Gas-Liquid Chromatographic Determination of Amino Acids in Some Korean Foods

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Gas-liquid chromatography에 의한 韓國 主要食品의 아미노酸 含量測定

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SUMMARY

The purpose of this study was to determine protein amino acid contents of some Korean foods by gas-liquid chromatography, and to evaluate this technique as a procedure for the quantitative determination of amino acids in foods. The crude protein content of foods was also estimated from the nitrogen content.

1. Nitrogen content of each food sample was determined previously to adjust the amount of sample for GLC analysis

2. In the analysis of 17 known amino acids, a linear relationship was found between the weight of 13 amino acids of 17 amino acids, the internal standard as well as the injection volume of a mixture and the detector responses for the derivatives of the amino acids. No response for arginine, cystein, histidine, and tyrosine was observed.

3. The relative molar response (RMR) values for the 13 amino acids of standard solution relative to glutamic acid as "1.00" were obtained under normal operating conditions with a hydrogen flame ionization detector.

4. The recovery of amino acids from their mixtures with natural food materials was carried out. The recoveries were essentially quantitative except threonine and serine. An overall mean recovery of 11 amino acids was 101.4±8.4 per cent before hydrolysis and 98.1±8.7 per cent after hydrolysis of samples.

5. The comparative analysis of the acid hydrolysates of two food samples by gas-liquid and ion-exchange chromatographic analysis were carried out. In white-bait pemmican, only threonine and asparagine amounts by GLC analysis had similar values to those obtained by ion-exchange chromatography. The other seven amino acids gave higher values as measured by GLC than by ion-exchange. With the food sample, soybean, alanine, valine, asparagine, and glutamic acid were in good agreement in two analysis, while leucine, proline, threonine, phenylalanine, and lysine were found in slightly higher concentrations in the GLC analysis.

6. Great variations of amino acid content were found among samples analyzed. The amino acid contents of each sample were compared with the values found in the literature.

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INTRODUCTION

The importance of having information on amino acid composition of foods was recognized when investigators identified amino acids and their biological roles. Even though many investigators have analyzed the amino acid contents of various foods, very few data were reported on the amino acid contents of Korean foods. In 1959 Chai et al. analyzed the ten essential amino acid contents of thirty Korean foods using the micro-biological method with acid and enzymatic hydrolyzates (2) Most of the determinations on food materials were done by the micro-biological or ion-exchange chromato-graphic methods.

The methods for amino acids determination in proteins and other samples of biological origin have been thoroughly reviewed by Block et al. (1). Investigations by Moore, Stein, and Spackman (8) and Hamilton (4) have developed ion-exchange chromatography into a refined method for the quantitative determination of amino acids. Very few determinations were done by using gas-liquid chromatography. The gas-liquid chromatography in amino acid is then relatively new, but it offers several possible advantages in the refined separation possible, small sample size required, and in the speed, accuracy, and simplicity of the method. Gas-liquid chromatography has been applied to a wide variety of substances volatile at temperatures up to approximately 280°C. Progress in the analysis of amino acids by this technique has been slow because they are not volatile owing to their zwitterion structure.

The present study was planned to determine protein amino acid contents of some Korean foods by gas-liquid chromatography, and to evaluate this technique as a procedure for the quantitative determination of amino acids in foods.

MATERIALS AND METHODS

A. Instrumentation

1. A gas-liquid chromatography, F&M Model 810, equipped with dual column, hydrogen flame detector, and temperature programmer made by F&M Division of Hewitt Pachard Co., Avondale, Penn.

2. Honeywell Electronik K-16 strip chart recorder, 0.1—1.0 millivolt 12 inch chart paper.

3. Column: $\frac{1}{4}$ inch outside diameter × 6 feet copper tubing.

B. Preparation of chromatographic columns

To have a stationary phase that will give good resolution for the solutes being analyzed DEGS or HI-EFF1 B (diethylene glycol succinate) and EGSS-X (Ethylene glycol succinate methyl silicone polymer), Applied Science Laboratories, Inc., State College, Penn., were used since complete resolution with these stationary phases has been reported for the *n*-butyl *N*-trifluoroacetyl easter derivatives of amino acids (5).

To have a solid support for GLC chromosorb W, acid washed, 60/80 mesh, Applied Science Lab., Inc., State College, Penn., was used.

Selection of the packing ratio and coating the solid support were done according to the method described by Gehrks et al. (6). Preparation of the column was accomplished in the following manner. Two sections of copper tubing were cleaned by passing through it, successively, 50 m*l*. portions of each of the following solutions, methanol, acetone, and chloroform. The tubes were then air-dried. Before filling, the bottom of the column was stoppered with a 1 cm. plug of glass wool. The column was supported in a vertical position, and a powder funnel was attached to the top with a short length of plastic tubing. The packing was placed in the funnel and sifted into place by vibrating the column with an electric sander and with gentle tapping. The column was filled to within 1 cm. of the top and plugged with glass wool. Approximately nine grams of packing material was packed into each column. The column was conditioned overnight at 180°C. with a flow rate of 50 ml. Helium/min.

C. The operation conditions for GLC:

Column temperature...Initial 65°—final 198°C.
Injection port temp ...120°±20°C.
Detector cell temp ...210°±10°C.
Program rate.....2°C./min.
Carrier gas, helium (NCG)...100ml./min. at 40Psig
Hydrogen (to detector)...100ml./min. at 20 Psig
Air (to detector)...800 ml./min. at 40 Psig

Chart speed.....1.016cm./min.

D. Identification of peak positions:

Retention time, retention volume, partition coefficient, specific retention volume, and relative retention volume were used to describe the position of a peak.

E. Quantification of GLC:

The method described by Fredericks et al. (3) was used to calculate the area of each peak produced. Namely, for each peak that is produced, the area was calculated by multiplying the peak height by the width of the curve at the midpoint of the altitude (estimated to within 0.2mm). In the case of overlapping peaks, division of the area was accomplished by drawing the vertical linefrom the lowest point between two peaks to the baseline.

F. Analytical preparation of n-butyl N-trifluo roacetyl esters was done according to the method of Gehrks et al. (6).

G. Preparation of food samples:

The following food samples for amino acid determination were supplied by the College of Agriculture, Seoul National University. 1. Three varieties of rice: Jae-geun, Pal-dal, and Suwon No. 82. These varieties were polished 100 per cent. 2. Two varieties of barley: Suwon No. 18 and Boo Heung. These were processed 75 per cent. 3. One variety of wheat, Jae-kwang. 4. One Itallian millet variety, China-sook. 5. One variety of small red-bean, Juk-sodoo. 6. One variety of mungbean, Suwon local. 7. Two varieties of soybean, Jangd-an-baik mok and a local obtained from the Office of Rural Development, Suwon. 9. One sorghum variety. Small sardine dried and white-bait pemmican dried were purchased at the city market in Suwon.

Determination of nitrogen in foods was carried out by the Official Micro-Kjeldahl method. Oven-dried food samples of known weight (nitrogen percentage basis) were hydrolyzed with one ml. 6N HCI, per five mg. protein in a sealed flask for 22 hours in the oven. The hydrolyzates were filtered and dried. The residues were then treated according to the analytical procedure for n-butyl N-trifluoroacetyl ester derivatives for gas chromatography.

RESULTS AND DISCUSSION

A. Nitrogen content of samples

Table 1 shows the per cent nitrogen and protein of foods. The crude protein content of foods was estimated from the nitrogen content. The average nitrogen content of protein in foods is frequently estimated as 16 per cent although it is recognized that this represents only an approximation. For comparison. of different materials, the conventional equation of $N \times 6.25 =$ crude protein is considered to be satisfactory. Jones (1931) derived special factors for converting N to protein in foods for which he considered the different N content of proteins and the various kinds of proteins in a food (9). Proteins of non legume plant origin, as a group, were shown to have the greatest deviation from the commonly used value of 16 per cent, averaging about 17.5 per cent nitrogen, while fish, meat, and egg proteins and those of legume plant seeds, averaged 16 per cent in nitrogen. Table 1 also includes the values calculated on the basis of the special factors for converting N to protein in foods as the figures for protein in tables of food composition issued by the U.S. Department of Agriculture have been calculated by use of the factors.

The accuracy and precision of the nitrogen analysis procedure was determined by carrying out assay of the nitrogen content of ammonium sulfate. It was shown that the per cent recovery of N in ammonium sulfate using the micro-Kjeldahl method is 96.3 per cent with digestion only, 96.5 per cent with digestion and distillation, and 99.7 per cent with the mixture of ammonium sulfate and food using the same procedure as described for the food nitrogen determination.

B. GLC with known amino acids

Results indicated a linear relationship between the weight of 13 amino acids among the 17 amino acids, the internal standard as well as the injection volume of a mixture and the detector responses for the derivatives of the amino acids. No response for arginine, cystein, histidine, and tyrosine was observed. The derivative of iso-leucine, which contained D-allo-iso-leucine as an impurity had

Table 1. Per cent Nitrogen and Crude Protein in Foods (Dried Weight Basis)

Foods	% Nitrogen	% Protein (N×6.25)	% Protein (N×N* conversion factor)
Rice: Jae-geun	1.09	6.80	6.47
Pal-dal	1.16	7.27	6.93
Suwon #82	1.14	7.16	6.81
Barley: Suwon #18	1.83	11.44	10.67
Boo-heung	2.18	13.72	12.71
Wheat: Jae-Kwang	1.91	11.97	11.16
Sorghum	2.24	13.99	13.99
Italian millet: China-sook	1.36	8.50	8.50
Small red bean: Jok-sodoo	3.93	24.56	24.56
Mungbean: Suwon local	4.40	27.50	27.50
Soybean: Jang dan baik mok	6.60	41.25	41.25
Local	6.49	40.56	40.56
Spring lobster, dried	11.06	69.12	69.12
Small sardine: #1	10.34	64.61	64.61
#2 Choo-by	11.72	73.25	73.25
#3 Ko-by	11.25	69.71	69.71
White-bait pemmican	11.60	72.50	72.50
Laver, dried	5.66	35.37	35.37
Red pepper seed	3.05	19.06	16.17
Sunflower seed	6.50	40.62	34.45
Peanut	4.91	30.72	26.84
Sesame seed	4.01	25.07	21.26
Pine nut	3.17	19.83	16.82
Pumpkin seed	5.74	35.86	30.41

* From Orr and Watt (9).

partially separated, two peaks, indicating that D-allo-iso-leucine has a different partition coefficient which is similar to that of iso-leucine. Lamkin and Gehrks (7) found no difference between the chromatographic behavior of derivatives prepared from L- and DL-amino acids.

C. Relative molar response of amino acids in mixture

The relative molar response (RMR) values for the 13 amino acids relative to glutamic acid as "1.00" were obtained under normal operating conditions with a hydrogen flame ionization detctor. The RMR values of alanine, valine, glycine, iso-leucine, leucine, methionine, and asparagine were greater than those obtained by Lamkin et al., while the values of proline, serine, threonine, and lysine were smaller. The RMR value for phenylalanine was the same, 1.12, as that of Lamkin's.

The derivatives of alanine, valine, iso-leucine, leucine, phenylalanine, asparagine and glutamic acid had a linear relationship between the peak areas and the concentrations of the derivatives, while occasional deviations from linearity were observed for gaycine, proline, threonine, serine, methionine, and lysine among the 13 amino acids. Tryptophan gave a single peak at the retention temperature 224°C. under two hours acylation, which was employed for the quantitative trifluoroacetylation of tyrosine. However, no response was shown for the derivatives of arginine, cystine-SH, cystine-S, histidine, and tyrosine.

D. Recovery of amino acids from mixtures with natural food material by GLC

The results presented in Table 2 indicate that

the recoveries were essentially quantitative except threonine and serine. An overall mean recovery of 11 amino acids from their mixture with the natural food material before hydrolysis and the standard deviation were 101.4±8.4 per cent. An over-all mean recovery of those combined with natural food material after hvdrolysis and the standard deviation were 98.1±8.7 per cent. The significant differences in the recoveries of threonine and serine were omitted from the overall means. These differences might be due to the breakdown of the EGSS-X.

There were no significant differences in the recoveries of 13 amino acids from their mixture with foods between the addition of amino acids before and after hydrolysis.

E. Comparison of GLC and ion-exchange chromatography

Data from the comparative analysis of the acid hydrolysates of two food samples are given in Table 3. In white-bait pemmican, only threonine and asparagine contents by GLC analysis had similar values to those obtained by ion-exchange chromatography. The other seven amino acids gave higher values as measured by GLC than by ion-exchange. With the food sample, soybean, alanine, valine, asparagine and glutamic acid were in good agreement, while leucine, proline, threonine, phenylalanine, and lysine were found in slightly higher concentrations in the GLC analysis.

The peaks for glycine and iso-leucine on the chromatograms were completely overlapped. After approximately 20 chromatographic runs, the iso-leucine and glycine peaks were partially resolved to allow the measurements of both areas. It was possible that the column required longer conditioning at higher temperature for better resolution of the iso-leucine and glycine peaks, though Gehrks et al. reported that prolonged conditioning at temperatures greater than 180°C. causes the resolution to be impaired. Another possible reason might be the different partition coefficients of two amino acids between two stationary phases (EGSS-X and DEGS).

Serine and methionine peaks were too small to be measured for their areas except for the larger quantity of the food sample of white-bait pemmican.

Table 2. Recovery of 13 Amino Acids from Mixture when Combined with Food Sample, White-bait Pemmican

Amino Acid	Peak Areas (cm.²)		Recovery (%)	Peak Areas(cm.²)	Recovery
	Food only (40mg. pro.) Avg.*±SD	Before hydro. food (40mg pro.) ±0.02m mole amino a Avg.±SD		After hydro. food (40mg. pro.) ±0.01 m mole amino a Avg.±SD	
Ala	2.0±0.081	3.3±0.025	93.0	2.7±0.000	97.7
Val	1.5±0.106	3.5±0.016	107.0	2.5±0.020	102.9
Gly	1.9±0.067	3.1±0.023	96.4	2.5±0.303	99.0
Ileu	1.8±0.015	4.2±0.046	102.5	2.9±0.010	98.8
Leu	2.7±0.020	4.8±0.011	90.8	4.0±0.017	94.4
Pro	1.5±0.057	3.4±0.037	92.3	2.6±0.013	98.9
Thr	0.6±0.505	2.9±0.095	159.6**	1.4±0.185	111.3
Ser	— —	1.0±0.003	298.0**	0.4 —	215.3**
Met	0.5±0.017	1.9±0.044	114.9	1.3±0.145	118.0
Phe	1.6±0.023	4.6±0.010	94.0	3.1±0.022	97.0
AspN	3.5±0.129	6.3±0.008	101.7	5.2±0.226	107.8
Glu	5.2±0.277	7.8±0.130	96.0	6.8±0.145	102.2
Lys	2.7±0.021	4.7±0.085	90.7	3.9±0.103	98.3
Average			98.1		102.6

* Average of 2 gas chromatographic runs on the same sample.
** Significant difference from the 100 per cent recovery.

Table 3. Comparison with Amino Acid Values obtained by Ion-Exchange Chromatography

Amino Acid	Amounts of Amino Acid (gm/16 gm nitrogen)			
	White-bait Pemmican		Soybean (local)	
	GLC[a]	ion-exch.[b]	GLC	ion-exch.
Ala	6.0±.14[d]	5.1	4.0±0.6	4.1
Val	3.4±.08	2.1	2.3±.06	2.2
Gly[c]	—	5.1	—	3.9
Ileu[c]	—	2.6	—	1.9
Leu	6.6±.18	5.1	6.1±.12	5.6
Pro	6.1±.35	3.4	6.7±.10	4.7
Thr	3.5±.80	3.1	2.9±.10	4.1
Ser	—	3.9	—	6.3
Met	—	1.3	—	trace
Phe	3.7±.05	2.7	4.1±.10	3.7
Asp	8.1±.43	7.2	9.2±.26	8.8
Glu	13.3±.38	10.9	17.0±.55	16.8
Lys	8.9±.33	5.8	7.6±.50	6.0
His	—	1.4	—	2.1
Arg	—	4.4	—	6.2
CysSH	—	3.6	—	1.0
Tyr	—	2.3	—	2.6

a: GLC condition described in RMR explanation
b: Single ion-exchange chromatographic analysis
c: Completely overlapped.
d: Means of four GLC analyses and the S.D. from the data in the comparison with amino acid values obtained by ion-exchange chromatography.

F. Determination of amino acid contents in foods

Table 4 summarizes the results of the amino acid analyses of food samples. It also includes the values in the literature for the comparisons, though differences in the origin and preparation of materials analyzed limit the comparison. Amino acids contents are expressed in gm. per 16 gm. nitrogen as determined by the micro-Kjeldahl analysis. The presented values express the means of four chromatographic analyses on a single hydrolysates unless otherwise stated, and they are given together with the estimate of the standard deviation of a single run.

It was evident from Table that the *N*-trifluoroacetyl *n*-butyl esters of serine and threonine were not stable with time as indicated by Lamkin et al. (7).

The thirteen amino acids contents determined from rice hydrolysates were lower than the values found in the literature except for methionine. This might be due to the difficulty of obtaining completely dried-hydrolysates, though the rice hydrolysates were treated with absolute ethanol and methylene chloride to remove water and then were dried over phosphorus pentoxide in a vacuum desciccator overnight. Consequently, it showed poor yields of amino acids derivatives. The high value of methionine might be due to the same retention temperature of the methionine and methionine sulfoxide. The values obtained by GLC for thirteen amino acids in small sardine, which contains high protein, were in good agreement with those given in the literature for similar materials.

It was quite interesting to find that one variety of soybean samples analyzed showed the highest

Table 4. Recovery of 13 amino Acids from Mixture when Comined with Food Sample, Soybean

Amino Acid	Peak areas (cm.²)		Recovery (%)	Peak areas (cm²)	Recovery (%)
	Food only (40mg pro.) Avg.*±SD	Before hydro. food(40mg pro.) +0.01m mole amino acid Avg.*±SD		After hydro. food(40mg pro.) +0.02m mole amino a Avg.*±SD	
Ala	1.6±0.006	2.4±0.037	100.8	2.9±0.082	91.8
Val	1.7±0.028	2.9±0.009	112.9	3.0±0.133	88.2
Gly	1.6±0.010	2.3±0.023	106.6	2.7±0.164	97.0
Ileu	1.9±0.053	3.5±0.070	116.0	3.5±0.180	83.9
Leu	3.2±0.041	4.9±0.078	108.1	5.2±0.103	89.7
Pro	2.1±0.037	3.4±0.054	105.6	3.9±0.160	91.4
Thr	1.0±0.035	1.7±0.095	104.7	2.6±0.613	117.3
Ser	0.4±0.016	0.5±0.001	86.9	1.0±1.398	143.1**
Met	0.5±0.011	1.1±0.011	93.7	1.7±0.125	98.9
Phe	2.2±0.036	4.1±0.014	106.5	5.0±0.106	91.2
AspN	5.0±0.190	7.1±0.011	112.8	7.4±0.180	97.1
Glu	7.9±0.027	10.2±0.030	108.2	9.6±0.386	88.5
Lys	3.1±0.047	4.3±0.247	99.8	4.9±0.178	89.5
Average			104.8		93.7
Overall mean±SD			101.4±8.4%		98.1±8.7%

* Average of 2 gas chromatographic runs on the same sample
** Significant difference from the per cent recovery.

amount of lysine of all samples tested. Varietal differences of amino acid contents were found in other cereal crops. This gives a suggestion that newly improved crops may be developed by breeding method in future.

CONCLUSION

There are a number of possible sources of errors in using the gas-liquid chromatographic method for the determination of amino acids in mixtures or food hydrolysates. Among factors of greater or lesser importance are the following:

1. The most important single factor affecting the accuracy of gas chromatographic method is the yield of derivatives. Gehrks et al. (6) indicated that by employing the proper reagents and reaction conditions it should be possible to esterify all carboxyl groups and to acylate all amino, hydroxyl, phenolic, sulfhydryl, imidazole, and guanido groups, thereby masking the polarity of all groups which would cause difficulty in gas chromatographic separations.

2. The hydrogen flame ionization detector has extreme sensitivity, and a wide linear response offering use over a wide range of sample concentration. For this problem Gehrks reported that the slope factor method for the calculation of amino acid concentration in protein hydrolysates or other complex biological samples e.g. foods might be the most applicable method for the interpretation of the analytical data.

3. The majority of difficulties in quantitation which arise in biological application are at least partially due to inadequate technique in the preparation of the column. It has been found in other studies in this laboratory that there were no tailing problems for the n-butyl N-trifluoroacetyl esters of amino acids, and the two peaks for glycine and isoleucine derivatives were almost completely separated when a 1.00m.×4mm i.d. U-shape borosilicate-glass column packed with neopentyl glycol sebacate on chromosorb B.A.W., 80-100 mesh was used.

4. The most difficult problem to control the

Table 5. Summaries of Amino acid Analyses of Food Hydralysates (F), with comparison to Available Literature Data (L)
(Grams amino acid per 16.0 nitrogen, average of four GLC run)

Food	Ala	Val	Gly	Ileu	Leu	Pro	Thr	Ser	Met	Phe
F1 Rice (Suwon No.82)	5.1	3.0	3.8	2.2	6.1	5.2	2.5	5.7	10.4	4.3
L White rice meal (7)		6.4		4.4	8.6		3.6		1.4	4.8
F2 Barley (Suwon No. 18)	3.4	3.1	3.3	2.8	6.0	14.2	2.7	5.2	6.0	5.5
F3 Barley (Boo-heung)	3.0	2.5	2.9	2.1	5.0	14.5	2.9	4.6	5.1	4.5
L Barley (7)	4.1	3.8	2.9	4.8	6.8	4.8	3.2	5.3	3.5	5.7
F4 Sorghum (local)	9.8	4.6	2.9	3.9	13.2	9.4	3.2	7.5	5.1	6.0
L Sorghum (7)		7.0		4.4	13.1		3.3		1.4	4.9
F5 Mungbean	4.0	4.6	3.8	3.6	7.4	3.7	4.0	7.0	2.6	6.0
L Avg. Mungbean (8)	3.0	5.9	1.8	5.5	9.0	4.4	3.1	3.0	1.1	4.8
F6 Soybean (ChangdanBaik Mok)	3.9	3.8	3.9	3.8	7.0	6.7	3.7	2.8	2.3	4.9
F7 Soybean (local)**	4.4	4.9	4.4	5.0	8.5	8.2	4.3	1.8	1.9	5.7
L Soybean (7)	5.7	4.8	1.7	4.3	7.2	4.3	2.6	5.2	1.1	3.8
F8 Small sardine(choo-by)	5.9	4.5	4.8	4.0	7.4	5.5	4.3	2.0	2.2	4.1
F9 Small sardine*	5.6	3.3	4.8	2.9	5.3	5.2	2.6		2.2	3.5
L Sardine (7)		5.1	5.1	4.4	7.0		4.7		2.8	3.6
F10 White-bait pemmican**	5.2	4.4	5.2	4.7	7.1	5.8	2.5	7.1	1.6	4.0
L Whole egg (7)		7.8	3.6	6.8	8.3		4.7		3.1	5.4

Table 5. Cont

Food	Asp	Glu	Lys
F1 Rice (Suwon No.82)	7.3	14.8	1.4
L White rice meal (7)		—	2.8
F2 Barley (Suwon No.18)	4.3	26.7	1.6
F3 Barley (Boo-heung)	4.0	21.7	2.6
L Barley (7)	2.5	22.0	3.3
F4 Sorghum (local)	5.7	1.6	1.1
L Sorghum (7)			1.5
F5 Mungeban	10.7	16.9	4.7
L Avg Mungbean (8)	8.6	11.5	7.6
F6 Soybean (Changdan-Baik Mok)	9.4	16.8	5.3
F7 Soybean (local)**	10.9	20.4	11.0
L Soybean (7)	6.7	16.0	5.3
F8 Small sardine (dhoo-by)	8.3	12.8	8.6
F9 Small sardine*	7.9	12.7	5.4
L Sardine (7)	8.3	12.8	8.2
F10 White-bait pemmican**	7.7	13.6	10.0
L Xhole egg (7)	5.6	11.9	6.7

* Day difference included (within three days)
** From the recovery study. Average of two GLG run

baseline stability was met throughout temperature programming specially at higher retention temperature than that of the serine derivatives. Initially, in an attempt to reduce the retention time, a column temperature, initial 70°, final 235°, had been used. Since the baseline was raised at high temperature, the column temperature was reduced to the initial 60°, the final 198°C. and the pressure of the air to the detector, 55 Psig instead of 40 Psig was used.

要 約

우리나라 주요 식품들의 단백질 아미노산 함량을 최근에 와서 크게 실용화되고 있는 GLC 방법에 의해 분석함에 있어서 GLC의 기술적 재평가를 하고자 시험하였든바

1. 우선 여러식품들의 단백질 질소함량을 조사하여 GLC 분석에 필요한 시료량을 정하였으며

2. GLC에 주입한 17종 아미노산들에 대하여 조사한 결과 Arginine, Cystine, Histidine, Tyrosine을 제외한 13종만이 주입한 량에 비례하여 detector response를 얻었다.

3. 13종 아미노산에 대한 RMR 値를 얻었다.

4. 아미노산에 대한 recovery 檢定을 하였든바 거의 100% 가까이 recover되었으며, 가수분해가 recovery에 미치는 영향을 조사하였든바 별 영향이 없었음을 알았다.

5. GLC와 ion-exchange chromatography 분석법을 비교시험하였든바 백어포 시료에 있어서는 Threonine과 Asparagine만이 두 방법에서 거의 같게 나왔고 다른 아미노산들은 GLC에서 보다 많은량이 나타났다.

6. 각식품들에 대한 단백질 아미노산함량을 측정하였든바 식품종류에 따라 그리고 보리 및 대두에 있어서는 품종에 따라서도 아미노산 함량이 다르게 나타났다.

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