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Determination of daidzein and genistein in soybean foods by automated on-line in-tube solid-phase microextraction coupled to high-performance liquid chromatography

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Abstract

An automated on-line method for the determination of the isoflavones, daidzein and genistein, was developed using in-tube solid-phase microextraction coupled to high-performance liquid chromatography (in-tube SPME–HPLC). In-tube SPME is a new extraction technique for organic compounds in aqueous samples, in which analytes are extracted from the sample directly into an open tubular capillary by repeated draw/eject cycles of sample solution. Daidzein, genistein and their glucosides tested in this study were clearly separated within 8 min by HPLC using an XDB-C_8 column with diode array detection. In order to optimize the extraction of these compounds, several in-tube SPME parameters were examined. The glucosides daidzin and genistin were analyzed as aglycones after hydrolysis because the glucosides were not concentrated by in-tube SPME. The optimum extraction conditions for daidzein and genistein were obtained with 20 draw/eject cycles of 40 μl of sample using a Supel-Q porous layer open tubular capillary column. The extracted compounds were easily desorbed from the capillary by mobile phase flow, and carryover was not observed. Using the in-tube SPME–HPLC method, the calibration curves of these compounds were linear in the range 5–200 ng/ml, with a correlation coefficient above 0.9999 ($n=18$), and the detection limits ($S/N=3$) were 0.4–0.5 ng/ml. This method was successfully applied to the analysis of soybean foods without interference peaks. The recoveries of aglycones and glucosides spiked into food samples were above 97%.

Keywords: Solid-phase microextraction; Automation; Soybean; Food analysis; Isoflavones; Daidzein; Genistein; Aglycones

1. Introduction

Recently, soy ingredients have received a great deal of attention all over the world as functional, healthy components [1–3]. Epidemiological studies have indicated that the consumption of soybeans may be associated with a reduction in the risk of breast cancer [3–5], prostate cancer [3,4,6], colon cancer [3,4], hyperlipidemia [3,7], cardiovascular disease [3,8,9] and osteoporosis [3,10]. In this respect, isoflavones in soybeans have been reported to have antioxidant [11], anticarcinogenic [2,4] and anti-osteoporosis [12,13] activities. Isoflavones including daidzein and genistein are found almost exclusively in soybeans, which contain daidzin and genistin as

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the main glucosides. Thus, isoflavones are useful as food supplements for the purposes of enhancing human health and preventing the above diseases. Daidzin and genistin are hydrolyzed to daidzein and genistein, respectively, by β-glucosidase in the gastrointestinal system. As shown in Fig. 1, they have a structure similar to the hormone estrogen and share some of its physiological properties. For these reasons, isoflavones are sometimes called phytoestrogens. Therefore, isoflavones may not only have a variety of desirable physiological effects on the body, but they may also act as endocrine disruptors [14,15]. Exposure to these phytoestrogens may pose a developmental hazard to infants, because soy products are becoming increasingly popular as infant foods. Though an accurate assessment of the evaluation of these contradictory health effects is difficult, knowing the existence and amount of isoflavones in foods is important.

The determination of daidzein and genistein in food samples has been carried out by gas chromatography (GC) [16,17], GC–mass spectrometry (GC–MS) [18–21], high-performance liquid chromatography (HPLC) [22–27], liquid chromatography–mass spectrometry (LC–MS) [28–33] and capillary electrophoresis (CE) [34]. These methods involve processes such as sampling, sample preparation, separation, detection and data analysis, and more than 80% of the analysis time is spent on sampling and sample preparation steps such as extraction, concentration, fractionation and isolation. For the extraction of daidzein and genistein from food samples, liquid–liquid extraction, counter-current chromatography and solid-phase extraction have been used. However, most of these techniques are complicated and time-consuming, and require relatively large volumes of organic solvent. More complicated pretreatment may cause error, and the use of large volumes of organic solvent may pose a health hazard to the analyst as well as cause environmental pollution. On the other hand, a solid-phase microextraction (SPME) method [33] using a fused-silica fiber coated on the outside with a stationary phase was recently developed as a solvent-free sample preparation method for the analysis of isoflavones. However, this technique is manual, and the fiber must be handled carefully because the coating is prone to being stripped off from the needle during insertion and removal from the desorption chamber [35]. Therefore, it is very important to develop an efficient sample pretreatment method. Automating the operation will ensure savings of labor and cost of the analysis work.

In-tube solid-phase microextraction (in-tube SPME) [35–41] is a preconcentration technique using an open tubular fused-silica capillary with an inner surface coating as the SPME device, which can be easily coupled on-line with HPLC and LC–MS. A technique using a GC capillary tube (also known as open-tubular trapping) can be coupled on-line with

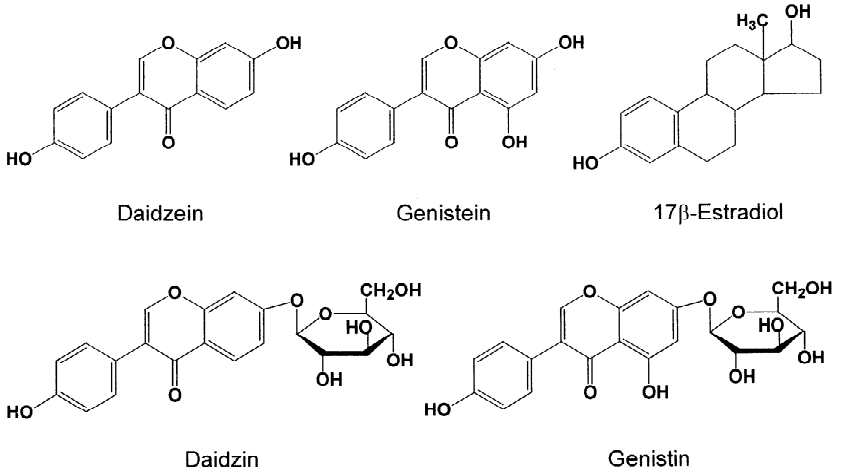

Fig. 1. Chemical structures of isoflavones and 17β-estradiol.

GC [42]. In-tube SPME allows for convenient automation of the extraction process, which not only shortens the analysis time, but also provides better accuracy, precision and sensitivity relative to off-line manual techniques. It has been successfully applied to a wide variety of compounds such as drugs, pesticides and endocrine disruptors when coupled with HPLC [43–47] or LC–MS [48–54].

In this paper, we report an automated on-line method for the determination of daidzein and genistein by in-tube SPME coupled with a HPLC photodiode array detection system. This method was also applied to the analysis of several food samples.

2. Experimental

2.1. Reagents

Fig. 1 shows the isoflavones used in this study. Daidzein was purchased from Sigma (St. Louis, MO, USA). Genistein, daidzin and genistin were purchased from Wako (Osaka, Japan). β-Naphthol as an internal standard (I.S.) and *tert*.-butylhydroxytoluene (BHT) as an antioxidant were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Each compound was dissolved in methanol to make separate stock solutions at a concentration of 1 mg/ml, except for BHT, which was prepared as a 1% solution in methanol. The solutions were stored at 4 °C and used after dilution with distilled water to the required working concentrations. All solvents and water used in this study were of HPLC grade. All other chemicals were of analytical grade.

2.2. Instrument and analytical conditions

The HPLC system was a Model 1100 series (Agilent Technologies, Boeblingen, Germany), which consisted of a binary pump, an on-line degasser, an autosampler, a column compartment, a photodiode array detection (DAD) system, and a HP ChemStation. A Zorbax Eclipse XDB-C_8 column (15 cm×4.6 mm I.D., 5 μm particle size) from Agilent Technologies was used for the HPLC separation. HPLC conditions were as follows: column temperature, 40 °C; mobile phase, programmed by a linear gradient from 40% to 70% methanol in water for a 6-min run and held at 70% for 2 min; flow-rate, programmed in a linear gradient at 1.0 to 1.5 ml/min for a 6-min run and held at 1.5 ml/min for 2 min. UV detection was performed at 255 nm. UV spectra from 190 to 400 nm were also recorded for peak identification. The peak height counts of isoflavones and the I.S. were measured, and the peak height count ratios against the I.S. were calculated to construct calibration curves.

2.3. In-tube solid-phase microextraction

A Supel-Q porous layer open tubular (PLOT) capillary column (60 cm×0.32 mm I.D., 12 μm film thickness, Supelco, Bellefonte, PA, USA) was used as the in-tube SPME device. It was placed between the injection loop and injection needle of the autosampler. The injection loop was retained in the system to avoid fouling of the metering pump. Capillary connections were facilitated by the use of a 2.5-cm sleeve of polyetheretherketone (PEEK) tubing of 1/16-in. thickness at each end of the capillary (1 in.=2.54 cm). A PEEK tubing internal diameter of 330 μm was found to be suitable to accommodate the capillary used. Normal 1/16-in. stainless steel nuts, ferrules and connectors were then used to complete the connections. The autosampler software was programmed to control the in-tube SPME extraction, desorption and injection. Vials (2 ml) were filled with 1 ml of sample in 20 m*M* acetate buffer (pH 4.0), which contains the I.S., and set into the autosampler programmed to control the SPME extraction and desorption technique. In addition, 1.5 ml each of methanol and water in 2-ml autosampler vials with septa were loaded into the autosampler. The capillary column was washed with methanol and then conditioned with water by two repeated draw/eject cycles (40 μl each) of these solvents prior to extraction. The extraction of daidzein and genistein onto the capillary coating was performed by 20 repeated draw/eject cycles of 40 μl of sample at a flow-rate of 100 μl/min with the six-port valve in the LOAD position. After washing the tip of the injection needle with one draw/eject cycle of 2 μl of methanol, the extracted compounds were desorbed from the capillary coating with mobile phase flow and then transported to the LC column by switching the six-port valve to the INJECT position. The

sample was then transferred to the DAD system by means of the mobile phase flow.

2.4. Hydrolysis and preparation of food samples

Several beans and soybean products were purchased from a local supermarket. All samples were stored in their original packaging under recommended conditions (either refrigerated or at room temperature) until use. Solid samples such as dry beans were homogenized with a blender. Semi-solid and liquid samples were used directly. An aliquot of each sample (5–500 mg) was weighed into a 10-ml Pyrex glass tube with a PTFE-lined screw-cap. One milliliter of 2 *M* hydrochloric acid–methanol (1:3) containing 0.05% BHT as an antioxidant was added to the sample. Then, the mixture was hydrolyzed with a loose fitting cap for 2 h at 100 °C in a dry block heater. To the resulting hydrolysate were added 0.05 ml of 10 μg/ml β-naphthol (I.S.) and 0.1 ml of 200 m*M* acetate buffer (pH 4.0), and the final volume was made up to 1 ml with water. The mixture was centrifuged at 3000 *g* for 10 min, then washed with 2 ml of *n*-hexane to remove lipid (if necessary), and the final supernatant was used for in-tube SPME–HPLC analysis.

3. Results and discussion

3.1. High-performance liquid chromatography

In order to select the monitoring wavelength for daidzein and genistein, UV absorption spectra were initially analyzed in the range of 190 to 400 nm by HPLC–DAD with direct liquid injection into the column. Maximum absorption wavelengths were observed at 208, 248 and 302 nm for daidzein, and at 196 and 260 nm for genistein. The wavelength selected for detection of these compounds was 255 nm because more interference is encountered at shorter wavelengths, and sensitivity is lower at longer wavelengths. LC separation of daidzein, genistein and their glucosides was performed using an XDB-C_8 column. As shown in Fig. 2, these compounds were clearly separated within 8 min by gradient elution using methanol–water as a mobile phase.

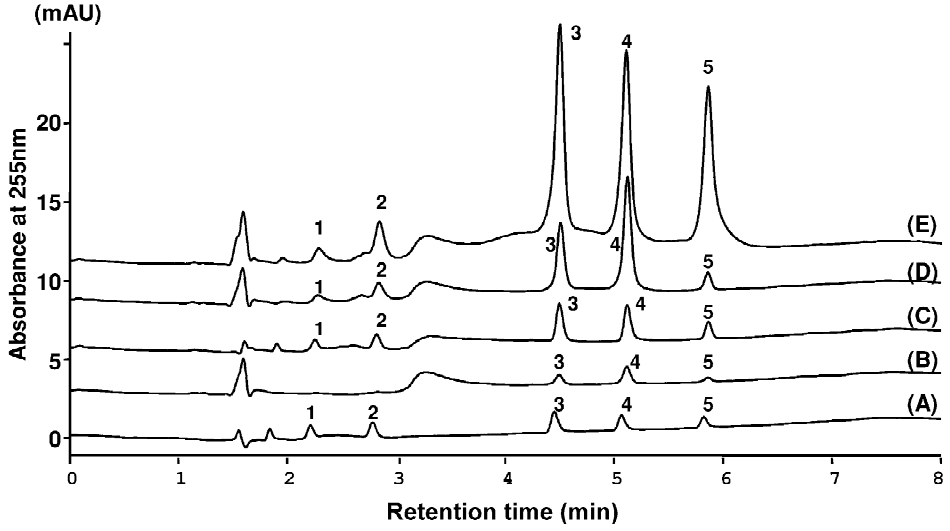

Fig. 2. Effects of capillary coatings on the in-tube SPME of isoflavones. (A) Standard direct injection, (B) DB-1 capillary in-tube SPME, (C) DB-17 capillary in-tube SPME, (D) Omegawax capillary in-tube SPME, (E) Supel-Q PLOT capillary in-tube SPME. Peak: 1, daidzin; 2, genistin; 3, daidzein; 4, genistein; 5, β-naphthol (I.S.). For standard direct injection, 10 μl of standard solution (containing 100 ng/ml of each compound) were injected. For in-tube SPME, each compound was extracted by 20 draw/eject cycles of 40 μl of standard solution at a flow-rate of 100 μl/min. HPLC conditions: see Experimental section.

3.2. Optimization of in-tube solid-phase microextraction and desorption

In order to optimize the extraction of daidzein, genistein and their glucosides by in-tube SPME, several parameters such as the stationary phase of the in-tube SPME capillary column and number and volume of draw/eject cycles were investigated. In this work, four different capillary columns (Omegawax 250, DB-17, DB-1 and Supel-Q PLOT) were evaluated for extraction efficiency. As shown in Fig. 2, the porous polymer-type capillary column (Supel-Q PLOT) gave superior extraction efficiency compared with the liquid phase type capillary columns (Omegawax 250, DB-17 and DB-1). Because the Supel-Q PLOT column has a large adsorption surface area, the extracted amount seemed to be greater than that with liquid phase type columns. The sample load and the amount of compounds extracted increased with increasing inside diameter and film thickness of the column. However, the area of liquid phase contact with the sample under constant draw/eject condition was found to have a greater effect on extraction efficiency than the film thickness of the column. In comparison with the direct injection method, the in-tube SPME method gave a higher concentration efficiency for the aglycones, daidzein and genistein. However, the glucosides, daidzin and genistin, were hard to concentrate using in-tube SPME because of their hydrophilic properties. Therefore, in this study, these glucosides were analyzed as their aglycones after hydrolysis, and the Supel-Q PLOT column was used as an extraction capillary.

In-tube SPME is the extraction method for the distribution of compounds between mobile and stationary phases, and therefore it is important to raise the distribution factor to the stationary phase in order to obtain quicker and higher recovery rates. The extraction time, flow-rate and sample pH are related to the amount of extracted compounds. In order to monitor the extraction time profiles of daidzein and genistein by in-tube SPME, the number of draw/eject cycles was varied from 0 to 20 using a Supel-Q PLOT capillary. As shown in Fig. 3, extraction equilibrium of these compounds was not obtained with 20 cycles; however, it could be analyzed reproducibly because the extraction conditions were fixed using an autosampler. A draw/eject rate of 100 μl/min was optimal for extraction. Below this level, extraction required an inconveniently long time, and above this level, bubbles forming inside the capillary reduced the extraction efficiency. The effect of pH of the sample matrix on the extraction of daidzein and genistein by in-tube SPME was examined using several buffer solutions. As shown in Fig. 4, acidic pH was more effective, and acetate buffer (pH 4.0) was selected because the capillary coating tends to deteriorate under strong acidic conditions. A buffer concentration of 20 m*M* was optimal for extraction. Moreover, extraction efficiency was not affected by alcohol (0–20%) in samples. The absolute amounts of daidzein and

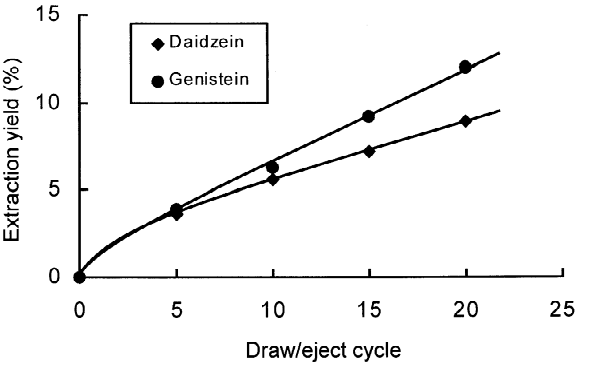

Fig. 3. Effect of number of draw/eject cycles on the in-tube SPME of isoflavones. Each compound was extracted by draw/eject cycles of 40 μl of standard solution using a Supel-Q PLOT capillary.

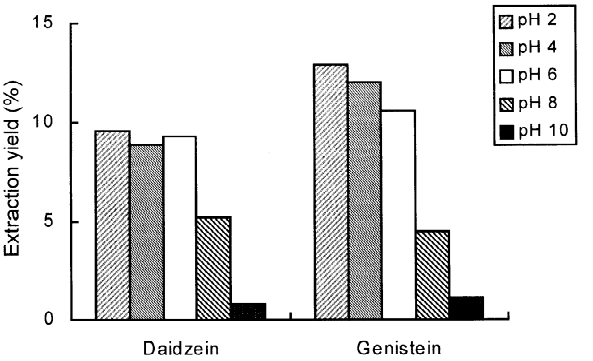

Fig. 4. Effect of sample pH on the in-tube SPME of isoflavones. Each compound was extracted by 20 draw/eject cycles of 40 μl of standard solution at a flow-rate of 100 μl/min using a Supel-Q PLOT capillary.

genistein extracted by the SPME capillary were calculated by comparing peak height counts with the corresponding direct injection of the sample solution onto the LC column. At the sample concentrations of 100 ng/ml, 8.9 ng (9%) of daidzein and 12.0 ng (12%) of genistein were extracted onto the Supel-Q PLOT column by in-tube SPME. Although the extraction yields of these compounds were low, their reproducibility was good (RSD$<$2.7%) due to the use of an autosampler.

The mobile phase was found to be suitable for desorption of daidzein and genistein extracted onto the stationary phase of the capillary column. Static desorption of these compounds from the capillary was achieved by loading the mobile phase onto the capillary column. The desorbed compounds were easily transferred to the LC column with mobile phase flow, and carryover was not observed because the capillary column was washed and conditioned by draw/eject cycles of methanol and mobile phase prior to extraction. The extraction and desorption of daidzein and genistein by the in-tube SPME method were accomplished automatically within 30 min, and automatic analysis of about 48 samples per day was also possible by operating the system overnight.

3.3. Detection limits and calibration curves

Daidzein and genistein gave excellent responses to UV detection, and the detection limits were 0.41 and 0.48 ng/ml, respectively (Table 1), with signal-to-noise ratios of three under our HPLC–DAD conditions. The in-tube SPME method gave 24–31 times higher sensitivity than the direct injection method (10 μl injection), because these compounds in the sample solution were concentrated in the capillary column during draw/eject cycles.

In order to test the linearity of the calibration curve, various concentrations of daidzein and genistein ranging from 5 to 200 ng/ml were analyzed. Calibration curves were constructed from the peak height counts. As shown in Table 1, a linear relationship was obtained for each compound in this range (six-point calibration). The correlation coefficients were above 0.9999, and relative standard deviations were 0.4–8.8% ($n=3$). Peak area counts can be used instead of peak height counts for quantification.

3.4. Optimization of hydrolysis of food samples

Isoflavones in soybean mainly exist as conjugates such as glucosides and their acetyl or malonyl forms [55]. The isoflavones are commonly analyzed as aglycones because standards of some conjugates are not available commercially. Furthermore, as mentioned above, the glucosides are poorly concentrated by in-tube SPME. Daidzin and genistin were hydrolyzed using hydrochloric acid under several conditions in order to release aglycones. As shown in Fig. 5, these glucosides were completely hydrolyzed at 100 °C for 90 min in 0.4 *M* hydrochloric acid. The peaks of the glucosides completely disappeared after hydrolysis, and only the peaks of daidzein and genistein were seen. As described below, the recoveries of daidzein and genistein from samples spiked with daidzin and genistin were about 100%. Therefore, we assume that these glucosides were completely hydrolyzed and quantitatively analyzed as daidzein and genistein. Addition of BHT (optimal concentration 0.05%) was effective to prevent oxidation during hydrolysis.

Table 1
Linear regression data and detection limits of isoflavones by in-tube SPME–HPLC

Compound	Range (ng/ml)	Regression line		Correlation coefficient[a]	Detection limit[b] (ng/ml)	
		Slope	Intercept	(r)	In-tube SPME	Direct injection
Daidzein	5–200	0.0095	0.0161	0.99993	0.41	9.9
Genistein	5–200	0.0106	−0.0003	0.99993	0.48	14.8

[a] $n=18$.
[b] $S/N=3$.

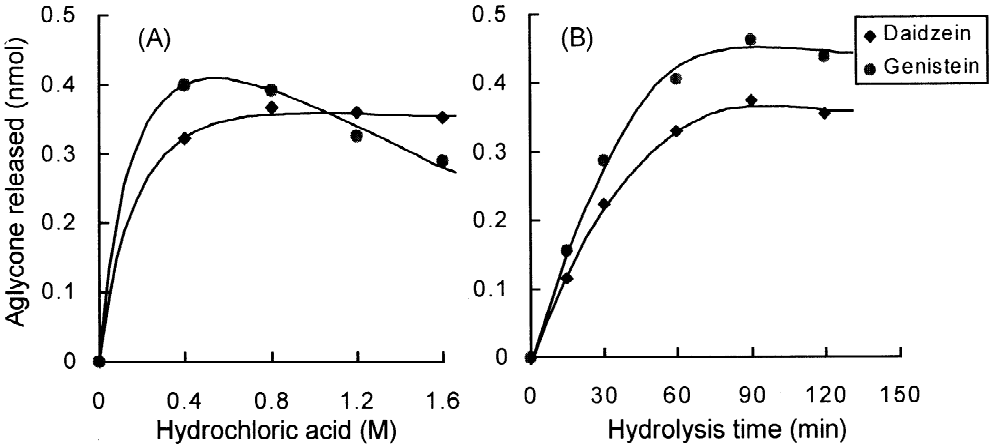

Fig. 5. Effects of (A) hydrochloric acid concentration and (B) hydrolysis time on the release of daidzein and genistein from daidzin and genistin, respectively, by acid hydrolysis.

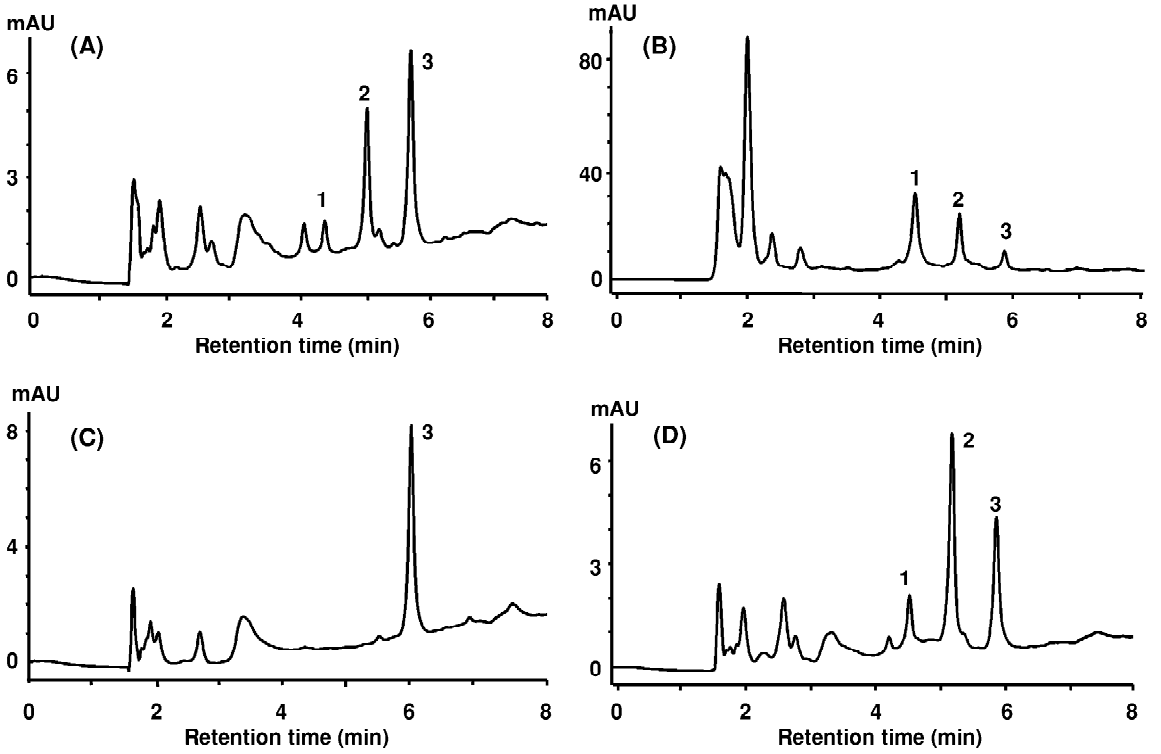

Fig. 6. Chromatograms obtained from several food samples. (A) Dried soybeans (5 mg), (B) soybean paste (500 mg), (C) adzuki beans (5 mg), (D) soy milk (100 mg). Samples were hydrolyzed in 2 *M* hydrochloric acid–methanol (1:3) containing 0.05% BHT at 100 °C. Peak: 1, daidzein; 2, genistein; 3, β-naphthol (I.S.). In-tube SPME–HPLC conditions: see Experimental.

Table 2
Recoveries of isoflavones spiked to dried soybeans

Compound	Sample (mg)	Spiked (μg/g)	Recovery[a] (%)
Daidzein	5.0	4.0	112.1±8.3
Genistein	5.0	4.0	97.8±3.2
Daidzin	5.0	20.0	116.8±6.7
Genistin	5.0	20.0	107.9±3.1

[a] Mean±SD ($n=3$).

3.5. Application to the analysis of food samples

Marketed beans and related processed foods were analyzed using our in-tube SPME–HPLC method. The sampling amount was adjusted so as not to exceed the calibration range. Prior to analysis, the samples were hydrolyzed, partitioned with *n*-hexane, and centrifuged to remove insolubles in the hydrolysate. As shown in Fig. 6, daidzein and genistein were detected from several food samples, and the peaks of these compounds were identified from absorption spectrum analysis with DAD. The concentrations of daidzein and genistein in foods were calculated by using their calibration curves. Daidzein and genistein were detected at high concentrations from the dried soybeans. In order to confirm the validity of this method, known amounts of daidzein, genistein, daidzin and genistin were spiked into food samples, and their recoveries were calculated. As shown in Table 2, the overall recoveries of these compounds were above 97%, and the relative standard deviations were below 2.9–7.4%. As shown in Table 3, daidzein and genistein were detected in soybeans and their processed foods, but not detected in other beans used in this study.

Table 3
Concentrations of isoflavones in several food samples

Food	Content (μg/g)/mean±SD ($n=3$)	
	Daidzein	Genistein
Dried soybeans	74.5±4.6	267.7±16.5
Dried black soybeans	156.0±21.3	435.5±43.5
Field peas	ND[a]	8.0±0.3
Dried broad beans	ND	ND
Dried adzuki beans	ND	ND
Fermented soybeans	66.7±6.2	114.2±11.2
Soy paste (Miso)	18.1±0.4	14.5±0.5
Tofu	9.0±0.9	22.2±1.6
Soy sauce	2.8±0.2	2.7±0.1
Soy milk	9.9±0.7	41.6±2.0

[a] Not detectable.

4. Conclusion

In-tube SPME is an ideal sample preparation technique because of its fast operation, ease of automation, reduced solvent use and low expense. The automated in-tube SPME–HPLC method developed in this study can continuously perform extraction of daidzein and genistein from aqueous samples, and the two compounds can then be analyzed by HPLC–DAD. This method is simple, rapid, selective and sensitive for analysis of these compounds, and it can be applied to the analysis of food samples. We believe that this method provides a useful tool for the screening and determination of daidzein and genistein in food analysis.

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References

[1] T.M. Badger, M.J. Ronis, R. Hakkak, J.C. Rowlands, S. Korourian, J. Nutr. 132 (2002) 559S.
[2] W.H. Watson, J. Cai, D.P. Jones, Annu. Rev. Nutr. 20 (2000) 485.
[3] C.R. Sirtori, Drug Saf. 24 (2001) 665.
[4] H. Adlercreutz, Lancet Oncol. 3 (2002) 364.
[5] H.L. Bradlow, D.W. Sepkovic, Ann. NY Acad. Sci. 963 (2002) 247.
[6] E.P. Castle, J.B. Thrasher, Urol. Clin. North Am. 29 (2002) 71.
[7] D.J. Jenkins, C.W. Kendall, C.J. Jackson, P.W. Connelly, T. Parker, D. Faulkner, E. Vidgen, S.C. Cunnane, L.A. Leiter, R.G. Josse, Am. J. Clin. Nutr. 76 (2002) 365.
[8] Y.T. van der Schouw, M.J. de Kleijn, P.H. Peeters, D.E. Grobbee, Nutr. Metab. Cardiovasc. Dis. 10 (2000) 154.
[9] J. Yamakoshi, M.K. Piskula, T. Izumi, K. Tobe, M. Saito, S. Kataoka, A. Obata, M. Kikuchi, J. Nutr. 130 (2000) 1887.
[10] M. Yamaguchi, J. Health Sci. 48 (2002) 209.

[11] Z. Djuric, G. Chen, D.R. Doerge, L.K. Heibrun, O. Kucuk, Cancer Lett. 172 (2001) 1.
[12] J.J.B. Anderson, S.C. Garner, Nutr. Res. 17 (1997) 1617.
[13] H. Ishida, T. Uesugi, K. Hirai, T. Toda, H. Nukaya, K. Yokotsuka, K. Tsuji, Biol. Pharm. Bull. 21 (1998) 62.
[14] H. Leffers, M. Naesby, B. Vendelbo, N.E. Skakkebaek, M. Jorgensen, Hum. Reprod. 16 (2001) 1037.
[15] J. Kanno, H. Kato, T. Iwata, T. Inoue, J. Agric. Food Chem. 50 (2002) 3883.
[16] L.J. Lu, L.D. Broemeling, M.V. Marshall, V.M. Ramanujam, Cancer Epidemiol. Biomarkers Prev. 4 (1995) 497.
[17] P. Ghosh, G.P. Fenner, J. Agric. Food Chem. 47 (1999) 3455.
[18] J. Liggins, L. Bluck, W.A. Coward, S.A. Bingham, Biochem. Soc. Trans. 26 (1998) S87.
[19] P.A. Murphy, T. Song, G. Buseman, K. Barua, G.R. Beecher, T. Traine, J. Holden, J. Agric. Food Chem. 47 (1999) 2697.
[20] J. Tekel, E. Daeseleire, A. Heeremans, C. van Peteghem, J. Agric. Food Chem. 47 (1999) 3489.
[21] J. Liggins, L.J. Bluck, S. Runswick, C. Atkinson, W.A. Coward, S.A. Bingham, Br. J. Nutr. 84 (2000) 717.
[22] C.H. Irvine, M.G. Fitzpatrick, S.L. Alexander, Proc. Soc. Exp. Biol. Med. 217 (1998) 247.
[23] S. Barnes, L. Coward, M. Kirk, J. Sfakianos, Proc. Soc. Exp. Biol. Med. 217 (1998) 263.
[24] Y. Kikuchi, Y. Shimamura, M. Hirokado, K. Yasuda, M. Nishijima, J. Food Hyg. Soc. Japan 40 (1999) 444.
[25] Y. Nakamura, S. Tsuji, Y. Tonogai, J. AOAC Int. 83 (2000) 635.
[26] S.P. Klump, M.C. Allred, J.L. MacDonald, J.M. Ballam, J. AOAC Int. 84 (2001) 1865.
[27] T. Nurmi, W. Mazur, S. Heinonen, J. Kokkonen, H. Adlercreutz, J. Pharm. Biomed. Anal. 28 (2002) 1.
[28] K.A. Barnes, R.A. Smith, K. Williams, A.P. Damant, M.J. Shepherd, Rapid Commun. Mass Spectrom. 12 (1998) 130.
[29] S. Barnes, L. Coward, M. Kirk, J. Sfakianos, Proc. Soc. Exp. Biol. Med. 217 (1998) 254.
[30] C.L. Holder, M.I. Churchwell, D.R. Doerge, J. Agric. Food Chem. 47 (1999) 3764.
[31] L.S. Hutabarat, H. Greenfield, M. Mulholland, J. Chromatogr. A 886 (2000) 55.
[32] A.P. Griffith, M.W. Collison, J. Chromatogr. A 913 (2001) 397.
[33] M. Satterfield, D.M. Black, J.S. Brodbelt, J. Chromatogr. B 759 (2001) 33.
[34] G. Chen, J. Zhang, J. Ye, J. Chromatogr. A 923 (2001) 255.
[35] H. Kataoka, Anal. Bioanal. Chem. 373 (2002) 31.
[36] J. Pawliszyn, Solid Phase Microextraction: Theory and Practice, Wiley–VCH, New York, 1997.
[37] H. Kataoka, S. Narimatsu, H.L. Lord, J. Pawliszyn, Chromatography 20 (1999) 237.
[38] H. Kataoka, H.L. Lord, J. Pawliszyn, J. Chromatogr. A 880 (2000) 35.
[39] H.L. Lord, J. Pawliszyn, J. Chromatogr. A 885 (2000) 153.
[40] H.L. Lord, J. Pawliszyn, J. Chromatogr. A 902 (2000) 17.
[41] H. Kataoka, H.L. Lord, J. Pawliszyn, in: I.D. Wilson, T.D. Adhard, C.F. Poole, M. Cook (Eds.), Encyclopedia of Separation Science, Vol. 9, Academic Press, London, 2000, p. 4153.
[42] E. Baltussen, C.A. Cramers, P.J.F. Sandra, Anal. Bioanal. Chem. 373 (2002) 3.
[43] Y. Gou, J. Pawliszyn, Anal. Chem. 72 (2000) 2774.
[44] Y. Saito, M. Kawazoe, M. Hayashida, K. Jinno, Analyst 125 (2000) 807.
[45] J. Wu, J. Pawliszyn, Anal. Chem. 73 (2001) 55.
[46] H. Kataoka, M. Ise, S. Narimatsu, J. Sep. Sci. 25 (2002) 77.
[47] Y. Saito, M. Kawazoe, M. Imaizumi, Y. Morishima, Y. Nakao, K. Hatano, M. Hayashida, K. Jinno, Anal. Sci. 18 (2002) 7.
[48] H. Kataoka, H.L. Lord, J. Pawliszyn, J. Chromatogr. B 731 (1999) 353.
[49] H. Kataoka, H.L. Lord, J. Pawliszyn, Anal. Chem. 71 (1999) 4237.
[50] H. Kataoka, J. Pawliszyn, Chromatographia 50 (1999) 532.
[51] H. Kataoka, H.L. Lord, J. Pawliszyn, J. Anal. Toxicol. 24 (2000) 257.
[52] J. Wu, H.L. Lord, J. Pawliszyn, H. Kataoka, J. Microcol. Sep. 12 (2000) 255.
[53] J. Wu, W. Xie, J. Pawliszyn, Analyst 125 (2000) 2216.
[54] M. Takino, S. Daishima, T. Nakahara, Analyst 126 (2001) 602.
[55] F. Yang, Y. Ma, Y. Ito, J. Chromatogr. A 928 (2001) 163.