Automatic Integration and Computation of Amino Acid Analyses[1]

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ABSTRACT

A versatile computer program has been developed for calculating amino acid analysis data. Peaks are identified by visual observation of chromatograms. The calculation was completely automated by recording the photometer output on a magnetic tape which is played back into an electronic integrator to produce a punched paper tape. The punched tape can be either fed directly to a computer or converted to punched cards. Calculations with the automatic system have been performed with data from a variety of hydrolysates, including pure proteins, chemically modified proteins, and feed meals. The computer program can be used with different interphase equipment. Computer output prints the results in several forms suitable for a variety of needs. Computation time is reduced more than 90% as compared to manual procedures.

Manual calculation of peak areas on charts from an amino acid analyzer is a laborious, time-consuming procedure and subject to considerable error. Since the entire process is routine in nature, it is readily adaptable to automation.

Complete automation of the process requires both automatic integration of peak areas and automatic calculations. Automatic calculation can be performed by a digital computer; however, integration of the photometer output is required before computerized calculations are made. This conversion may be done either by (a) manual integration and punching of cards; (b) a data logger, where the integration is carried out by the computer; or (c) an electronic integrator. Generally a data-logging system has been applied to automation of calculations (1–4). However, an intermediate electronic integrator allows output to be used for either manual or computerized calculations. A computer program has been developed that accepts integrated values for peak areas, performs the required calculations, and prints the results in tabular form.[2] This paper contains a general description of the program.

MATERIALS AND METHODS

Equipment

A flow chart of the complete analysis process for both automatic integration and calculation is shown in Fig. 1. The calculation procedure requires the following stages between detection and results: The photometer output of an amino acid analyzer is converted from a logarithmic function to a linear one and recorded on a magnetic tape by means of an Infotronics tape recorder; the tape recording is played into an Infotronics electronic integrator equipped with automatic peak detection and baseline drift corrector (5), which produces a punched paper tape; the punched tape is read into an IBM

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[2]The complete listing of the program in Fortran IV language with detailed instructions can be supplied on request.

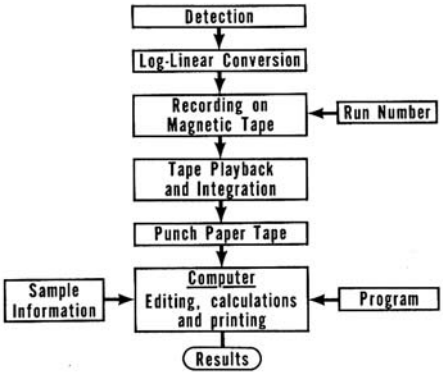

Fig. 1. Flow diagram for automatic integration and calculation of amino acid analyses.

1130 computer, which edits the information, carries out the calculations, and prints the results.

Computer Programs

A general program was developed to calculate all analyses (Fig. 2). In addition, the general program was modified, in order to calculate color constants from standard amino acid mixtures, and an abbreviated version of the general program was adopted for short analyses. The programs are written in Fortran IV language for an IBM 1130 computer. An internal repeat is incorporated into the program; this permits calculation of any number of analyses without the necessity of reloading the program. The program terminates when the data are exhausted.

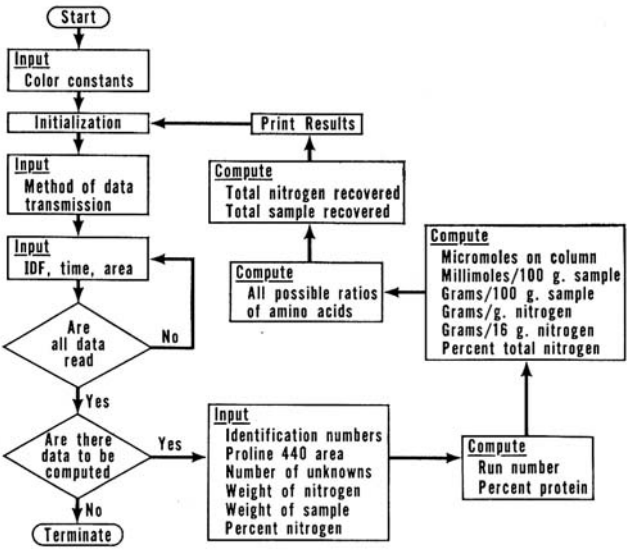

Fig. 2. Flow diagram for computer program logic.

Input Information

Peak identifications are assigned manually as follows: The strip chart from an amino acid analyzer is inspected visually and numbers 1 through 18 are assigned to the 18 amino acids found in standard mixtures according to their elution positions (Lys. 1, His. 2, etc.). Numbers 19 through 30 are assigned to unknown peaks and number 0 to extraneous peaks, such as buffer changes. The number designation (0 to 30) identifies the amino acid and determines the type of calculation to be performed. The input information is divided into the following parts: start, set color constants, computation-print, and termination. All parts are executed only once during a computer run, except the computation-print portion which can be recycled to accommodate the number of analyses to be calculated. The information being transmitted to the computer consists of control, analysis data, and sample information. Control information indicates the sequence and type of operations to be performed. Only analysis data can be transmitted by punched tape and consists of a control digit, elution time, and peak area. Sample information consists of peak identification numbers, proline 440 curve area, number of unknowns, sample weight in mg., sample nitrogen in mg., and percentage nitrogen in sample.

Output Information

A sample output form is illustrated in Fig. 3. This is a reproduction of a computer output, with the exception of sample identification and hydrolysis information which are not printed by the computer. Amino acids are listed in the order of their elution times except for unknowns, which are listed after the last known amino acid. Micromoles on column for each amino acid are converted into mM/100 g. sample, g./100 g. sample, g./g. nitrogen, g./16 g. nitrogen, and percentage total nitrogen. From these data the computer calculates percent nitrogen recovered and percent sample weight as amino acids. The lower portion of the report consists of a permutation of molar ratios for all amino acids.

DISCUSSION

The automatic calculation procedure for amino acid analyses is more accurate and less time-consuming than most manual procedures. It is estimated that the totally automatic procedure achieves a time saving of more than 90%. The equipment set-up may be modified in a variety of ways to meet individual needs. For example, the recording and integration stages could be combined. If no interphase equipment is available, manual integration values could be punched into cards for computer calculation. The replacement of the equipment described by a data logger would necessitate an additional program for integration by the computer.

The computer programs outlined are versatile, since the logic and operations permit easy modification to meet a variety of individual needs, including other chromatographic procedures such as gas chromatography. Along with sample information, the computer requires only a reading of peak area to perform calculations. The visual identification of peaks could be replaced by a computer identification sequence; however, this would make the program less versatile and subject to error. The operator identification step assumes special significance in analyses of hydrolysates of new or chemically modified

RUN NO. 141 MOORE AND STEIN SYSTEM

SAMPLE...Wheat Shorts.....

PERCENT NITROGEN= 2.88
PERCENT PROTEIN 16.41 (WHEAT) 18.00 (OTHER CEREALS)

HYDROLYSIS METHOD...Sealed Tube.....
MG. N/ML...0.292.....
MG.SAMPLE/ML...10.00.....
HUMIN...2.439 μg./mg.....

WEIGHT OF SAMPLE= 4.989 WEIGHT OF NITROGEN= 0.145

AMINO ACID	PEAK NO.	ELUTION TIME IN MIN.	MICROMOLES ON COLUMN	MILLIMOLES PER 100G. SAMPLE	GRAMS PER 100G. SAMPLE	GRAMS PER GRAM NITROGEN	GRAMS PER 16 G. NITROGEN	PERCENT TOTAL NITROGEN
LYSINE.....	1.	22.7	0.291	5.836	0.853	0.291	4.665	5.585
HISTIDINE.....	2.	26.0	0.155	3.112	0.482	0.165	2.640	4.467
AMMONIA.....	3.	29.2	1.152	23.092	0.392	0.134	2.146	11.049
ARGININE.....	4.	40.9	0.359	6.812	1.186	0.405	6.489	9.779
ASPARTIC.....	7.	30.8	0.496	9.948	1.324	0.452	7.241	4.760
THREONINE.....	8.	37.8	0.253	5.074	0.604	0.206	3.305	2.428
SERINE.....	9.	41.1	0.350	7.029	0.738	0.252	4.040	3.363
GLUTAMIC.....	10.	52.0	1.165	23.347	3.435	1.174	18.785	11.171
PROLINE.....	11.	56.5	0.442	8.858	1.019	0.348	5.577	4.238
GLYCINE.....	12.	76.1	0.654	13.119	0.984	0.336	5.385	6.277
1/2CYSTINE.....	14.	101.1	0.083	1.676	0.201	0.068	1.101	0.802
VALINE.....	15.	109.7	0.353	7.082	0.829	0.283	4.537	3.388
METHIONINE.....	16.	120.8	0.078	1.567	0.233	0.079	1.278	0.749
ISOLEUCINE.....	17.	128.6	0.216	4.332	0.568	0.194	3.107	2.073
LEUCINE.....	18.	133.2	0.420	8.426	1.105	0.377	6.044	4.031
TYROSINE.....	19.	152:2	0.144	2.898	0.525	0.179	2.871	1.386
PHENYLALANINE..	20.	160.8	0.204	4.106	0.678	0.231	3.709	1.964
UNKNOWN.....	5.	12.7	0.131	2.644	0.370	0.126	2.024	1.265
UNKNOWN.....	6.	24.7	0.114	2.292	0.320	0.109	1.755	1.096

PERCENT NITROGEN RECOVERED= 84.89
PERCENT SAMPLE WEIGHT AS AMINO ACIDS= 16.78

RATIO OF AMINO ACIDS

	/LYS	/HIS	/NH3	/ARG	/ASP	/THR	/SER	/GLU	/PRO
LYS	1.000	1.875	0.252	0.856	0.586	1.150	0.830	0.249	0.658
HIS	0.533	1.000	0.134	0.456	0.312	0.613	0.442	0.133	0.351
NH3	3.956	7.420	1.000	3.389	2.321	4.550	3.285	0.989	2.606
ARG	1.167	2.189	0.294	1.000	0.684	1.342	0.969	0.291	0.769
ASP	1.704	3.196	0.430	1.460	1.000	1.960	1.415	0.426	1.122
THR	0.869	1.630	0.219	0.744	0.510	1.000	0.721	0.217	0.572
SER	1.204	2.258	0.304	1.031	0.706	1.385	1.000	0.301	0.793
GLU	4.000	7.502	1.011	3.427	2.346	4.600	3.321	1.000	2.635
PRO	1.517	2.846	0.383	1.300	0.890	1.745	1.260	0.379	1.000
GLY	2.247	4.215	0.568	1.925	1.318	2.585	1.866	0.561	1.480
ALA	1.794	3.365	0.453	1.537	1.052	2.063	1.490	0.448	1.182
CYS	0.287	0.538	0.072	0.246	0.168	0.330	0.238	0.071	0.189
VAL	1.213	2.275	0.306	1.039	0.711	1.395	1.007	0.303	0.799
MET	0.268	0.503	0.067	0.230	0.157	0.308	0.222	0.067	0.176
ILEU	0.742	1.392	0.187	0.635	0.435	0.853	0.616	0.185	0.489
LEU	1.443	2.707	0.364	1.236	0.847	1.660	1.198	0.360	0.951
TYR	0.496	0.931	0.125	0.425	0.291	0.571	0.412	0.124	0.327
PHE	0.703	1.319	0.177	0.602	0.412	0.809	0.584	0.175	0.463
UNK	0.453	0.849	0.114	0.388	0.265	0.521	0.376	0.113	0.298
UNK	0.392	0.736	0.099	0.336	0.230	0.451	0.326	0.098	0.258

	/GLY	/ALA	/CYS	/VAL	/MET	/ILEU	/LEU	/TYR	/PHE
LYS	0.444	0.557	3.481	0.824	3.724	1.347	0.692	2.013	1.421
HIS	0.237	0.297	1.856	0.439	1.985	0.718	0.369	1.073	0.757
NH3	1.760	2.204	13.774	3.260	14.736	5.330	2.740	7.968	5.624
ARG	0.519	0.650	4.063	0.961	4.347	1.572	0.808	2.350	1.659
ASP	0.758	0.949	5.933	1.404	6.348	2.296	1.180	3.432	2.422
THR	0.386	0.484	3.026	0.716	3.238	1.171	0.602	1.750	1.235
SER	0.535	0.671	4.192	0.992	4.485	1.622	0.834	2.425	1.711
GLU	1.779	2.229	13.926	3.296	14.898	5.388	2.770	8.056	5.686
PRO	0.675	0.845	5.284	1.250	5.653	2.044	1.051	3.056	2.157
GLY	1.000	1.252	7.825	1.852	8.372	3.028	1.556	4.526	3.195
ALA	0.798	1.000	6.247	1.478	6.683	2.417	1.243	3.613	2.550
CYS	0.127	0.160	1.000	0.236	1.069	0.386	0.198	0.578	0.408
VAL	0.539	0.676	4.224	1.000	4.519	1.634	0.840	2.443	1.724
MET	0.119	0.149	0.934	0.221	1.000	0.361	0.185	0.540	0.381
ILEU	0.330	0.413	2.584	0.611	2.764	1.000	0.514	1.494	1.055
LEU	0.642	0.804	5.026	1.189	5.377	1.944	1.000	2.907	2.052
TYR	0.220	0.276	1.728	0.409	1.849	0.668	0.343	1.000	0.705
PHE	0.312	0.392	2.449	0.579	2.620	0.947	0.487	1.416	1.000
UNK	0.201	0.252	1.577	0.373	1.687	0.610	0.313	0.912	0.644
UNK	0.174	0.218	1.367	0.323	1.462	0.529	0.272	0.791	0.558

Fig. 3. Typical report produced by computer for an amino acid analysis.

proteins which may contain novel ninhydrin-positive compounds. Without visual identification, new peaks and analyzer inconsistencies might go undetected.

The output report (Fig. 3) presents results in several forms usually encountered in cereal protein research. Simple changes in the program permit calculation of any number of additional parameters. The reported total nitrogen recovery gives a check on the analysis. The ratio of amino acid values is useful in compositional studies of cereal and other proteins. Inspection of the ratio columns reveals differences in amino acid composition of closely related proteins or decreases in certain amino acids in chemically modified proteins.

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