its bearing on protein evaluation. In Meeting Protein needs of infants and children, pub.843. National Academy of Sciences-National Research Council,Washington D.C.,p.424(1961).

11- McCance,R.A. and Widdowson,E.M.V. The Composition of Foods. Med, Res. Council.Spec.Ser. No. 297 , London HMSO (1978).

12- Mitchell,H.H. Biological value of proteins and amino acid interaction. Quarter master Food and Container Inst. Surveys, National Research Council, Washington, D.C. (1954).

13- Mitchell,H.H. and Block,R.J. Some relationships between the amino acid contents of proteins and their nutritive values for rat. J. Biol.Chem.163:599(1946).

14- Mohammadiha,H. and Mostafavi,P. Estimation of methionine and lysine in food protein by thin-layer chromatography.(1984).

15- Moore,S, Spackman,D.H. and Stein,W. H. Chromatography of amino acids on sulfonated polystyrene resin. An improved system.Anal. Chem. 30: 1185,(1959).

16- Oser, B.L. Method for integrating essential amino acid content in the nutritional evaluation of protein J. Am. Dietet. A. ,27: 396,(1951).

17- Pearson,W.N. and Darby,W.J. Protein nutrition. In. Annual Review of Biochemistry. Annual Reviews Inc . Palo. Alto. California, p. 325,(1961).

18- Sheffner,A.L., Eckfeldt,G.A.and Spector,H.The pepsin-digest-residue(PDR) amino acid index of net protein utilization. J. Nutrition, 60: 105,(1956).

19- Sommer,E.E. The biological value of milk and egg protein in young and mature rats. J. Nutrition, 16: 129, (1938).

20- World Health Organisation,WHO Technical Report.Series No. 522. (1973).

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REFERENCES

1 - Akeson,W.R. and Stahmann,M.A. A pepsin pancreatin digest index of protein quality evaluation. J. Nutrition, 83: 257-261 (1964).

2 - Bender,A.E. Determination of nutritive value of protein by chemical analysis. In Meeting protein needs of infants and children, Pub.843. National Academy of Sciences-National Research Council,Washington,D.C. p. 407 (1961)

3 - Bender,A.E.,Mohammadiha,H. and Almas,K Digestibility of legumes and avilable lysine content.Qualitas Plantarum, Plant Foods for Human Nutrition 29:1-2,219-228 (1979).

4 - Block,R.J. and Mitchell,H.H. The correlation of amino acid composition of proteins with their nutritive value. Nutrition Abstr. Rev. 16: 249 (1946).

5 - Campbell,J.A. A critical appraisal of methods for evaluation of proteins in food. R. 1 Add. 37 WHO,FAO, Unicef-PAG, United Nations, New York(1961).

6 - Food and Agriculture Organisation (FAO) of the United Nations/World Health Organisation. Report Series No. 512,(1971).

7 - Ford,J.E. and Salter,D.N. Analysis of enzymically digested food proteins by Sephadex-gel filtration.Br. J. Nutr. 20: p. 843 (1966).

8 - Frost,D.V. Measuring the nutritive value of proteins. In Protein and Amino Acid Nutrition. ed., A.A. Albanese. Academic Press,New York p. 255(1959).

9 - Grau,C.R. and Carroll,R.W. Evaluation of protein quality. In Processed Plant Protein Foodstuffs,eds.,A.M. Alschul. Academic Press, New York,p.153 (1959).

10- Mauron,J. The concept of amino acid availability and

Table 5

The Elastase amino acid index and biological value and nitrogen digestibility of some protein foods

Food protein	Nitrogen digestibility	Biological value %	Elastase digest index %	
			Comparison with acid hydrolysis	N content of supernatant and residue
White Kidney beans	55 (1)	50 (1)	55	49
Arkasoy	58 (1)	57 (2)	57	50
Over-heated Arkasoy	60 (1)	63 (2)	58	53
Skimmed milk powder	85 (3)	84 (3)	75	88

(1) Bender and Mohammadiha. 1979.

(2) Block and Mitchell. 1946.

(3) Sommer. 1938.

TABLE 4

Distribution of amino acids in some logume's protein ?

Amino acids	Pigeon pea & (1)	(2)	Chick pea (3)	Soyabeans (4)	White Kidney beans (5)	Black beans (6)	Cow pea (7)
Lys.,His. & Arg.	15.7	13.8	16.7	16.3	16.0	17.3	14.7
Thr. & Asp.	15.3	12.9	17.2	17.2	11.2	20.1	19.8
Ser. & Glu.	27.4	20.6	25.3	12.1	26.5	24.3	30.2
Val. & Ala.	10.1	12.1	13.0	10.3	8.6	6.4	9.8
Ile., Met. & Cys.	6.5	5.4	7.5	5.4	6.9	7.3	7.2
Tyr. & Leu.	9.5	8.9	13.1	13.5	15.1	13.8	10.5
Phe. & Try.	7.2	8.5	5.9	5.8	8.2	8.0	11.9

(1),(4) and (6) McCance and Widdowson (1978).

(2) and (5) FAO (1971).

(3) and (7) WHO (1973).

TABLE 3
(%)

Distribution of amino acids in enzyme hydrolyzed supernatant and its residue after acid hydrolysing.

Sample / Amino acids	Cooked white Kidney beans		Processed soy bean flour		Over-heated soy-bean flour		Skimmed milk powder	
	Sup.	Res.	Sup.	Res.	Sup.	Res.	Sup.	Res.
Lys.	65	35	56	44	58	42	86	14
Thr.+Asp.	54	46	60	40	57	43	75	25
Ser.+Glu.	52	48	63	37	60	40	86	14
Val.+Ala.	54	46	55	45	55	45	80	20
Met.+Cys.	62	38	47	53	50	50	60	40
Tyr.+Leu.	56	44	52	48	38	62	68	32
Phe.+Try.	70	30	62	38	75	25	79	21

Sup. = Supernatant *Res. = Residue*

TABLE 2

Amino acids explain as par cent of total recovered

Sample	Treatment	Lys. His. % Arg.	Thr. Asp. %	Ser. Glu. %	Val. Ala. %	Ilou. Met. % Cys.	Tyr. Leu. %	Phe. Try. %
Arkasoy n=8	Acid Hyd.	12.0(.6)	13.4(1.1)	11.9(.3)	15.1(2.1)	15.5(.8)	12.9(2.8)	15.2(1.1)
	Enz. Hyd.	13.7(.5)	13.8(1.5)	11.4(.9)	12.3(1.9)	15.3(.7)	17.1(1.1)	18.8(2.3)
	Residue	12.1(.9)	10.7(.8)	9.6(.5)	14.7(2.5)	16.4(2.6)	9.9(2.3)	14.1(1.5)
over-heated Arkasoy n=7	Acid Hyd.	14.2(1.4)	15.6(1.7)	13.6(1.5)	11.4(1.8)	16.4(1.1)	10.9(0.9)	18.6(2.8)
	Enz. Hyd.	15.4(1.3)	17.3(0.9)	13.1(0.7)	12.2(0.8)	10.3(0.7)	15.8(1.2)	19.8(2.3)
	Residue	13.5(0.8)	12.5(1.1)	10.4(0.5)	13.2(3.5)	13.5(3.1)	12.5(1.9)	11.7(2.2)
Skimm-ed milk powder n=8	Acid Hyd.	12.8(0.6)	14.8(0.5)	11.4(0.6)	9.1(0.4)	18.3(1.9)	12.7(0.9)	15.0(4.6)
	Enz. Hyd.	16.0(2.1)	14.3(3.1)	7.9(1.2)	10.2(3.5)	16.2(2.7)	17.8(1.2)	21.5(1.7)
	Residue	13.2(0.8)	13.4(2.6)	0.9(0.6)	8.1(4.6)	20.6(8.1)	18.2(4.3)	2.8(1.4)
White kidney beans n=7	Acid Hyd.	13.6(1.3)	13.4(2.6)	16.4(2.4)	18.6(2.1)	12.9(0.8)	12.9(1.9)	15.9(2.1)
	Enz. Hyd.	15.7(1.6)	9.9(1.1)	16.5(1.8)	10.1(0.9)	21.4(1.7)	10.0(0.8)	18.7(3.0)
	Residue	12.0(0.9)	14.4(3.3)	12.4(1.1)	14.5(1.7)	15.3(4.3)	10.5(2.2)	11.1(2.7)

( )= S.D. Hyd. = Hydrolyezed Enz. = enzyme

In another form of calculation the area of similar amino acids were compared with each other and the percentage of them in term of the whole areas of supernatant and residue of enzyme hydrolyzed for each sample were calculated. Table 3 shows the results.

As three of the examined samples are leguminous seeds and in this kind of protein food the pattern of amino acids content is more or less similar , the results from the literature on Table 4 is a good comparison.

The Elastase amino acid index calculated are shown on Table 5. The biological values and nitrogen digestibilities as reported in the literature for examined proteins are shown in columns 1 and 2. For Elastase digest index no correlation factor was used.

TABLE 1

Position and Rf of amino acids on TLC chromatogram

No. of spots	Amino acids	Rf.
1st.	Lysine	0.027
2nd.	Histidine	0.053
3rd.	Arginine	0.070
4th.	Threonine & Asp.	0.420 & 0.451
5th.	Serine and Glu.	0.458 & 0.515
6th.	Glycine	0.548
7th.	Valine & Alanine	0.586 & 0.600
8th.	Isoleucine, Meht. & Cys.	0.655, 0.662 & 0.675
9th.	Tyrosine and Leucine	0.724 & 0.737
10th.	Phenyl-alanine and Try.	0.800 & 0.820

The per cent of amino acids in supernatant and residue of enzyme digest(the residue was hydrolyzed by 6 N HCl)is shown in following Table:

Sample	Supernatant%	Residue%
White kidney beans	55.2	44.8
Arkasoy	58.6	41.4
Over-heated Arkasoy	57.7	42.3
Skimmed milk powder	75.0	25.0

The differences of results of 10 determinations were not more than 10%.

Determination of nitrogen content of supernatant and residue after hydrolyzing showed following results:

Sample	N% of Supernatant	N% of Residue
White kidney beans	49	51
Arkasoy	50	50
Over-heated Arkasoy	53	47
Skimmed milk powder	88	12

The results of nitrogen more or less approved the previous results.

As it is shown in Figure 1 more than 13 spots were separeted on TLC technique of those four samples,but only 10 spots of them were clearly separated and were not overlapped with other spots.If the spots to be numerated from the bottom according to the chromatogram of different amino acids, the fist spot would be lysine , the second histidine and etc.

Table 1 shows the position of separation of different amino acids and their Rfs.

For each amino acid or some amino acids together area in term of per cent was calculated and the results on Table 2 are the percentage of amino acid or amino acids which was released of each sample by the action of HCl or Elastase.

Although the density of colour of each amino acid is slightly different with the other one, and the leucine is always used as a standard(Moore, Spakman and Stein 1959). Since the above calculation was on the base of comparison of similar amino acid, the results would be acceptable.

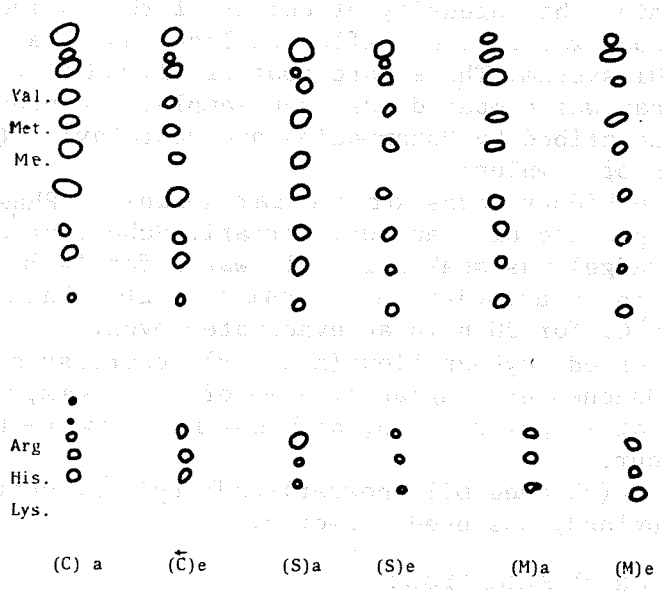
The area of whole spots of each sample was read by digital chromscan and the results were proportionately equal to digestibility of each sample by enzyme or acid.

The comparison of enzyme digestibility of supernatant and residue for all form samples were as follows:

Per cent of amino acids release with reference to 6 N HCl hydrolysate as 100%

Figure 1

Chromatogram of acid (a) and enzyme (e)hydrolysis of casein(c) Soybeab(S) and skimmed milk powder(N) on silica gel G.



Sample	Enzyme hydrolysis%	%of undigested residue hydrolyzed by 6N HCl
White kidney beans	72.1	57.3
Cooked Arkasoy	49.8	54.0
Over-heated Arkasoy	61.4	45.5
Skimmed milk powder	65.6	21.8

Using a 10 µl Hamilton syringe, with a cut point niddle No. 50, 2-6 µl of the sample solution was applied on TLC plate.

The plates were first developed in ethanol - water solvent system 70:30 (V/V) 3 h. At the end of this period the plates are removed from the chamber and dried, then redeveloped in phenol-water solvent system 78:22 (V/V) using the same ascending technique for 4 h . The plates were redeveloped for the third time in methyl ethyl ketone/pyridine/distilled water/ acetic acid solvent system , 90:30:30:3 (V/V), using the same ascending technique for 2 h.

After drying, the plates were sprayed with ninhydrin reagent and the colour was developed by incubating at 80° C. for 15 min. The intensity of colour of the amino acid ninhydrin spot was read by reflected light using a Chromscan 200/201 system. The square root of digital reading by Chromoscan was compared for each sample. The procedure was fully described by Mohammadiha and Mostafavi (14).

Preparation of Samples:

1- White kidney beans or Marmar beans (Phaseolus vulgaris), produce of Iran (Dr. Sarrafi, School of Agriculture, Karadge) was soaked in cold water for 14 h , and was cooked in an autoclave at 15 psi for 20 min., then dried in 60° C. for 20 h in an evaccuated oven.

2- Processed soybean flour(Arkasoy) (courtesy of Arkady Mills Manchester, England). Some of this sample was autoclaved at 15 psi for 2 h, and used as over- heated soybean flour.

3- Marvel (skimmed milk powder)(Cadbury's production , Reading, England) was used directly.

Results and Discussion:

Figure 1 shows the comparative chromatogram of acid and enzyme hydrolysis of white kidney beans , Arkasoy and Marvel. As it is shown in this figure,number 1 stands for acid hydrolyzed, 2 for supernatant of enzyme hydrolyzed , and 3 for residue of enzyme hydrolyzed after being hydrolyzed by HCl from dried white kidney beans, nos. 4,5, and 6 stand the same pattern for cooked soybean flour (arkasoy), 7,8 and 9 are for over-heated Arrkasoy,and 10,11 and 12 stand for Marverl.

Recent reviews of laboratory methods for protein quality estimation has been given by various authors(1, 5,8, 9,10,17). For protein quality evaluation, " the chemical score" was used by Mitchell and Block (13) and Bender (2) and "the essential amino acid index" by Oser (16) and Mitchell (12). Sheffner et al,(18) developed "the pepsin-digest-residue amino acid index" which combined the pattern of essentail amino acids released by in vitro pepsin digestion with the amino acids pattern in the reminder of the protein. Akeson and Stahmann (1) used " a pepsin pancreatin-digest-index ".Ford and Salter (7)used pronase (an enzyme preparation from Streptomyces griseus),pepsin, pancreatin and erepsin, pepsin and papain in-vitro to digest freeze-dried cod fillet.

The procedure to be described in the present paper gives the relationship between the proportion of amino acids released by pancreatopeptidase (Elastase) and the acid hydrolysis of protein of white kidney beans (phaseolus vulgaris), cooked and over heated soybean powder (Arkasoy) and skimmed milk powder.

Materials and Methods

Enzyme digest was prepared by incubating 50 mg sample with 0.1 ml of pancreatopeptidase solution (10mg Elastase from Sigma No. 3,4,21,11) in 5 ml of buffer phosphate (0.05 M Na2HP04-HCl, pH 8.2) for 24 h. at 40 C. then 0.1 ml of toluene was added to cover the solution during incbation and shaken continiously.

At the end of the indicated incubation period 0.2 ml of N perchloric acid was added to digest and left for 15 min. The undigested residue was separated for acid hydrolysis by centrifugation.

Acid hydrolysis for the estimation of the total amino acids content in the protein samples and in the enzyme - digest-residue was prepared by autoclving of 50 mg of sample or the whole residue in a sealed evacuated tube with 6 N HCl at 110±2°C. for 24 h. After removing the HCl by vaccu-evaporation and dissolvig in 5 ml of 10%propanol and adjusting the pH to 7-7.2 by N/100 NaOH , the samples were ready for thin-layer chromatography(TLC).

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ENZYME DIGEST AND ACID HYDROLYZED INDEX OF PROTEIN QUALITY EVALUATION.

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Key Words: Protein Guality - Enzyme Hgdro Lyeation of protein-Acid Hydrolyzation of Protein

ABSTRACT

A pancreatopeptidase (Elastase) digest index was devised for a rapid and accurate estimation of protein quality. This index was calculated on the basis of all the amino acids released by an in-vitro Elastase digestion , acid hydrolysis of same sample and the residue of enzyme hydrolyzed. The amino acids were determined by Thin-Layer Chromatography. Samples used were cooked white kidney beans, cooked and over-heated soybean powder , and skimmed milk powder. Good correlation was observed between Elastase index value and their biological values reported in the literature from feeding trials. The pattern of amino acids released by acid and by enzyme hydrolysis were about the same.

INTRODUCTION

Although the nutritional quality of protein must , in final analysis, be stablished with feeding traials , in - vitro methods of protein evaluation are useful in screening new protein sources and processing methods because of their rapidity.

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