CHAPTER-IV

STUDIES ON PROTEINS AND AMINO ACIDS

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4.1 ISOLATION OF PROTEINS FROM PLANT SEEDS :[1]

The defatted powdered seeds were repeatedly extracted with cold alkaline 10% brine containing 0.25% of sodium hydroxide for several hours. The contents obtained were filtered and proteins were precipitated from the filtrate by mainting the pH value between 3.5 to 4.5, by the addition of 10% aqueous acetic acid solution and heating it at 80°C for 30 minutes. The precipitate so obtained was filtered and washed successively with water and then with acetone and dried. The protein contents present in the seed were estimated subsequently. The protein percentages, thus obtained and that calculated are given in table No. 4.1.

Table No. 4.1

The Percentages of Proteins in Various Seeds

S. No.	Name of the Seeds	Percentages of Proteins	
		Estimated	Calculated
1	Annona Squamosa Linn.	18.84	21.64
2	Citrullus Vulgaris Var. fistulosus	30.72	33.6
3	Brassica Oleracea Linn (Gongylodes Group)	34.28	36.76
4	Moringa Oleifera Lm.	28.86	30.22

4.2. PREPARATION OF PROTEIN HYDROLYSATES : [2-4]

1.0 gm each of protein isolates were refluxed with a mixture (150-200 ml) of 6N-HCl and 80% formic acid for 12-16 hours for their complete hydrolysis. Excess of acid was removed by evaporating it under mild conditions and the volume of the contents was reduced to about 5.0 ml. It was diluted to 25 ml with distilled water and was filtered to remove humin. The filtrate was evaporated to a syrupy mass and was dried in vacuum. The residue was dissolved in 30% iso-propanol and stored in brown bottles in cold to prevent photochemical decomposition of amino acids.

Mixture of 6N-HCl and 80% formic acid used for hydrolysis of proteins, was found to be more useful in two ways,-

(i) as it reduces time of hydrolysis of proteins to 12 to 16 hours, from 18-24 hours required in case of 6N-hydrochloric acid, and

(ii) secondly, the mixture of hydrochloric acid and formic acid prevents the losses of amino acids by checking their oxidative degradation these are - Threonine, methionine, lysine and tryptophan.

4.3 EXAMINATION OF SEED PROTEIN HYDROLYSATES BY PAPER AND THIN LAYER CHROMATOGRAPHY : [5,6]

Various standard methods have been used for the identification of amino acids. In the present investigation ascending paper and cellulose thin layer chromatographic techniques have been used to identify the amino acid profiles present in the seeds. Whatman filter paper No. 1 (Chromatographic grade) for paper chromatography and chromatographic grade cellulose for preparation of thin layer plates were used during these studies.

Whatman filter paper No. 1 or Cellulose TLC plates were spotted with either authentic samples of amino acids or protein

hydrolysates of various seeds under study and these loaded paper strips or plates were dried in hot air. These paper chromatograms or TLC plats were first equilibrated and developed by vertical run techniques using suitable solvent systems. The solvent systems used were,

(i) n-Butanol, acetic acid, water (4:1:1.6, v/v)

(ii) n-Butanol, acetic acid, water (4:1:1.6, v/v)

(iii) Tert-Butanol, acetic acid, Water (4:1:1.6, v/v)

(iv) n-Butanol, Pyridine, water (1:1:1, v/v)

(v) n-Butanol, pyridine, water (6:4:3, v/v)

The developed chromatograms were dried in hot air and stained with various staining reagents for visualisation and subsequent identification of separated amino acid spots.

4.4 STAINING REAGENTS USED FOR IDENTIFICATION OF AMINO ACIDS : [7-10]

1. Ninhydrin reagent,

0.2-0.5% ninhydrin solution was prepared in water saturated with n-Butanol. All α-amino acids gave purple colour with ninhydrin except proline and hydroxy proline, which appear yellow in colour. Ninhydrin stained heated chromatogram was after sprayed with 1.0% aqueous sodium bicarbonate solution only. Phenyl alanine gives stable blue colour. When this chromatogram was further sprayed with 1N-HCl solution, yellow spot of hydroxy proline turned red.

2. Isatin reagent,

0.2% solution of isatine was prepared in acetone containing 4% of acetic acid. Chromatograms were stained with this solution

and were heated at 100°C for 10 minutes in an oven. Blue, lavender or blue green coloured spots were produced by proline, hydroxy proline, phenyl alanine, tyrosine, tryptophan, glutamic acid, arginine, lysine, methionine, histidine and cystine on the chromatogram.

3. Folin's reagent,

0.3 gm of β-naphthoquinone-4-sodium sulphonate was dissolved in 10 ml of water and the contents were diluted to 300 ml with acetone. Folin's reagent stained chromatogram was heated at 100°C for 3-5 minutes in an oven. Amino acids imparted pink colour.

4. Pauley's reagent,

Salution - I,

It was prepared by dissolving 0.9 gm of sulphanilic acid in 9.0 ml of concentrated hydrochloric acid and volume was made upto 100 ml with distilled water.

Solution - II,

It was prepared by dissolving 5.0 gms of sodium nitrite in 100 ml of distilled water.

Solution - III,

10% aqueous solution of sodium carbonate in distilled water.

Staining procedure of Chromatograms,

Solution I and II were mixed in equal volumes and were kept standing for 5 minutes below 20°C. 2.0 mls of solution III were added to above mixed solution. The developed chromatograms were stained with this mixed reagent. Histidine and tyrosine appeared as red spots.

4.5 EXPERIMENTAL :

UNIDIMENSIONAL ASCENDING PAPER CHROMATOGRAPHY OF AMINO ACIDS AND DETERMINATION OF THEIR R_f VALUES IN VARIOUS SOLVENT SYSTEMS : [11]

Authentic amino acid samples (chromatographic grade) were dissolved in 10% iso-propanol and were stored for an overnight. Whatman filter paper No. 1 (chromatographic grade) were cut in rectangular sheets of required size. Amino acids sample solutions were spotted at various positions on these sheets. These spots were dried in hot air. The loaded sheets were equilibrated with the solvent vapours and developed with the solvent systems with multiple (×5) ascending run technique. The developed chromatograms were air dried and stained with ninhydrin solution. R_f values of authentic amino acids were determined in following solvent systems and the results are given in Table No. 4.2.

(i) n-Butanol, acetic acid, water (4:1:1.6, v/v)

(ii) iso-Butanol, acetic acid, water (4:1:1.6, v/v)

(iii) Tert-Butanol, acetic acid, water (4:1:1.6, v/v)

(iv) n-Butanol, pyridine, water (1:1:1, v/v)

(v) n-Butanol, pyridine, water (6:4:3, v/v)

A perusal of table No. 4.2 indicates that Isobutanol, acetic acid, water system gives better separation of amino acids than the other four solvent systems. Separation sequence of amino acids in these solvent systems is given below,

Leucine and isoleucine > phenylalanine > Methionine + Valine + Tryptophan > Tyrosine > Proline + Alanine > Glutamic acid + Threonine > Hydroxy proline + Glycine > Serine + Aspartic acid > Arginine > Histidine + Lysine > Cystine.

Table No. 4.2

R_f Values of Various Amino Acids at 30 ± 1°C
(Uni-dimensional ascending run method)

(Length of solvent run 28 cms)

S. No.	Amino acid	n-Butanol : Acetic acid : water (4:1:1.6)	iso-butanol : Acetic acid : water (4:1:1.6)	tert-Butanol : Acetic acid : water (4:1:1.6)	n-butanol : pyridine : water (1:1:1)	n-butanol : pyridine : water (6:4:3)
1	Arginine	0.204	0.342	0.444	0.405	0.330
2	Aspartic acid	0.218	0.430	0.586	0.302	0.218
3	Alanine	0.393	0.639	0.730	0.568	0.470
4	Aspargine	-	0.308	0.478	-	-
5	Cystine	0.072	0.107	0.158	0.288	0.212
6	Glycine	0.238	0.422	0.521	0.441	0.378
7	Glutamic acid	0.304	0.491	0.563	0.304	0.300
8	Histidine	0.106	0.332	0.419	0.453	0.376
9	Hydroxy proline	0.300	0.450	0.603	0.548	0.468
10	Leucine	0.884	0.924	0.931	0.708	0.730
11	Lysine	0.088	0.248	0.401	0.340	0.297
12	Methionine	0.711	0.845	0.860	0.682	0.691
13	Phenyl alanine	0.851	0.902	0.894	0.712	0.728
14	Proline	0.430	0.689	0.742	0.590	0.532
15	Serine	0.198	0.409	0.501	0.503	0.440
16	Threonine	0.319	0.530	0.651	0.574	0.476
17	Tyrosine	0.554	0.724	0.721	0.686	0.688
18	Tyrptophen	0.706	0.807	0.810	0.700	0.710
19	Valine	0.714	0.834	0.836	0.640	0.680
Mean of five values						

4.6 ASCENDING CELLULOSE THIN LAYER CHROMATOGRAPHY OF AMINO ACIDS AND DETERMINATION OF THEIR R_f VALUES IN VARIOUS SOLVENTS SYSTEMS : [11]

10.5 gms of Chromatographic grade cellulose was shaken thoroughly with 35.0 ml of distilled water for 5 minutes. A slurry thus obtained was uniformly spread over three glass plates (20 x 20 cm) using a "Stahl type" TLC applicator, adjusting the layer thickness to 0.350 mm. these cellulose coated plates were air dried for an overnight. The TLC plates were spotted with the authentic samples of amino acids and dried in air for 5 to 6 hours. The loaded TLC plates were equilibrated with solvent vapours and were developed by ascending multiple run (x 5) technique in following solvent systems,

(i) n-butanol, acetic acid, water (4:1:1.6, v/v)

(ii) n-butanol, pyridine, water (1:1:1, v/v) and

(iii) Pyridine, water (4:1, v/v)

The developed chromatograms were dried in air and finally stained with ninhydrin solution. The R_f values of various amino acids were determined and the results are given in Table No. 4.3.

Table No. 4.3

R_f Values of Various Amino Acids resolved on cellulose TLC plates at 30 ± 1°C

(Uni-dimensional ascending run technique)

S. No.	Amino acid	R_f values in Solvent Systems		
		n-Butanol : Acetic acid : water (4:1:1.6)	n-butanol : pyridine : water (1:1:1)	Pyridine : water (4:1)
1	Arginine	0.106	0.173	0.110
2	Aspartic acid	0.134	0.150	0.264
3	Alanine	0.353	0.380	0.412
4	Cystine	0.042	0.050	0.040
5	Glycine	0.161	0.220	0.270
6	Glutamic acid	0.226	0.169	0.260
7	Histidine	0.079	0.218	0.272
8	Hydroxy proline	0.183	0.402	0.430
9	Isoleucine	0.730	0.763	0.706
10	Leucine	0.731	0.738	0.704
11	Lysine	0.070	0.112	0.086
12	Methionine	0.557	0.624	0.640
13	Phenyl alanine	0.664	0.724	0.696
14	Proline	0.364	0.413	0.452
15	Serine	0.163	0.300	0.364
16	Threonine	0.256	0.373	0.530
17	Tyrosine	0.415	0.656	0.623
18	Tyrptophan	0.532	0.673	0.707
19	Valine	0.588	0.580	0.620
Mean values of five determinations				

The separation trend of amino acids by ascending paper and cellulose thin layer chromatographic methods were found identical.

4.7 UNIDIMENSIONAL DESCENDING PAPER CHROMATOGRAPHY OF AUTHENTIC AMINO ACIDS AND THEIR R_f VALUES DETERMINATION : [12]

Authentic amino acid solutions were spotted on rectangular sheet (47 cm x 57 cm) of Whatman chromatographic grade filter paper No. 1 and it was dried in hot air. The loaded sheet was equilibrated with solvent vapours and was developed by unidimensional descending run technique using four different solvent systems. The solvent systems used were,

(i) n-butanol, acetic acid, water (4:1:1.6, v/v)

(ii) Isobutanol, acetic acid, water (4:1:1.6, v/v)

(iii) Pyridine, water (4:1, v/v)

(iv) n-butanol, pyridine, water (1:1:1, v/v)

The separated amino acids present in developed chromatograms were identified by staining with ninhydrin solution.

R_f values of various amino acids determined are given in Table No. 4.4.

Table No. 4.4

R_f Values of Authentic Amino Acids in different solvents at 30 ± 1°C

(Uni-dimensional descending run method)

S. No.	Amino acid	R_f valus in Solvent System			
		n-Butanol : Acetic acid : water (4:1:1.6)	iso-Butanol : Acetic acid : water (4:1:1.6)	Pyridine : water (4:1)	n-butanol : pyridine : water (1:1:1)
1	Arginine	0.15	0.14	0.27	0.20
2	Aspartic acid	0.19	0.21	0.17	0.18
3	Alanine	0.35	0.38	0.41	0.42
4	Cystine	0.05	0.07	0.05	0.06
5	Glycine	0.23	0.26	0.28	0.25
6	Glutamic acid	0.26	0.28	0.20	0.22
7	Histidine	0.08	0.16	0.29	0.24
8	Hydroxy proline	0.25	0.27	0.40	0.44
9	Isoleucine	0.84	0.90	0.72	0.86
10	Leucine	0.83	0.89	0.71	0.85
11	Lysine	0.08	0.12	0.14	0.16
12	Methionine	0.64	0.68	0.61	0.70
13	Phenyl alanine	0.73	0.76	0.58	0.83
14	Proline	0.43	0.47	0.37	0.46
15	Serine	0.21	0.22	0.35	0.30
16	Threonine	0.32	0.34	0.39	0.43
17	Tyrosine	0.47	0.51	0.59	0.74
18	Tyrptophan	0.59	0.66	0.56	0.76
19	Valine	0.66	0.71	0.60	0.65
Time of run		20 Hrs	20 Hrs	12 Hrs	12 Hrs
Length of solvent		38 cm	38 cm	28 cm	28 cm

Mean values of five determinations

4.8 TWO DIMENSIONAL DESCENDING PAPER CHROMATOGRAPHY OF AMINO ACIDS OF SEED PROTEIN HYDROLYSATES : [12]

To dimensional descending paper chromatographic method was also used for the separation of amino acids from the seed protein hydrolysates. The starting point was marked at a distance of 6-7 cm from each of the two adjacent edges on a Whatman filter paper chromatographic grade No. 1 sheet (47 cm x 57 cm). The seed protein hydrolysate solution was spotted and dried in hot air. This chromatogram was developed with n-butanol, acetic acid, water (4:1:1.6, v/v) solvent system by descending run technique in a chromatographic chamber. When the solvent front moved almost upto the other edge of the sheet, it was removed from the chromatographic chamber and dried in unpolluted atmosphere. The chromatogram was then run with another solvent system i.e. either in n-butanol, pyridine, water (1:1:1, v/v) or in pyridine, water (4:1, v/v) at right angle to the previous direction of the solvent in another chromatographic chamber. The developed chromatogram was dried and was stained with ninhydrin and amino acids present were identified by comparison of their R_f values with those of authentic amino acid samples chromatographed under identical experimental conditions.

4.9 TWO DIMENSIONAL ASCENDING CELLULOSE THIN LAYER CHROMATOGRAPHY OF VARIOUS SEED PROTEIN HYDROLYSATES : [12]

10.5 gms of chromatographic grade cellulose was shaken thoroughly with 35.0 ml of distilled water for five minutes. The slurry thus obtained was uniformly spread over (20 cm x 20 cm) glass plates using a "Stahl type" TLC applicator adjusting the layer thickness to 0.350 mm. The coated plates were activated by drying in air for an overnight. A starting point at a distance of 2.0 cm from two adjacent edges was marked and protein hydrolysate solution was spotted at this point and dried in hot air. The loaded TLC plate was first developed with n-butanol, acetic acid, water (4:1:1.6, v/v) by ascending run technique in solvent vapours saturated chromatographic chamber. When the solvent front moved almost upto the other edge of the plate, it was removed from the chamber and was dried in air for 2 to 3 hours. The TLC plate was then run in another chamber in the second solvent i.e. n-butanol, pyridine, water (1:1:1, v/v) at right angle to the previous direction of the solvent in another chromatographic chamber. The developed cellulose plate was dried in air and was stained with ninhydrin for visualisation of spots and amino acids were identified by comparision of their R_f values with authentic samples of amino acids chromatographed under identical conditions.

4.10 QUALITATIVE INVESTIGATION OF SEED PROTEIN HYDROLYSATES AND IDENTIFICATION OF AMINO ACIDS PRESENT IN THEM : [13]

The subjected seed protein hydrolysates were examined qualitatively by using uni- and two dimensional techniques of paper and cellulose thin layer chromatography. The solvent systems for separation of amino acids present in hydrolysates used were (i) n-butanol, acetic acid, water (4:1:1.6, v/v), (ii) n-butanol, pyridine, water (1:1:1, v/v), (iii) n-butanol, pyridine, water (6:4:3, v/v) and (iv) Pyridine, water (4:1,v/v). Various staining reagents as described earlier were used for the identification of amino acids. These analyses revealed the presence of sixteen common amino acids viz., cystine, lysine, histidine, arginine, aspartic acid, glycine, glutamic acid, serine, alanine, proline, threonine, methionine, phenyl alanine, valine, leucine, iso-leucine in protein hydrolysates of Annona squamosa linn, Citrullus vulgaris var fistulosus, Brassica oleracea linn and Moringa oleifera lam. In fact sixten amino acids in Annona squamosa, seventeen in Citullus vulgaris, Eighteen in Brassica oleracea linn and fourteen amino acids were detected in Moringa oleifera lam. seed protein hydrolysates. Tryptophan was detected in brassica oleracea seed protein isolate only. Traces of alanine, cystine, leucine, phenyl alanine, proline, threonine and valine were recorded in Annona squamosa seed protein hydrolysate. The results of qualitative chromatographic studies of various seed protein hydrolysates are given in Table No. 4.5.

Table No. 4.5

Results of Qualitative Chromatography of Seed Protein Hydrolysates Indicating Presence of Various Amino Acids

S. No.	Amino acid	Annona Squamosa Linn	Citrullus Vulgaris Var. fistulosus	Brassica Oleracea Linn (Gongylodes Group)	Moringa Oleifera Lam.
1	Arginine	+	+	+	+
2	Aspartic acid	+	+	+	+
3	Alanine	+	+	+	+
4	Cystine	+	+	+	-
5	Glycine	+	+	+	+
6	Glutamic acid	+	+	+	+
7	Histidine	+	+	+	+
8	Hydroxy proline	-	-	-	-
9	Isoleucine	+	+	+	+
10	Leucine	+	+	+	+
11	Lysine	+	+	+	-
12	Methionine	+	+	+	+
13	Phenyl alanine	+	+	+	-
14	Proline	+	+	+	+
15	Serine	+	+	+	+
16	Threonine	+	+	+	+
17	Tyrosine	-	-	+	+
18	Tyrptophan	-	+	+	-
19	Valine	+	+	+	+

4.11 ESTIMATION OF AMINO ACIDS IN VARIOUS SEED PROTEIN HYDROLYSATES :

Survey of literature reveals that a number of techniques have been used by different workers for the quantitative estimation of amino acids in seed protein hydrolysates.

Ninhydrin stained paper and thin layer chromatograms can be directly quantitated with a photo electric densitometer after imparting transparency to the stained chromatogram by immersing it for 5 minutes in anisole, benzyl alcohol or in a mixture of α-bromonaphthalene and liquid paraffin (24:46, v/v).

Ninhydrin colours of amino acids after elution from the paper or thin layer chromatogram in solvents n-butanol, water, aqueous ethanol etc. have been estimated photometrically.

Hirs et al.[14] have separated amino acids by ion exchange resin column chromatography. Gordon et al.[15] have resolved amino acids by Silica gel partition column chromatography. Resolved amino acids were estimated by ninhydrin photometric method.

Moore and Stein[16] have estimated amino acids by using column chromatographic technique using starch as adsorbent and (i) n-butanol, propanol, 0.1N-HCl (1:2:1, v/v) and (ii) n-Propanol, 0.5N-HCl (2:1, v/v) as eluents. The resolved acids were determined by ninhydrin photometric method.

Moore and Stein[17,18] have developed an automatic amino acid analyser for determination of amino acids in the seed protein

hydrolysates. This method is based upon the principle of ion exchange resin column chromatography and subsequent determination of resolved components by ninhydrin photometric method.

Recently developed technique of high performance (or pressure) liquid chromatography (HPLC) has been used by various workers for the quantitation of amino acids in seed protein hydrolysates.[19-20]

In the present investigation two dimensional paper chromatography in combination with spectrophotometric determination of ninhydrin colours after elution with 75% aqueous ethanol has been used for the quantitation of the amino acids. These seed protein hydrolysates were also subjected to HPLC analysis using SUPELCO make HPLC system.[20]

4.12 EXPERIMENTAL :

Two dimensional chromatography was done on acid (3% HCl) washed Whatman chromatographic grade No. 1 filter paper sheets (47 cm x 57 cm) and MN-Gel (300) TLC plates. 2μl of cation free protein hydrolysate solution was spotted on the filter paper or TLC plate with the help of a graduated micro pipette. Desalting of each subjected protein hydrolysate was done by ion exchange resin column chromatography method as described below,

Desalting of Protein Hydrolysate :

A column (25 x 1 cm) filled with DOWEX-50×8 (H^+) cation exchange resin of size 200-400 mesh seive was prepared. The protein

hydrolysate was evaporated under mild conditions to a syrupy liquid, which was vacuum desiccated. The residue so obtained (10-15 mg) was dissolved in 5-10 ml of 80% ethanol and was further percolated through the column at a rate of about 2-3 ml per minute. The unabsorbed material in the column was washed with 30-35 ml of 80% ethanol. The column was eluted successively with 60 ml of 0.4N ammonium hydroxide in 80% ethanol; 25 ml of 80% ethanol; 30 ml of distilled water, 40 ml of 4N ammonium hydroxide and finally with 30 ml of distilled water. The combined elutriate was evaporated to a syrupy mass and finally dried in vacuum. The residue was dissolved in 10% iso-propanol. This solution was used for spotting on the paper or thin layer chromatographic plates.

4.13 ESTIMATION OF AMINO ACIDS IN SEED PROTEIN HYDROLYSATES BY QUANTITATIVE TWO DIMENSIONAL CHROMATOGRAPHY :

Cation free protein hydrolysate solution was spotted on Whatman chromatographic grade filter No. 1 filter paper sheet (47 cm x 57 cm) and MN-Gel (300) coated TLC plates (20 cm x 20 cm) and was dried in hot air. The loaded chromatograms were equilibrated with the solvent vapours and were first developed with n-butanol, acetic acid, water (4:1:1.6, v/v) till the solvent front reached almost upto the maximum i.e. upto the other edge of sheet/plate. The developed chromatograms were dried under fan for several hours. Now these chromatograms were developed with the second solvent system i.e. either with pyridine, water (4:1, v/v) or n-butanol, pyridine, water (1:1:1, v/v) at right angle to the direction of previous run. These developed

chromatograms were again properly dried and were stained with ninhydrin dissolved in water saturated n-butanol and heated to 70-80°C for 15 minutes and then kept in dark for 24 hours for complete development of colours. Ninhydrin colours of individual amino acids were separately eluted with 10.0 ml of 75% ethanol and estimated spectrophotometrically at 570 nm using systronics make spectrophotometer model-169. The results of spectrophotometric estimation of amino acids are given in Tables No. 4.6 to 4.9.

4.14 HPLC ANALYSES OF AMINO ACID COMPOSITION OF VARIOUS SEED PROTEIN HYDROLYSATES :

Protein hydrolysates of Annona squamosa linn, Citrullus vulgaris var. fistulosus, Brassica oleracea linn (Gongylodes Group) and Moringa oleifera lam. seeds were also subjected to HPLC analysis for the determination of percentage profile of different amino acids. The details of instrument used for these analyses are as below,

HPLC Instrument details,

Make : SUPELCO Company

Column : C-18 (High Purity) Amino acid column

Diameter : 250 x 4.6 mm

Particle size : 5 μ

A fixed wavelength detector operating at 254 nm was used. HPLC curves for various seed protein hydrolysates are shown in Fig. 4.1 to 4.4 and the results of the quantitation of amino acids present in seed protein hydrolysates are given in Tables No. 4.6 to 4.9.

Table No. 4.6

Amino Acid Composition of Seed Protein Hydrolysate of Annona Squamosa Linn.

(Amino Acid Percentages Expressed in g/100 gm of Protein)

S. No.	Amino acid	Percentage Composition	
		From HPLC Analysis	From Spectrophoto-meter Analysis
1	Arginine [e, +]	0.183	0.172
2	Aspartic acid	1.011	0.810
3	Alanine	0.069	Traces
4	Cystine [e]	0.038	Traces
5	Glycine [++]	0.432	0.388
6	Glutamic acid	0.147	0.138
7	Histidine [e]	88.4	87.24
8	Iso-leucine [e]	8.063	7.86
9	Leucine [e]	0.152	Traces
10	Lysine [e]	0.243	0.222
11	Methionine [e]	0.418	0.368
12	Phenyl alanine [e]	0.033	Traces
13	Proline [+++]	0.026	Traces
14	Serine	0.572	0.488
15	Threonine [e]	0.053	Traces
16	Tyrosine	-	-
17	Tyrptophan	-	-
18	Valine	0.162	Traces

e = Essential amino acids,

+ = Indispensable for rats and chicks both

++ = Indispensable for Chicks

+++ = Optical density measured at 440 nm

Table No. 4.7

Amino Acid Composition of Seed Protein Hydrolysate of Citrullus Vulgaris Var. Fistulosus (Stocks)

(Amino Acid Percentage Expressed in g/100 gm of Protein)

S. No.	Amino acid	Percentage Composition	
		From HPLC Analysis	From Spectrophoto-meter Analysis
1	Arginine [e +]	0.654	0.603
2	Aspartic acid	6.111	5.84
3	Alanine	0.662	0.586
4	Cystine [e]	0.556	0.501
5	Glycine [++]	8.979	8.68
6	Glutamic acid	17.564	16.942
7	Histidine [e]	1.586	1.36
8	Iso-leucine [e]	0.845	0.788
9	Leucine [e]	1.998	1.72
10	Lysine [e]	1.116	0.886
11	Methionine [e]	17.852	17.346
12	Phenyl alanine [e]	1.676	1.06
13	Proline [+++]	8.102	7.94
14	Serine	11.714	11.36
15	Threonine [e]	7.345	7.08
16	Tyrosine	1.637	1.310
17	Tyrptophan	-	-
18	Valine	11.602	11.214

e = Essential amino acids,

+ = Indispensable for rats and chicks both

++ = Indispensable for Chicks

+++ = Optical density measured at 440 nm

Table No. 4.8

Amino Acid Composition of Seed Protein Hydrolysate of Brassica Oleracea Linn (Gongylodes Group)

(Amino Acid Percentage Expressed in g/100 gm of Protein)

S. No.	Amino acid	Percentage Composition	
		From HPLC Analysis	From Spectrophoto-meter Analysis
1	Arginine e +	9.461	8.842
2	Aspartic acid	13.298	12.76
3	Alanine	0.762	0.722
4	Cystine e	0.459	Traces
5	Glycine ++	13.952	13.464
6	Glutamic acid	5.874	5.380
7	Histidine e	21.874	20.750
8	Iso-leucine e	2.183	1.780
9	Leucine e	3.94	3.388
10	Lysine e	0.481	0.401
11	Methionine e	1.282	1.066
12	Phenyl alanine e	0.980	0.816
13	Proline +++	6.543	6.140
14	Serine	2.871	2.468
15	Threonine e	2.179	1.876
16	Tyrosine	2.93	2.81
17	Tyrptophan	0.943	0.768
18	Valine	5.686	5.034

e = Essential amino acids,
+ = Indispensable for rats and chicks both
++ = Indispensable for Chicks
+++ = Optical density measured at 440 nm

Table No. 4.9

Amino Acid Composition of Seed Protein Hydrolysate of Moringa Oleifera Lam

(Amino Acid Percentage Expressed in g/100 gm of Protein)

S. No.	Amino acid	Percentage Composition	
		From HPLC Analysis	From Spectrophoto-meter Analysis
1	Arginine e +	16.392	15.804
2	Aspartic acid	23.96	22.843
3	Alanine	4.681	3.568
4	Cystine e	-	-
5	Glycine ++	6.172	5.78
6	Glutamic acid	8.432	7.68
7	Histidine e	2.009	1.68
8	Iso-leucine e	1.927	1.460
9	Leucine e	2.303	1.782
10	Lysine e	-	-
11	Methionine e	6.811	5.88
12	Phenyl alanine e	-	-
13	Proline +++	0.705	0.584
14	Serine	20.523	19.66
15	Threonine e	4.665	4.33
16	Tyrosine	0.674	0.522
17	Tyrptophan	-	-
18	Valine	0.747	0.635

e = Essential amino acids,

+ = Indispensable for rats and chicks both

++ = Indispensable for Chicks

+++ = Optical density measured at 440 nm

Fig. 4.1 HPLC CURVE OF SEED PROTEIN HYDROLYSATE OF ANNONA SQUAMOSA LINN

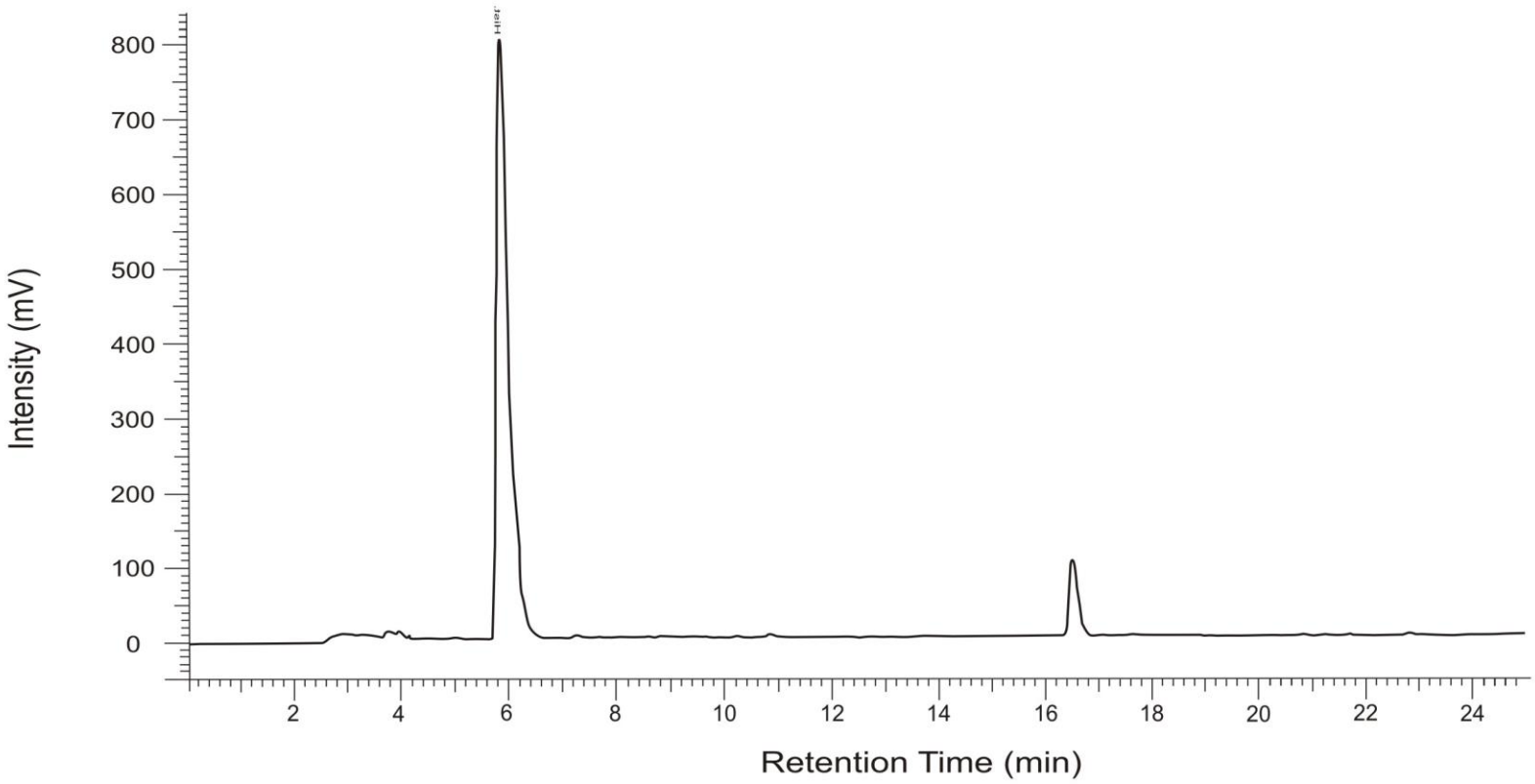


Fig. 4.2 HPLC CURVE OF SEED PROTEIN HYDROLYSATE OF CITRULLUS OF VULGARIS VAR. FISTULOSUS (STOCKS)

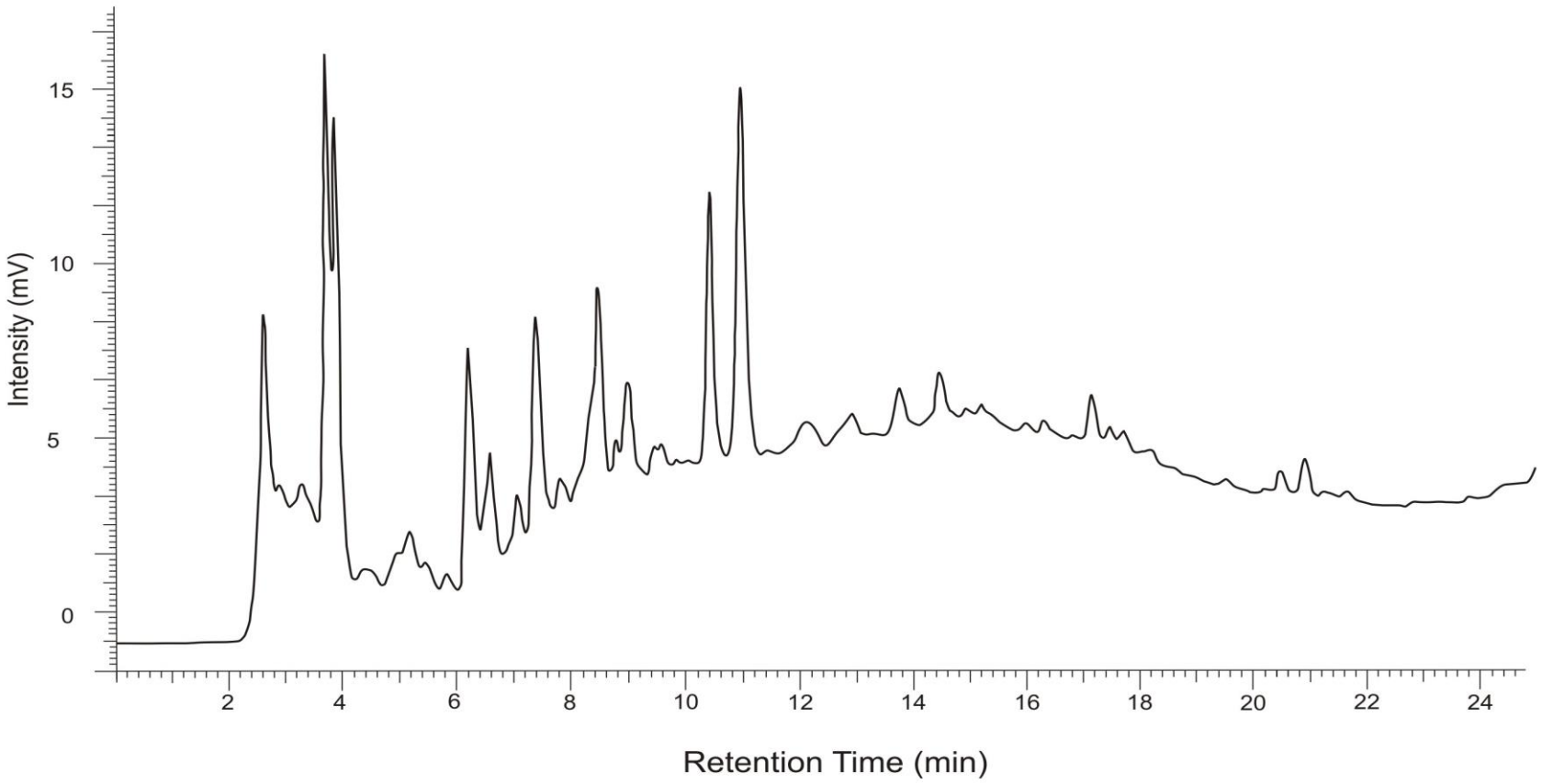


Fig. 4.3 HPLC CURVE OF SEED PROTEIN HYDROLYSATE OF BRASSICA OLERACEA LINN (GONGYLODES GROUP)

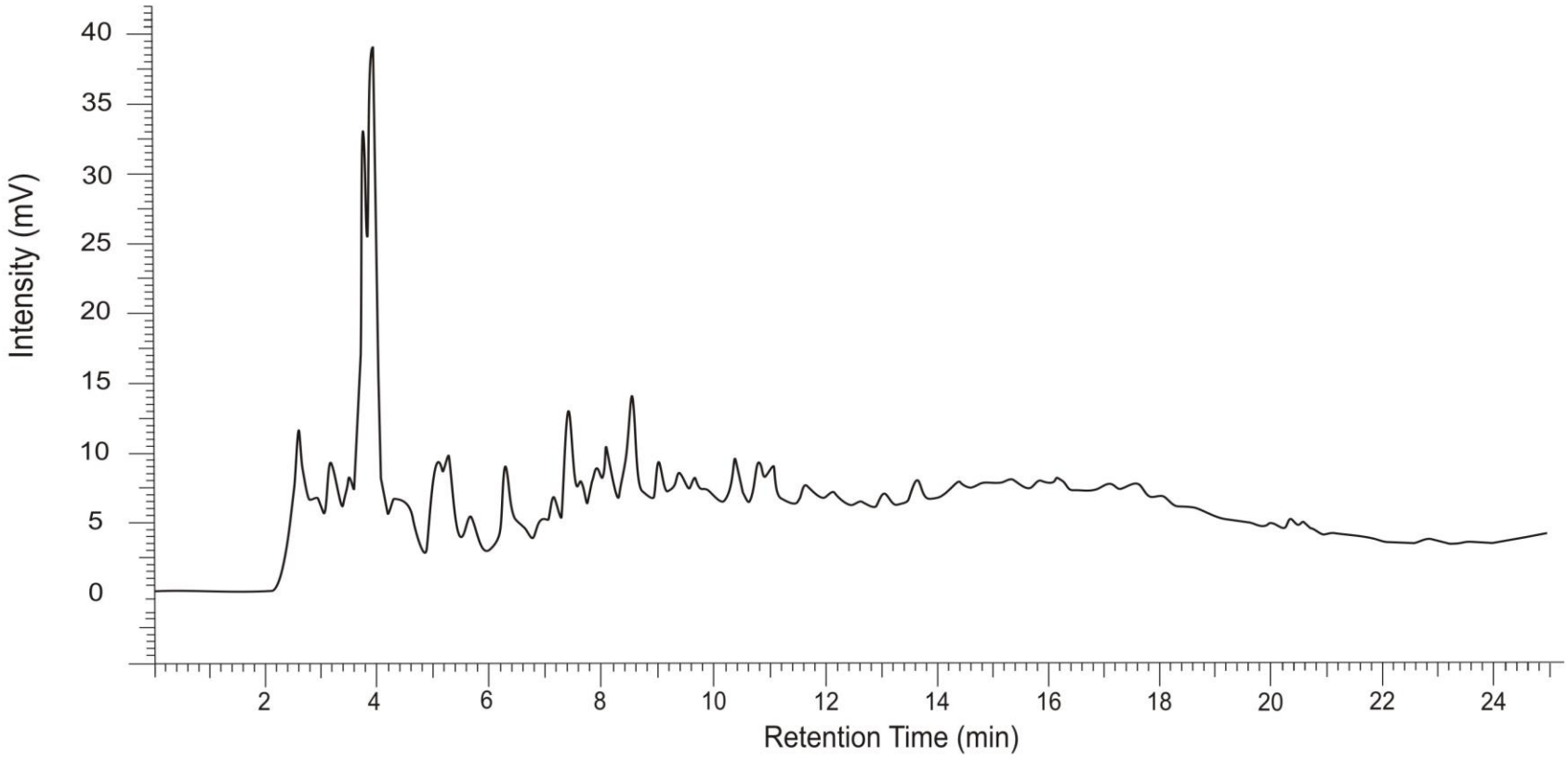
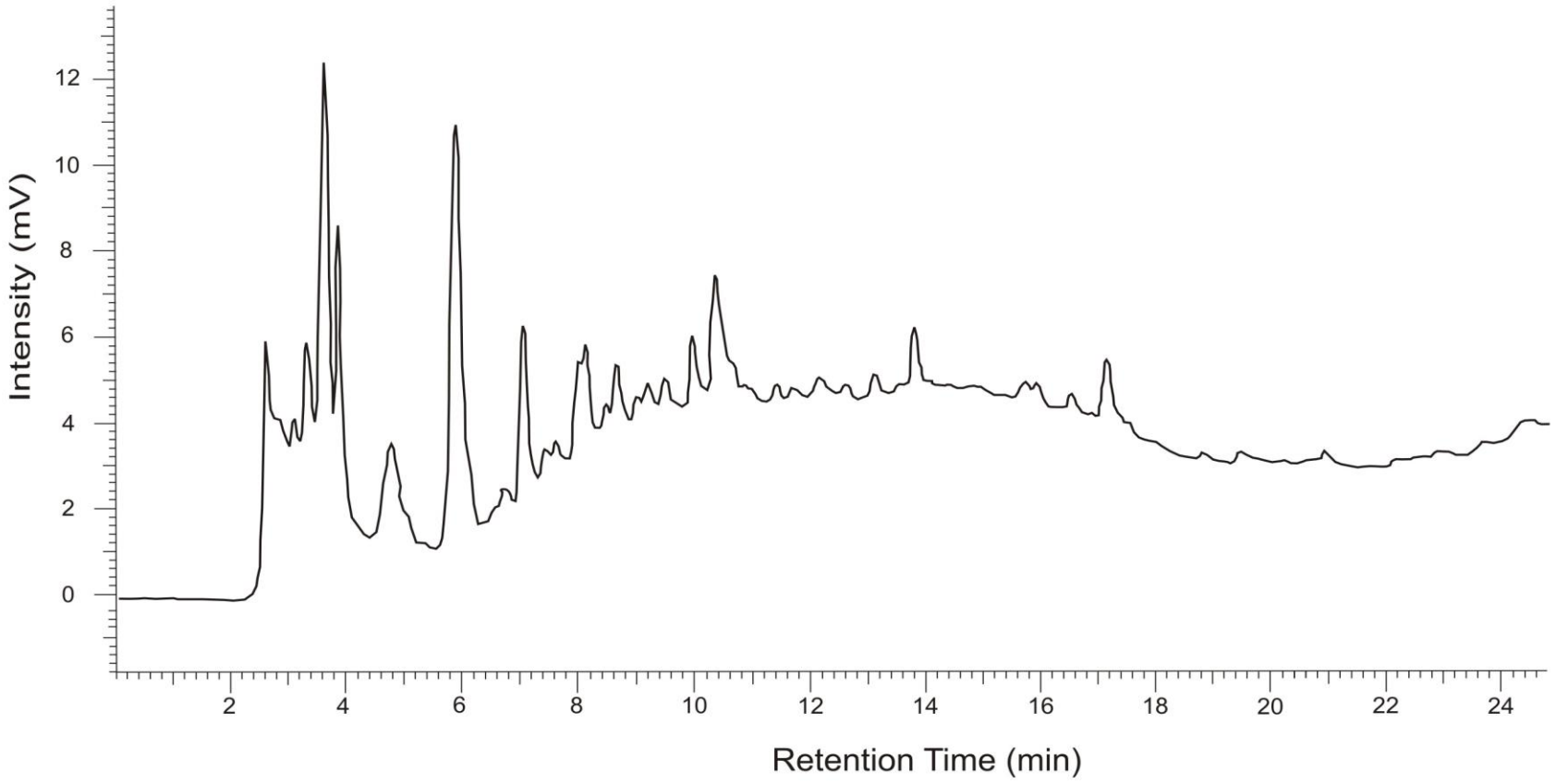


Fig. 4.4 HPLC CURVE OF SEED PROTEIN HYDROLYSATE OF MORINGA OLEIFERA LAM



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