Comparative Study between Gas Chromatography and Ion-exchange Chromatography on Amino Acid Analysis of Foods[†]

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Received May 1, 1978

Ion-exchange chromatography is so far the most widely used method of amino acid analysis. On the other side, a different technique, gas chromatography was studied by other investigators.¹⁻¹⁵ The advantages of gas chromatography are obviously the low cost and the much greater versatility of the instrument compared with specialized amino acid analyzers. Moreover, gas chromatography offers a simple means of combining the analytical system with mass spectrometry. The use of gas chromatography to determine amino acids in several foods, such as oliseed¹⁶ and mushroom,²⁰ has been reported. Their procedure, however, include the complicated clean-up as pre-treatment for derivatization of amino acids. In this study, we examined amino acids in many kinds of foods by gas chromatographic analysis excluding clean-up, and the comparative study between gas chromatography and ion-exchange chromatography was carried out.

Eight kinds of foods, pork, chicken, tuna (lean), milk, rice, wheat (flour), soybean, and potato were used. Samples were freeze-dried, fat-extracted and made to powders. Nitrogen contents of samples were determined by semi-micro Kjeldahl method. Samples were hydrolyzed in 6 N HCl at 110°C for 22 hr.

For the gas chromatographic analysis of amino acid, various derivatives have been developed such as N-acetyl methyl esters,¹ N-acetyl *n*-propyl esters,² N-TFA (trifluoroacetyl) methyl esters,^{3,4} N-TFA *n*-butyl esters,⁵⁻¹³ N-HFB (heptafluorobutyl) *n*-propyl es-

ters,¹⁴⁻¹⁶ N-isoBOC (butyloxycarbonyl) methyl esters¹⁷ and TMS (trimethylsilyl) derivatives¹⁸ etc. In this study, N-HFB *n*-propyl ester, which was possible to separate the protein amino acids on a single column in short time, was selected. The procedure for derivatization was according to the method appeared in the references.¹⁴⁻¹⁶ For chromatography, a gas chromatograph, Shimadzu model GC-6A, equipped with a flame ionization detector, was used. The column was a 3 m × 3 mm I.D. glass column packed with 3% OV-1 on Chromosorb W(HP), 80~100 mesh. Peak area on chromatogram was measured by digital integrater, Shimadzu model ITG-4AX.

For ion-exchange chromatographic analysis of amino acids, an amino acid analyzer, Hitachi model KLA-3B, was used.

Food samples were hydrolyzed and the each hydrolysate was divided into two equal portions. One portion was analyzed by gas chromatography and the other was analyzed by ion-exchange chromatography. The samples were hydrolyzed three times and gas and ion-exchange chromatography were carried out on each hydrolysate.

The average values and respective standard deviations along with relative standard deviation percentages as the results of analysis are given in Tables I and II. In this study, tryptophan and cystine which are destroyed during hydrolysis were not determined. Furthermore, data for histidine analysis by gas chromatography were dismissed in several cases because of poor reproducibility.

Although Tables I and II show some differences in the amounts of several amino acids determined by the two methods of analysis, for example, Leu in tuna, Met in pork, chicken and milk, Tyr in pork, Thr in rice and Pro in soybean, it can be concluded that the analytical value obtained by the two methods are comparable. Table III shows the average of relative standard deviation of amino acid determined by the two methods. The average of RSD of all amino acid determined by gas chromatography was between 6.50 and 13.6% and that of ion-exchange chromatography was between 6.17 and 15.9%. Total average of the average of RSD by the two methods were equivalent 10.6%. This fact indicates that precision of amino acid determination by the two methods are identical. So, it may be concluded that gas chromatography can be used for amino acid analysis of foods as similar to ion-exchange chromatography.

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[†] Gas Chromatographic Analysis of Amino Acids in Biological Materials. Part I.

This report was presented at the Annual Meeting of Japanese Society of Analytical Chemistry, Yamaguchi-shi, Oct. 11, 1977.

TABLE I. AMINO ACID ANALYSIS OF FOODS BY GAS CHROMATOGRAPHY (GC) AND ION-EXCHANGE CHROMATOGRAPHY (IEC) (mg/gN)

Amino acid	Pork						Chicken						Tuna						Milk					
	GC			IEC			GC			IEC			GC			IEC			GC			IEC		
	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD
Ileu	244	13	5.3	242	21	8.7	206	47	22.8	207	27	13.0	236	11	4.7	201	23	11.4	368	16	4.3	346	13	3.8
Leu	504	26	5.2	480	32	6.7	398	56	14.1	399	29	7.3	432	4	0.9	373	20	5.4	782	50	6.4	663	47	7.1
Lys	451	104	23.1	518	47	9.1	395	13	3.3	436	28	6.4	383	70	18.3	429	14	3.3	557	112	20.1	553	25	4.5
Met	127	20	15.7	162	6	3.7	92	18	19.6	139	13	9.4	133	29	19.5	56	76	136	143			179	4	2.2
Phe	233	48	20.6	256	18	7.0	192	19	9.9	204	40	19.6	193	7	3.6	204	18	8.8	336	20	5.9	356	12	3.4
Tyr	168	23	13.7	214	15	7.0	144	14	9.7	178	23	12.9	198	28	14.1	171	14	8.2	295	30	10.2	317	13	4.1
Thr	261	37	14.2	278	24	8.6	197	16	8.1	237	17	7.2	224	25	11.2	227	14	6.2	289	11	3.9	311	11	3.5
Val	270	7	2.6	276	58	21.0	270	38	14.1	245	8	3.3	334	28	8.4	191	6	3.1	469	48	10.2	449	98	21.8
Arg	419	47	11.2	406	27	6.7	382	31	8.1	335	30	8.5	284	81	28.5	279	10	3.6	275	17	6.2	238	8	3.4
His				223	23	10.3	91	6	7.0	187	15	8.0	214	23	10.7	373	14	3.8				190	7	3.7
Ala	400	24	6.0	392	32	8.2	379	31	8.2	359	82	22.8	325	11	3.4	280	10	3.6	271	26	9.6	233	19	8.1
Asp	601	90	15.0	553	33	6.0	480	61	12.7	479	31	6.5	485	8	1.6	466	30	6.4	539	41	7.6	542	38	7.0
Glu	827	92	11.1	911	82	9.0	777	104	13.4	784	63	8.0	704	36	5.1	655	32	4.9	1509	60	4.0	1413	88	6.2
Gly	441	44	10.0	384	27	7.0	438	22	5.0	376	36	9.6	253	24	9.5	228	11	4.8	185	25	13.5	140	20	14.3
Pro	296	30	10.1	279	28	10.0	303	30	9.9	258	13	5.0	187	6	3.2	170	20	11.8	769	72	9.4	673	33	4.9
Ser	245	27	11.0	264	21	8.0	207	18	8.7	229	15	6.6	219	10	4.6	203	15	7.4	370	25	6.8	408	45	11.0

SD : standard deviation, RSD : relative standard deviation.

TABLE II. AMINO ACID ANALYSIS OF FOODS BY GAS CHROMATOGRAPHY (GC) AND ION-EXCHANGE CHROMATOGRAPHY (IEC) (mg/gN)

Amino acid	Rice						Wheat						Soybean						Potato					
	GC			IEC			GC			IEC			GC			IEC			GC			IEC		
	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD	Aver- age	SD	RSD
Ileu	206	41	19.9	206	27	13.1	209	19	9.1	171	25	14.6	232	9	3.9	218	9	4.1	217	54	24.9	181	9	5.0
Leu	481	69	14.3	481	41	8.5	487	38	7.8	367	57	15.5	386	11	2.8	391	32	8.2	298	18	6.0	298	18	6.0
Lys	184	30	16.3	214	46	21.5	120	28	23.3	120	21	17.5	265	44	16.6	315	40	12.7	393	101	25.7	342	10	2.9
Met	125	16	12.8	141	63	44.7	79	14	17.7	86	22	25.6	51	4	7.8	47	24	51.1	72	10	13.9	82	21	25.6
Phe	260	35	13.5	294	17	5.8	295	16	5.4	263	32	12.2	243	9	3.7	263	23	8.7	239	23	9.6	228	1	0.4
Tyr	266	38	14.3	323	19	5.9	166	26	15.7	157	35	22.3	190	25	13.2	185	15	8.1	212	37	17.5	212	12	5.7
Thr	162	12	7.4	199	12	6.0	161	0	0	149	23	15.4	216	8	3.7	211	15	7.1	189	16	8.5	205	16	7.8
Val	282	37	13.1	258	98	38.0	259	28	11.2	209	28	13.4	222	10	4.5	193	65	33.7	323	32	9.9	290	24	8.2
Arg	402	55	13.7	420	146	34.8	235	25	10.6	202	42	20.8	470	102	21.7	377	38	10.1	372	36	9.7	336	12	3.6
His	45	6	14.4	130	16	12.3				112	22	19.6	59			133	15	11.3				118	21	17.8
Ala	364	75	20.6	308	19	6.2	240	4	1.7	175	39	22.3	236	5	2.1	221	18	8.1	196	6	3.1	172	18	10.5
Asp	447	56	12.5	544	56	10.3	251	19	7.6	231	32	13.9	524	18	3.4	595	68	11.4	1211	183	15.1	1265	55	4.3
Glu	1095	139	12.7	956	61	6.4	2159	78	3.6	1868	312	16.7	900	10	11.1	939	94	10.0	1064	41	3.9	994	51	5.1
Gly	275	31	11.3	260	16	6.2	246	72	29.3	196	29	14.8	213	9	4.2	221	20	9.0	171	31	18.1	176	16	9.1
Pro	290	12	4.1	253	12	4.7	899	55	6.1	717	15	2.1	251	19	7.6	315	19	6.0	190	63	33.2	230	32	13.9
Ser	297	33	11.1	308	22	7.1	299	46	15.4	284	25	8.8	261	3	1.1	289	27	9.3	232	14	6.0	224	29	12.9

SD: standard deviation, RSD: relative standard deviation.

TABLE III. AVERAGE OF RELATIVE STANDARD DEVIATION (%) FOR ALL AMINO ACIDS OF EACH SAMPLES

	Gas chromatography	Ion-exchange chromatography
Pork	11.6	8.55
Chicken	11.2	10.9
Tuna	9.26	6.17 ^a
Milk	8.44	6.82
Rice	13.2	14.5
Wheat	11.0	15.9
Soybean	6.50	13.0
Potato	13.6	8.69
Average	10.6	10.6

^a RSD for Met was eliminated.

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