J. Chem. Soc. Pak. Vol. 2, No. 2, 1980

Amino acid Composition of Protein Hydrolysates of some Lentils of Common use.

T. IQBAL AND G.H. KAZI

National Centre of Excellence in Analytical Chemistry, University of Sind, Jamshoro, Pakistan.

(Received 27th Dec. 1979)

Summary: The chemical investigations of seeds of *Cicer arictinum, Lens esulenta, Phascolus radiatus, Phaseolus mungo* and *Cajanus indicus* have been carried out using T.L.C., paper chromatographic and colorimetric methods. Amino acid composition of the acid hydrolyzates of proteins extracted from lentils is reported here. Lysine, methionine and tryptophan have been recognized to play an important role in nutritional effectiveness of various foods. The lentils investigated are an important source of proteineous foods available to people in Asia, therefore much emphasis was laid down on estimation of the amino acids present in them and in turn evaluating various physico-chemical parameters such as protein score and net dietary protein percentage. The results are consolidated in Table I - IX.

Introduction

The physico-chemical investigation of five lentil seeds were carried out using T.L.C., paper chromatography and colorimetric methods. The main emphasis was laid on the separation of the proteins from these seeds and their amino acid composition. The amount of amino acids present has direct impact on the nutritional effectiveness of the available food. The seeds investigated are consumed by a large number of people in the Asian continent as a substitute for milk, meat and eggs. Most of the seed proteins on hydrolysis or in metabolic systems break up into amino acids. Lysine, methionine cystine and tryptophan have nutritional importance and are required for the normal functioning and growth of living beings.

The work reported in this paper mainly focussed on analytical investigation and estimation of different amino acids and correlating their sulphur and nitrogen content with net dietary protein content of the seeds. The results presented in tables I-IX can be utilized to provide additional information to laboratory workers and end users of these lentils to select seeds in mixed diets for obtaining the best possible nutritional benefit out of them. For example, normal minimum daily requirement of L-tryptophan for human subjects is 0.25 g. However a two fold excess (0.5g) intake of tryptophan is recommended because the absorption level of amino acid and intake level are not essentially identical. Therefore, the requiste amount of L-tryptophan may be obtained from eating 200-300g of lentil seeds or preparing a recipe containing meat, vegetables and some proportionate amount of lentils.

Experimental

The isolation of proteineous material was accomplished as follows:

The powdered lentil samples were subjected to extraction with petroleum ether, benzene, acetone, ethylacetate and chloroform so as to remove fats and lipids. Thereafter the carbohydrates were hydrolyzed[1] under mild conditions, i.e. with crystalline bacterial amylase at pH 7.0 and the unreacted insoluble proteineous material filtered. The proteineous material was further subjected to acid hydrolysis by refluxing with 6N HCl for various intervals ranging between 6 to 24 hours. The proteins were transformed into a mixture of free amino acids which were purfied by column chromatography using ion exchange resin IR-120-X8[2]. The purified mixture of amino acids was subjected to paper and thin layer chromatography using n-butanol-water-acetic acid or phenol-isopropanol solvent[3] systems. After elution was complete, the paper or plates were dried and sprayed with ninhydrin in acetone followed by dilute ammonia spray and warmed to develop the coloured bands of different amino acids present in the protein hydrolyzate.

Table I

Quantitative and qualitative estimation of various inorganic and organic contents of lentils

S.No.	Lentil	N	Grams per 100 g sample				mg per 100 g sample		
			Moisture	Ash	Lipid	Carbohydrate	Calcium	Sulphate	Chloride
1.	L_1	20	9.60 ± 1.50	4.30 ± 0.31	2.22 ± 0.26 (1.30)*	60.25 (63.0)	240.7 ± 24.82	285.0 ± 4.00	365.0 ± 7.00
2.	L_2	20	8.82 ± 1.02	2.22 ± 0.33	1.44 ± 0.25 (1.20)	67.31 (65.0)	78.0 ± 3.21	285.0 ± 4.00	198 ± 0.00
3.	L_3	20	8.78 ± 1.71	3.78 ± 0.39	1.40 ± 0.16 (1.50)	62.04 (63.70)	128.0 ± 15.50	337.0 ± 2.39	265.0 ± 0.04
4.	L_4	20	9.68 ± 1.43	3.32 ± 0.29	1.43 ± 0.50 (1.30)	61.91 (68.80)	140.0 ± 8.48	185.3 ± 1.30	205.0 ± 0.00
5.	L_5	20	7.40 ± 0.12	3.82 ± 0.26	1.38 ± 0.16	68.40 (72.0)	137.0 ± 9.90	491.0 ± 16.26	224.0 ± 16.97

Quantitative Analysis:- Cations. Fe^{3+}, Al^{3+}, Ca^{2+}, Na^{+}, and K^{+}
Anions. Cl^{-}, SO_4^{2-}, PO_4^{3-}, and CO_3^{2-}.
Also Mn^{2+} is present in L_1, L_2 and L_3,
and Mg^{2+} is present in L_3, L_4 and L_5.

Key:- N = No. of measurements for statistical analysis.
L_1 = Gram (Cicer arictinum).
L_2 = Masure (Lens esculenta).
L_3 = Mash (Phaseolus radiatus).
L_4 = Mung (Phaseolus mungo).
L_5 = Arhar (Cajanus indicus).
*(Values in parenthesis are literature values).

Table II

Quantitative estimation of percentage protein content of the lentils using different methods.

S No.	Lentils	Percentage N_2 (Kjeldhal's Method).	Percentage Protein x 6.25	Percentage protein Separation method.
1	L_1	3.88 ± 0.04	24.25 ±0.28	23.90 ± 0.66
2	L_2	3.21 ± 0.00	19.94 ±0.90	20.15 ± 0.00
3	L_3	3.77 ± 0.00	23.26 ±1.11	24.00 ± 1.15
4	L_4	3.46 ± 0.21	22.29 ±0.16	23.66 ± 1.15
5	L_5	2.85 ± 0.00	18.13 ±0.60	19.00 ± 1.25

The R_f values were noted for hydrolyzate bands and were compared with R_f values obtained for a series of mixtures of the known amino acids run under identical conditions. Eighteen to twenty amino acids were identified in the lentil samples.

The quantitative estimation of the amino acids present was also accomplished by colorimetric methods as decribed for histidine by the Knoop-Kapeller-Alder reaction[4], lysine by Kibrick[5], tyrosine and tryptophan, phenylalanine by Block and Bolling6, cystine by the Winterstein Folin method, methionine by the Sullivan-McCarthy Method, and proline by modified Chinard method[7].

The experimental values were obtained for at least six measurements of each amino acid under investigation. Calibration curves where necessary were drawn and results analysed statistically, employing the straight line relationship y = mx + c. The amount of amino acids was thus calculated in mg per 100g of each lentil sample.

Materials used

All the chemicals were of A.R. grade. The solutions were freshly prepared whenever needed. Phenol was purified by charcoalising and repeated distillation to remove coloured material. Isopropanol was also distilled to constant boiling point.

Results and Discussion

The percentages of amino acids present in lentils have been reported here from the protein residues obtained after removal of carbohydrates, lipids and fats. The same have also been verified from the calculations based on the experimentally estimated amount of nitrogen in each sample multiplied by 6.25. The mode

Table III

Slopes and intercepts of linear graphs of Galvano meter deflection versus concentration for standard solutions of inorganic ions and unknown lentil samples by colorimetric methods.

S.No.	Ion	N	Statistical analysis Y= mx + c			Sample concentration of ions (ppm)				
			m (slope)	c (Intercept)	"r" correlation coefficient	L_1	L_2	L_3		L_5
1.	Sodium	5	2.26	0.87	0.999	4.48	5.13	3.60	4.04	4.48
2.	Potassium	5	3.04	0.35	0.999	7.35	5.15	5.14	6.14	4.99
3.	Phosphorous	5	1.76	0.23	0.995	439.54	83.12	152.70	201.06	78.36

Table IV

Comparative R_f values of amino acids identified in circular paper chromatography of lentil hydrolyzates using solvent system I (n-butanol: acetic acid: water:: 4: 1: 5).

Experimental amino acids in R_f value for known solvent system I:		R_f values of amino acids identified in hydrolyzates				
Amino acid	R_f value	L1	L_2	L_3	L_4	L_5
1. Cys	0.20±0.02	0.22	0.22	0.22	0.20	0.20
2. –	–	0.25	0.26	0.27	0.27	0.26
3. His	0.31±0.03	–	0.32	0.31	0.32	0.31
4. Arg	0.31±0.10	0.33	–	–	–	–
5. Lys	0.35±0.03	–	–	–	–	–
6. Ser	0.37±0.02	0.37	0.37	0.38	0.37	0.37
7. Gly	0.42±0.01	0.41	0.41	0.41	0.42	0.42
8. Asp	0.43±0.01	0.44	–	0.44	0.44	–
9. Thr	0.43±0.02	–	0.44	–	–	0.44
10. Glu	0.46±0.01	0.47	–	0.47	0.47	–
11. Pro	0.46±0.02	–	0.47	–	–	0.47
12. Ala	0.46±0.01	–	–	–	–	–
13. –	–	–	0.52	0.51	0.51	0.51
14. Tyr	0.58±0.01	0.57	0.56	0.57	0.56	0.56
15. Met	0.62±0.02	0.61	0.61	0,61	0.63	0.61
16. Try	0.65±0.02	0.67	0.66	0.67	0.67	0.65
17. Val	0.71±0.02	0.72	0.70	0.71	0.71	0.70
18. Phe	0.77±0.01	0.77	0.74	0.76	0.76	–
19. Leu	0.78±0.02	0.82	–	0.81	0.81	0.8

Table V

Comparative R_f values of amino acids, identification on thin layer chromatography of using solevent system I. (n-butanol-acetic acid: Water: 4: 1: 5).

Experimental R_f values for: known amino acids.			Comparative R_f value of amino acids identified in Lentil hydrolyzates.				
	Amino acid	R_f value	L_1	L_2	L_3	L_4	L_5
1.	Cys	0.07	0.08	–	0.08	0.10	0.10
2.	His	0.15	0.13	0.12	–	0.15	0.13
3.	Arg	0.22	0.20	0.21	0.19	0.21	0.20
4.	Asp	0.22	–	0.23	–	–	–
5.	Lys	0.25	0.25	–	0.26	0.24	–
6.	Thr	0.28	–	0.28	–	–	0.28
7.	Ser	0.29	–	–	–	0.30	–
8.	Gly	0.32	0.32	–	0.31	0.32	–
9.	Ala	0.33	–	0.33	0.33	–	–
10.	Met	0.36	0.36	0.35	0.36	–	0.37
11.	Glu	0.44	0.43	0.43	0.44	0.44	0.43
12.	–	–	0.46	0.47	0.47	0.47	0.46
13.	Val	0.50	0.50	0.50	0.50	–	–
14.	Le	0.51	–	–	–	–	0.51
15.	Tyr	0.52	–	0.52	–	0.52	–
16.	Trp	0.56	0.56	0.56	0.56	0.57	0.56
17.	Phe	0.58	–	0.60	–	0.58	–
18.	–	–	0.66	0.70	–	–	–

Table VI

Comparative R_f values of amino acids identified in circular paper chromatography of Lentil hydrolyzates using solvent system II (Isopropanol: Phenol: Water:: 5: 1: 1).

S.No.	Experimental R_f values for: Known amino acids.		Comparative R_f value of amino acid identified in hydrolyzate				
	Amino Acid	R_f value	L_1	L_2	L_3	L_4	L_5
1.	Cys	0.10±0.00	0.12	0.14	0.15	–	–
2.	Asp	0.20±0.02	0.19	0.17	0.20	–	0.18
3.	Glu	0.22±0.01	0.21	0.21	–	0.21	–
4.	Lys	0.22±0.01	–	–	–	–	–
5.	–	–	0.24	0.25	0.24	0.24	–
6.	Ser	0.31±0.01	0.31	0.32	0.31	0.29	0.28
7.	Gly	0.34±0.02	–	–	–	–	–
8.	His	0.37±0.01	–	–	–	–	–
9.	Arg	0.38±0.00	–	0.40	–	0.41	0.38
10.	Thr	0.42±0.01	0.42	0.43	0.42	–	0.44
11.	Ala	0.45±0.00	0.46	0.46	0.45	0.45	–
12.	Tyr	0.49±0.02	0.50	0.52	–	0.48	0.50
13.	–	–	–	0.57	0.56	0.57	0.55
14.	Val	0.60±0.02	0.61	0.63	0.62	0.60	0.61
15.	Tryp	0.64±0.00	–	–	0.66	–	–
16.	Meth.	0.67±0.00	0.70	0.68	–	0.68	0.70
17.	Phe	0.77±0.02	0.75	0.76	–	–	0.75
18.	Pro	0.80±0.02	0.81	–	–	–	–
19.	Leu	0.83±0.02	–	0.85	–	–	0.83

Table VII

Comparative R_f values of amino acids identified in thin layer chromatography of Lentil hydrolyzates using solvent system II (Isopropanol: Phenol: Water 25: 40: 35).

Experimental R_f values for: known amino acids.			Comparative R_f values of amino acids identified in lentil hydrolyzates				
Amino acid		R_f values	L_1	L_2	L_3	L_4	L_5
1.	Cys	0.08	0.08	–	0.09	0.08	0.08
2.	Lys	0.10	0.10	0.10	0.10	–	0.10
3.	Asp	0.13	0.13	0.13	0.14	0.13	–
4.	His	0.15	–	–	–	–	–
5.	Glu	0.19	0.18	0.18	–	0.17	0.17
6.	Arg	0.20	0.22	0.20	0.21	–	–
7.	Ser	0.24	–	0.24	–	0.23	0.23
8.	Gly	0.25	–	0.24	–	–	–
9.	Thr	0.25	0.26	–	0.25	0.26	–
10.	–	–	0.33	0.30	0.30	–	0.30
11.	Ala	0.34	–	–	0.35	0.35	0.34
12.	Try	0.43	0.45	0.43	0.43	0.41	0.45
13.	Trp	0.50	0.51	0.46	0.46	0.50	0.47
14.	Meth	0.52	0.53	0.54	0.54	0.53	0.53
15.	Leu	0.62	0.62	–	0.64	0.60	–
16.	Pro	0.68	0.69	–	–	–	0.69
17.	Val	0.68	–	–	–	–	–
18.	Phe	0.77	–	–	–	–	0.74

Table VIII

Slope and intercepts of linear graphs of Galvonometer deflection (Q) versus concentration of standard solution of the unknown Amino acids by colorimetric method.

S.No.	Amino acid	N*	Y = m x + c m	c	"r"	Concentration of amino acid in lentils mg/100 g. L_1	L_2	L_3	L_4	L_5
1.	Tryp	6	763.00	0.05	0.999	162.20±	138.24± 0.00	177.80± 0.00	130.45± 0.00	109.80± 0.00
2.	Cys	6	255.38	0.69	0.996	239.20± 3.97	241.36± 1.52	254.04± 17.45	150.98± 17.38	111.89± 2.39
3.	His	6	643.78	0.01	0.999	290.61± 1.84	273.06± 1.50	293.96± 3.04	324.58± 3.04	250.9 ± 2.63
4.	Lys	6	314.87	0.16	0.999	735.49±19.16	661.94±44.73	510.84± 27.2	677.43± 8.21	517.41± 0.00
5.	Meth	6	482.70	0.05	0.999	276.74±20.97	272.65±34.00	343.81± 17.80	329.71± 31.77	240.85± 17.03
6.	Phe	6	219.75	0.02	0.999	739.98±10.80	594.98±28.37	740.48±37.30	564.68±16.50	561.35± 6.24
7.	Tyr	6	211.00	0.09	0.999	644.48± 0.00	507.10±	615.03± 0.00	483.94± 0.00	520.70± 0.00
8.	Pro	6	174.48	0.22	0.999	886.94±10.29	902.02± 9.20	1097.99±10.50	999.91± 9.69	685.16± 5.61

N = Number of measurement

Table IX

Net dietary protein calories data for lentils.

S.No.	Lentil	"S" Sulphur content (mg)	"N" Nitrogen content (mg)	Protein score = 100S/N	Pm = 400 Protein Score	NDP-Calories	REMARKS.
1.	L_1	1.80	34.22	52.30	7.60	815.67	*Sulphur containing amino acids not detected.
2.	L_2	1.71	124.30	13.68	29.25	376.79	
3.	L_3	9.28	483.36	19.20	20.84	416.0	
4.	L_4	–	121.70	–	–	–	
5.	L_5	7.13	184.07	38.73	10.33	589.72	

of degradation of proteineous material into free amino acids has been considered important to evaluate the percentages of various amino acids. In fact the extent of hydrolysis[8] is dependent on the pH of the solution and duration allowed for refluxing. However, experimentally these factors are controlled so as to break various peptide linkages.

The resolution of amino acids by paper and thin layer chromatographic techniques has been reported by many workers. However in the present work the technique described by Irwin and Safer[9] has been employed and has been found satisfactory. Twenty amino acids have been identified in protein hydrolyzates using various mixed solvents and closely controlling the experimental factors such as ratio of solvents in eluent, pH and temperature. The separation of amino acids on T.L.C plates using silica support was found to have low resolution and required more time and this was comparatively less satisfactory than that obtained in paper chromatography under similar condition.

The quantitative results obtained by colorimetric methods for amino acids (Table VIII) indicate that the amount of various amino acids required for daily intake by human being is available from these seeds if used together in correct proportions.

Acknowledgement.

The award of fellowship by University of Sind and University Grants Commission to one of the authors (T. Iqbal) is gratefully acknowledged.

References

1. P. Alexander and R.J. Block A laboratory manual of Analytical methods of Protein Chemistry, Pergamon Press, 2, 5, (1960).
2. F. Busson and P. Guth, New Techniques of Chromatography and Paper Electrophoresis, Academic Press, New York, 1958, p 833.
3. S.M. Partidge, *Biochem. J*, **42**, 238, (1947).
4. A. Hunter, *J. Biol. Chem.*, **216**, 391 (1952).
5. A.D. Kbrick, *Arch. Biochem.*, **6**, 425, (1949).
6. R.J. Block and D. Bolling, The Amino Acid Composition of Proteins and Foods. Thomas Publishers Springfield, Illinois p 576 (1951).
7. F.P. Chinard, *J. Biol. Chem.*, **199**, 91 (1952).
8. J.M. Mclaughlan, C.G. Rogers, D.G. Chapman and J.A. Campbell., *Canad. J. Biochem. Physiol.*, **37**, 1293 (1959).
9. I. Oreskes and A. Saifer, *Anal. Chem.*, **27**, 854, (1955).
10. M.I.D. Chughtai and A. W. Khan, A monograph of Nutritive value of food stuffs and planning of satisfactory Diets in Pakistan. Part I (Composition of Raw-Food stuff) Punjab University 1960, p 5.
11. A.H. Harold, Review of Physiological Chemistry, Lange Medical Publications, Los Altos, California U.S.A. 1971, 474.