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Determination of isoflavones in soy products by capillary electrophoresis with electrochemical detection

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Abstract

Simultaneous determination of daidzein and genistein in soy products by capillary electrophoresis with electrochemical detection (CE–ED) was reported. The effects of working electrode potential, running buffer pH, separation voltage and injection time on CE–ED were investigated. Under the optimum conditions, the analytes could be separated in a 100 mmol/l borate buffer (pH 11.0) within 20 min. A 300 μm diameter carbon disk electrode has a good response at +0.70 V (vs. SCE) for the analytes. The response was linear over three orders of magnitude for the analytes. This method has been satisfactorily used for the determination of daidzein and genistein in several actual soy samples.

Keywords: Capillary electrophoresis; Electrochemical detection; Daidzein; Genistein; Soy products

1. Introduction

Isoflavones are a group of naturally occurring heterocyclic phenols found mainly in soybean (*Glycine max*) and have been credited with performing several health-promoting functions. The lower incidence of certain diseases has been reported in Asian countries where soybean consumption is high (average intake of isoflavones is 40–80 mg per day) (Aldercreutz et al., 1995; Pariza, Aeschbacher, Felton, & Sato, 1990). Soy foods are suggested to provide a protective effect on the breast, intestine, liver, bladder, prostate, skin and stomach from cancer development (Messina & Barnes, 1991; Messina, Persky, Setchell, & Barnes, 1994). The major active components in soybean are isoflavones such as genistein and daidzein, their molecular structures are shown in Fig. 1. Genistein, which possesses weak estrogenic activity, has been shown to act in animal models as an anti-estrogen and, therefore, may play a protective role in hormonally influenced cancers, such as breast cancer (Hendrich & Lee, 1993; Zava & Duwe, 1997).

Experiments on animals and observations on human being have shown that soybean protein has hypocholesterolemic and anti-atherogenic property (Carroll, 1991). Recently, in an analysis of the effects of soy protein intake on serum lipids, it was observed that soy protein may significantly decrease serum concentrations of total cholesterol, low density lipoproteins (LDLs) cholesterol and triglycerides when compared with protein of animal origin (Anderson, Ambrose, & Garner, 1995; Potter, 1998). Studies in Primates indicate that soy protein may exert its anti-atherogenic effects via associated isoflavones (Anthony, Clarkson, Weddle, & Wolfe, 1995). Soy isoflavones also have antioxidant properties, which may protect LDL from oxidation (Wei, Bowen, Cai, Barnes, & Wang, 1995). Consumption of 25 g of soybean protein per day can contribute to the lowering of serum cholesterol levels and the prevention of heart disease (Food & Drug Administration, 1999). This health claim places soy foods among a selected category of ''functional foods'' possessing unique medicinal, as well as, nutritional value.

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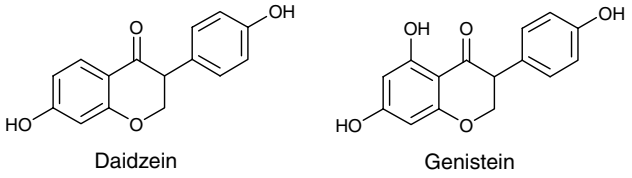

Fig. 1. Chemical structures of the analytes.

Epidemiological studies have shown that women who have higher intake of soy foods have lower rates of osteoporosis (Messina et al., 1994). As isoflavones are weak estrogens, ingestion of soybean foods has been proposed as an alternative to hormone replacement therapy for postmenopausal women (Cassidy, Bingham, & Setchell, 1994).

Given the strong association between diet and disease, the potential implications of ingesting biologically active compounds, such as phytoestrogens requires further examination. This is particularly the case with the increasing use of soy-based products for human consumption, and therefore it is important to develop a suitable method for the detection of these compounds in diets.

Several analytical methods including gas chromatography (GC) (Ghosh & Fenner, 1999), high-performance liquid chromatography (HPLC) (Hutabarat, Mulholland, & Greenfield, 1998; Shirota-Matsumoto, Aiyama, & Yokokura, 2000; Tarbin & Sharman, 2001) have been employed for this purpose. Capillary electrophoresis (CE) with UV detection (Shihabi, Kute, Garcia, & Hinsdale, 1994) has also been employed, the drawback of this approach being its low sensitivity. HPLC, regarded as a prime separation method, has some shortcomings in analysis of food samples, including long analysis time, low resolution and short column lifetime owing to numerous co-existent interferences. Recently, CE is becoming increasingly recognized as an important analytical separation technique for its speed, efficiency, reproducibility, ultra-small sample volume, and minimal consumption of solvent. In addition, with electrochemical detection (ED), CE–ED offers high sensitivity and good selectivity for electroactive analytes, and the major limitation of CE–ED is that it can only be used to analyze electroactive species. This method has been applied to analyze isoflavones in plants. (Cao, Lou, Zhang, Chu, Fang, & Ye, 2002). To our knowledge, so far CE–ED method has not been applied to determine isoflavones such as daidzein and genistein in soy products. In this work, we first developed a simple, rapid and dependable method for the determination of daidzein and genistein in actual soybean products.

2. Experimental

2.1. Apparatus

The laboratory-built CE–ED system has been described previously (Chen, Ye, & Cheng, 2000). A ±30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, Shanghai, China) provided a voltage between the ends of the capillary. The inlet of the capillary was held at a positive potential and the outlet end of the capillary was maintained at ground. The separations were undertaken in a 70 cm length of 25 μm i.d. and 360 μm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA). Samples were injected electrokinetically at 12 kV for 6 s.

A three-electrode cell system consisting of a 300-μm diameter carbon disk working electrode, a platinum auxiliary electrodes, and a saturated calomel electrode (SCE) as the reference electrode, was used in combination with a BAS LC-3D amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA). Before use, the carbon disc electrode was polished with emery paper, then sonicated in doubly distilled water, and finally positioned carefully opposite the outlet of the capillary and arranged in a wall-jet configuration (Ye & Baldwin, 1994). The electropherograms were recorded using a chart record (XWTD-164, Shanghai Dahua Instrument Factory, China).

2.2. Reagents and solutions

Daidzein and genistein were purchased from Sigma (St. Louis, MO, USA). Soybean milk powder and soy powder were purchased from supermarket. *Seme sojae preparatum* was purchased from a drug store in Shanghai. Stock solutions of two analytes (1.0×10^{-3} g/ml each) were prepared in anhydrous ethanol (A.R. grade) and were diluted to the appropriate concentration with running buffer (100 mmol/l $Na_2B_4O_7$–NaOH, pH 11.0) for the construction of calibration curves. Before use, all solutions were filtered through 0.22 μm nylon filters.

2.3. Sample preparation

All the samples were ground into power and accurately weighed, respectively. Each weighed sample (about 2 g) was extracted with 10 ml 70% ethanol for 2 h in an ultrasonic bath. Next each of the samples was filtered through filter paper first, then through a 0.22 μm syringer filter. Sample solutions were stored at 4 °C in the dark.

3. Results and discussion

3.1. Effect of the potential applied to the working electrode

The potential applied to the working electrode directly affects the electrochemical response of the analytes. In order to obtain best detection results, optimum potential applied to the working electrode should be selected, therefore, hydrodynamic voltammetry experi-

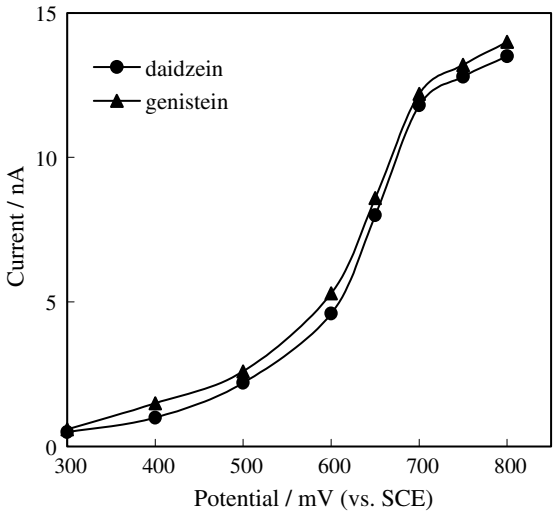

Fig. 2. Hydrodynamic voltammograms (HDVs) for daidzein and genistein (2.0×10^{-5} g/ml each). Fused-silica capillary: 25 μm i.d.×65 cm; working electrode: 300 μm diameter carbon disc electrode; running buffer: 100 mmol/l borate buffer (pH 11.0); separation voltage: 12 kV; electrokinetic injection: 6 s (at 12 kV).

ment was conducted to find this optimum potential. As shown in Fig. 2, when the applied potential exceeds +0.50 V (vs. SCE), oxidation currents of daidzein and genistein increase rapidly; when the applied potential passes +0.70 V (vs. SCE), however, the peak currents of the two analytes increase much more slowly. Although an applied potential greater than +0.70 V (vs. SCE) results in higher peak currents, both the baseline noise and the background current increase substantially due to the solvent oxidation. The high background current leads to an unstable baseline, which is a disadvantage for sensitive and stable detection. The potential applied to the working electrode was, therefore, maintained at +0.70 V (vs. SCE) where the background current is not too high and the signal-to-noise (S/N) ratio is the highest.

3.2. Effects of the running buffer pH

The acidity of the running buffer plays an important role in CE for its effect on zeta potential (ζ), the electroosmotic flow (EOF), as well as the overall charge of all the analytes, affects the migration time and the separation of the analytes. Therefore, it is important to study its influence on CE in order to obtain optimum separations. Based on experiments, 100 mmol/l borate buffer (pH 11.0) was finally chosen as the running buffer in considering the peak current, resolution and the analysis time.

3.3. Effects of separation voltage and injection time

Fig. 3 illustrates the influence of separation voltage on the migration time of the two analytes. Increasing the

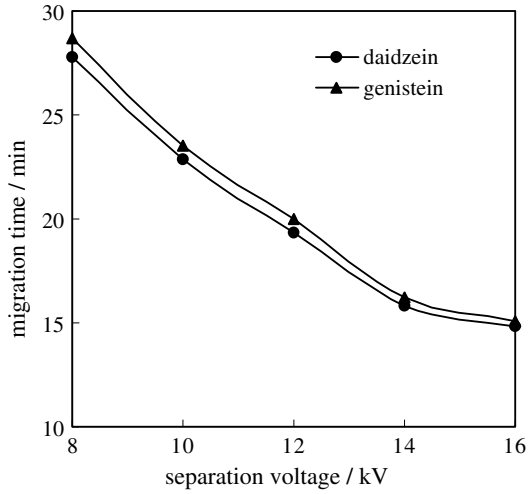

Fig. 3. Effect of separation voltage on the migration time of the analytes. Working potential: +0.70 V (vs. SCE); other conditions as in Fig. 2.

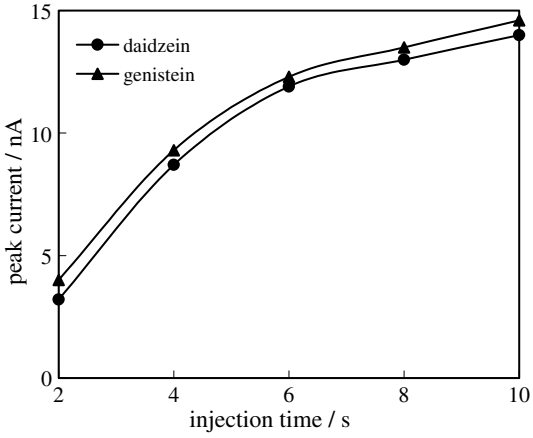

Fig. 4. Effect of injection time on the peak current of the analytes. Working potential: +0.70 V (vs. SCE); other conditions as in Fig. 2.

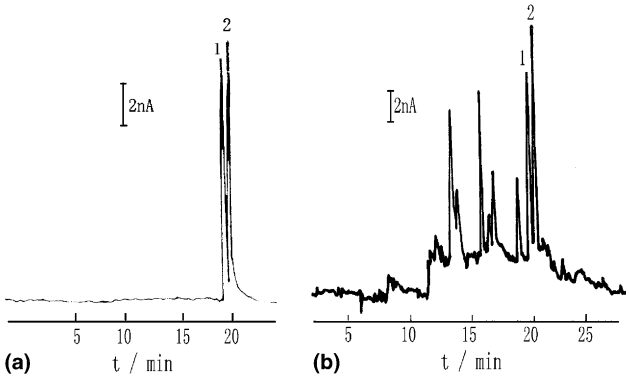

Fig. 5. Electropherogram of standard mixture solution of daidzein and genistein (2.0×10^{-5} g/ml each) (a), and typical electropherogram of *Seme sojae preparatum* sample (dilution: 1:20) (b). Peak identification: (1) daidzein; (2) genistein. Working potential: +0.70 V (vs. SCE); other conditions as in Fig. 2.

Table 1
The results of regression analysis on calibration and the detection limits[a]

Compound	Regression equation $y = ax + b$[b]	Correlation coefficient	Linear range (μg/ml)	Detection limit (g/ml)
Daidzein	$y = 599000x + 0.055$	0.9996	0.2–100	1×10^{-7}
Genistein	$y = 599714x + 0.13$	0.9998	0.2–100	1×10^{-7}

[a] Working potential is +0.70 V (vs. SCE). Other conditions as in Fig. 2.
[b] In the regression equations, the x value is the concentration of analytes (g/ml), the y value is the peak current (nA).

separation voltage gives shorter migration time, however, it is not beneficial to the resolution of the anatytes, on the other hand, too low separation voltage will increase the analysis time considerably, which in turn cause peak broadening. Based on experiments, 12 kV was chosen as the optimum separation voltage to accomplish a good compromise.

The effect of injection time on CE separation was investigated by varying the sampling time (2, 4, 6, 8, 10 s at a voltage of 12 kV, as shown in Fig. 4). The injection time determining the amount of sampling affects both peak current and peak shape. It was found that the peak current increases with increasing sampling time as we can see from Fig. 4, and it was also found that the peak width increases with increasing time. When injection time is longer than 6 s, peak current levels off and peak broadening becomes severe. In this experiment, 6 s (12 kV) is selected as the optimum injection time.

Through the experiments above, the optimum conditions for the determination of daidzein and genistein were decided. The typical electropherogram for a standard solution of the analytes is shown in Fig. 5(a), as we can see baseline separation can be achieved within 20 min.

3.4. Reproducibility, linearity and detection limits

The reproducibility of peak current and migration time in this experiment was determined by injecting a standard solution of a mixture of daidzein and genistein (2.0×10^{-5} g/ml each) into the system under the optimum conditions ($n = 7$). The relative standard deviations (RSDs) of peak current and migration time were 3.5% and 1.2%, respectively, for daidzein, and 2.6% and 0.9%, respectively, for genistein. The high reproducibility indicates that this method is suitable for the analysis of real samples.

A series of standard solutions of daidzein and genistein ranging from 1.0×10^{-7} to 1.0×10^{-3} g/ml in concentration were tested to determine the linearity of the determination. Results from regression analysis of both calibration curves and detection limits are listed in Table 1. The detection limits were evaluated on the basis of a single-to-noise ratio of 3.

Table 2
Assay results of the analytes in soy products ($n = 3$)[a]

Sample	Daidzein (μg/g)	Genistein (μg/g)
Soy bean	5.00	nd
Soybean milk powder	24.6	28.8
Seme sojae preparatum	436	507

[a] Working potential is +0.70 V (vs. SCE). Other conditions as in Fig. 2.

Table 3
Determination results of the recovery for this method ($n = 3$, 10^{-4} g/ml)[a]

Compound	Original amount	Added amount	Found amount	Recovery (%)	RSD (%)
Daidzein	4.36	5.00	9.11	95	2.6
Genistein	5.07	5.00	9.87	96	3.5

[a] Working potential is +0.70 V (vs. SCE). Other conditions as in Fig. 2.

3.5. Sample analysis and recovery

Daidzein and genistein in soy products were determined by CE–ED under the optimum conditions. Typical electropherogram of *Seme sojae preparatum* is shown in Fig. 5(b). By a standard addition method, the active ingredients in samples can be identified and determined. The assay results are listed in Table 2, as we can see, the content of daidzein and genistein is much higher in *Seme sojae preparatum* than in soy bean. The concentration of genistein and daidzein found in this work agrees with the literature values (Wang & Liu, 1998). The recovery and reproducibility experiments under the optimum conditions were also conducted to evaluate the precision and accuracy of the method. Recovery was determined by standard addition method, and the results are listed in Table 3. The above assay results indicate that this method is simple, rapid and dependable, providing a useful qualitative method for the analysis of food samples.

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