

Molecules

Supplementary Materials

Determination of D- and L-Amino Acids in Garlic Foodstuffs by Liquid Chromatography–Tandem Mass Spectrometry

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1 Chemicals and reagents

L-Alanine (Ala), L-arginine (Arg), L-asparagine (Asn), L-aspartic acid (Asp), L-citrulline (Cit), γ -aminobutyric acid (GABA), L-glutamine (Gln), L-glutamate (Glu), glycine (Gly), L-histidine (His), L-isoleucine (Ile), L-leucine (Leu), L-lysine (Lys), L-methionine (Met), L-phenylalanine (Phe), L-proline (Pro), L-serine (Ser), L-tryptophan (Trp), L-threonine (Thr), L-tyrosine (Tyr), L-valine (Val), and L-ornithine (Orn) were obtained from Kyowa Hakko Bio Co., Ltd. (Tokyo, Japan). D-Ala, D-Phe, D-Trp, D-Ser, LC-MS-grade CH₃OH, HPLC-grade formic acid, and APDSTAG[®] Wako Amino Acids Internal Standard Mixture Solution were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). *N,N*-Dimethylaminopyridine (DMAP), D-Ala, D-Arg, D-Asn, D-Asp, D-Gln, D-Glu, D-His, D-Ile, D-Leu, D-Lys, D-Thr, and D-Val were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), and D-kynurenine (KYN), L-KYN, D-Met, D-Tyr, DL-Orn, D-Pro, and ammonium formate were procured from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). DL-Cit was purchased from Matrix Scientific (Columbia, SC, USA), and LC-MS-grade CH₃CN was obtained from Kanto Kagaku Co., Ltd. (Tokyo, Japan). The anion-exchange syringe-type cartridge, InertSep[®] NH₂ (50 mg/mL), was purchased from GL Sciences Inc. (Tokyo, Japan), and the water used was purified using a Milli-Q Labo system (Nihon Millipore Co. Ltd., Tokyo, Japan). Millex[®]-

LG filters (0.20 μm) were purchased from Merck Ltd. (Darmstadt, Germany).

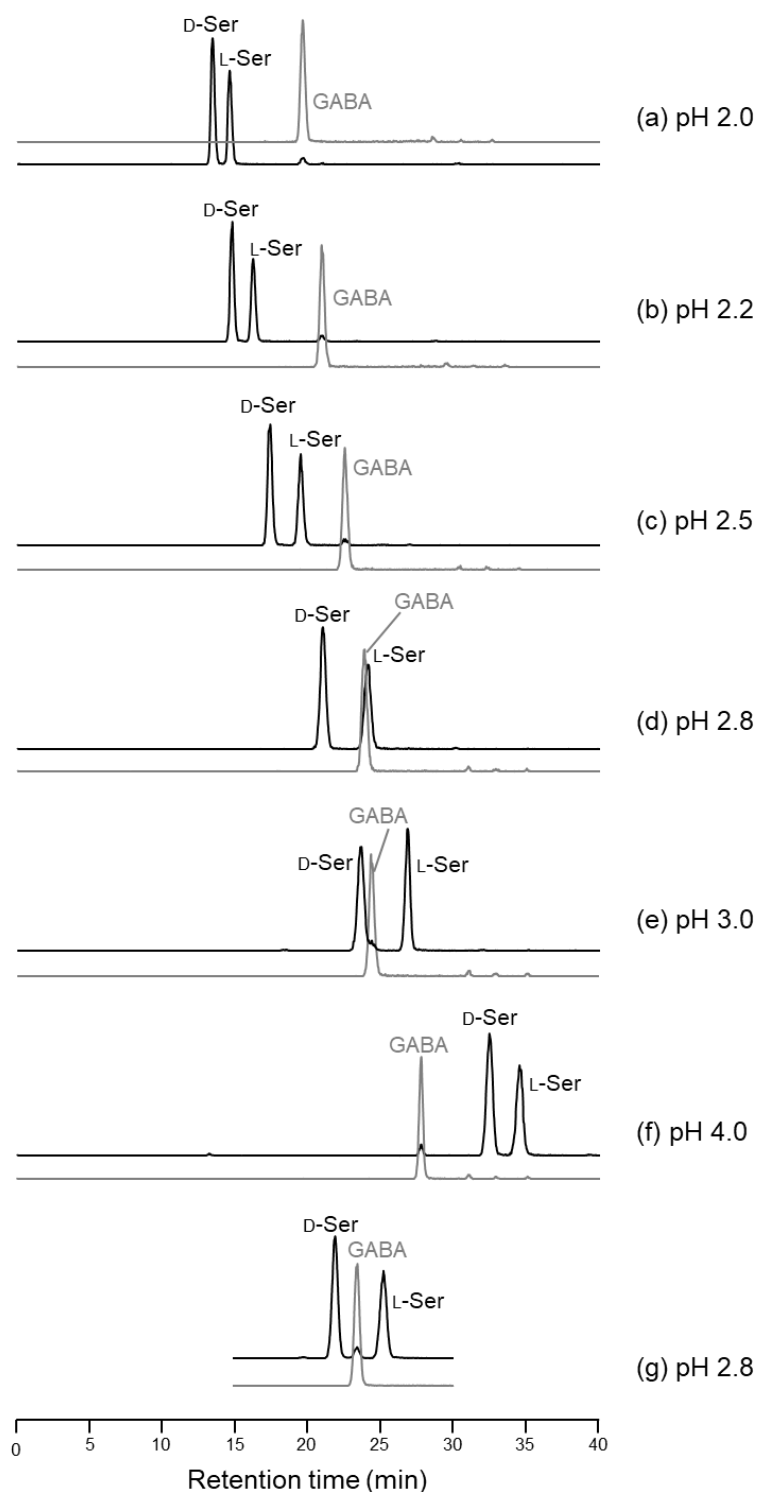


Figure S1 Chromatograms of DL-Ser and GABA obtained using mobile phase A at different pH levels. Mobile phase A) (a–f): H₂O/MeOH/10 mM ammonium formate (1/1/3, v/v/v), (g) H₂O/MeOH/10 mM ammonium formate (pH: 2.8, (5/2/3, v/v/v), B) 10 mM ammonium formate in [H₂O/MeOH (3/7, v/v)]. The time program for gradient elution of A) and B) is described in the text.

Table S1 Transitions for multiple-reaction monitoring (MRM) of amino acids and the corresponding internal standard (IS)

Amino acid				IS			
	Precursor		Product		Precursor		Product
Asn	407.30	>	91.10	Asn-IS	413.30	>	91.10
Ala	364.20	>	91.10	Ala-IS	367.20	>	91.10
Cit	450.10	>	91.10	Cit-IS	454.10	>	91.10
Gln	421.20	>	91.10	Gln-IS	428.20	>	91.10
Ser	380.10	>	91.10	Ser-IS	384.10	>	91.10
Gly	350.20	>	91.10	Gly-IS	353.20	>	91.10
GABA	378.05	>	91.15	GABA		—	
Thr	394.10	>	91.10	Thr-IS	398.10	>	91.10
Glu	422.10	>	91.10	Glu-IS	428.10	>	91.10
Asp	408.10	>	91.10	Asp-IS	411.10	>	91.10
His	430.20	>	91.10	His-IS	439.20	>	91.10
Pro	390.10	>	91.10	Pro-IS	396.10	>	91.10
Val	392.10	>	91.10	Val-IS	398.10	>	91.10
Met	424.10	>	91.10	Met-IS	430.10	>	91.10
Arg	449.20	>	91.10	Arg-IS	453.20	>	91.10
KYN	483.30	>	91.10	KYN-IS		—	
Ile	406.10	>	91.10	Ile-IS	413.10	>	91.10
Leu	406.10	>	91.10	Leu-IS	409.10	>	91.10
Trp	479.30	>	91.10	Trp-IS	492.10	>	91.10
Phe	440.10	>	91.10	Phe-IS	450.10	>	91.10
Orn	681.40	>	91.10	Orn-IS	686.10	>	91.10
Lys	695.40	>	91.10	Lys-IS	703.10	>	91.10
Tyr	730.20	>	91.10	Tyr-IS	736.20	>	91.10

GABA-IS and KYN-IS were not included in APDSTAG[®] Wako Amino Acids Internal Standard Mixture Solution. Thus, Gly-IS and Met-IS were used as IS for GABA and KYN, respectively.

Table S2 Limit of detection (LOD, $S/N = 3$) for amino acids (fmol/injection).

	D-Amino acid		L-Amino acid	
	Previous study	This study	Previous study	This study
Asn	—	4.47	10.5	4.56
Ala	17.4	3.28	3.83	5.40
Cit	—	3.90	—	3.30
Gln	—	4.38	238	3.71
Ser	5.75	3.30	9.68	3.43
Thr	—	3.29	1.54	4.20
Glu	—	4.89	36.0	7.42
Asp	—	10.4	17.8	13.7
His	—	5.35	8.31	3.79
Pro	—	4.85	22.4	3.30
Val	—	2.93	2.12	4.28
Met	—	3.11	1.8	3.87
Arg	—	2.65	4.52	2.42
KYN	—	3.10	—	3.38
Ile	—	3.48	0.501	4.07
Leu	—	2.94	0.628	3.87
Trp	—	2.49	14.4	2.68
Phe	—	3.23	4.20	2.94
Orn	—	2.41	2.39	2.77
Lys	—	2.41	4.07	2.55
Tyr	—	2.07	4.25	2.66

	Previous study	This study
GABA	—	5.04
Gly	12.6	3.27
β-Ala	—	3.92

