

Application News

No. C156

Liquid Chromatograph Mass Spectrometry

Analysis of Chiral Amino Acids within Fermented Beverages Utilizing a Column Switching System

With the exception of glycine, the 20 types of amino acids that make up proteins occur as D and L optical isomers. In recent years, it has been found that D-amino acids are contained in various foods such as fruits and vegetables, although much less abundant than L-amino acids. Particularly, it is known that fermented foods contain a number of D-amino acids.

The D-amino acids contained in foods have various effects. D-alanine, D-leucine, and D-phenylalanine are known to taste sweeter than their L-amino acid counterparts. For this reason, the amount of D-amino acids contained in a fermented food is considered to affect the taste of the food, and high sensitivity and highly selective analysis methods for D-amino acids in fermented foods are gaining attention.

This article introduces an example analysis of fermented beverages using a column switching system (introduced in Application News No. C149) which alternates between two types of chiral columns using high-pressure column switching valves (FCV).

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Sample Pretreatment

Three types of black vinegar beverages and two types of yogurt beverages were used as samples. Each sample was pretreated by liquid-liquid extraction using water, methanol, and chloroform^{*1}. Fig. 1 indicates the protocol.

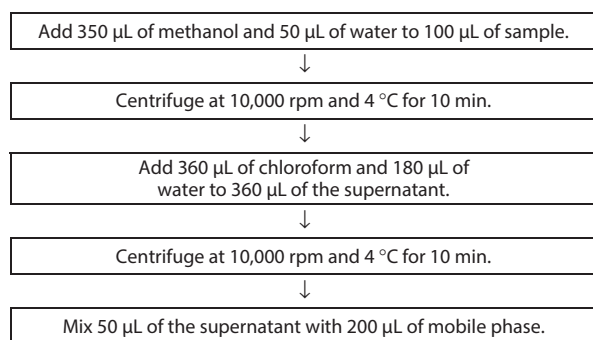


Fig. 1 Pretreatment Protocol

Table 1 Measurement Conditions

Column	: CROWNPAK CR-I(+)/CR-I(-) (3 mm × 150 mm, 5 µm)
Mobile phase	: acetonitrile/ethanol/water/TFA = 80/15/5/0.5
Flow rate	: 0.6 mL/min.
Injection volume	: 1 µL
Oven temperature	: 20 °C
Ionization mode	: ESI (Positive)
Probe voltage	: +4.0 kV
Nebulizing gas flow	: 3.0 L/min.
Drying gas flow	: 15.0 L/min.
Heating gas flow	: 5.0 L/min.
Interface temperature	: 250 °C
DL temperature	: 250 °C
Block heater temperature	: 300 °C

Analysis Results of Black Vinegar Beverages

We verified that D-amino acids are contained in all three types of black vinegar beverages (Table 2). The obtained D/L ratios indicate that black vinegar beverages B and C contain D-Ala in relatively large amounts.

Table 2 D/L Ratio of Amino Acids in Black Vinegar Beverages

	Black Vinegar Beverage A		Black Vinegar Beverage B		Black Vinegar Beverage C	
	Area	Ratio of D/L	Area	Ratio of D/L	Area	Ratio of D/L
D-Ala	7127	3.8 %	54094	20.5 %	26505	15.5 %
L-Ala	187083		263547		171483	
D-Arg	23703	0.6 %	81626	2.4 %	106896	1.7 %
L-Arg	3945110		3353883		6214029	
D-Asn	7047	1.3 %	11213	3.4 %	13135	3.0 %
L-Asn	547867		333152		433012	
D-Asp	6934	1.5 %	7086	2.3 %	8248	2.2 %
L-Asp	476730		302901		370152	
D-Cys	(N.D.)	-	(N.D.)	-	(N.D.)	-
L-Cys	(N.D.)	-	(N.D.)	-	(N.D.)	-
D-Gln	4153	56.1 %	5013	128.1 %	5738	17.3 %
L-Gln	7399		3912		33155	
D-Glu	11658	0.7 %	36502	2.2 %	7575	1.1 %
L-Glu	1635202		1675657		713130	
Gly	2375	-	6382	-	3163	-
D-His	(N.D.)	-	(N.D.)	-	(N.D.)	-
L-His	351973	-	410895	-	232228	-
D-Ile	1262	0.3 %	(N.D.)	-	1861	0.6 %
L-Ile	392041		580580		330869	
D-allo-Ile	1816	50.3 %	(N.D.)	-	2519	136.9 %
L-allo-Ile	3612		4357		1840	
D-Leu	3255	0.5 %	4698	0.5 %	4198	0.9 %
L-Leu	691108		1031536		493487	
D-Lys	13921	1.4 %	4446	0.4 %	28009	5.1 %
L-Lys	965688		1220610		548517	
D-Met	(N.D.)	-	(N.D.)	-	(N.D.)	-
L-Met	22647	-	48753	-	13151	-
D-Phe	2738	0.4 %	3587	0.7 %	3634	0.9 %
L-Phe	746758		549410		419561	
DL-Pro	301069	-	683984	-	549718	-
D-Ser	10568	9.3 %	8036	7.5 %	4653	8.5 %
L-Ser	113543		106729		54472	
D-Thr	2646	1.7 %	4374	2.3 %	2036	1.2 %
L-Thr	159723		193429		170581	
D-allo-Thr	1973	91.6 %	3538	120.7 %	1297	66.6 %
L-allo-Thr	2153		2932		1946	
D-Trp	2098	23.2 %	2195	39.1 %	4159	39.6 %
L-Trp	9045		5609		10506	
D-Tyr	7314	1.7 %	2495	0.8 %	4026	1.4 %
L-Tyr	437963		314522		297401	
D-Val	3046	0.5 %	3186	0.4 %	3613	0.9 %
L-Val	573054		870777		387972	

■ Analysis Results of Yogurt Beverages

We verified that D-amino acids are contained in both types of yogurt beverages (Table 3). The obtained D/L ratios indicate that D-Ala, -Arg, -Asn, -Asp, -Glu, -Lys, and -Ser amino acids are contained in relatively large amounts. Particularly, D-Glu is contained in both yogurt beverages by over 40 times more than L-Glu.

Table 3 D/L Ratio of Amino Acids in Yogurt Beverages

	Yogurt D		Yogurt E	
	Area	Ratio of D/L	Area	Ratio of D/L
D-Ala	140959	164.0 %	37900	40.2 %
L-Ala	85940		94190	
D-Arg	81779	6.9 %	95602	36.5 %
L-Arg	1192614		262060	
D-Asn	60836	43.2 %	3209	16.5 %
L-Asn	140872		19416	
D-Asp	47149	38.1 %	2441	15.3 %
L-Asp	123860		16003	
D-Cys	(N.D.)	-	(N.D.)	-
L-Cys	(N.D.)		(N.D.)	
D-Gln	4743	0.6 %	5157	19.8 %
L-Gln	856603		26021	
D-Glu	412572	4091.1 %	163715	5069.6 %
L-Glu	10085		3229	
Gly	957	-	1106	-
D-His	(N.D.)		(N.D.)	
L-His	839834	-	175326	5.2 %
D-Ile	1428		1366	
L-Ile	176626	0.8 %	130832	1.0 %
D-allo-Ile	2225	59.4 %	1247	39.3 %
L-allo-Ile	3744		3172	
D-Leu	4042	1.0 %	(N.D.)	-
L-Leu	403567		132923	
D-Lys	1151264	73.5 %	24797	3.5 %
L-Lys	1565451		698677	
D-Met	463	0.9 %	(N.D.)	-
L-Met	54490		(N.D.)	
D-Phe	1600	0.5 %	1799	1.5 %
L-Phe	313615		117732	
DL-Pro	2094819	-	888155	-
D-Ser	14619		8332	
L-Ser	101651	14.4 %	28395	29.3 %
D-Thr	1711	1.5 %	3314	4.6 %
L-Thr	112074		71653	
D-allo-Thr	1973	42.5 %	1020	23.7 %
L-allo-Thr	4647		4294	
D-Trp	3039	1.9 %	1879	13.3 %
L-Trp	155899		14086	
D-Tyr	4882	2.1 %	5876	107.4 %
L-Tyr	230926		5470	
D-Val	1241	0.4 %	1277	0.9 %
L-Val	285792		148323	

■ Comparison of D/L Amino Acid Amounts in Fermented Beverages

We next compared the amount of amino acids in the black vinegar and yogurt beverages with regard to the amino acids which were found in relatively large amounts as D isomers (Fig. 2). This comparison shows that the yogurt beverages contain more types of D-amino acids compared to the black vinegar beverages. D-Ala and D-Ser were found in both the black vinegar and the yogurt beverages, but the yogurt beverages contain more D-Ala than the black vinegar beverages. The L-Glu contained in the yogurt beverages is far less in amount than that contained in the black vinegar beverages. Furthermore, the amount of D-Glu contained in the yogurt beverages is relatively large considering the amount of L-Glu contained in the black vinegar beverages.

Yogurt beverage D contains various types of D-amino acids in relatively large amounts. Particularly, D-Ala and D-Lys were contained in large amounts in comparison with the black vinegar beverages.

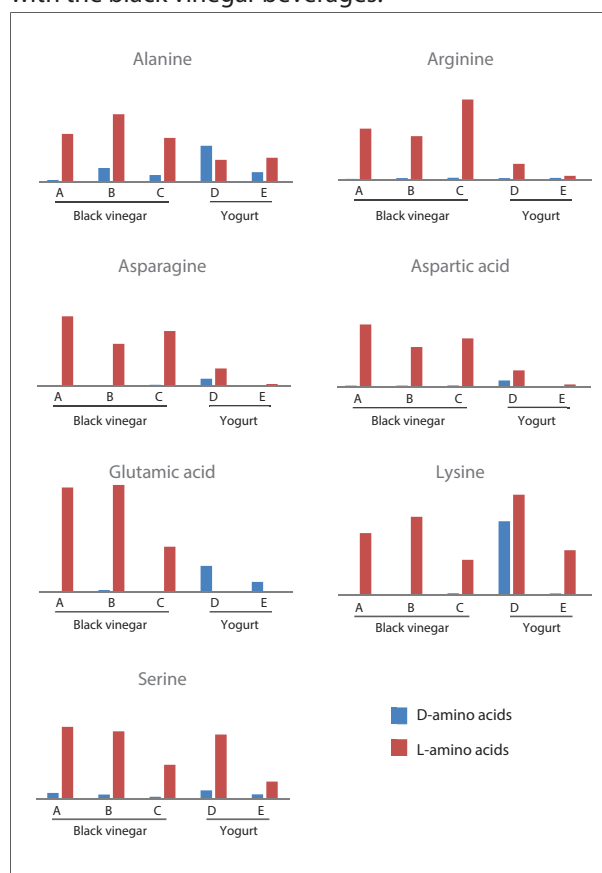


Fig. 2 Comparison of D/L Amino Acids in Black Vinegar Beverages and Yogurt Beverages

These analysis results indicate that actual samples can be effectively analyzed and that this column switching system is useful for the analysis of chiral amino acids.

Reference

*1 Nakano, Y., Konya, Y., Taniguchi, M., Fukusaki, E., Journal of Bioscience and Bioengineering, 123, 134-138 (2016)

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