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Short communication

Simultaneous analysis of tyrosol, tryptophol and ferulic acid in commercial sake samples by micellar electrokinetic chromatography

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Abstract

Micellar electrokinetic chromatography (MEKC) was applied to the simultaneous analysis of tyrosol, tryptophol and ferulic acid in sake, using an uncoated fused-silica capillary with sodium dodecyl sulfate solutions in borate buffers at pH 8.5 and UV detection at 280 nm. Compared to HPLC, the MEKC method is advantageous due to its low running cost, and shorter analysis time requirements. Several commercial sake samples were analyzed by the developed technique for quantitation of the three compounds.

Keywords: Beverages; Food analysis; Tyrosol; Tryptophol; Ferulic acid

1. Introduction

Sake is the most popular alcoholic beverage in Japan. The raw materials of sake are rice and water, and the most characteristic features of brewing sake are the use of rice koji (a mold, produces the diastatic enzyme, which converts starch to sugar, and the proteolytic enzyme, which breaks down proteins, as well as more than fifty other enzymes that are responsible for the flavor and taste of sake) and parallel fermentation. Sake has a good flavor due to alcoholic, etheric and phenolic compounds contained therein. However, tyrosol, tryptophol and ferulic acid in sake taste bitter, and the bitter taste lowers the quality of the sake [1]. These constituents have been analyzed by high-performance liquid chromatography (HPLC) and gas chromatography (GC) [2]. The compounds are produced from raw materials by the yeast and rice koji [3]. Tyrosol is a major alcoholic bitter constituent found in alcoholic beverages and is produced from tyrosine by the yeast, as shown in Fig. 1A. Tryptophol is an alcoholic con-

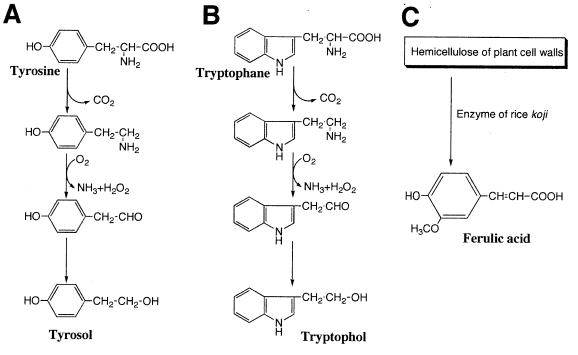

Fig. 1. Schematic of the mechanism for the production of tyrosol (A), tryptophol (B) and ferulic acid (C) during sake brewing.

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stituent found in sake and generated from tryptophan by the yeast, as shown in Fig. 1B, and has a bitter taste. Ferulic acid, initially bound to the hemicellulose of plant cell walls, is liberated by hydrolytic enzymes from koji mold, and has been found to possess anti-oxidant property, as shown in Fig. 1C.

Capillary electrophoresis (CE) has high separation efficiency and requires a small amount of sample. Micellar electrokinetic chromatography (MEKC), first reported by Terabe et al. in 1984 [4], can separate neutral analytes by the difference in their partitioning with the micelle. Recently, we reported [5–10] the analysis of food additives and principal ingredients in food by the MEKC method. This paper describes simultaneous analysis of tyrosol, tryptophol and ferulic acid in sake by MEKC.

2. Experimental

2.1. Chemicals

Sake samples were purchased from a market. Three constituents in sake employed were commercial products: ferulic acid (4-hydroxy-3-methoxycinnamic acid) (Nacalai Tesque, Kyoto, Japan), tyrosol (*p*-hydroxyphenethyl alcohol) and tryptophol (3-(2-hydroxyethyl)indole) (Wako, Osaka, Japan). Sodium dodecyl sulfate (SDS) from Fluka (Buchs, Switzerland) was used as the anionic micellar pseudo-stationary phases in MEKC. All other chemicals and solvents were of analytical reagent grade, supplied by Wako. Pure water was prepared by purifying distilled water with a Milli-Q SP system (Millipore, Bedford, MA, USA) prior to use.

2.2. Apparatus

The capillary electrophoretic system was a BioFocus 3000 CE system (Bio-Rad, Richmond, CA, USA) equipped with a pre-packed cartridge of an uncoated fused-silica capillary of 50 μm I.D. and a total length of 36 cm. The effective length of the capillary was 31.4 cm. 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). For analytical separation an L-column ODS packed column, 150 mm×4.6 mm I.D. (Kagakuhin Kensa Kyokai, Tokyo, Japan) was used, and a shim-pack PREP-ODS packed column, 250 mm×20.0 mm I.D. (Shimadzu, Kyoto, Japan) was used for preparative separation.

2.3. Sample preparation

Tyrosol, tryptophol and ferulic acid samples in sake were prepared by a solid-phase extraction (SPE) cartridge. A 20 ml sample of sake was evaporated to dryness at 40°C. The residue was dissolved in 10 ml pure water (concentrated sake sample). A 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 pure water prior to use. A 10 ml aliquot of the concentrated sake sample was applied to the SPE cartridge and the SPE cartridge was washed with 20 ml of pure water. These three constituents were eluted with 1 ml of methanol. Eluents were diluted with pure water and analyzed by HPLC and MEKC.

2.4. HPLC separation and preparation

Isocratic elution was performed for tyrosol, tryptophol and ferulic acid with a mixture of pure water–acetonitrile (85:15) 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.

2.5. MEKC separation

A 20 m*M* SDS solution in 30 m*M* borate buffer at pH 8.5 was used for the MEKC method. A potential of 15 kV was applied, detection was carried out by UV absorbance at 280 nm, and the capillary was thermostated at 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 60 s and distilled water for 60 s then the running buffer was introduced into the capillary before each injection. The capillary was stored after filling with distilled water overnight and was rinsed with 1.0 *M* NaOH solution and distilled water at the start of analysis.

3. Results and discussion

3.1. HPLC and MEKC analysis

In HPLC and MEKC analysis, tyrosol, tryptophol and ferulic acid in sake samples were extracted by SPE. Both HPLC with isocratic elution and MEKC with SDS solutions in borate buffers were successful for the separation of these constituents in sake, but retention or migration order was different between tryptophol and ferulic acid, as shown in Fig. 2. The pH of the HPLC mobile phase was acidic and

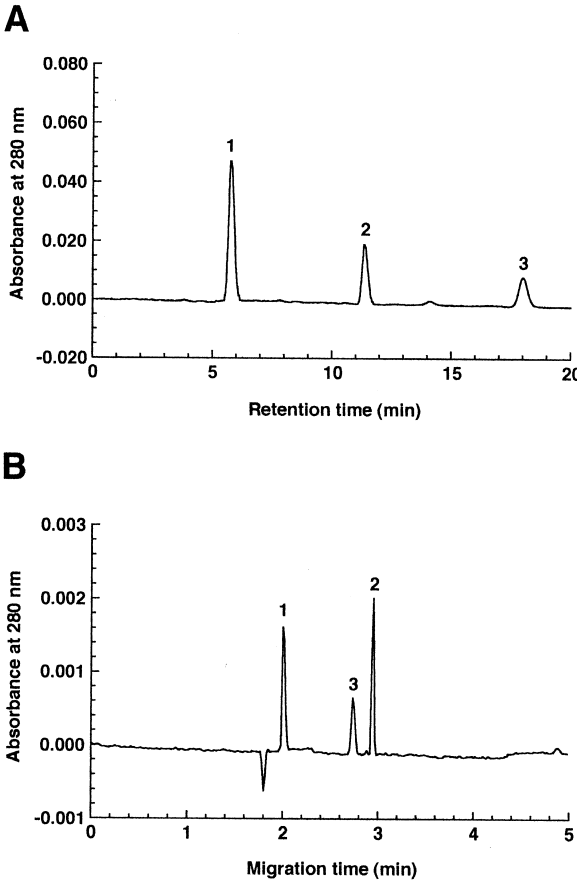

Fig. 2. Separation of tyrosol, tryptophol and ferulic acid by HPLC (A) and MEKC (B). (A) HPLC conditions: mobile phase: water containing 0.05% TFA: acetonitrile (85:15); flow-rate: 1.0 ml/min; column temperature: 40°C; detection: 280 nm. (B) MEKC conditions: capillary: 36 cm×50 μm I.D.; running solution: 20 m*M* sodium dodecyl sulfate solution in 30 m*M* borate buffer (pH8.5); applied voltage: 15 kV; temperature: 20°C; detection: 280 nm. Peaks: 1=tyrosol; 2=tryptophol; 3=ferulic acid.

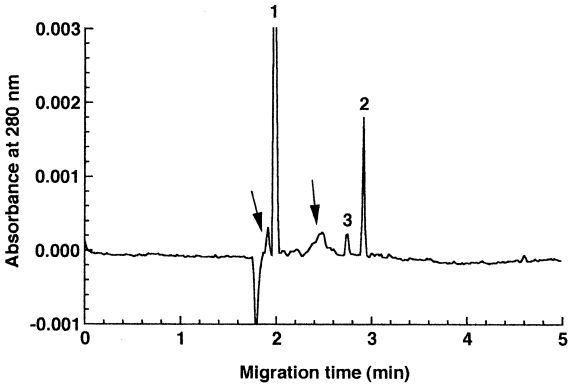

Fig. 3. Separation of tyrosol, tryptophol and ferulic acid in commercial sake sample by MEKC. Conditions and peaks as in Fig. 2. Other peaks (indicated by arrows) were not identified.

therefore, the dissociation of ferulic acid was suppressed, causing a higher retention, whereas pH 8.5 in the MEKC solution generated the ferulate ion which will not interact with the anionic SDS micelles. In Fig. 2A the fact that HPLC method can be applied to the routine quality control of sake is shown, employing an isocratic elution and needing an analysis time longer than 20 min. However, the MEKC method required about 8 min for one analysis including the capillary conditioning, as shown in Fig. 2B. In Fig. 3 an electropherogram of these constituents in commercial sake samples, which were prepared without SPE method is shown. The peaks were easily identified as tyrosol, tryptophol and ferulic acid. Other peaks (indicated by arrows in Fig. 3) were not identified, but these constituents did not taste bitter.

Calibration curves obtained with tyrosol, tryptophol and ferulic acid from 0.1 to 200.0 μg/ml, showed straight lines (tyrosol; $r=0.995$: tryptophol; $r=0.996$: ferulic acid; $r=0.997$). The detection limits of these chemicals were 0.05 μg/ml. Three constituents in sake were determined with these calibration curves. The repeatability ($n=5$) of the method was also evaluated for both intra- and inter-day RSDs, as shown in Table 1.

3.2. Determination of tyrosol, tryptophol and ferulic acid in commercial sake samples

Tyrosol, tryptophol and ferulic acid in ten commercial sake samples were determined by this meth-

Table 1
Repeatability in intra- and inter-day migration time and determination of standard tyrosol, tryptophol and ferulic acid[a]

	Migration time		Determination	
	Mean/min	RSD/%	Mean/μg ml^{-1}	RSD/%
Intra-day (n=5)				
Tyrosol	2.04	0.35	10.10	0.31
Tryptophol	2.79	0.32	10.13	0.69
Ferulic acid	2.98	0.18	10.17	0.53
Inter-day (n=5)				
Tyrosol	2.06	1.54	10.09	0.98
Tryptophol	2.81	0.81	10.16	0.94
Ferulic acid	3.01	0.69	10.20	1.09

[a] Conditions are the same as given in Fig. 2B.

od, as shown in Table 2. Commercial sake samples A, B, C, and D were called 'junmai-shu' in Japanese. These are pure rice sake in which only rice, rice koji and water are used as ingredients, with no additions of ethanol, sugar, or anything else. Sake samples E, F and G were called 'honjohzo-shu' in Japanese. In these samples, not more than 120 ml of 100% ethanol per kg of white rice and no sugar have been added during the sake-brewing process. Sake samples H, I and J were called 'ginjo-shu' in Japanese. This is a special type of 'junmai-shu' or 'honjohzo-shu', and professional tasters are almost unanimous in regarding it as the ultimate achievement of the brewer's art. The concentration of tyrosol was the highest obtained, and the concentration of tryptophol was the most constant obtained in all commercial sake samples. Samples C, D and G were unheated during the manufacturing process of sake which is called 'nama-shu' (brew draft sake) in Japanese. These sake are usually consumed after refrigeration. The commercial sake samples A, B, E, F, H, I and J were heated twice during the manufacturing process. These sake are usually consumed under warm condition. The concentration of ferulic acid was found to be lower in the heated sake samples, probably due to the conversion to volatile 4-vinylguaiacol [11,12] by heating during the manufacturing process. Ferulic acid has been found to possess anti-oxidant properties, and an analytical method for ferulic acid is thus important. The repeatability (n=5) for the determination of these commercial sake samples were acceptable with RSD values less than 1.7%.

Table 2
Repeatability in intra-day determination of tyrosol, tryptophol and ferulic acid in commercial sake samples by MEKC[a]

	Tyrosol		Tryptophol		Ferulic acid	
	Mean/μg ml^{-1}	RSD/%	Mean/μg ml^{-1}	RSD/%	Mean/μg ml^{-1}	RSD/%
Junmai-shu (n=5)						
Sample A[b]	89.67	1.43	0.89	1.23	1.24	1.15
Sample B[b]	107.68	1.34	1.06	1.24	1.06	1.18
Sample C[c]	165.67	1.28	1.08	1.08	13.54	1.04
Sample D[c]	138.68	1.16	2.65	1.06	10.84	1.02
Honjohzo-shu (n=5)						
Sample E[b]	106.42	1.08	1.36	0.99	0.48	0.95
Sample F[b]	96.78	1.35	0.85	1.15	1.15	1.07
Sample G[c]	105.46	1.67	1.08	1.37	0.94	1.08
Ginjo-shu (n=5)						
Sample H[b]	72.65	1.24	0.65	1.35	0.34	1.25
Sample I[b]	88.68	1.32	0.87	1.26	0.42	0.97
Sample J[b]	95.86	1.14	0.76	1.15	0.57	1.38

[a] Conditions are the same as given in Fig. 3.
[b] Heated during the manufacturing process of sake.
[c] Unheated during the manufacturing process of sake.

4. Conclusions

MEKC is found to be a useful technique for the analysis of tyrosol, tryptophol and ferulic acid in several commercial sake samples. The MEKC method has several advantages over the HPLC method from viewpoints of low capillary costs, low running costs, small sample amounts, low production of waste materials, and short analysis times. In sake brewing, the analysis of tyrosol, tryptophol and ferulic acid from sake is very important for quality control. This MEKC method can be applied to the routine quality control of sake brewing.

References

[1] M. Moriwaki, Foods Food Ingred. (Jpn.) 155 (1993) 64.
[2] S. Sato, M. Tadenuma, K. Takahashi, J. Brew. Soc. Japan 70 (1975) 513.
[3] K. Aso, T. Nakayama, M. Maki, Hakkokogaku Zasshi 31 (1953) 43.
[4] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Trends Anal. Chem. 56 (1984) 111.
[5] T. Watanabe, N. Hasegawa, A. Yamamoto, S. Nagai, S. Terabe, Bunseki Kagaku 45 (1996) 765.
[6] T. Watanabe, N. Hasegawa, A. Yamamoto, S. Nagai, S. Terabe, Biosci. Biotech. Biochem. 61 (1997) 1179.
[7] T. Watanabe, A. Yamamoto, S. Nagai, S. Terabe, Anal. Sci. 13 (1997) 571.
[8] T. Watanabe, A. Yamamoto, S. Nagai, S. Terabe, J. Chromatogr. A 793 (1998) 409.
[9] T. Watanabe, A. Yamamoto, S. Nagai, S. Terabe, Food Sci. Technol. Int. Tokyo 4 (1998) 54.
[10] T. Watanabe, A. Yamamoto, S. Nagai, S. Terabe, Anal. Sci. 14 (1998) 839.
[11] T. Koseki, Y. Ito, S. Furuse, K. Ito, K. Iwano, J. Ferment. Bioeng. 82 (1996) 46.
[12] T. Koseki, Y. Ito, K. Ito, K. Iwano, M. Tadenuma, J. Brew. Soc. Japan 89 (1994) 408.