Techniques for Leaf Sampling and Automated Analysis of Sugarbeet Leaf Amino Acids[1]

G. W. Maag, R. J. Hecker, and P. A. Whitaker[2]

Received for publication September 20, 1971

The leaf is the major site of synthesis in the plant and contains a vast array of chemical mechanisms for syntheses of the most varied character. Included in the metabolic activities of leaves are the changes in nitrogenous compounds. Among the outstanding nitrogenous compounds are the amino acids, the building blocks for enzymes, peptides, proteins and other related components of the leaf.

Experiments were planned to detect possible relationships of sugarbeet leaf amino acids with *Cercospora* leaf spot resistance. Review of the literature showed no record of previous automated analysis for sugarbeet leaf amino acids; therefore it was necessary to devise leaf sampling, sample preparation, and analysis techniques before the studies could be made.

In field studies of three or more replications of several sugarbeet cultivars, sample bulk and number of samples prohibited sampling each leaf of each plant. Also extensive leaf sampling would deter further growth of the plant. Therefore a survey study was made to determine the leaf section and the stage of leaf maturity which would give the best representative sample of the free amino acids in the leaves. We analyzed three transverse sections of medium aged leaves and the mid-transverse section of three ages of leaves from four sugarbeet cultivars to aid us in the selection of a representative sampling technique.

Materials and Methods

Sugarbeets used for this experiment were planted April 16, 1968 and grown under irrigation at the Colorado State University Agronomy Research Center at Fort Collins, Colorado.

Four populations, two heterogeneous and two inbred cultivars, covering a wide range of *Cercospora beticola* leaf spot resistance, were planted in a randomized complete block design with four

[1] Joint contribution of the Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture; the Beet Sugar Development Foundation, and the Colorado State University Experiment Station. Published with the approval of the Director of the Colorado State University Experiment Station as Scientific Series No. 1679.

[2] Research Chemist and Research Geneticist, Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, and Assistant Chemist, Colorado State University, Fort Collins, Colorado 80521.

replications. The plants were grown under relatively disease-free conditions. Leaf harvests were made on two dates: leaf section study, August 23, different aged leaves, August 31.

The populations used for both studies were:

1. US 201, homozygous for high leaf spot resistance (LSR) and relatively heterogeneous for other characters.
2. GWI-29, leaf spot resistant (LSR), inbred.
3. R & G Pioneer, leaf spot susceptible (LSS), heterogeneous.
4. 52-407, leaf spot susceptible (LSS), inbred.

Four leaves from each plant in each of the four populations were harvested for amino acid sampling of leaf sections. The leaves selected were intermediate between the small young leaves and the largest old leaves (showing signs of senescence) and represented the most typical leaf on each plant. The leaves were stacked carefully, by replication, tip to tip and petiole to petiole, and placed in a plastic bag with proper labeling.

The amino acid samples were prepared in the laboratory immediately after harvest. Each replication of stacked leaves was cut transversely into three sections—base, mid and tip. Each section was weighed and ground at high speed in a Waring[3] blendor for 5 minutes in 4 ml of 10% sulfosalicylic acid solution (w/v in glass distilled water) per gram of fresh leaf. Previously we had tested different proportionate amounts of several grinding media (2,9)[4] and we selected 4 ml of 10% sulfosalicylic acid per gram fresh leaf as the medium which best deproteinized the leaf sample with minimum dilution and still dissolved the free amino acids. The resulting sample required no further purification except centrifugation before it was placed on the analyzer column. High quality chemicals and glass distilled or deionized water were used to prepare samples and buffer solutions which pass through the analyzer column resin beads (8,10,12). Certain metallic ions such as copper, zinc and iron strongly adhere to the column sulfonated resin beads and cause appreciable loss in sensitivity (11). As the metallic ion concentration increases on the beads the amino acid chromatogram peak elution pattern is destroyed. Impurities can also cause unstable base lines on the chromatogram (8,12). After 5 minutes of grinding, the slurry was poured into a glass or plastic container. When the liquid layer separated, and aliquot (10-15 ml) was centrifuged at ca. 15,000 rpm for 10 minutes. To reduce the number of time consuming analyses, we pooled the replications from each population by pipet-

[3] Mention of a proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and it does not imply approval to the exclusion of other products that may be suitable.

[4] Numbers in parentheses refer to literature cited.

ting 5 ml of each replicate sample into a plastic vial. The 20 ml pooled sample was taken to a pH 2.0 (from ca pH 1.2) with 40% NaOH and stored at -20 C until the automated analysis.

For amino acid sampling of different aged leaves, four old, four medium aged (equivalent to those used above), and four young partially expanded leaves were selected from each plant, stacked by replication, and bagged. Immediately after harvest the mid-transverse section was cut from each replicate sample, weighed and prepared as described above. The prepared samples were again pooled by population, adjusted to pH 2.0, and frozen.

A two-column Technicon AutoAnalyzer[3] was used for the amino acid analysis (4,7,8). The original Technicon procedure (6) was modified along with the arrangement of the modules so two 21-hour analyses could be made simultaneously (3). At analysis time each sample was thawed and again centrifuged to remove any sediment or excess sulfosalicylic acid. A 1.5 ml sample was placed on each of the two 140 x 6 cm columns filled with Technicon[3] type B, 8% cross-linked sulfonated resin beads (1,5). The column temperature was maintained at 56.6 C. Gradient buffers (10,15), ranging from pH 2.875 to 5.000, were pumped through the columns at a rate of 0.5 ml per minute, along with the sample, under a pressure of 300-350 psi for ca 21 hours. The amino acids, separated as the sample and gradient buffers passed through the ion exchange resin (4), were later mixed with a buffered methyl cellosolve ninhydrin solution and an aqueous hydrazine sulfate solution in the closed AutoAnalyzer system. To aid mixing and to prevent oxidation, the solution was segmented into small volumes by repurified nitrogen. The ninhydrin solution must contain some hydrindantin before reaction with the amino acids in order that the color formed is linear with concentration, stable, and of the requisite sensitivity (12). We used hydrazine sulfate (138 mg per liter) to reduce the ninhydrin to hydrindantin in place of adding hydrindantin directly to the ninhydrin solution (15) or using either stannous chloride or potassium cyanide as the reducing agent as suggested by Rosen *et al.* (7). This eliminated most of our problems with the color-sensitivity constant which we experienced earlier when hydrindantin or other reducing agents were used. The amino acids and other ninhydrin reacting substances (e.g. ammonia) react with the ninhydrin-hydrindantin mixture to produce a colored compound (12). The color factor for each amino acid is proportional to the amount of that amino acid present. The optical density was read at 440 and 570 mμ and recorded as chromatogram peaks (14).

Before sample analysis, three or four duplicate analyses were made using Technicon's 18 amino acid standard[3] plus any additional amino acid standard(s) needed and an internal standard. We used a carefully chosen amino acid as the internal standard to check the reproductivity of the ninhydrin-hydrindantin color reaction. This internal standard must not occur in a sample, must elute on the chromatogram where no sample amino acid will interfere, and should give a representative peak. Norleucine is often used as an internal standard but we could not use it since it eluted in the immediate area of 3,4-dihydroxyphenylalanine (Dopa), sometimes present in sugarbeet samples. Therefore, we chose L-α—amino-β-guanidinopropionic acid as our internal standard. Each standard solution contained 1.0 micromole (μM) per ml of each amino acid in a 12.5% sucrose solution (pH 2.0). The concentrated standard solutions (including Technicon's 18 AA standard), which contained 2.5 μM per ml of each amino acid, were diluted to 1.0 μM per ml by using 2.0 ml of the standard and 3.0 ml of a 20.8% sucrose solution in 0.01N HCl (w/v). A 0.2 ml aliquot (0.2 μM each amino acid) was used for each standard analysis. The quantitative amount of each sample amino acid was calculated in reference to the standard areas (13). The internal standard (0.2 μM) was added with each sample so adjustments could be made for any variation between analyses due to sensitivity changes in the color producing reagents. The amino acids were reported as μM per 100 g fresh leaf.

Results and Discussion

Twenty-one free amino acids and two amides were quantitatively determined in the fresh leaf samples (Tables 1,3). Several unknown amino acid peaks also showed on the chromatograms. Since completion of this study some unknowns have been indentified as citrulline, α-aminoadipic acid, α-amino-n-butyric acid, and possibly iso-valine and pipecolic acid. Work on identification of other unknowns is in progress. The amino acid serine and the two amides, glutamine and asparagine, eluted on the chromatogram as occluded peaks (peaks not separated). This occluded peak was calculated as one peak using serine as the standard. Tests showed this occluded peak was mainly serine in leaves. Threonine, eluted just prior to the larger serine-glutamine-asparagine peak, was sometimes also partially occluded to the larger peak. With a change in temperature and buffer pH these peaks could be separated, but peaks of other amino acids would then occlude. The temperature and buffer pH chosen gave the best separation of the peaks for the majority of the amino acids in the samples. Sometimes proline occluded to the larger glutamic acid peak which eluted on the chromatogram immediately prior to proline.

Table 1.—Amino acids (μM/100g) in base, mid, and tip sections of fresh leaves of four sugarbeet populations.

Amino acids μM/100g	US 201			GWI-29			R & G Pioneer			52-407		
	Base	Mid	Tip	Base	Mid	Tip	Base	Mid	Tip	Base	Mid	Tip
1. ASP[a]	119.1	200.4	283.9	195.8	194.5	261.0	178.0	205.5	302.4	157.3	207.6	290.1
2. THR	12.8	15.2	11.2	15.6	15.3	9.9	13.4	19.4	16.1	12.3	18.0	22.2
3. SER*	94.5	117.4	119.5	96.4	110.4	85.4	69.2	105.5	98.1	65.6	92.6	111.4
4. GLU	220.2	263.9	389.7	356.8	298.6	410.3	313.8	325.3	464.8	283.1	241.5	433.2
5. PRO	21.2	27.8	occ.**	38.6	21.9	occ.	23.3	19.9	occ.	22.8	18.0	39.6
6. GLY	6.6	8.2	10.6	7.1	10.2	13.0	4.4	7.9	10.2	3.2	6.8	7.3
7. ALA	72.9	66.0	98.6	56.2	52.8	41.6	37.3	61.3	66.7	21.0	44.7	49.0
8. VAL	13.4	14.8	25.2	11.4	13.0	13.4	8.6	13.9	23.5	5.6	10.4	14.4
9. CYS	2.2	Trace	6.7	3.1	Trace	5.0	2.5	Trace	11.7	1.5	Trace	4.5
10. MET	Trace	Trace	2.6	Trace	Trace	Trace	Trace	Trace	2.4	Trace	Trace	2.2
11. ILE	7.3	6.2	13.0	7.3	5.9	8.2	6.7	7.5	14.9	4.1	6.0	9.5
12. LEU	9.7	6.2	7.6	9.7	7.5	7.6	6.9	5.1	8.2	5.7	5.6	8.8
13. DOPA	1.3	Trace	1.5	Trace	Trace	Trace	Trace	Trace	Trace	1.5	Trace	2.2
14. TYR	9.8	10.1	18.4	8.7	8.7	10.9	9.6	11.5	18.5	5.6	6.8	10.9
15. PHE	4.9	8.6	7.5	7.0	7.9	6.6	5.4	7.1	6.8	3.4	5.6	6.8
16. GABA	136.3	60.7	140.9	114.3	38.4	60.0	67.5	54.9	91.0	72.6	55.1	121.5
17. ORN	Trace	Trace	Trace	1.4	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
18. LYS	4.1	2.7	3.6	3.7	2.8	3.5	2.4	2.0	2.9	2.1	3.2	2.7
19. HIS	2.4	2.7	2.5	2.1	Trace	1.6	2.4	2.1	2.0	1.4	2.0	2.3
20. TRY	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	1.6
20. ARG	3.6	1.7	2.4	1.4	Trace	1.0	1.4	1.2	1.6	2.6	1.7	1.5

* Includes glutamine and asparagine.

** Occluded peaks.

[a] 1. aspartic 2. threonine 3. serine 4. glutamic 5. proline 6. glycine 7. alanine 8. valine 9. cystine 10. methionine 11. isoleucine 12. leucine 13. 3,4-dihydroxyphenylalanine 14. tyrosine 15. phenylalanine 16. gamma-aminobutyric acid 17. ornithine 18. lysine 19. histidine 20. tryptophan 21, arginine.

The free amino acids (μM/100 g fresh leaf) in the three transverse sections of the medium aged leaves for the four populations are presented in Table 1. Some differences were noted in the relative amounts of certain amino acids in the four populations; sometimes the two heterogeneous or the two inbred lines showed similarities. The two LSR populations contained more gamma-aminobutyric acid (GABA) in the base section than did the LSS populations. US 201, the highly LSR population, contained the most GABA in each section at this stage of leaf growth. Since no statistical analysis was made because of insufficient replications, we do not know whether these are significant differences. Glutamic acid ranked highest in quantity in all leaf sections of each population and the tip section contained the largest amount. The LSS heterogeneous R & G Pioneer had the highest average amount of glutamic acid and US 201 (LSR) the lowest average amount. Aspartic acid ranked second quantitatively in all populations; the tip section again contained the most and the base section the least except for the LSR inbred, GWI-29, in which the base and mid-section quantities were almost the same. The serine-glutamine-asparagine combination, GABA, and alanine were present in significant quantities in all populations. All other amino acids were present in lesser quantities which varied in the three sections and in the different populations.

Table 2 gives the percent of the leaf section amino acids in each quantitative rank for all populations. Out of a maximum of 84 possible amino acid values (4 populations x 21 amino acids) for each leaf section, 60.7% of the tip amino acid quantities ranked highest; the highest percentage (42.9) of the mid-section amino acids ranked intermediate, while in the basal section the highest percent (39.3) ranked lowest in quantity.

Table 2.—The percent of amino acids in highest, intermediate, and lowest quantities in three sugarbeet leaf sections for all populations.

Relative amino acid quantity	Leaf section		
	Tip	Mid	Base
Highest	60.7%	11.9%	16.7%
Intermediate	16.7	42.9	34.5
Lowest	10.7	35.7	39.3
Not ranked*	11.9	9.5	9.5

* Not ranked because of trace amounts or occluded peaks.

The individual amino acid quantities (μM/100 g) in the three different aged leaves for the four populations are given in Table 3. These leaves were harvested eight days later (Aug. 31) than those for the leaf study (Aug. 23) but at approximately the same time of day (10 AM). Relative amino acid content on the two dates showed considerable variation possibly because of change

Table 3.—Amino acids (μM/100g) in young, medium, and old sugarbeet leaves of four populations

Amino acids μM/100g	US 201			GWI-29			R & G Pioneer			52-407		
	Young	Medium	Old	Young	Medium	Old	Young	Medium	Old	Young	Medium	Old
1. ASP	182.9	111.3	221.7	81.8	150.5	158.4	134.2	117.6	189.5	135.6	216.5	154.5
2. THR	15.7	11.8	29.9	17.2	18.8	25.9	17.9	13.4	21.2	30.0*	35.0	19.0
3. SER**	132.4	55.2	66.6	112.2	100.4	67.4	94.8	48.6	65.0	110.0*	112.9	62.6
4. GLU	309.1	192.3	271.9	218.5	314.3	222.2	233.1	231.0	257.6	240.7	329.3	217.0
5. PRO	29.9	43.7	19.8	27.3	86.7	40.0	18.4	22.5	27.5	46.5	63.0	35.7
6. GLY	6.1	3.0	5.6	4.9	3.9	3.8	4.9	3.2	2.8	4.5	3.9	4.2
7. ALA	62.0	38.0	45.9	61.6	53.3	29.5	58.0	41.1	30.1	45.3	39.2	21.1
8. VAL	13.6	8.3	18.4	11.5	9.4	26.4	12.2	7.7	17.9	14.3	19.8	16.7
9. CYS	Trace	4.9	10.7	3.6	3.2	3.9	Trace	5.1	6.3	Trace	7.0	6.0
10. MET	Trace	Trace	1.6	Trace	2.8	Trace	Trace	Trace	1.5	2.2	3.4	2.7
11. ILE	9.7	5.1	11.2	9.8	6.8	13.4	53.4	7.2	10.0	14.0	11.6	8.6
12. LEU	9.0	8.1	17.1	13.8	9.7	34.6	15.0	7.0	20.0	18.3	16.0	13.7
13. DOPA	3.1	1.7	Trace	1.2	Trace	1.1	1.5	Trace	Trace	1.6	1.9	1.5
14. TYR	15.8	5.0	8.3	10.9	4.5	9.8	27.9	4.2	8.1	9.7	8.9	7.4
15. PHE	4.7	4.5	8.7	5.2	6.6	16.0	5.0	4.6	9.4	5.8	12.3	9.6
16. GABA	69.6	61.4	72.2	127.2	107.6	82.5	82.3	65.1	86.3	83.2	84.8	51.2
17. ORN	0.8	Trace	Trace	0.7	Trace	Trace	0.8	0.8	Trace	Trace	Trace	Trace
18. LYS	5.6	5.0	6.4	8.1	5.2	7.4	8.1	4.1	7.0	8.6	8.7	5.3
19. HIS	17.1	2.4	2.9	4.0	1.4	5.9	83.2	1.5	4.4	25.0	6.8	4.0
20. TRY	4.2	Trace	3.0	4.3	Trace	5.2	2.3	Trace	3.5	2.4	12.0	5.0
21. ARG	5.2	2.3	3.3	3.3	2.9	5.3	5.9	2.2	7.0	18.5	30.9	3.4

* Partially occluded peaks, approximate values only.

** Includes glutamine and asparagine.

in metabolic rate due to difference in moisture, nitrogen nutrition, temperature, and light conditions. Generally the amino acid values were higher on August 23 except for population 52-407. We have no record of relative moisture conditions but on the 2 days previous to August 23 the high temperatures were 82 and 83 F, the low 52 and 52 F, and the solar radiation 505 and 476 gram calories (g cal). On the two days previous to August 31 the high temperatures were 61 and 75 F, the low 47 and 39 F, and the solar radiation 162 and 472 g cal, respectively. The 162 g cal indicated a dark cloudy day. The lower temperatures and solar radiation before the August 31 date possibly lowered the metabolic rate; other genetic factors may have affected the relative amino acid content of 52-407.

Glutamic acid was again the dominant amino acid in all aged leaves, with aspartic, the serine-glutamine-asparagine combination, GABA, and alanine next in rank. The comparative quantity of each amino acid in each age leaves in each population varied. For example, the medium aged leaves of the two inbred lines contained the largest amount of glutamic acid while the medium aged leaves of the two heterogeneous lines had the least amount of glutamic acid. The aspartic acid content was highest in the old leaves except for 52-407 in which the medium aged leaves had the greatest amount.

The frequency percents of the quantitative rank of the individual amino acids in the three different aged leaves for the four populations are given in Table 4. The young leaves had the greatest percent of the amino acid quantities which ranked highest and intermediate, the medium age leaves the greatest number which ranked lowest, and the old leaves the greatest number which ranked highest.

Table 4.—The percent of the amino acids in the highest, intermediate, and lowest quantity in three ages of sugarbeet leaves for 4 populations.

Relative amino acid quantity	Leaf age		
	Young	Medium	Old
Highest	39.3%	22.6%	38.1%
Intermediate	38.1	21.4	29.8
Lowest	16.7	48.4	26.2
Not ranked*	5.9	7.2	5.9

* Not ranked because of trace values or occluded peaks.

All amino acids in this study were calculated on the fresh leaf weight basis. Later work indicated that calculations based on dry weight would probably be more accurate. We determined the percent dry matter on three transverse sections of medium aged and three different aged leaves from three populations (3 reps) grown in the greenhouse. The average percent dry matter

in the transverse sections was: tip, 16.1%; mid section, 15.5%; and base 15.1%. A greater variation in the median dry matter percentages was found in different aged leaves—young, 15.5%; medium aged, 15.0%; and old leaves 12.4%. Field grown medium aged leaves from six populations (3 reps) harvested July 30, August 10, and August 19, 1970, showed differences in percent dry matter among populations and harvests ranging from 9.4% to 15.1% with the higher percentage found later in the growing season. This indicated leaf moisture content may depend not only upon moisture available but population and leaf and plant maturity.

Conclusion

Relative quantities of individual amino acids in different sections of medium aged sugarbeet leaves showed considerable variation among leaf sections and populations. Similarities were sometimes apparent in the amino acid content of the two inbred or the two heterogeneous sugarbeet cultivars. Also the two LSR lines showed some quantitative differences in certain amino acids from that found in the LSS lines.

Comparison of the relative quantities of each amino acid in the three transverse sections of medium aged leaves for all populations showed over 60% of the tip section amino acids ranked highest quantitatively; in the mid-section the highest percent (42%) ranked intermediate; while in the basal section the highest percent ranked lowest quantitatively. This suggests that the mid-section of the medium aged leaves was probably the most representative section for amino acid determinations. Relative amounts of individual amino acids in the three aged leaves (young, medium, and old) did not give a good indication of the best choice. Both the young and old leaves had the highest percent of their amino acids which ranked highest in relative quantity while the medium aged leaves had the highest percent which ranked lowest in quantity. Since the amino acids were calculated on the fresh weight basis, relative water content of the leaves probably had an effect. It was found in later work that percent leaf dry matter was dependent not only upon moisture available but upon sugarbeet cultivar, leaf and plant maturity.

As a result of this study we make the following recommendations for leaf amino acid sampling and automated analysis:

1. If the entire leaf or leaves can be used, the medium aged leaf is probably best. If only a leaf section can be used, the mid-transverse section of the medium aged leaf seemed to be most representative.
2. Leaf harvests should be made at the same time of day by the same individual(s) to eliminate as much variation as possible in choosing samples.

3. Samples should be prepared immediately after harvest to minimize chemical changes due to enzymatic action.
4. Quantitative calculations should be made on dry leaf weight basis to avoid quantitative errors resulting from variations in percent moisture (or percent dry substance) due to available moisture, sugarbeet cultivar, and maturity of leaf and plant.
5. Ten percent sulfosalicylic acid (4 ml/g fresh leaf) is recommended for a grinding medium to deproteinize the sample, inactivate the enzymes, and dissolve the free amino acids. After centrifugation and adjustment of pH the sample is ready to analyze.
6. Deionized or glass distilled water should be used for preparation of samples or buffer solutions because of the adverse effect of heavy metallic ions on the performance of the column sulfonated resin beads.
7. High quality chemicals and carefully prepared buffers with accurate pH are required for good peak separation and resolution and a stable base line on the chromatographic chart.
8. For greater stability of the color factor, hydrazine sulfate is recommended as the reducing agent with ninhydrin in the analyzer closed system.
9. Prepared samples may be stored frozen until analysis.

Acknowledgments

The authors are indebted to the Agronomy and Horticulture Departments, Colorado State University, Fort Collins, Colorado, for the daily temperature and radiation data.

Literature Cited

(1) BENSON, J. V. and J. A. PATTERSON. 1965. Accelerated automatic chromatographic analysis of amino acids on a spherical resin. Anal. Chem. 37: 108.

(2) BLOCK, WALTER D., MARA E. MARKOUS and BETTY F. STEELE. 1966. Comparison between free amino acid levels in plasma deproteinated with picric acid and sulfosalicylic acid. Proc. Soc. for Exp. Biology and Medicine 122: 1089-1091.

(3) DUS, K. M., M. DEKKER and R. M. SMITH. 1965. Quantitative spectrophotometric amino acid submicro analysis with a multicolumn system. Anal. Biochem. 11: 312.

(4) HAMILTON, PAUL B. 1963. Ion exchange chromatography of amino acids. A single column, high resolving, fully automatic procedure. Anal. Chem. 35: 2055.

(5) MOORE, S. and W. H. STEIN. 1951. Chromatography of amino acids on sulfonated polystyrene resins. J. Biol. Chem. 192: 663.

(6) PIEZ, K. A. and L. MORRIS. 1960. A modified procedure for the automatic analysis of amino acids. Anal. Biochem. 1: 187.

(7) ROSEN, H., C. W. BERNARD and S. M. LEVENSON. 1962. A simplified procedure for automatic amino acid analysis. Anal. Biochem. 4: 213.

(8) SCHMIDT, DORIS I., ed. 1966. Techniques in animo acid analysis. Technicon Instruments Booklet, p. 17-25. Technicon Instruments Company, Ltd., Chertsey, England.

(9) SCHMIDT, DORIS I., ed. 1966. Ibid. p. 38-42.

(10) SCHMIDT, DORIS I., ed. 1966. Ibid. p. 93-106.

(11) SCHMIDT, DORIS I., ed. 1966. Ibid. p. 124-125.

(12) SCHMIDT, DORIS I., ed. 1966. Ibid. P. 127-140.

(13) SCHMIDT, DORIS I., ed. 1966. Ibid. P. 142-156.

(14) SPACKMAN, D. H., W. H. STEIN and S. MOORE. 1958. Automatic recording apparatus for use in chromatography of amino acids. Anal. Chem. 30: 190.

(15) TECHNICON AUTOANALYZER RESEARCH BULLETIN No. 20. 1967. p. 2-39. Technicon Corporation, Ardsley, New York.