

RELATION BETWEEN SOME AMINO ACIDS CONTENT IN PLANT SEED PROTEINS AND ISO-ELECTRIC POINT OF PROTEIN.

By

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Amino acids content and iso-electric point (IEP) of six plant proteins ,namely fenugreek , lupine, horsebean, sorghum, barely and rice were determined. Data obtained from chemical analysis as well as other data obtained from literature were used to establish mathematical equation to deduce the IEP of protein with information about glutamic + asparatic (A) and lysine + arginine (B) amino acids content as follows:

$$\text{IEP} = 3.73 + 1.79 \text{ Log. } A/B$$

Where ; IEP is the iso-electric point of true protein.

A is glutamic + asparatic amino acids .

B is lysine + arginine amino acids .

3.73 and 1.79 are constants.

Differences between values calculated from above suggested equation and those obtained from chemical analysis are not significant . So, with information about amino acids content , it could be calculate the IEP of protein by use of such equation as a substituent of titration curves , consequently we can get information about the adequate IEP for precipitating protein .

Informations obtained from above mentioned equation are valid up to 2.88 of A/B ratios .

INTRODUCTION

Apparently the first suggestion that amphoteric compounds (compounds that contain both acidic and basic groups) exist in a special was made by Adam in 1916. He pointed out that acetic acid exists almost exclusively as inner salt with probably less than one part of million in the true amino acid. Bjerrum (1932) , in more complete discussion of the constitution of ampholytes confirmed and extended Adams theory out that all the amino acids exist in salt like (dipolar ions) and therefore they

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are not true amino acids but rather ionized internal ammonium salts. Bjerrum denominated this particular type of substance " zwitterions" .

Felix (1963) reported that since amino acids migrate in alkaline solutions to the anode and in acid solution to the cathode , there is a pH value at which no migration occurs . this pH value has been called the iso-electric point (IEP) and it can be calculated from the ionization constants according to the migration equation :

$$\text{pH} = \frac{\text{PK1} + \text{PK2}}{2}$$

The same author added also that the iso-electric point is a state of hydrogen ion concentration where the ampholyte exists at the maximum degree at a zwitterions and the positive charges on the zwitterion exactly balance the negative charges of the molecule.

Recently , iso-electric point is determined by titration curve of isolated protein after precipitation at various pH . Cluskey et al , (1973);reported that the minimum solubility of Oat protein concentrate was about pH 5. Rhee et al . (1973) studied the effect of processing pH on the properties of protein isolates during precipitation. Maximum recovery was peanut isolates during precipitation . Maximum recovery was obtained at precipitation pH of 4.5 for product, while subsequent at showed that optimum precipitation pH would be 4,0 +- 0.25 . El-Hawwary (1982) found that maximum precipitation of protein isolate occurred at pH 4.0 for sorghum and lupine, pH 3.5-4.0 for barely and at pH 4.5 for fenugreek .

This study is an attempt to use the results of the chemical analysis of the more acidic amino acids glutamic and asparatic , the more basic amino acids lysine and arginine and pH of IEP of precipitated proteins with other data obtained from literature to establish mathematical equation to facilitate calculation of IEP of protein with information about above mentioned amino acids .

MATEIALS AND METHODS

Six seed varieties , namely , fenugreek (Giza 2) , lupine (Giza 2) , sorghum (Giza 114) , horsbean (Giza 1), barely (Giza 119), and rice (Giza 172) constituted the materials used in this work to determine amino

acids content. Analysis of amino acids was carried out according to Block et al . (1958).

Protein of seed varieties was extracted by using alkaline solution. 10 gm of ground seeds (particle size of 1.0 and 0.4 mm .) were used to sodium hydroxide for 30 min. , extraction time . The supernatant was considered to have 100% soluble nitrogen . Aliquots of 20 ml for every sample were adjusted to pH values ranging from 1.0 to 13 and left for 3 hours under refrigeration for equilibration . Volumes of suspensions were brought to 50 ml. with distilled water and centrifuged at 3000 r. p. m. for 15 min. Supernatants were analyzed for their nitrogen content and results were expressed as percent of total extracted nitrogen in the initially prepared alkaline extract .

The iso- electric point was determined by titration curves of protein isolated according to the methods of Smith et al . (1959).

All the statistical analysis were carried out according to Snedecor & Cochran (1967).

RESULTS AND DISCUSSION

Essential amino acids amino and aspartic , glutamic , lysine and arginine non essential amino acids of fenugreek , lupine , sorghum , rice, horsebean and barely were determined . values of amino acids are presented in table 1 and 2 . From table (I) it can be seen that essential amino acids were found in different ratios in the six types of seeds . Lupine and fenugreek contained the highest values of total essential amino acids (36.93%) compared with other seeds.

On other hand , it can be seen also from data presented in table (I) that fenugreek seeds contained the highest value of aspartic and glutamic non essential amino acids compared with other seeds .

The titration curves of protein extracted from different seeds (Table 2) showed that the iso-electric points were around pH 4.0 for lupine , sorghum , horsebean and rice , around 4.5 and pH 3.5-4.0 for fenugreek and barely respectively .

Four amino acids had been chosen to establish the relation between the amino acids and the iso-electric point of protein, these amino acids were ; the acidic amino acids glutamic (pKa = 4.00) and aspartic

(pKa – 3.50) and the more basic amino acids lysine (pKa =12) and arginine (pKa = 10.46).

Total of above-mentioned four amino acids in different seeds is presented in table (2) . From table (2) it can be seen that fenugreek protein contained the highest total values of asparatic + glutamic and lysine + arginine amino acids (48.53%) while sorghum protein contained the lowest value (32.76%) compared with other seed proteins.

From table (2) , it can be seen also that ratios between the more acidic amino acids (A) and more basic amino acids (B) showed the highest value in barely (2.47) while lupine and horsebean showed the lowest values (1.57 and 1.58 respectively).

Table (1) : Essential amino acids , asparatic and glutamic non essential amino acids of fenugreek, lupine ,sorghum, rice horsebean and barley proteins (gm/100 gm protein).

Amino acids	Fenugreek	Lupine	Sorghum	Rice	Horsebean	Barely
Lysine	6.66	4.38	1.75	5.08	5.40	2.81
Histidine	1.87	1.71	2.20	2.28	3.50	2.47
Arginine	10.60	11.58	8.70	8.69	9.70	7.80
Thereonine	3.58	3.08	2.20	3.62	2.40	3.51
Methionine	0.48	0.48	2.12	1.66	0.90	2.70
Valine	5.00	5.00	5.00	6.09	5.40	4.30
Phenylalanine	4.80	4.18	3.35	6.00	4.40	3.30
Leucine+ isole.	19.30	21.97	12.75	10.56	14.31	10.42
Tryptophan	1.21	1.35	1.05	1.05	1.10	0.90
Total of essential amino acids	53.50	53.73	36.92	48.13	47.11	37.71
Asparatic	11.17	9.59	6.70	8.15	9.30	6.62
Gutamic	20.10	15.55	15.71	14.16	14.60	19.65
Total of Asparatic + glutamic	31.27	25.14	22.41	22.31	23.90	26.27

Semi logarithmic relation between log . A/b ratios on the abscissa axis and ios-electric points on the ordinate axis was proved . Hence , log. A/B ratios and IEP of proteins obtained from chemical analysis as well as other data obtained from literature were used to establish mathematical equation to calculate the IEP of protein with information about asparatic + glutamic (A) and lysine +arginine (B) amino acids .

Table (2): Asparatic + glutamic, Lysine + arginie amino acids and iso-electric points of fenugreek , lupine , sorghum, rice horsbean and barely proteins.

Samples	Amino Acids (gm/100 protein)		Total	A/B ratio	IEP
	Asparatic + Glutamic (A)	Lysine + Arginine (B)			
Fenugreek	31.27	17.26	48.53	1.8120	4.5
Lupine	25.1	15.96	41.10	1.570	4.0
Sorghum	22.31	10.45	32.76	2.130	4.0
Rice	22.31	13.77	36.08	1.630	4.0
Horsbean	23.90	15.10	39.00	1.580	4.0
Barely	26.27	10.60	36.87	2.470	3.5-4.0

Table (3): Comarison between experimental and calculated IEP of proteins at different A/B ratios .

A/B ratios	IEP Experimental pH	IEP calculated pH	Confidence limits	Source of protein
1.57	4.00	4.04		lupine
1.58	4.00	4.04		horsbean
1.63	4.00	4.10		rice
1.81	around 4.50	4.20		fenugreek
2.13	4.00	4.30		sorghum
2.47	3.5-4.0	4.43		barely
1.66	4.28	4.12		horsbean *
1.70	2.50	4.14	+ -0.315	peanut *
1.83	4.25	4.20		horsbean *
2.06	4.50	4.49		peanut *
2.11	4.50	4.31		peanut *
2.17	4.50	4.34		peanut *
2.23	around 4.50	4.27		peanut *
2.40	Around 5.00	4.51		Oat *
2.47	4.00	4.43		barely *
2.48	Around 5.00	4.44		Oat *
2.58	Around 5.00	4.45		Oat *
2.60	Around 5.00	4.48		Oat *
2.88	Around 5.00	4.55		Oat *

* Data obtained from literature .

Relation between $\log . A/B$ ratios and IEP of proteins was linear , therefore , straight line equation was used to establish the suggested equation as follows :

$$\text{IEP} = 3.73 + 1.79 \log . A/B$$

Where ; IEP is iso-electric point of true protein ,
 A is asparatic + glutamic amino acids ,
 B is lysine + arginine amino acids ,
 3.73 and 1.79 are constants obtained by straight line equation .

Values of IEP of proteins calculated from above suggested equation at various A/B ratios and IEP of proteins obtained from chemical analysis are presented in table (3) . As it can be seen from data presented in table(3) , differences between calculated IEP of proteins and IEP of proteins and IEP obtained from chemical analysis are not signisificant , and confidence limits were ± 0.315 .

The minimum and maximum of A/B ratios and IEP of protein are summarized in table(4) . From data presented in this table it can be seen that as the range of acidic to basic amino acids is low , the iso-electric range is also low .Otherwise , increasing of IEP of protein is due to increases of the range of acidic to basic amino acids.

Table (4): Effect of A/B ratio of true protein.

A/B ratio		IEP Of protein		A/B max. - A/B min. IEP max. – IEP min. ratio	
Min.	Max.	Min.	Max.		
1.57	2.88	4/04	4.55	$\frac{2.88}{4.55} - \frac{1.57}{4.04}$	= 2.57

A/B IEP ratio was also calculated for true protein (table 4) . From obtained results it can be seen that every one pH effected on 2.57% of A/B ratio , where IEP was expressed as pH value .

Summerising the results obtained in this study it can be supposed that the iso- electric point of protein could be calculate mathematically by use of the proposed equation if the asparatic, glutamic , lysine and arginine amino acids content is available . So ,by use of such equation we can get information about the adequate IEP for precipitating protein

as a substituent of titration curves. Differences between calculated values of IEP of protein and values of IEP obtained by chemical analysis are not significant. Calculated values of are valid up to 2.88 of acidic / basic amino acids ratios.

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