To date, the concentrations of catechins obtained from drinking matcha have not been investigated. Is there more EGCG obtained from matcha than normal green tea? This question has remained unanswered.

Separation and identification of the components of green teas has been traditionally performed using liquid chromatography. However, these analyses are typically slow, requiring complex and time consuming gradients [2,3,20–23]. Early use of capillary electrophoresis in the zone mode [24] for the separation of catechins in green tea suffered from poor peak resolution. A complimentary method for the determination of catechins is the micellar electrokinetic chromatography (MEKC) mode of capillary electrophoresis. MEKC offers greater efficiency, selectivity, and speed compared with LC for catechins [25–31]. For example, Barroso and co-authors [29] separated the catechins in a green tea sample in 14 min using MEKC compared to the 30 min required for a green tea sample as reported by Merken et al. with LC [20].

Although MEKC works well for green tea that is extracted with water from the whole or broken leaves, matcha is a powder and not prepared in the same manner as typical green teas. Our goal was to determine the quantity of catechins available upon consumption of the powdered leaves which requires a more complete extraction of catechins from the tea. We planned to separate the catechins in both a widely available green tea and matcha, and compare both concentration results. The green tea was to be

extracted with water and catechins were expected to be in the range reported in the literature [21,27]. The extraction of the matcha was to be accomplished with methanol in order to obtain as much catechins as possible to simulate the amount of catechins available from the powder.

To our knowledge, this paper is the first to report the separation of catechins in matcha using MEKC. This work presents a comparison between the concentration of catechins available from matcha and those of a common commercial green tea available in the United States. Results indicate that drinking matcha provides greater than 100 times more EGCG than drinking China Green Tips green tea based upon a mg catechin/g dry leaf comparison.

2. Experimental

2.1. Chemicals

Caffeine (CAF), (−)-epicatechin (EC), (+)-catechin (C), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin-3-gallate (EGCG), and 4-amino-2-hydroxybenzoic acid (AHBA) where purchased from Sigma (St. Louis, MO, USA) and used as received. Sodium hydroxide, sodium phosphate dibasic, sodium dodecyl sulfate (SDS), and HPLC-grade methanol where purchased from Fisher Scientific (Fairlawn, NJ, USA) and used as received. Deionized water was obtained daily using a Corning Mega-Pure system MP-190 (Corning, NY, USA).

2.2. CE instrumentation

Experiments where performed using an Agilent Technologies (Waldbronn, Germany) capillary electrophoresis unit with diode array detection. After initial experiments were performed to determine the optimum wavelength for detection, the detector wavelength was fixed at 200 nm. An 80.5 cm (effective length of 72.0 cm)×50 μm I.D. fused-silica bubble capillary (Agilent Technologies, Germany) was used as the separation capillary. At the beginning of the day, the capillary was flushed with 1 M NaOH for 10 min, followed by deionized water for 15 min, and then with the run buffer for 15 min.

Injection was performed using pressure at 50 mbar/ s, for 3 s. Pre-conditioning of the capillary between runs was done with 1 M NaOH for 1 min, deionized water for 1 min, and finally the run buffer for 1 min. At the end of the day, the capillary was flushed with deionized water and the capillary ends where placed in water vials overnight.

2.3. Preparation of standards and tea samples

Standard solutions (C, EC, ECG, EGC, EGCG, CAF, AHBA) where initially prepared at 2 mg/ml by dissolving the appropriate amount of solid in 100% methanol. The standards were then diluted to 150 μg/ml or 60 μg/ml using methanol, or deionized water. China Green Tips (Tazo, Portland Oregon) tea was purchased at a local Starbucks Coffee house. This tea was prepared by immersing a bag of tea in a beaker of 75.0 ml of boiling deionized water for 10 min. The mass of the tea leaves available from the bag was 2.1290±0.0372 g (n=3).

Matcha was acquired from Polaris Health (Spera, Stamford, CT) and used as received. The matcha sample was stored at −20 °C in the freezer when not in use. Matcha samples extracted with water were prepared by the traditional Japanese method [16] using a bamboo bowl and wisk. A small amount of hot water was added to the bowl to warm the bowl. The bowl was then dried and a half teaspoon (1.0 g) of matcha was added to the bowl. This was followed by adding 85.0 ml of 85 °C water and stirring with the bamboo whisk to make a frothy green suspension of the tea.

Matcha samples dissolved in methanol were prepared using 1.0 g of matcha dissolved in 25.0 ml of methanol. The sample was stirred on a stir plate for 10 min to extract all of the components of the tea. The beaker was covered during this time to minimize loss of methanol. All samples of green tea and matcha were filtered with 0.2 μm nylon syringe filters before use. Experiments run with and without filtering with nylon filters did not show significantly different results for samples of China Green Tips green tea. It was particularly necessary to filter the matcha samples since the leaves precipitated in the sample tubes and clogged the capillaries in unfiltered samples. AHBA was used as an internal standard

[27,28] and all green tea and matcha samples were spiked with 60 μg/ml AHBA before analysis. All tea samples prepared in water were done so using deionized water. Identification of the compounds was performed using sample spiking as well as UV spectra from the diode-array detector.

3. Results and discussion

3.1. Separation of catechin standards by MEKC

MEKC methods for the separation and identification of green teas have been reported using buffers with a pH anywhere between 2 and 9 [27–31]. Recently, Worth et al. found the greatest stability of catechin standards at acidic pH [27]. Worth et al. flushed their capillaries with NaOH followed by HCl and ran their experiments at a pH of 2.5 with addition of both SDS and methanol to the run buffer. Unfortunately, we were unable to reproduce their results in our laboratory. At pH 2.5 it took approximately 50 min for catechin and caffeine to elute, even without the addition of SDS. This result was clearly unacceptable and a new buffer system was needed.

Since polyphenolic compounds are known to become negatively charged and reactive above pH 7.5, we chose a pH of 7 for our run buffer to ensure catechin stability [27,31]. Starting with a 25 mM phosphate buffer at a pH of 7.0 we increased the micelle concentration from 0 to 150 mM using a 150 μg/ml standard mixture. At 0 mM SDS there was little separation of the catechins and no peaks were resolved. With addition of SDS, both the migration times and resolution increased. The limiting factor was the resolution between caffeine and catechin. Above 40 mM SDS there was little improvement in resolution for caffeine and catechin. Therefore, 20 mM SDS was chosen as the optimum surfactant concentration under these conditions.

The typical concentrations of catechins in green tea range from 2 to 200 μg/ml [25,27], or 0.6–21 mg catechin/g dry leaf [21], depending on the source and preparation. Therefore, we wanted to make our separation and standards perform well at a broad range of concentrations and chose a lower concentration (60 μg/ml) to run our tea samples than

we used to set up the general separation method. The rationale behind this is that we found experimentally that the green tea samples had concentrations of catechins on the lower end of the normal range. However, the matcha samples had catechin concentrations that were at the high end of the typical range for green teas. A lower concentration standard ensures that the separation works at both high and low concentrations. Fig. 2 shows an electropherogram for a 60 μg/ml mix of standard catechins, caffeine and internal standard (AHBA) with the addition of 20 mM SDS to the run buffer. At 60 μg/ml the resolution of the standards was greater than 1.5. At 150 μg/ml all peaks were separated at baseline resolution. Using a 25 mM phosphate buffer at a pH of 7.0 with the addition of 20 mM SDS, detection limits of the seven compounds using the bubble capillary ranged from 0.1 to 1 μg/ml, which is below the concentrations necessary for analysis of green tea.

3.2. Analysis of green tea

China Green Tips green tea was used as it is widely available from Starbucks Coffees and is made of *Camellia Sinensis* tea leaves with no additional plant mater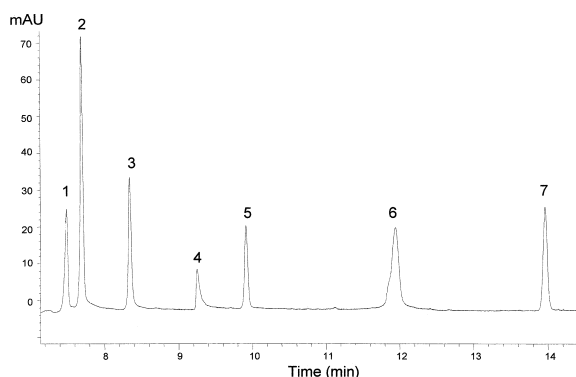ials. Samples were prepared in a manner typical to what one might do when planning to drink the tea. Unfortunately, matrix effects were observed with the EGCG peak which is not well resolved from its neighboring peak. As a result, we could not accurately quantitate the EGCG in this sample.

The migration time, migration order, and selectivity can be affected by the use of an organic modifier [27,28,30,32–35]. Using this approach, methanol was added to improve the separation of catechins in the tea samples. Fig. 3 presents an electropherogram for the separation of a green tea sample with the addition of 5% methanol. Since baseline separation was obtained, additional methanol was not added to the run buffer as it would only increase analysis time. Table 1 presents the concentrations of caffeine and catechins for the green tea sample shown in Fig. 3. These values are within the range typically reported for catechins in green teas [21,30].

3.3. Analysis of matcha

Matcha was prepared in the traditional manner [16] in water and syringe filtered before analysis. Samples of the water portion of the matcha were analyzed in order to compare those results to the water extracted portion of the China Green Tips green tea. Fig. 4 shows an electropherogram of the matcha sample prepared in water under the separation conditions optimized for the standards. Caf-
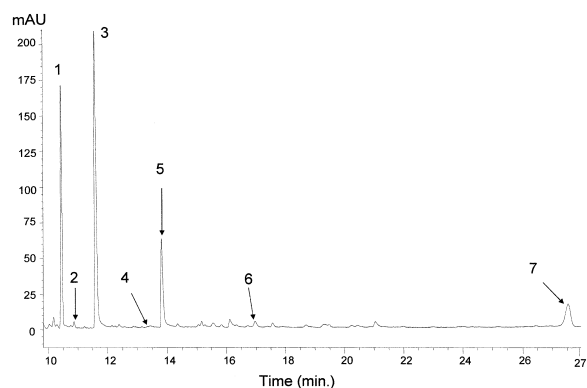

Fig. 2. Electropherogram of 60 μg/ml catechin standards and caffeine with internal standard, AHBA. Electrophoretic conditions: 80.5 cm (77.0 cm effective length)×50 μm I.D. bubble capillary; run buffer 25 mM phosphate buffer pH 7.0–20 mM SDS, electrophoresis voltage 27 kV, detection at 200 nm. Sample is in deionized water. Peak identification: (1) caffeine (CAF); (2) catechin (C); (3) epigallocatechin (EGC); (4) epigallocatechin gallate (EGCG); (5) epicatechin (EC); (6) epicatechin gallate (ECG); (7) internal standard (AHBA).

Fig. 3. Electropherogram of a green tea sample prepared in deionized water. Electrophoretic conditions: 80.5 cm (72.0 cm effective length)×50 μm I.D. bubble capillary; run buffer 25 mM phosphate buffer pH 7.0–20 mM SDS 5% methanol, electrophoresis voltage 27 kV, detection at 200 nm. Peak identification: see Fig. 2.

Table 1
Comparison of catechins in green tea and matcha green tea (in mg catechin/g dry leaf)

Compound	Green Tea (mg/g)	Matcha MeOH extract (mg/g)	Matcha water extract (mg/g)
Caffeine (CAF)	3.9 (0.35)	6.4 (1.6)	23.9 (0.34)
Catechin (C)	6.2 (0.01)	0.83 (0.26)	n.d.
Epigallocatechin (EGC)	6.2 (0.05)	12.6 (3.4)	0.75 (0.04)
Epigallocatechin gallate (EGCG)	0.42 (0.05)	57.4 (17.5)	0.32 (0.03)
Epicatechin (EC)	4.4 (0.30)	4.0 (0.77)	2.4 (0.11)
Epicatechingallate (ECG)	0.10 (0.02)	12.8 (2.6)	0.25 (0.03)

Preparation of teas: green tea-extraction of China Green Tips green tea with 75.0 ml of boiling water using a tea bag. Matcha (MeOH extract)-extraction of 1.0 g matcha with 25.0 ml of 100% methanol. Matcha (water extract)-extraction of 1.0 g of matcha using the traditional Japanese method with 85.0 ml of water at 85 °C. The number in parenthesis is the standard deviation for triplicate runs. n.d.=not determined (under limits of detection).

feine was the largest peak and had the highest concentration of any of the compounds in the water extract (Table 1). The concentration of catechin (C) was under the limit of detection and lower concentrations of catechins were present in this fraction than in the China Green Tips tea. This result was expected since matcha is consumed almost immediately after preparation, whereas other green teas are steeped for a few minutes.

Despite the fact that matcha is prepared in water, the tea powder itself is ingested. To get a more quantitative idea of the amount of catechins available
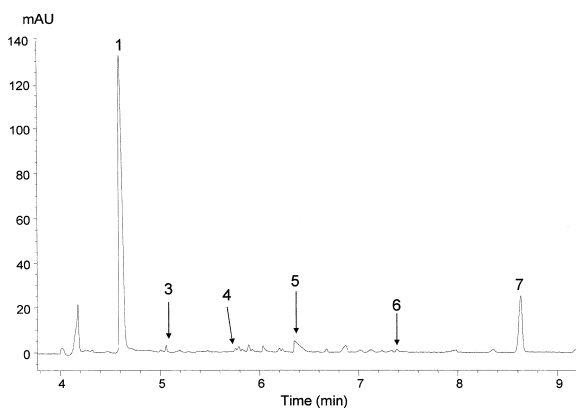

Fig. 4. Electropherogram of a matcha tea sample prepared in deionized water using the traditional Japanese method. Electrophoretic conditions and peak identification: see Fig. 2.

in matcha upon its ingestion, the matcha sample was extracted with methanol. Sample standards which were prepared in 100% methanol not only had the migration times increased compared to the results in Fig. 2, but the peaks were skewed and broad (not shown). This effect was expected as a result of "destacking", where the methanol in the sample increases the viscosity and slows migration in the methanol zone [36]. Analytes move slower in the methanol zone than in the run buffer. When they hit the run buffer they will speed up broadening the peaks.

In order to optimize the extraction of the catechins in the matcha sample, extraction with 30 and 60% methanol were performed and compared to the electropherograms from the 100% methanol extractions. As expected, sample standards prepared in less methanol were improved in peak shape and decreased in migration time compared to those prepared with 100% methanol. However, matcha that had been extracted with 30% methanol did not result in improved extraction compared with the water fractions of matcha presented in Fig. 4. Matcha prepared with 60% methanol resulted in increased extraction of catechins compared to the 30% methanol extraction. Still, the peaks from the 100% methanol extraction produced the maximum response obtained.

For matcha samples extracted with 100% metha-

nol the EGCG peak was the largest peak in the electropherogram. However, matrix effects were evident for the EC peak. In order to improve the peak shape and resolution, 5% methanol was added to the run buffer to slow down the electroosmotic flow and improve the separation. The result of addition of methanol to the run buffer is shown in Fig. 5. EC has the same fronting as in the sample standards, but it is separated from the rest of the sample matrix.

Compared to the method used by Goto et al. [18] for the separation of matcha using LC, this method does not require gradient elution, nor long extraction times. If concentrations in μg/ml are compared, the amount of EGCG in the water extracted China Green Tips green tea was 11.8 μg/ml, whereas the EGCG concentration from the methanol extracted matcha was 2,297 μg/ml. Therefore, there was approximately 200 times more EGCG available from the matcha than the other green tea. However, this comparison does not take into account the amount of catechins per dry weight of the leaves. The concentration of the catechins and caffeine present in matcha extracted using 100% methanol are presented in Table 1 as mg catechin/g dry leaf. Using this comparison, these results indicate that the concentration of EGCG obtained from matcha is 137 times greater than from China Green Tips green tea. The concentration of the majority of the catechins present were increased compared to those obtained from China Green Tips green tea as well. In addition, the concentration of EGCG was dramatically increased compared with even the highest concentrations of this catechin reported in green teas [21,27].

4. Conclusions

We have presented the first analysis of the Japanese ceremonial green tea, matcha, by MEKC. Addition of methanol was necessary in order to overcome matrix effects and produce baseline resolution of all important peaks. The additional catechins obtained upon drinking matcha compared with other green teas may be the result of the manner in which people consume a typical green tea compared with matcha. These results suggest that drinking matcha will result in dramatically greater intake of EGCG compared to drinking other types of green tea.

Acknowledgements
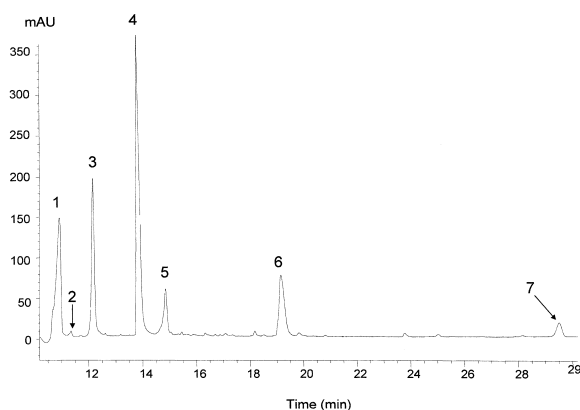
Fig. 5. Electropherogram of a matcha sample prepared in 100% methanol. Electrophoretic conditions: 80.5 cm (72.0 cm effective length)×50 μm I.D. bubble capillary; run buffer 25 m*M* phosphate buffer pH 7.0–20 m*M* SDS–5% methanol, electrophoresis voltage 27 kV, detection at 200 nm. Peak identification: see Fig. 2.

References

[1] V.E. Steele, G.J. Kelloff, D. Balentine, C.W. Boone, R. Mehta, D. Bagheri, C.C. Sigman, S. Zhu, S. Sharma, Carcinogenesis 21 (2000) 63.
[2] H. Horie, K. Kohata, J. Chromatogr. A 881 (2000) 425.
[3] J.J. Dalluge, B.C. Nelson, J. Chromatogr. A 881 (2000) 411.
[4] I.C.W. Arts, P.C.H. Hollman, E.J.M. Feskens, H. Bas Bueno de Mesquita, D. Kromhout, Am. J. Clin. Nutr. 74 (2001) 227.
[5] M.G.L. Hertog, P.C.H. Hollman, B. van de Putte, J. Agric. Food Chem. 41 (1993) 1242.
[6] M. Serafini, A. Ghiselli, A. Ferro-Luzzi, Eur. J. Clin. Nutr. 50 (1996) 28.
[7] G. Cao, E. Sofic, R. Prior, J. Agric. Food Chem. 44 (1996) 3426.
[8] M. Richelle, I. Tavazzi, E. Offord, J. Agric. Food Chem. 49 (2001) 3438.
[9] L. Bravo, Nutr. Rev. 56 (1998) 317.

[10] N.C. Cook, S. Samman, Nutr. Biochem. 7 (1996) 66.

[11] J. Jankun, S.H. Selman, R. Swiercz, E. Skrzypczak-Jankun, Nature 387 (1997) 561.

[12] J.E. Klaunig, Y. Xu, C. Han, L.M. Kamendulis, J. Chen, C. Heiser, M.S. Gordon, E.R. Mohler, Proc. Soc. Exp. Biol. Med. 220 (1999) 249.

[13] K. Imai, D. Litt, K. Suga, K. Nakachi, Preventative Med. 26 (1997) 769.

[14] H. Fujiki, M. Suganuma, S. Okabe, E. Sueoka, K. Suga, K. Imai, K. Nakachi, Cancer Detect. Prevention 24 (2000) 91.

[15] M.A. Lea, Q. Xiao, A.K. Sadhukhan, S. Cottle, Z.-Y. Wang, C.S. Yang, Cancer Lett. 68 (1993) 231.

[16] A. Weil, R. Daley, The Healthy Kitchen, Alfred A. Knopf, New York, 2002,44.

[17] J. Pettigrew, The Tea Companion, Quintet Publ, NY, 1997, p. 168.

[18] T. Goto, Y. Yoshida, K. Masaaki, H. Nagashima, J. Chromatogr. A 749 (1996) 295.

[19] S. Suematsu, Y. Hisanobu, H. Saigo, R. Matsuda, Y. Komatsu, Nippon Shokuhin Kagaku Kogaku Kaishi 42 (1995) 419.

[20] H.M. Merken, G.R. Beecher, J. Chromatogr. A 897 (2000) 177.

[21] J.J. Dalluge, B.C. Nelson, J.B. Thomas, L.C. Sander, J. Chromatogr. A 793 (1998) 265.

[22] W.E. Bronner, G.R. Beecher, J. Chromatogr. A 805 (1998) 137.

[23] J.-K. Lin, C.-L. Lin, Y.-C. Liang, S.-Y. Lin-Shiau, I.-M. Juan, J. Agric. Food Chem. 46 (1998) 3635.

[24] H. Horie, T. Mukai, K. Kohata, J. Chromatogr. A 758 (1997) 332.

[25] T. Watanabe, R. Nishiyama, A. Yamamoto, S. Nagai, S. Terabe, Anal. Sci. 14 (1998) 435.

[26] H. Horie, K. Kohata, J. Chromatogr. A 802 (1998) 219.

[27] C.C. Worth, M. Wieβler, O.J. Schmitz, Electrophoresis 21 (2000) 3634.

[28] D. Stach, O.J. Schmitz, J. Chromatogr. A 924 (2001) 519.

[29] M.B. Barroso, G. van de Werken, J. High Resolut. Chromatogr. 22 (1999) 225.

[30] B.C. Nelson, J.B. Thomas, S.A. Wise, J.J. Dalluge, J. Microcol. Sep. 10 (1998) 671.

[31] P.J. Larger, A.D. Jones, C. Dacombe, J. Chromatogr. A 799 (1998) 309.

[32] M. Idei, I. Mezo, Z. Vadasz, A. Horvath, O. Teplan, G.Y. Keri, J. Liq. Chromatogr. 15 (1992) 3181.

[33] S.M. Lunte, D.M. Radzik (Eds.), Pharmaceutical and Biomedical Applications of Capillary Electrophoresis, Elsevier, New York, 1996, p. 345.

[34] R.O. Cole, M.J. Sepaniak, W.L. Hinze, J. Gorse, K. Oldiges, J. Chromatogr. 557 (1991) 113.

[35] D.J. Weiss, C.E. Lunte, Electrophoresis 21 (2000) 2080.

[36] S.H. Hansen, J. Tjornelund, I. Bjornsdottir, Trends Anal. Chem. 15 (1996) 175.