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Determination of polyphenols in apple juice and cider by capillary electrophoresis with electrochemical detection

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Abstract

A simultaneous determination of phloridzin, (−)-epicatechin, chlorogenic acid and myricetin in apple juices and ciders by capillary electrophoresis with electrochemical detection (CE-ED) was reported. Effects of several important factors such as the pH and concentration of running buffer, separation voltage, injection time and detection potential were investigated to acquire the optimum conditions. Under the optimum conditions, the analytes could be separated within 20 min in a 75 cm length capillary at a separation voltage of 18 kV in a 50 mmol/l borate buffer (pH 8.7). A 300 μm diameter carbon disk electrode generates good response at +0.90 V (vs. SCE) for all analytes. The relationship between peak currents and analyte concentrations was linear over about three orders of magnitude with detection limits ($S/N = 3$) ranging from 1×10^{-7} to 5×10^{-7} g/ml for all analytes. This proposed method demonstrated long-term stability and reproducibility with relative standard deviations of less than 4% for both migration time and peak current ($n = 7$). It has been successively used for the determination of the analytes in apple juices and ciders, and the assay result was satisfactory.

Keywords: Capillary electrophoresis; Electrochemical detection; Polyphenols; Apple juice and cider

1. Introduction

Phenolic compounds in apples are of undoubted importance because of their contribution to the colour, taste and flavour characteristics of apples and its derivative products (Lea & Arnold, 1978). Recently, polyphenols have been the object of increasing interest because of their biological properties, namely anti-inflammatory, anti-histaminic and anti-tumor activities, and as free radical scavengers and protection against cardiovascular diseases (Vinson, Dabbagh, Serry, & Jang, 1995; Vinson & Hontz, 1995). There is now increasing evidence to suggest that age-related human diseases, such as heart disease, cancer, immune system decline and brain dysfunction are result of cellular damage by free radicals. The antioxidants in our diet could play an important role in such disease prevention (Aruoma, 1998), which has fueled much public interest in natural antioxidants and has led to an extensive search for effective antioxidants in nature (Frankel, Huang, Aeschbach, & Prior, 1996; Madsen & Bertelsen, 1995), especially those are present naturally in human diets (Vinson, Hao, Su, & Zubik, 1998; Wang, Cao, & Prior, 1996). Apple fruits are rich in quercetin glycosides, epicatechin, chlorogenic acid, procyanidins and dihydrochalones (such as phloridzin), which have been shown to exert strong antioxidant activity in vitro (Lu & Foo, 2000). Therefore, it is necessary to establish some simple, economical and accurate methods for the

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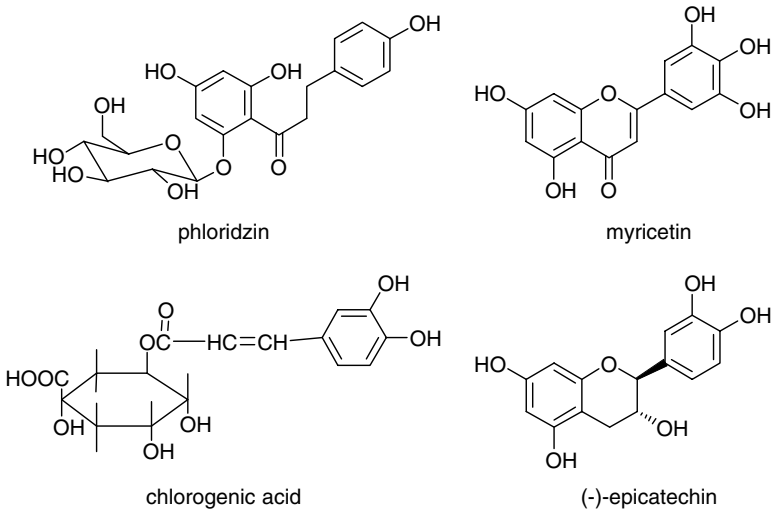

Fig. 1. Chemical structures of the analytes.

determination of polyphenols in apples and its derivative products.

The isolation and quantitation of polyphenols in processed apple products is difficult due to the chemical complexity. Several analytical methods including high-performance liquid chromatography (HPLC) (Delage, Bohuon, Baron, & Drilleau, 1991; Oleszek, Amiot, & Aubert, 1994; Spanos, Wrolatad, & Heatherbell, 1990), GC–MS (Lu & Foo, 1998) and high-speed counter-current chromatography (Shibusawa, Yanagida, Ito, Ichihashi, & Shindo, 2000) have been employed for the determination of polyphenols in apples and processed apple products. It is true that HPLC is the widely used method for the analysis of dietary polyphenols, and combined with MS, the structural information of the analytes can be obtained and confirmation of identity could be achieved further (Tsao, Yang, Young, & Zhu, 2003). However, HPLC has some shortcomings including long analysis time (Delage et al., 1991; Tsao et al., 2003) and short column lifetime owing to numerous co-existent interferences and column contaminations. Capillary electrophoresis (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 can provide higher selectivity as only electroactive substances can be detected. Another important advantage of CE as an alternative technique for the analysis of foods over the more commonly used HPLC is that the capillary is much easier to wash to acquire high reproducibility. CE-ED has been applied to analyze some phenols in foods (Cao, Zhang, Ding, Fang, & Ye, 2001a; Cao, Zhang, Fang, & Ye, 2001b). To our knowledge, so far CE-ED method has not been applied for the simultaneous determination of phloridzin, (−)-epicatechin, chlorogenic acid and myricetin (the molecular structures are shown in Fig. 1) in apple juices and ciders.

In this work, an alternative method for the determination of phloridzin, (−)-epicatechin, chlorogenic acid and myricetin in apple juices and ciders by using CE-ED approach is described, which has been proven to be simple and convenient, as well as sensitive and selective.

2. Experimental

2.1. Apparatus

In this work, a CE–ED system has been constructed and is similar to that described previously (Ye, Jin, Zhao, & Fang, 1998). 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 75 cm length, 25 μm i.d. and 360 μm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA).

A three-electrode electrochemical cell consisting of a 300 μm diameter carbon disc working electrode, a platinum auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode, was used in combination with a BAS LC-3D amperometric detector (Biochemical System, West Lafayette, IN, USA). Before use, the carbon disc electrode was polished with emery paper and sonicated in doubly distilled water, and finally carefully positioned opposite the outlet of the capillary with the aid of a micro-manipulator (Correct, Tokyo, Japan) and arranged in a wall-jet configuration

(Ye & Baldwin, 1994). The distance between the tip of the working electrode and the capillary outlet was as close as possible so that the CE effluent directly impinged upon the electrode surface. The electropherograms were recorded using chart recorder (Shanghai Dahua Instrument Factory, China). CE was performed in a 50 mmol/l borate buffer (pH 8.7) used as the running buffer at a separation voltage of 18 kV. The potential applied to the working electrode was +0.90 V (vs. SCE). Samples were injected electrokinetically at 18 kV for 6 s.

2.2. Reagents and solutions

Phloridzin, (−)-epicatechin and myricetin were purchased from Sigma (St. Louis, MO, USA), chlorogenic acid was purchased from Aldrich (Milwaukee, WI, USA). Stock solutions of phloridzin, (−)-epicatechin, chlorogenic acid and myricetin (1.0×10^{-3} g/ml each) were prepared in anhydrous ethanol (AR grade), stored in the dark at 4 °C, and was diluted to the desired concentrations with the running buffer (50 mmol/l borate buffer, pH 8.7), in which carbon working electrode shows excellent response to phenolic compounds. Before use, all solutions were filtered through 0.22 μm nylon filters.

2.3. Sample preparation

The real samples were Changyu sparkling cider, Grand Dragon sparkling cider, Suntory apple juice and Weiquan apple juice, respectively. All the samples were purchased from a supermarket in Shanghai. One millilitre of each sample was neutralized with NaOH, then diluted with the running buffer to 2.00 ml. After filtered through 0.22 μm nylon filters, the samples could be injected electrokinetically without pre-concentration. 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

In amperometric detection the potential applied to the working electrode directly affects the sensitivity, detection limit and stability of this method. Therefore, the effect of working electrode potential on the peak current (calculated by measuring the peak height) of the analytes was investigated to obtain optimum detection. Fig. 2 illustrates the hydrodynamic voltammograms of phloridzin, (−)-epicatechin, chlorogenic acid and myricetin. When the applied potential reaches +0.60 V (vs. SCE), the peak currents increase rapidly, except for chlorogenic acid, which started to generate current at +0.80 V (vs. SCE). However, when the potential exceeds +0.90 V (vs. SCE), the peak currents of all the analytes level off. Although applied potential greater than +0.90 V (vs. SCE) results in larger peak current, the background current of the working electrode increases sharply. Hence, the applied potential of the working electrode was maintained at +0.90 V (vs. SCE), where the background current is not too high and the *S*/*N* ratio is the highest.

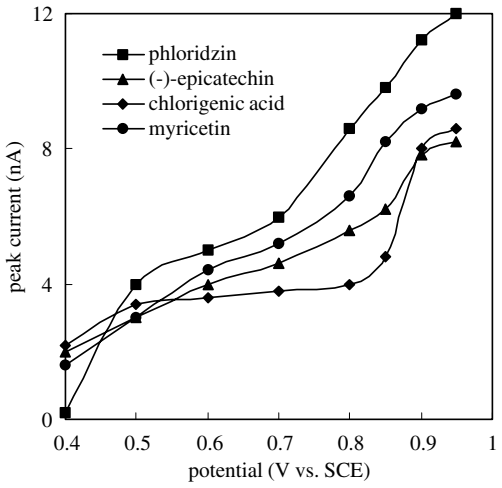

Fig. 2. Hydrodynamic voltammograms (HDVs) for phloridzin, (−)-epicatechin, chlorogenic acid and myricetin in CE. Working conditions: Fused-silica capillary, 25 μm i.d. × 75 cm; working electrode, 300 μm diameter carbon disk electrode; running buffer, 50 mmol/l borate buffer (pH 8.7); separation voltage, 18 kV; electrokinetic injection: 6 s (18 kV); concentrations, 2.0×10^{-5} g/ml for (−)-epicatechin, 5.0×10^{-5} g/ml for phloridzin, chlorogenic acid and myricetin.

3.2. Effects of buffer pH and concentration

The acidity and concentration of the running buffer play an important role in CE for the effects on zeta potential (ζ), the electroosmotic flow (EOF), as well as the overall charge of all the analytes, which affect the migration time and the separation of the analytes. Therefore, it is important to study their influences on CE in order to obtain optimum separations. The effect of the running buffer pH on the migration time of the investigated analytes is shown in Fig. 3. The running buffer is 50 mmol/l borate buffer at five different pH values (8.0, 8.4, 8.7, 9.0 and 9.5). As shown in Fig. 3, the resolution of the four analytes is poor at pH 8.0. When the running buffer pH increases, the resolution of all compounds is improved with increasing migration time. It is also found that the peak current is low and the peak shape became poor at pH value above 8.7. So 50 mmol/l borate buffer with pH 8.7 was chosen as the running buffer in considering the peak current, resolution and the analytical time.

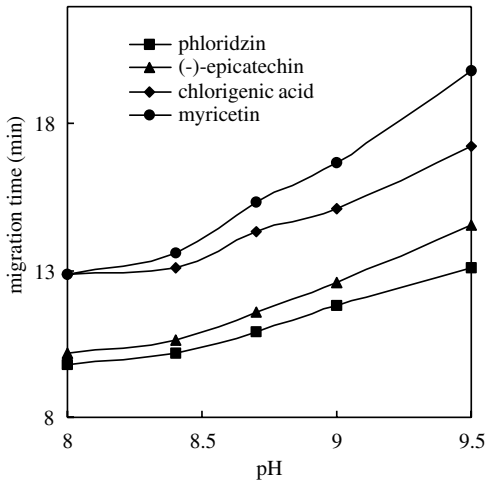

Fig. 3. Effect of buffer pH on the migration time of phloridzin, (−)-epicatechin, chlorogenic acid and myricetin. Working potential: +0.90 V (vs. SCE); other conditions as in Fig. 2.

As the buffer concentration influences the viscosity coefficient of the solution, the diffusion coefficient of analytes and the ζ-potential of the inner surface of capillary tube as well, it affects not only the resolution and migration time of the analytes, but also the peak current. The migration time and the resolution increase with increasing buffer concentration. However, if buffer concentrations is too high (>50 mmol/l), it will also cause a negative effect on the detection limits because the peak currents of all analytes decrease and the effect of Joule heat becomes more pronounced, so 50 mmol/l borate buffer (pH 8.7) was chosen as the running buffer in considering the peak current, resolution and the analytical time and buffer capacity.

3.3. Effect of separation voltage and injection time

The separation voltage affects the electric field strength, which in turn affects the EOF and the migration velocity of charged particles, which determine the migration time of the analytes. Moreover, higher separation voltage may result in higher Joule heating. The effect of separation voltage on the migration time of the analytes is shown in Fig. 4(a), increasing the voltage gives shorter migration times but also increases the background noise, resulting in a higher detection limit. Although the resolution of analytes can be improved to some extent, too low a separation voltage will increase the analytical time considerably, which in turn causes severe peak broadening. Based on experiments, 18 kV was chosen as the optimum voltage to accomplish a good compromise.

The effect of injection time on separation was investigated by different sampling time (2, 4, 6, 8, 10 s at a voltage of 18 kV, as shown in Fig. 4(b)). The injection time determining the amount of sampling affects both peak

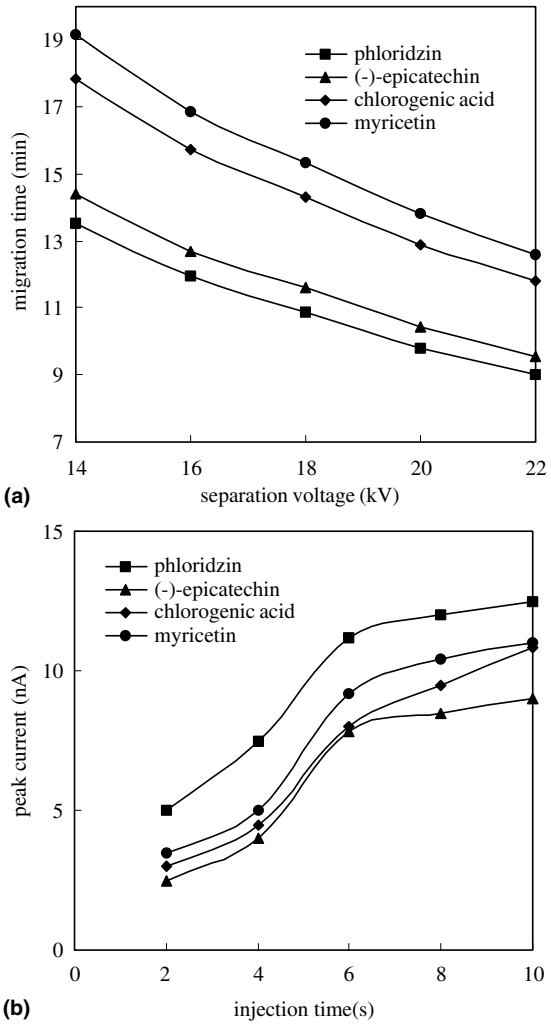

Fig. 4. Effect of separation voltage on the migration time of phloridzin, (−)-epicatechin, chlorogenic acid and myricetin and effect of injection time on the analytes peak current. Working potential: +0.90 V (vs. SCE); other conditions as in Fig. 2.

current and peak shape. It was found that the current increases with increasing sampling time as we can see from Fig. 4(b), and it was also found that the peak width increases with increasing time. When the injection time is more than 6 s, the peak current levels off and peak broadening becomes more severe. 6 s (18 kV) was, therefore, selected as the optimum injection time.

A typical electropherogram for the standard mixture solution under the optimum conditions is shown in Fig. 5(a). Baseline separation for all analytes can be achieved within 20 min.

3.4. Reproducibility, linearity and detection limits

A standard mixture solution of 2.0×10^{-5} g/ml (−)-epicatechin, 5.0×10^{-5} g/ml phloridzin, chlorogenic acid and myricetin was analyzed for seven times to determine the reproducibility of peak current and migration time

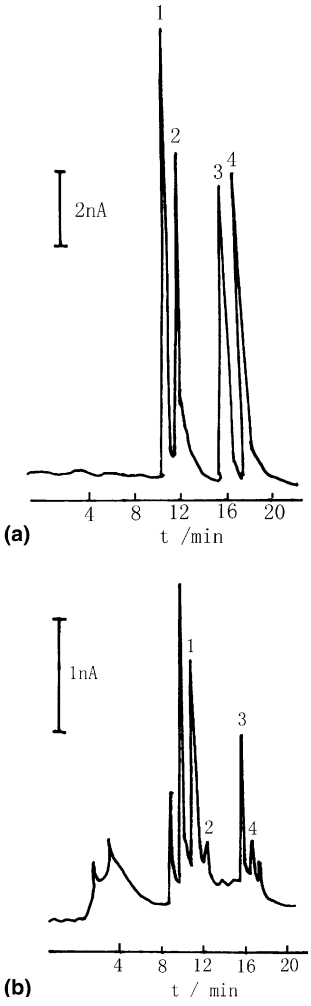

Fig. 5. The electropherogram of a standard mixture solution (2.0×10^{-5} g/ml for (−)-epicatechin, 5.0×10^{-5} g/ml for phloridzin, chlorogenic acid and myricetin) (a), and the typical electropherogram of the real sample (b), (Changyu sparkling cider, dilution: 1:2). Working potential: +0.90 V (vs. SCE); other conditions as in Fig. 2. Peak identification: 1, phloridzin; 2, (−)-epicatechin; 3, chlorogenic acid; 4, myricetin.

for all analytes under the optimum conditions in this experiment. The relative standard deviations (RSDs) of peak current and migration time are 2.5% and 0.9% for (−)-epicatechin, 3.8% and 1.2% for phloridzin, 2.0% and 0.6% for chlorogenic acid, 3.0% and 0.8% for myricetin, respectively.

A series of the standard mixture solutions of phloridzin, (−)-epicatechin, chlorogenic acid and myricetin with a concentration range of 5.0×10^{-7}–1.0×10^{-3} g/ml were tested to determine the linearity for all analytes at the carbon disc electrode in this method. The results of regression analysis on calibration curves and detection limits are presented in Table 1. The determination limits are evaluated on the basis of a signal-to-noise ratio of 3. The calibration curves exhibit excellent linear behavior over the concentration range of about three orders of magnitude with the detection limits ranging from 1×10^{-7} to 5×10^{-7} g/ml for all the analytes.

3.5. Sample analysis and recovery

Under optimum conditions, the determination of phloridzin, (−)-epicatechin, chlorogenic acid and myricetin in real samples was carried out according to the procedures described earlier. Typical electropherogram of sparkling cider sample is shown in Fig. 5(b). By comparing with the electropherogram of the standard solution (Fig. 5(a)), the active ingredients namely phloridzin, (−)-epicatechin, chlorogenic acid and myricetin can be determined. The assay results are listed in Table 2, which agree with the literature (Dupont, Bennett, Mellon, & Williamson, 2002). It was observed that each variety showed the same phenolic acid and flavonoid pattern, but the amounts of the phenolics were different in different juices and ciders. The results agree with literatures (Delage et al., 1991; Gomis, Palomino, & Alonso, 2001; Tsao et al., 2003), which suggest that the levels of phenolic compounds in apple juices and ciders depend strongly on the fruit varieties and the process.

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.

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

Compound	Regression equation $y = a + bx$[b]	Correlation coefficient R	Linear range (μg/ml)	Detection limit[c] (10^{-7} g/ml)
Phloridzin	$y = 221943x + 0.13$	0.9998	1–1000	5
(−)-Epicatechin	$y = 390857x - 0.04$	0.9998	0.5–500	1
Chlorogenic acid	$y = 161314x - 0.055$	0.9999	1–1000	5
Myricetin	$y = 1841114x - 0.03$	0.9998	1–1000	5

[a] Working potential is +0.90 V (vs. SCE). Other conditions as in Fig. 2.
[b] The y and x are the peak current (nA) and concentration of the analytes (g/ml), respectively.
[c] The detection limits corresponding to concentrations giving signal to noise ratio of 3.

Table 2
Assay results of the analytes in apple juices and ciders ($n = 3$, 10^{-6} g/ml)[a]

Sample	Phloridzin	(−)-Epicatechin	Chlorogenic acid	Myricetin
Changyu sparkling cider	8.4 (2.5)[b]	0.77 (3.2)	8.9 (2.8)	2.2 (3.0)
Grand Dragon sparkling cider	16.8 (2.0)	1.0 (3.8)	2.2 (2.5)	1.2 (3.6)
Suntory apple juice	17.8 (3.5)	5.2 (3.4)	1.4 (3.5)	3.6 (2.8)
Weiquan apple juice	12.6 (3.6)	1.4 (2.6)	4.0 (3.8)	2.4 (3.2)

[a] Working potential is +0.90 V (vs. SCE). Other conditions as in Fig. 2.
[b] The data in the brackets refer to the RSD.

Table 3
Results of the recovery of this method ($n = 3$)[a]

Compound	Original (10^{-6} g/ml)	Added (10^{-6} g/ml)	Found (10^{-6} g/ml)	Recovery (%)	RSD (%)
Phloridzin	8.4	8.0	16.24	98	2.5
(−)-Epicatechin	0.77	1.0	1.72	95	1.8
Chlorogenic acid	8.9	8.0	16.58	96	3.6
Myricetin	2.2	2.0	4.1	95	2.0

[a] Working potential is +0.90 V (vs. SCE). Other conditions as in Fig. 2.

4. Conclusion

It has been demonstrated that CE-ED is characterized by high resolution and sensitivity, satisfactory reproducibility, low operating expense and minimal sample volume requirement. CE-ED has been successfully used for the determination of polyphenols in apple juice and cider. It could be concluded that CE-ED is a powerful technique for the study of electroactive constituents of foods and beverages and has become an alternative, competitive and supplementary method for HPLC, because of its special attributes.

Acknowledgements

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