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AMINO ACID ANALYSIS OF FOOD SAMPLES BY GAS LIQUID CHROMATOGRAPHY

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Due to the increase of interest in the nutritional evaluation of foodstuffs, chemical diagnosis of metabolic disturbances and determination of amino acid sequence and primary structure of isolated enzymes and other functional proteins, need for rapid and sensitive methods of amino acid analysis was experienced. A complete assessment of amino acid pattern requires hydrolysis of the peptide linkages before separation and detection. The most common methods of amino acid analysis are dealt with in earlier publications (Blackburn, 1968). Ion exchange chromatography (Moore and Stein, 1954) is so far the widely used procedure. Nevertheless because of its speed, sensitivity and high precision, gas chromatography could be the technique of choice for routine analysis of amino acid composition when a large number ot samples have to be handled, for instance in plant breeding, clinical studies or nutritional surveys.

Amino acids though volatile at temperatures about 250°C have too low vapour pressure to be suitable for gas chromatography analysis. Moreover, many of them decompose at these temperatures and require conversion of them to derivatives. Among the many derivatives like 2,4–dinitrophenyl (DNP), N-acetyl–n–propyl, phenylthiohydantoin (PTH), trimethyl silyl (TMS) and N-trifluoro acetyl-n–butyl (TAB), produced and evaluated for gas chromatographic purposes, TAB derivatives appear to be the most suitable. Recently, procedure for manufacturing trimethyl silyl derivatives, heptafluorobutyryl derivatives and phenylthiohydantoin derivatives has been improved and exploited for gas chromatographic purposes, though not yet widely used. The experimental conditions for the quantitative derivatisation and chromatographic requirements for their separation are described by Gehrke and coworkers (Gehrke *et al*, 1968).

Materials and Methods

Derivatisation of amino acids to N–trifluoroacetic n–butyl derivatives

The amino acid mixture is collected in small culture tubes with bakelite cap having teflon packing. The tubes are placed on an oil bath and evaporated to dryness by blowing dry nitrogen into them. To the dry sample 3 *N* HCl–butanol is added and the closed tubes are kept under 100°C for 30 minutes for esterification. When the esterification is complete the samples are dried as above and acylated with trifluoroacetic anhydride under 100°C for 30 minutes in closed tubes using methylene-chloride as solvent. The 3 *N* butanol is manufactured by dissolving clean dry hydrogenchloride in *n* - butanol. Butanol and methylene-chloride were refluxed with calcium chloride and distilled in an ail-glass apparatus. Trifluoroacetic anhydride, methylene-chloride and butanol are stored in all-glass bottles kept in desiccator to protect from atmospheric moisture.

Gas chromatographic separation

The GLC separation is done on a glass column (1700 x 4 mm) containing 0 65 w/w% stabilized ethylene glycol adipate on high performance Chromosorb W 80 100 mesh which is acid washed and dimethylchlorosilane treated. The column is conditioned over night under 230°C with a flow of 50 ml/min argon. Derivatives of histidine and arginine are separated on a mixed phase column (1500 x 4 mm) containing 1.0 w/w% OV-17 and 2.0 w/w% OV–210 on Chromosorb G 100/120 mesh using butylstearate as internal standard. This column isconditioned over night at 250°C with a flow of argon (50 ml/min).

Four microliters of the sample containing about 0.2 ng of each amino acid is injected into the gas chromatograph and separated by programming the temperature of the column oven from 50" to 220°C by an increase of 4°C/minute. The peaks are detected with a flame ionisation detector. First a standard solution of amino acids with an internal standard are carried through the procedure for calibration and the determination of relative molar response (RMR) factors for each amino acid. Then these factors are used for calculation of the amino acid content in unknown samples.

Hydrolysis of the protein in samples

Before the amino acids can be derivatised for separation on a gas chromatograph they have to be released from the protein by hydrolysis. The most common method of protein hydrolysis involves treatment of the sample with acid. Dry samples containing approximately 5 mg of protein are weighed into a hydrolysis tube (16 x 160 mm Duran. 50 test tubes). Five ml of 6 *N* HCl containing 10 g of phenol per liter is added to the tubes. After adding the adequate amount of internal standard solution (ω-amino caprylic acid) the tubes are kept to freeze in a mixture of solid CO_2 and ethanol. The frozen samples are placed in a desiccator. By applying vacuum for a period of 20 minutes, the dissolved air and O_2 are removed. Dry N_2 is let in to release the vacuum. The tubes are then sealed and kept constantly rotating in a hot air oven under 110°C for 24 hours.

Ion-exchange clean up

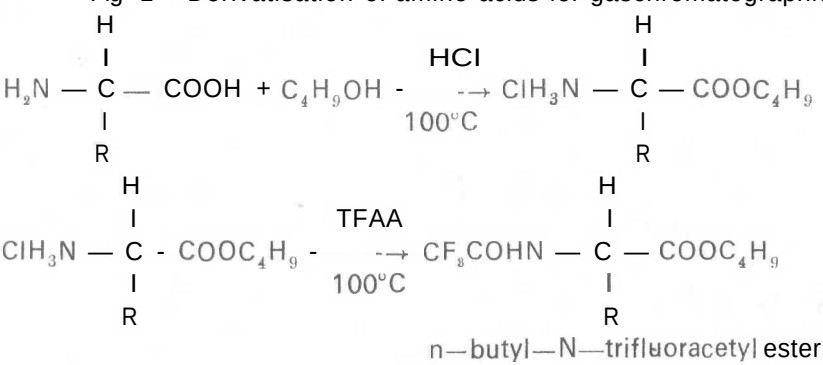
To remove the impurities which might interfere with the GLC-separation it is necessary to clean the hydrolysate through an ion-exchange resin column. Amberlite-IR 120, a strong cation exchange resin in hydrogen form. is treated as follows before filling it into the columns. The resin is washed with 7*N* NH_4OH by stirring it with a magnetic stirrer for an hour. This is repeated a couple of times before the resin was washed with deionised water till it was neutral to litmus paper Then excess of 3 *N* HCl is added and stirred over a magnetic stirrer very slowly. After an hour the HCl is poured away and the resin is washed with deionised water to neutral pH. The resin treated as above is filled into the columns (10 cm x 0.5cm) carefully without pockets and air bubbles. The hydrolysate is evaporated first and

dissolved then into 0.1 *N* HCl. One ml of this solution is placed over the ion-exchange column without disturbing the resin bed. The impurities are washed out by passing 5 ml of deionised water through the column. The amino acids are then eluted with 5ml of 7 *N* NH_4OH followed by 5 ml of deionised water. The column is regenerated with excess of 3 *N* HCl before further use. The eluate is collected in small tubes with bakelite cap having teflon packing. The tubes are placed on oil bath and evaporated to dryness by blowing dry nitrogen into them, before it is subjected to esterification with 3*N* HCl butanol, trifluoroacetic anhydride and further injection into gas chromatograph.

Results and Discussion

Table 1 shows the relative molar response of n-butyl-N–trifluoroacetyl derivatives df amino acids and Figure 2 presents a typical chromatogram of human serum albumin amino acid derivatives separated on an Ega-Column. For the sake of comparison a sample of human serum albumin was subjected to amino acid analysis by GLC and ion-exchange chromatography. Table 2 presents the results obtained showing good agreement with each other. Table 3 shows the amino acid composition of some 1–day mixed food diet samples. The diet samples are fat extracted and lyophillised before hydrolysis and subjected to ion-exchange clean up, derivatisation and GLC-separation (gas chromatography). GLC–value is an average of seven analyses and IE (ion-exchange) value is the result of one determination of a pooled sample containing seven samples. From the data presented it seems to be clearthat amino acid determination for the evaluation of food protein can be carried out by the above-described method of gas-liquid chromatography.

Fig 1 Derivatisation of amino acids for gaschromatographic analysis



Summary

A method for determination of amino acids by gas-liquid chromatography is described, applied and evaluated. For the sake of comparison a standard sample—human serum albumin—was analysed along with a number of one-day mixed food diet samples both on gas-liquid chromatography and ion-exchange chromatography. The results show that the gas chromatographic method is useful for this purpose.

Table 1

Relative molar response of n-butyl N-trifluoroacetyl esters of aminoacids

Amino acid	Rel. mol. response (Flame ionisation detector)		
	Replication 1	Replication 2	Mean
ALA	0.536	0.514	0.53
VAL	0.692	0.664	0.68
GLY	0.428	0.433	0.43
ILEU	0.786	0.801	0.79
LEU	0.764	0.790	0.78
PRO	0.701	0.740	0.72
THR	0.615	0.633	0.62
SER	0.531	0.548	0.54
CYSH	0.523	0.501	0.51
MET	0.685	0.707	0.70
HYDRO	0.755	0.766	0.76
PHE	1.127	1.114	1.12
ASP	0.940	0 898	0.92
GLU	1.000	1.000	1.00
TYR	1.014	1.029	1.02
LYS	0.886	0.830	0.86
HIS	0.572	0.553	0.56
CYS	0.415	0.410	0.41

Table 2

Amino acid analysis of human serum albumin by GLC and by ion-exchange chromatography

Amino acid	W/W%	
	GLC	IEC
ALA	6.25	6.42
VAL	5.69	5.34
GLY	1.35	1.13
ILUE	1.12	1.17
LEU	10.20	9.26
PRO	3.78	3.42
THR	3.56	3.88
SER	3.11	2.81
PHE	5.37	5.87
ASP	9.54	8.46
GLU	15.90	14.32
TYR	3.90	3.88
LYS	11.19	10.06

Table 3

Amino acid composition of 1 day mixed food diet samples: Five men
(Values are expressed as g/day)

	D 18		D 19		D 20		D 21		D 22	
Amino acid	GLC	IE	GLC	IE	GLC	IE	GLC	IE	GLC	IE
Alanine	1.85	1.55	2.14	2.35	1.71	2.53	1.85	2.44	1.86	2.21
Valine	2.16	2.19	2.52	2.74	2.11	2.80	2.21	2.83	2.04	2 69
Glycine	1.65	1.73	2.22	2.27	1.78	2.18	2.00	2.10	1.78	1.90
Isoleucine	1.75	1.49	1.98	2.23	1.73	2.27	1.95	2.23	1.61	2.17
Leucine	3.30	3.10	4.25	3.94	3.34	3.88	4.17	4.17	3.35	3.78
Proline	3.57	2.76	4.81	3.38	4.10	3.58	4.19	3.17	3.46	4.01
Threonine	1.47	1.44	1.73	2.07	1.66	1.97	1.92	1.96	1.51	1.92
Serine	2.43	1.74	2.74	2.04	2.68	2.32	3.16	2.21	2.69	2.53
Phenylalanine	1.47	1.75	1.61	2.20	1.87	2.20	1.91	2.46	1.63	2.22
Aspartic acid	3.19	2.95	3.93	4.15	3.76	4.42	3.93	4.37	304	3.79
Glutamic acid	8.92	7.70	10.57	9.97	8.27	9.86	10.51	9.92	8.33	9.90
Tyrosine	1.72	1.11	1.70	1.56	2.36	1.64	2.04	1.33	1.75	1.46
Lysine	3.02	2.95	3.68	4.06	3.90	2.78	2.84	4.41	2.63	2.94

IE, ion exchange chromatography
GLC, gas-liquid chromatography

Fig 3 Separation of aminoacids from a one-day mixed food diet sample as their TAB derivatives from an EGA column (for chromatographic conditions see figure 2)

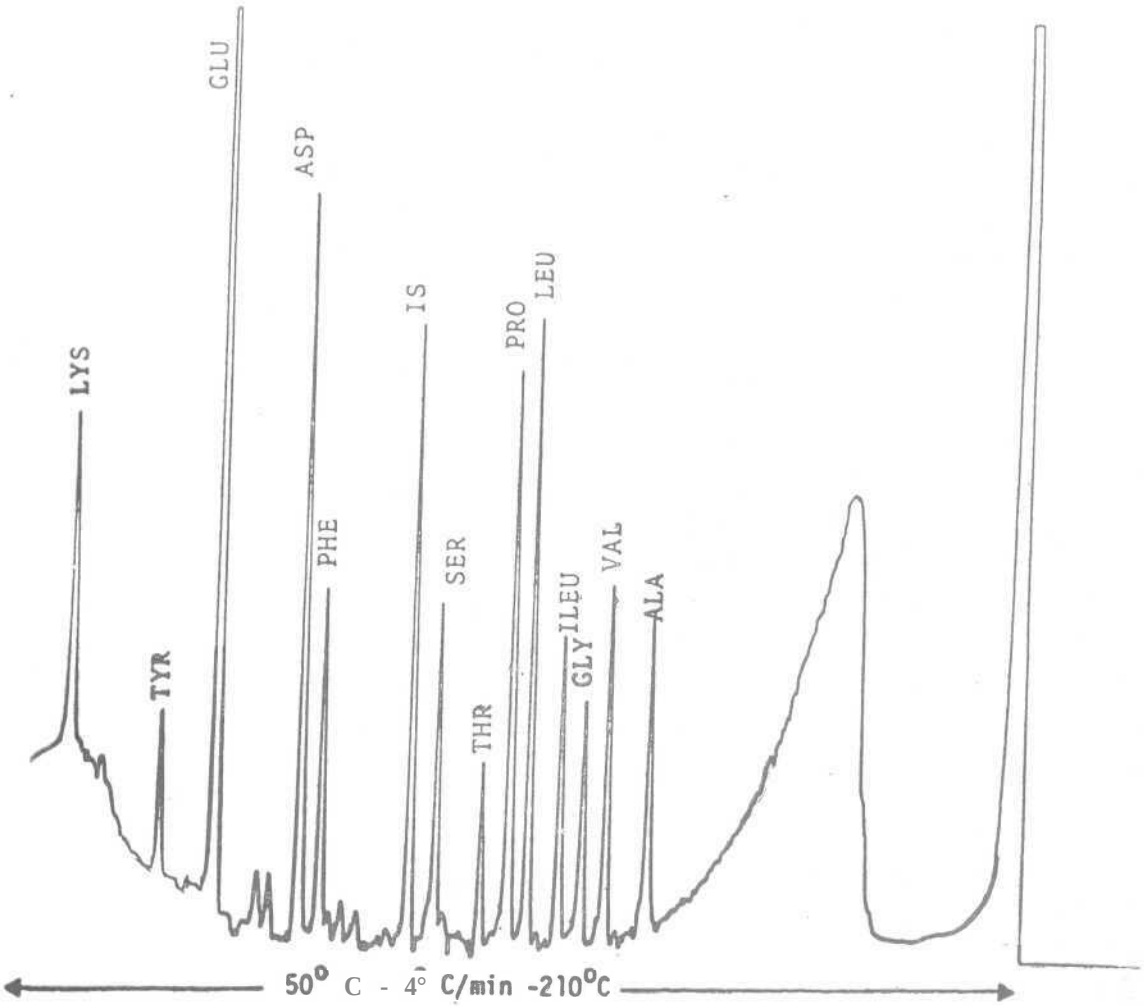
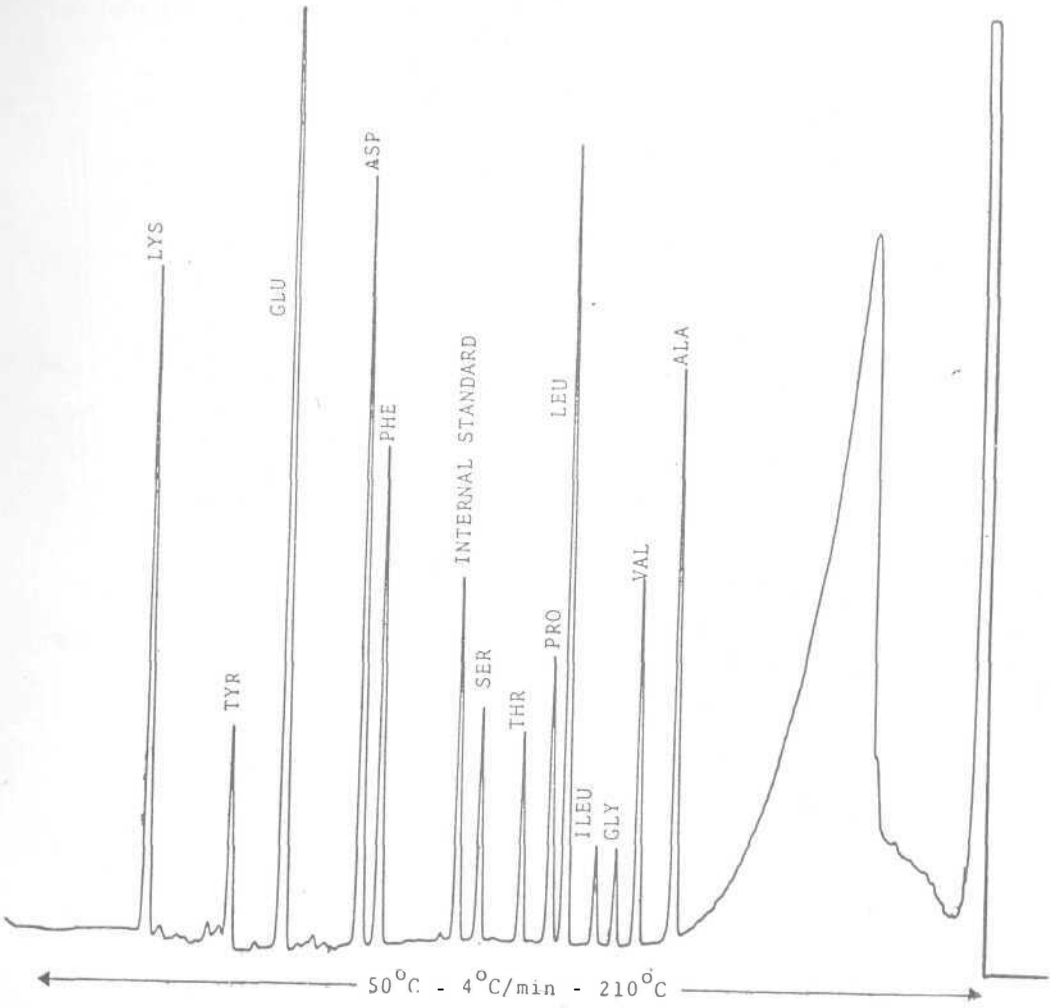


Fig 2 Separation of aminoacids from human serum albumin as their n-butyl-N-trifluoroacetyl esters on a EGA column (1500 mm x 4 mm glass, 0.65% EGA on 80/100 mesh Chromosorb WHP, carrier gas (argon) 5 ml min FID detectors)



സംഗ്രഹം

വാതക–ദ്രാവക–വർണരേഖാവിശ്ലേഷണം **(GLC)** ഉപയോഗിച്ച് ജൈവവസ്തുക്കളിലെ raroffllamo അമ്ളങ്ങൾ തിട്ടപ്പെടുത്തുന്ന മാർഗ്ഗം ഒരു താരതമ്യ പഠനത്തിൽക്കൂടി വിലയിരുത്തുകയുണ്ടായി. അയോൺ–വിനിമയ–വർണരേഖാ വിശ്ലേഷണംപോലെതന്നെ ജി. എൽ. സി. യും അമിനോ അമ്ളങ്ങൾ തിട്ടപ്പെടുത്തുന്നതിന് ഉപയോഗിക്കാമെന്ന് തെളിഞ്ഞു.

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