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Short communication

Micellar electrokinetic chromatography as an alternative to high-performance liquid chromatography for separation and determination of phenolic compounds in Japanese spirituous liquor

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Abstract

A capillary electrophoretic method has been developed for the separation and determination of vanillin, ferulic acid, vanillic acid and 4-vinylguaiacol in Japanese spirituous liquor. These phenolic compounds were extracted from Japanese spirituous liquor by solid-phase extraction and were successfully separated by micellar electrokinetic chromatography (MEKC) with 25 m*M* sodium dodecyl sulfate. MEKC required short analysis times and it could be an alternative technique for the analysis of vanillin, ferulic acid, vanillic acid and 4-vinylguaiacol in Japanese spirituous liquor. These phenolic compounds in five Japanese spirituous liquor samples were determined by this method. No significant difference in separation patterns and amounts was observed among the repeated analysis. © 1998 Elsevier Science B.V.

Keywords: Beverages; Phenolic compounds; Vanillin; Ferulic acid; Vanillic acid; Vinylguaiacol

1. Introduction

Japanese spirituous liquor, *shochu*, is the most popular distilled alcoholic beverage in Japan. Japanese spirituous liquor is brewed from rice and barley as raw materials, and has a good flavor due to the presence of phenolic compounds. Recently, flavored compounds in Japanese spirituous liquor have been analyzed by high-performance liquid chromatography (HPLC) [1,2]. Phenolic compounds found in alcoholic beverages are produced by the yeast from raw materials [3]. Ferulic acid, vanillin, and vanillic acid are major phenolic compounds found in alcoholic beverages [4]. In particular, vanillin is an important compound contributing to the fragrance of commercial foods such as cakes, cookies, candies and vanilla ice cream. Vanillin is also important for a flavor and taste of Japanese spirituous liquor. A mechanism for the conversion of ferulic acid to vanillin in Japanese spirituous liquor was proposed [1,2] as shown in Fig. 1. Ferulic acid, initially bound to the hemicellulose of plant cell walls [5–7], is liberated by hydrolytic enzymes from *Aspergillus awamori* and *Aspergillus kawachii*, and converted to 4-vinylguaiacol by acid and heat during distillation [1]. The 4-vinylguaiacol is then transformed into vanillin and vanillic acid by aging and oxidation during storage [8] (Fig. 1).

Phenolic compounds that are found in Japanese spirituous liquor has been analyzed by HPLC [1].

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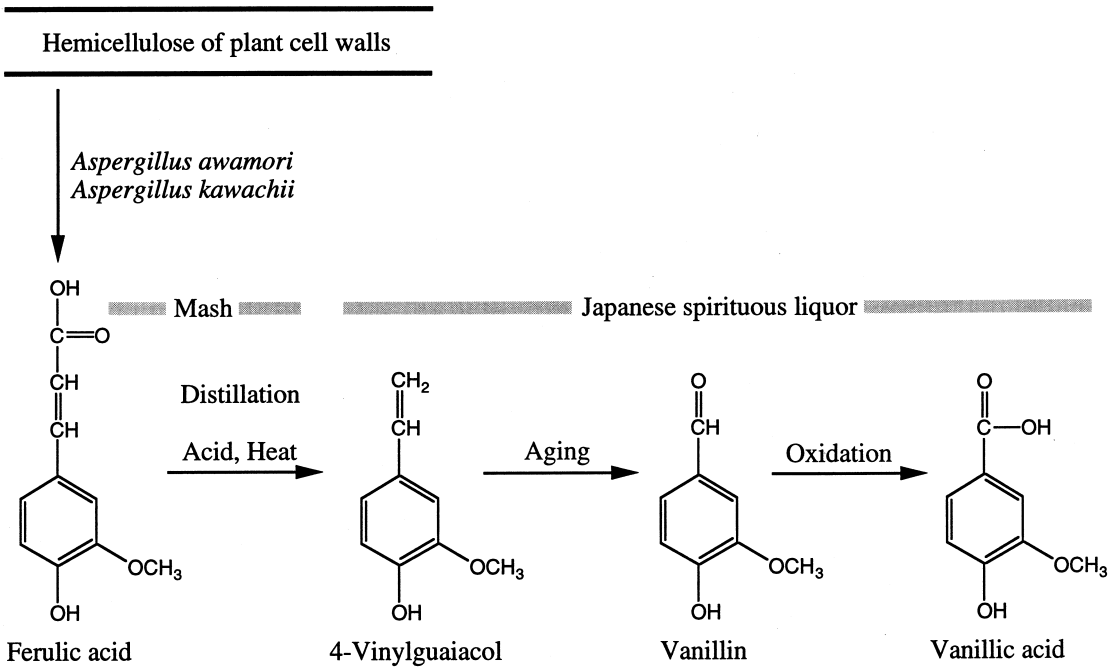

Fig. 1. Proposed mechanism for the conversion of ferulic acid in Japanese spirituous liquor. Mash: Mash is called *moromi* in Japan which is brewed from rice and barley as raw materials by fungus *Aspergillus* and yeast *Saccharomyces*. Japanese spirituous liquor: Japanese spirituous liquor is the most popular distilled alcoholic beverage in Japan.

However, the HPLC analysis is not applied to routine quality control because it is a time consuming process. Capillary electrophoresis (CE) is a relatively new separation technique; it has high separation efficiency and requires a small amount of samples. Micellar electrokinetic chromatography (MEKC), first reported by Terabe et al. in 1984 [9], can separate neutral analytes by the difference in their partitioning to the micelle.

The objective of our work is to provide an alternative methodology based on MEKC for the analysis of the phenolic compounds in Japanese spirituous liquor. The separation by MEKC was compared with that by HPLC, in order to assess MEKC as an alternative technique for determining the phenolic compounds in Japanese spirituous liquor.

2. Experimental

2.1. Chemicals

The following phenolic compounds were used: vanillin, ferulic acid, vanillic acid (Nacalai Tesque, Kyoto, Japan); 4-vinylguaiacol (4-hydroxy-3-methoxystyrene) (Lancaster, Lancashire, UK). Sodium dodecyl sulfate (SDS) from Fluka (Switzerland) was used as anionic micellar pseudo-stationary phases in MEKC. All other chemicals and solvents were of analytical reagent grade, supplied by Wako (Osaka, Japan). Pure water was prepared by purifying distilled water with a Milli-Q SP system (Millipore, Bedford, MA, USA) prior to use.

2.2. Apparatus

MEKC using UV–Vis detection was performed by a BioFocus 3000 CE system (Bio-Rad, Richmond, CA, USA) with a pre-packed cartridge of an uncoated fused-silica capillary of 36 cm (31.4 cm to the detector)×50 μm I.D. HPLC separation and purification were performed by a Beckman Gold HPLC system with a programmable solvent module 125 and programmable detector module 166 (Fullerton, CA, USA) using an L-column ODS packed column, 150×4.6 mm I.D. (Kagakuhin Kensa Kyokai, Tokyo, Japan).

2.3. HPLC separation

A linear gradient elution was used and the mobile phase was changed from 50 m*M* acetate buffer (pH 4.0)–acetonitrile (95:5) to 50 m*M* acetate buffer (pH 4.0)–acetonitrile (35:65) in 60 min at a flow-rate of 1.0 ml/min. The wavelength of the UV detector was set at 280 nm. The column temperature was 40°C.

2.4. Sample preparation

Phenolic samples from Japanese spirituous liquor were prepared by solid-phase extraction (SPE): Japanese spirituous liquor sample was diluted with distilled water in ten-fold, because it contained ethanol. The SPE cartridge (Bond Elut, 500 mg/6 ml, Varian, Harbor, CA, USA) was pre-conditioned with 10 ml of methanol and then with 20 ml of distilled water prior to use. A 50 ml volume of the diluted sample was applied to the SPE cartridge and the SPE cartridge was washed with 20 ml of distilled water. Phenolic compounds were eluted with 1 ml of methanol. Eluents were analyzed by HPLC and MEKC.

2.5. MEKC

A 25 m*M* SDS solution in 25 m*M* phosphate–50 m*M* borate buffer (pH 7.0) was used for MEKC. A potential of 15 kV was applied, detection was performed by measuring absorbance at 280 nm, and the capillary temperature was 20°C. The samples were injected by pressure at 350 mbar for 1.0 s. The capillary was rinsed with a 1.0 *M* NaOH solution for 120 s and distilled water for 120 s before each run.

3. Results and discussion

3.1. HPLC analysis

Recovery of the phenolic compounds from Japanese spirituous liquor by SPE as described above was found to be 95% by HPLC determination. A linear gradient HPLC method was successful for the separation of vanillic acid, vanillin, ferulic acid and 4-vinylguaiacol, as shown in Fig. 2A. The retention times of vanillic acid, vanillin and ferulic acid were shorter than that of 4-vinylguaiacol. With Japanese spirituous liquor sample, vanillin and 4-vinylguaiacol were detected but neither ferulic acid nor vanillic acid, as shown in Fig. 2B. Probably, ferulic acid was not distilled into the spirituous liquor and 4-vinylguaiacol was already converted to vanillin during aging.

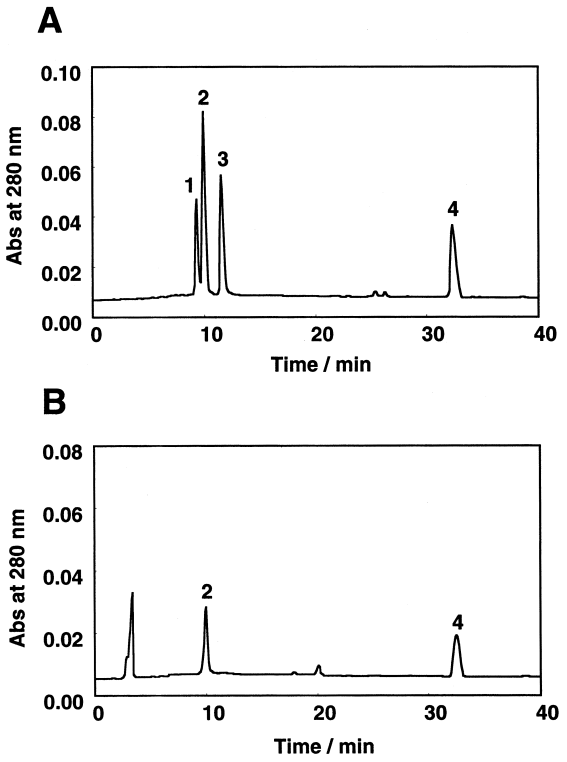

Fig. 2. Separation of standard phenolic compounds and Japanese spirituous liquor sample by HPLC. (A) Standard phenolic compounds. (B) Japanese spirituous liquor sample. Peak identification: 1=vanillic acid; 2=vanillin; 3=ferulic acid; 4=4-vinylguaiacol. HPLC conditions: mobile phase: changed from 50 m*M* acetate buffer (pH 4.0) in water–acetonitrile (95:5) to 50 m*M* acetate buffer (pH 4.0) in water (35:65) in 60 min; flow-rate: 1.0 ml/min; column temperature: 40°C; detection: 280 nm.

3.2. MEKC analysis

In the same way as in HPLC analysis, Japanese spirituous liquor samples were prepared by SPE. MEKC with SDS solutions in phosphate–borate buffers were successful for the separation of vanillin, ferulic acid, vanillic acid and 4-vinylguaiacol, as shown in Fig. 3. The migration times of all peaks in

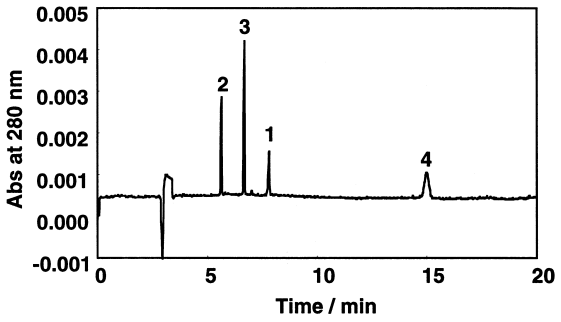

Fig. 3. Separation of standard phenolic compounds by MEKC. MEKC conditions: capillary: 36 cm×50 μm I.D.; running solution: 25 m*M* SDS solution in 25 m*M* phosphate–50 m*M* borate buffer (pH 7.0); applied voltage: 15 kV; temperture: 20°C; detection: 280 nm. Peak identification as in Fig. 2.

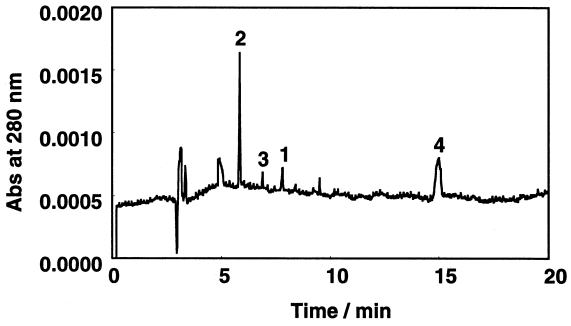

Fig. 4. Separation of Japanese spirituous liquor sample by MEKC. Conditions as in Fig. 3. Peak identification as in Fig. 2.

MEKC were shorter than those in HPLC analysis except for vanillic acid.

Standard curves obtained with vanillin, ferulic acid, vanillic acid and 4-vinylguaiacol from 0.1 to 5.0 μg/ml, showed straight lines (vanillin; $r=0.998$: ferulic acid; $r=0.997$: vanillic acid; $r=0.998$: 4-vinylguaiacol; $r=0.996$). The detection limit of vanillin, ferulic acid and vanillic acid was 0.04 μg/ml, and that of 4-vinylguaiacol was 0.08 μg/ml. The phenolic compounds in Japanese spirituous liquor were determined with these standard curves.

The repeatability of the method was also evaluated for both intra- and inter-day variabilities, as shown in Table 1. As shown in Fig. 4, Japanese spirituous liquor samples were successfully analyzed by this method. Ferulic acid and 4-vinylguaiacol were detected in Japanese spirituous liquor samples, immediately after distillation. However, vanillin, ferulic acid, vanillic acid and 4-vinylguaiacol were detected in Japanese spirituous liquor on the market, probably a portion of 4-vinylguaiacol was converted into vanillin and vanillic acid by aging and oxidation.

3.3. Determination of phenolic compounds in Japanese spirituous liquor

The reported method has been used for the determination of vanillin, ferulic acid, vanillic acid and 4-vinylguaiacol in five Japanese spirituous liquor samples. The liquor samples A, B and C were used without aging, and the samples D and E were used after aging in barrels. The repeatability ($n=3$) for the determination of five Japanese spirituous liquor samples were acceptable with relative standard deviation (R.S.D.) values less than 1.7% (Table 2). In

Table 1
Repeatability for intra- and inter-day migration time and determination of vanillin, ferulic acid, vanillic acid and 4-vinylguaiacol

		Migration time		Determination	
	n	Mean (min)	R.S.D. (%)	Mean (μg ml^{-1})	R.S.D. (%)
Intra-day					
Vanillin	5	5.52	0.15	0.21	1.26
Ferulic acid	5	6.24	0.18	0.21	0.98
Vanillic acid	5	7.74	0.23	0.20	1.34
4-Vinylguaiacol	5	14.98	0.41	0.22	1.19
Inter-day					
Vanillin	3	5.61	0.87	0.23	2.89
Ferulic acid	3	6.35	1.05	0.23	2.57
Vanillic acid	3	7.82	1.11	0.23	2.55
4-Vinylguaiacol	3	15.06	1.67	0.25	3.46

Table 2
Repeatability for intra-day determination of phenolic compounds in Japanese spirituous liquor samples

	n	Vanillin		Ferulic acid		Vanillic acid		4-Vinylguaiacol	
		Mean ($\mu g\ ml^{-1}$)	R.S.D. (%)	Mean ($\mu g\ ml^{-1}$)	R.S.D. (%)	Mean ($\mu g\ ml^{-1}$)	R.S.D. (%)	Mean ($\mu g\ ml^{-1}$)	R.S.D. (%)
Without aging									
Sample A	3	0.012	0.84	N.D.[a]		0.010	1.25	0.42	1.28
Sample B	3	0.021	0.84	N.D.[a]		N.D.		0.31	1.14
Sample C	3	0.014	0.97	N.D.[a]		0.013	1.04	0.67	1.07
Aging in barrel									
Sample D	3	0.15	1.43	0.013	0.64	0.024	1.49	0.021	1.25
Sample E	3	0.17	1.28	0.012	0.73	0.021	1.67	0.032	1.41

N.D.: Not detected.
[a] No ferulic acid was detected in this sample, although it was slightly found in Fig. 4.

the samples without aging (A, B and C), 4-vinylguaiacol was found in higher amounts but little vanillin. In contrast, the aged samples (D and E) contained vanillin as the major component with a minor amount of 4-vinylguaiacol, suggesting that 4-vinylguaiacol was converted to vanillin during aging in the barrel. Ferulic acid detected in the aged Japanese spirituous liquor samples must come from the barrel, because ferulic acid contained in the freshly distilled liquor is easily converted to 4-vinylguaiacol. These phenolic compounds in the aged Japanese spirituous liquor samples were not successfully separated from impurities originated from the barrel by capillary zone electrophoresis with borate buffer at pH 9.0.

4. Conclusions

In conclusion, MEKC is found to be a useful technique for the analysis of vanillin, ferulic acid, vanillic acid and 4-vinylguaiacol found in Japanese spirituous liquor. Compared to the HPLC method, MEKC method is advantageous due to its low running cost, and shorter analysis time requirements. Vanillin, ferulic acid, vanillic acid and 4-vinylguaiacol were completely separated by MEKC. This MEKC method can be applied to routine quality control of Japanese spirituous liquor.

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