Food Chemistry **15** (1984) 285–306

Release of Peptides and Amino Acids from Dietary Proteins during Sequential Enzymatic Digestion *in vitro* with Pepsin, Pancreatin + Trypsin and Erepsin

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(Received: 4 June, 1984)

ABSTRACT

Six plant proteins and five animal proteins were subjected to sequential enzymatic digestion in vitro *with pepsin, pancreatin + trypsin and erepsin and the release of peptides and amino acids (aa) was determined after separating the enzymatic digests on columns of copper Sephadex G25. The dipeptide content of the peptide fractions was determined by a simple Biuret method and the amino-acid composition of the different fractions was determined in an automatic amino-acid analyser. There were no significant differences between plant and animal proteins with respect to the quantities of aa released as total peptides and as free aa. However, the release of small peptides (especially the dipeptides) was significantly higher with animal proteins than plant proteins. The quality of the different fractions as judged by their essential amino acids/non-essential amino acids ratios or their essential amino-acid composition indicated the smaller peptide fraction (P_2) of animal proteins to be of better quality than all the other peptide fractions obtained while the free aa fraction was the best. There was a positive correlation between the dipeptide content of the enzymatic digests of proteins and their quality.*

INTRODUCTION

The capacity of the mammalian small intestine to digest and absorb small peptides, especially the di- and tri-peptides is well known (Newey & Smyth, 1959; Silk, 1981). Several mechanisms have been advanced to

Food Chemistry 0308-8146/84/$03·00

explain the various observations made regarding peptide transport and hydrolysis in the intestine (Matthews, 1975; Das & Radhakrishnan, 1976; Radhakrishnan, 1977). The quantitative importance of peptide uptake as opposed to free amino-acid uptake by the small intestine still remains to be determined (Silk *et al.*, 1982).

Dipeptide uptake by the mammalian intestine has been well studied and it has been shown that dipeptide absorption sites precede the free amino-acid absorption sites (Rubino & Guandalini, 1977). The kinetic advantage of peptide absorption as compared to free amino-acid absorption is well known (Matthews *et al.*, 1969; Adibi, 1971). Further, the mammalian intestinal lumen has been shown to contain a complex mixture of peptides (of 2 to 6 amino-acid (aa) residues) and amino acids during protein digestion, in which peptides predominate (Chung *et al.*, 1979). However, it is not clear whether qualitative and quantitative differences exist between different proteins regarding the release of peptides during their digestion and, if so, their relevance to the quality of the proteins. With a view to understanding the relationship, if any, between protein quality on the one hand and the quantity of small peptides released during its digestion on the other, an *in vitro* sequential enzymatic digestion protocol, simulating conditions *in vivo*, was used in the present investigation to study the release of peptides and free amino acids during digestion of different proteins, viz. five animal proteins and six plant proteins.

MATERIALS AND METHODS

Casein, α-lactalbumin, egg albumen, bovine serum albumin (BSA), wheat gluten and corn zein were purchased from Sigma Chemical Company, USA. Soya protein isolate (SPI) and groundnut protein isolate (GNPI) were obtained from the Central Food Technological Research Institute (CFTRI), Mysore, India. Protein isolates of rice and redgram dhal were prepared by the isoelectric precipitation procedure (Arthur, 1953), washed with acetone and dried. Goat meat was homogenised, lyophilised and powdered. Most of the protein sources had more than 60% by weight of protein (N × 6·25 or a factor suitable for that source of protein) and some of them even had around 85%.

1-Fluoro, 2,4-dinitro-benzene (FDNB), pepsin (hog stomach mucosa), pancreatin (hog pancreas), trypsin (porcine) were obtained from Sigma

Chemical Company, USA. Erepsin (hog duodenum) was obtained from Nutrition Biochemical Corporation, USA. Other chemicals used were all of analytical grade and glass distilled water was used in all the experiments.

Total nitrogen was determined by the micro-Kjeldahl method (Oser, 1965) and the amino nitrogen (AN) was estimated by the FDNB method (Goodwin, 1968).

Protein samples, whose total nitrogen and total AN were determined, were suspended in 0·05N HCl; their pH was adjusted to 1·8 with NaOH and they were subjected to sequential enzymatic digestion *in vitro* in conical flasks, according to the following protocol (Ford & Salter, 1966).

Protein

↓ (1 g substance suspended in 100 ml of 0·05N HCl, pH adjusted to 1·8) pepsin 100 mg/g of N taken — 37 °C, 24 h

Peptic digest (pH adjusted to 8·2 with 1N NaOH)

↓ Pancreatin (100 mg/g of N taken) + Trypsin (50 mg/g of N taken) — 37 °C, 24 h

Peptic, pancreatic + tryptic digest (pH checked and readjusted to 8·2)

↓ Erepsin (7·5 ml/g of N taken); erepsin solution was prepared by grinding 2 g of erepsin with 40 ml of 0·02M phosphate buffer, pH 7·6 for 10 min at room temperature, centrifuging and filtering the supernatant through a cotton plug. — 37 °C, 24 h

Sequential enzymatic digest of protein

During digestion with each enzyme, 1·0 ml aliquots were drawn at 0, 1, 2, 4, 8 and 24 h intervals; undigested protein was precipitated with tungstate and AN was determined in the supernatant. At the end of digestion with all the enzymes, the total enzymatic digest was lyophilised, redissolved in a minimum volume of water and stored frozen at −20 °C until further analysis. Amino nitrogen content of an aliquot of the enzymatic digest was estimated before and after acid hydrolysis. Acid hydrolysis of the proteins, their enzymatic digests and the various peptide

fractions obtained during digestion was done according to the method described by Pellet & Young (1980). After hydrolysis, in sealed, evacuated ampoules with 6N HCl for 20–22 h at 110 °C, the acid was removed by repeated flash evaporation and the residue was finally dissolved in a minimum volume of 0·2M citrate buffer (pH 2·2). Its amino nitrogen content was determined by the FDNB method. The amino nitrogen content of the acid hydrolysate of a fraction is referred to as total amino nitrogen throughout this paper.

An aliquot of the enzymatic digest (4–10 mg of total AN) was loaded onto a 1·5 × 35 cm column of copper Sephadex G25 (Cu-Seph-G25) and eluted (Fazakerley & Best, 1965); first 35 ml with 0·05M sodium tetraborate (pH 11) and then with 0·2N HCl until all the copper was eluted out of the column. Fractions (2·0 ml) of the acid eluent were collected and their optical absorbances at 530 and 620 nm read in a Gilford 250 spectrophotometer. The elution patterns of enzymatic digests of animal and plant proteins are given later in Figs 1 and 2. The fractions containing peptides (P1, P2 or P) and free amino acids as their copper chelates were pooled and acidified with a few drops of conc. HCl to pH 3–4; gaseous H_2S was passed through them and the copper sulphide precipitate was removed by centrifugation at 2000 rpm for 10 min at room temperature. Excess H_2S in the clear supernatants was removed by nitrogen flushing; the fractions were lyophilised and the lyophilisates were redissolved in minimum volumes of water and stored frozen at −20 °C until further analysis. The AN content of the different fractions was determined, before and after acid hydrolysis. Their amino-acid compositions were determined on an automatic amino-acid analyser (Beckman, Model CL 119). The dipeptide content of the various peptide fractions was assayed using the Biuret method developed in our laboratory for this purpose (Raghunath & Narasinga Rao, 1983) as follows. 1·0 ml of peptide solution (mixture of dipeptides, mixture of oligopeptides, mixture of dipeptides and oligopeptides or peptide fractions from enzymatic digests of protein) was mixed well with 4 ml of the normal Biuret reagent, allowed to stand at room temperature for 30 min and then the absorbance at 620 and 530 nm of the complexes was read in a spectrophotometer. By the use of a simple simultaneous equation of the type given below, the molar concentrations of peptide bonds contributed by dipeptides in the peptide fraction (hereafter called dipeptide bonds) were calculated:

$$A_{620} = (\varepsilon_X^{620}X) + (\varepsilon_Y^{620}Y)$$

$$A_{530} = (\varepsilon_X^{530}X) + (\varepsilon_Y^{530}Y)$$

where ε_X and ε_Y are the molar absorption coefficients of the peptide bonds in the Biuret complex of di- and oligo-peptides, respectively, at the given wavelengths, and X and Y are the molar concentrations of the peptide bonds of di- and oligo-peptides in the mixture of peptides or in the peptide fractions. The actual equation used was:

$$A_{620} = 44{\cdot}67X + 12{\cdot}48Y$$

$$A_{530} = 22{\cdot}75X + 32{\cdot}40Y$$

Estimation of dipeptide bonds in standard mixtures of di- and oligo-peptides indicated a consistent and satisfactory correlation between expected and estimated values, and also good recovery of the standard dipeptide mixtures added to various peptide fractions. Assuming that 1 mole of dipeptide gives 2 moles of AN on acid hydrolysis, the molar concentration of dipeptide bonds thus obtained was multiplied by a factor of 28 (i.e. a factor of 2 multiplied by a factor of 14 (equivalent to the atomic weight of nitrogen)) to get the total AN content contributed by the dipeptide in the peptide mixture or in the peptide fraction. Statistical analysis of the data was done using the appropriate methods (Snedecor & Cochran, 1967).

RESULTS AND DISCUSSION

The total nitrogen, total AN and the percentages of total AN released during the sequential enzymatic digestion *in vitro* of the different proteins are given in Table 1. It was observed that during sequential digestion of the proteins a higher percentage of AN was released during digestion with trypsin + pancreatin (16–22%), while only a small proportion was released during digestion with pepsin (4–6%) and erepsin (5–7%). To check whether there was any significant contribution to the release of AN by the enzymes themselves becoming digested, blank digests were run simultaneously, wherein these enzymes were incubated sequentially at concentrations actually used with protein samples. It was observed that the contribution by the blank digests was low and ranged from 5·3 to 8·6% in the sequential enzymatic digests of proteins. The percentage of total AN released during enzymatic digestion, although slightly higher in animal proteins, was not significantly different from that in plant proteins. However, the release of AN during pepsin digestion was significantly higher with plant proteins than animal proteins. Although not statistically significant, the converse trend was observed with

TABLE 1

Total Nitrogen and Total Amino Nitrogen (AN)[a] Contents of Some Protein Isolates, Release of Total Amino Nitrogen During Sequential Enzymatic Digestion *in vitro* and Its Distribution in the Peptide and Free Amino Acid Fractions

Protein	*Total nitrogen (g/100 g sub)*	*Total[a] amino nitrogen (g/100 g sub)*	*Release of total amino nitrogen by*							
			Pepsin		*Pancreatin + trypsin*		*Erepsin*		*Sequential enzyme digest*	
			g/100 g	*% of total*	*g/100 g*	*% of total*	*g/100 g*	*% of total*	*g/100 g*	*% of total*
Animal proteins										
Bovine serum albumin (BSA)	13·3	12·5	0·49 (0·037)	3·9	2·56 (0·193)	20·5	0·89 (0·067)	7·1	3·94 (0·297)	31·5
Casein	13·3	11·9	0·36 (0·027)	3·0	2·29 (0·172)	19·3	0·36 (0·027)	3·0	3·01 (0·226)	25·3
Egg albumen	14·6	10·9	—	—	3·79 (0·260)	34·8	0·69 (0·047)	6·3	4·48 (0·307)	41·1
α-Lactalbumin	10·8	9·7	0·29 (0·027)	3·0	2·01 (0·186)	20·7	0·60 (0·056)	6·2	2·90 (0·269)	29·9
Meat lyophilisate	11·9	7·9	0·52 (0·044)	6·6	1·35 (0·113)	17·1	0·81 (0·068)	10·2	2·68 (0·225)	33·9
Mean ± SEM (with meat)				4·1 ± 0·85		22·5 ± 3·15		6·6 ± 1·15		32·3 ± 2·60
Mean ± SEM (without meat)				—		—		—		—

Plant proteins										
Wheat gluten	13·0	11·7	0·54 (0·042)	4·6	1·67 (0·129)	14·3	0·30 (0·023)	2·6	2·51 (0·193)	21·5
Zein	12·5	11·2	0·67 (0·054)	6·0	0·95 (0·076)	8·5	0·76 (0·061)	6·8	2·38 (0·191)	21·3
Soya protein isolate (SPI)	10·0	8·5	0·58 (0·058)	6·8	1·26 (0·126)	14·8	0·18 (0·018)	2·1	2·02 (0·202)	23·7
Groundnut protein isolate (GNPI)	14·8	12·4	0·77 (0·052)	6·2	1·91 (0·129)	15·4	0·29 (0·020)	2·3	2·97 (0·201)	23·9
Rice protein isolate	14·4	8·3	0·57 (0·040)	6·9	1·79 (0·124)	21·6	0·54 (0·038)	6·5	2·90 (0·202)	35·0
Redgram protein isolate	13·8	8·6	0·71 (0·051)	8·3	1·70 (0·123)	19·8	0·70 (0·051)	8·2	3·11 (0·225)	36·3
Mean ± SEM				*6·51 ± 0·49[c]		15·7 ± 1·88		4·7 ± 1·11		26·9 ± 2·79
Blank digest[d]	0·628	0·047 8	0·000 75	1·57	0·002 85	5·96	0·012 8	26·7	0·016 4	34·23

TABLE 1—*contd.*

Protein	*Recovery of total AN from Cu-Seph column*		*Total amino nitrogen of enzyme digest recovered in different fractions*							
			Peptide 1 (P_1)		*Peptide 2 (P_2)*		*Peptide P or ($P_1 + P_2$)*		*Free aa*	
	mg of total AN recovered	*% Recovery*	*mg in[b] fraction*	*% of total recovered*	*mg in[b] fraction*	*% of total recovered*	*mg in[b] fraction*	*% of total recovered*	*mg in[b] fraction*	*% of total recovered*
Animal proteins										
Bovine serum albumin (BSA)	6·06	87·2	1·38	25·9	1·82	34·1	3·20	60·0	2·14	40·0
Casein	6·05	84·7	2·06	34·0	2·74	45·3	4·80	79·3	1·25	20·7
Egg albumen	0·74	78·2	—	—	—	—	0·44	59·7	0·30	40·3
α-Lactalbumin	3·70	70·5	1·21	32·8	1·59	43·0	2·80	75·8	0·90	24·2
Meat lyophilisate	6·12	71·4	3·55	58·0	1·00	16·3	4·55	74·3	1·57	25·7
Mean ± SEM (with meat)		78·4 ± 3·38		*37·7 ± 7·00[c]		34·7 ± 6·59		69·8 ± 4·15		30·2 ± 4·15
Mean ± SEM (without meat)		—		30·9 ± 2·52[c]		***40·8 ± 3·42[c]		—		—

Plant proteins										
Wheat gluten	5·75	77·8	—	—	—	—	4·08	70·9	1·67	29·1
Zein	6·18	89·4	4·82	78·0	0·74	12·0	5·56	90·0	0·62	10·0
Soya protein isolate (SPI)	4·27	74·2	—	—	—	—	2·99	70·1	1·28	29·9
Groundnut protein isolate (GNPI)	3·21	74·4	—	—	—	—	2·01	62·7	1·20	37·3
Rice protein isolate	7·72	84·4	4·65	60·2	1·54	20·0	6·19	80·2	1·53	19·8
Redgram protein isolate	7·88	98·6	4·58	58·1	1·32	16·7	5·90	74·8	1·98	25·2
Mean ± SEM		83·1 ± 3·94		65·4 ± 6·31		16·2 ± 2·32		74·8 ± 3·85		25·2 ± 3·85
Blank digest[d]	0·249	74·8	—	—	—	—	0·168	67·5	0·081	32·5

[a] Amino nitrogen content of an acid (6N HCl, 120 °C, 24 h) hydrolysate of the protein (for details see text).
[b] Milligrams of the total amino nitrogen recovered from the copper Sephadex G25 column, that is present in the fraction. Values in parentheses are g of total amino nitrogen released from amount of protein isolate containing 1 g of total nitrogen.
[c] * $P < 0.05$, *** $P < 0.01$ between plant and animal protein by Student's 't' test.
[d] Nitrogen and amino nitrogen content of the enzymes used for digesting a protein isolate containing 1 g of total nitrogen and the release of AN during blank digestion.

pancreatin + trypsin and erepsin digestion. It would thus appear that plant and animal proteins differ in their susceptibility to digestion with different enzymes of the gastrointestinal tract. Further, it was observed (data not given here) that the rate of AN release, during digestion of different proteins with each enzyme, followed an essentially similar pattern, plateauing after 8–12 h of digestion (however, digestion with each enzyme was continued for 24 h) indicating that the differences in this parameter between different proteins would probably have been detected if only initial rates of reaction were measured. However, the aim was only to subject the proteins to near complete digestion with each enzyme of the gastrointestinal tract to simulate, to the maximum extent possible, the situation *in vivo*.

Recovery of the total AN from different enzymatic digests loaded on Cu-Seph-G25 columns was quite satisfactory and ranged from 71 to 98 % (Table 1). The elution patterns of the enzymatic digests of animal and plant proteins are given in Figs 1 and 2. It was observed that the enzymatic digests of seven proteins (BSA, casein, α-lactalbumin, meat lyophilisate, zein and protein isolates of rice and redgram) resolved into two peptide fractions (P_1 and P_2) and a free aa fraction, while the other four proteins (egg albumen, gluten, SPI and GNPI) yielded only one peptide fraction (P) and an aa fraction, indicating the differences in the type of digestion products released from different proteins and also that peptide fraction P_1 contained higher quantities of larger peptides than the P_2 fraction which contained larger quantities of dipeptides as evidenced by their higher absorbances at 530 and 620 nm, respectively.

The approximate mean size of the peptides in the different peptide fractions was deduced from the AN content of the fractions before and after acid hydrolysis. Under the assumption that 1 mole of dipeptide, tripeptide, tetrapeptide, etc., on acid hydrolysis yields 2, 3, 4 moles, etc., of FDNB reactive amino nitrogen, respectively, a two-, three- or four-fold increase in AN on acid hydrolysis would thus mean that the peptides consist of two, three or four aa, respectively. Thus:

$$\text{Approximate mean size of the peptides in the fraction} = \frac{\text{Total AN content of the fraction (assessed after acid hydrolysis)}}{\text{Unsubstituted AN content of the fraction (assessed before acid hydrolysis)}}$$

These values, given in Table 2, indicate that in cases where two peptide

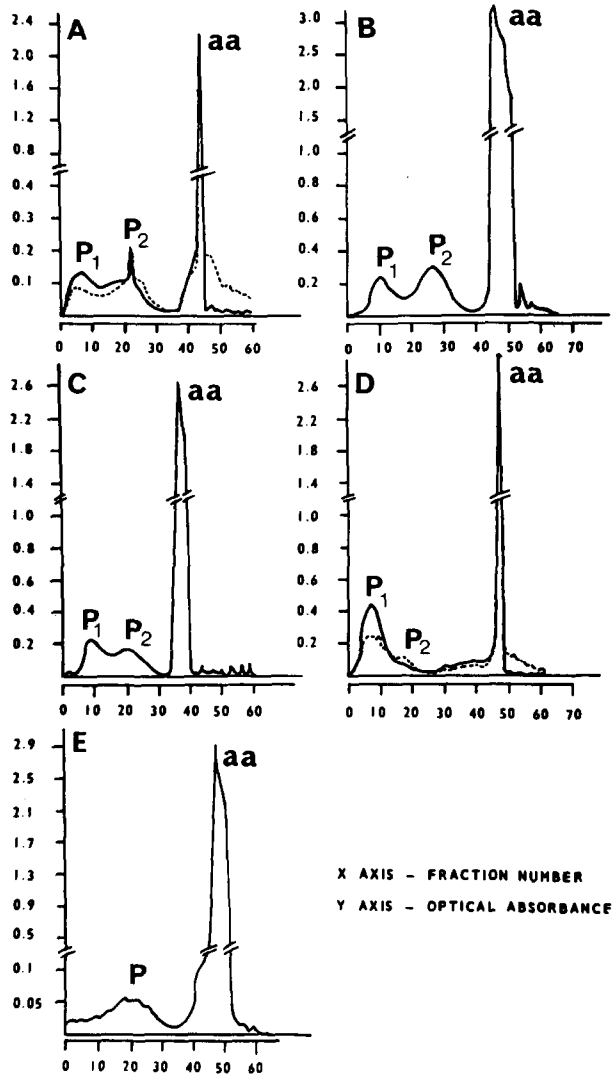

Fig. 1. Elution pattern of peptides and amino acids of the enzymatic digests of animal proteins. (A) BSA; (B) casein; (C) α-lactalbumin; (D) meat lyophilisate; and (E) egg albumen on copper Sephadex G25 column (refer to text for details). ——, Absorbance at 530 nm; – – –, absorbance at 620 nm; P_1, peptide fraction 1 (large peptides); P_2, peptide fraction 2 (small peptides); aa, free amino acid fraction.

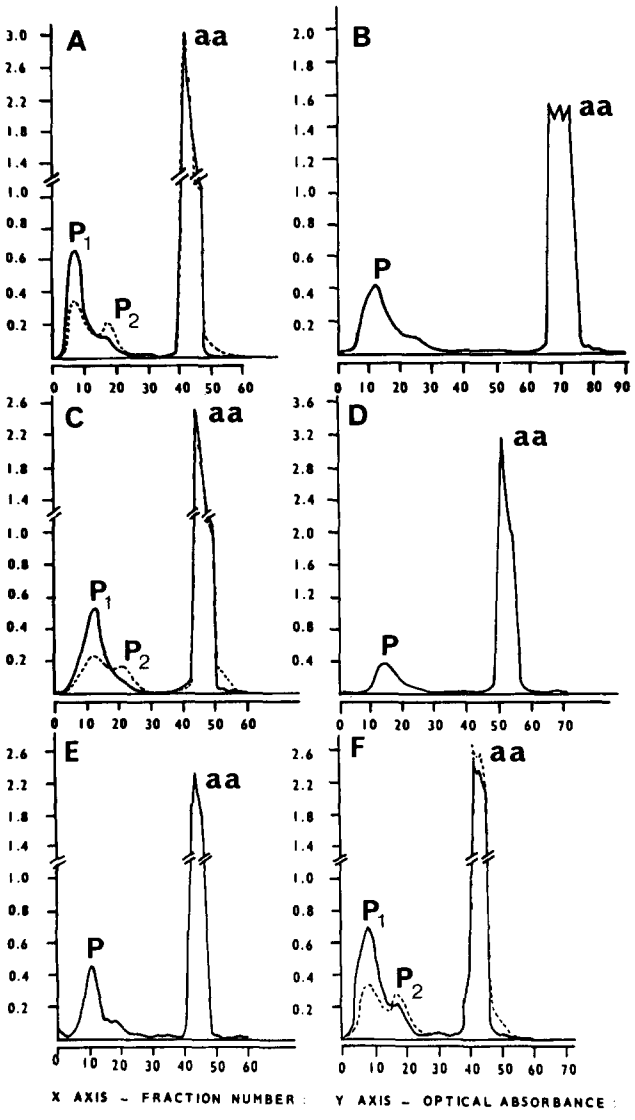

Fig. 2. Elution pattern of peptides and amino acids of the enzymatic digests of plant proteins. (A) Redgram protein isolate; (B) gluten; (C) zein; (D) soya protein isolate; (E) groundnut protein isolate; and (F) rice protein isolate on copper Sephadex G25 column (refer to text for details). ——, Absorbance at 530 nm; – – –, absorbance at 620 nm; P_1, peptide fraction 1 (large peptides); P_2, peptide fraction 2 (small peptides); aa, free amino acid fraction.

TABLE 2
Approximate Size of the Peptide Obtained During Sequential Enzymatic Digestion of Proteins *in vitro*

Protein	*Peptide 1* (P_1)	*Peptide 2* (P_2)	*Peptide* (P)	*Enzyme digest*
Animal proteins				
Bovine serum albumin	4·45[a]	2·45	—	2·62
Egg albumen	—	—	3·0	2·13
Casein	7·43	3·05	—	2·56
α-Lactalbumin	5·94	3·40	—	3·25
Meat lyophilisate	4·81	3·16	—	2·31
Mean ± SEM	5·66	3·02	—	2·57
	±0·67	±0·20		±0·19
Plant proteins				
Rice protein isolate	4·3	2·73	—	2·12
Redgram protein isolate	5·23	2·69	—	2·24
Zein	5·00	2·40	—	2·39
Wheat gluten	—	—	4·88	2·45
Soya protein isolate	—	—	3·56	2·18
Groundnut protein isolate	—	—	3·37	2·16
Mean ± SEM	4·84	2·61	3·94	2·26
	±0·28	±0·10	±0·47	±0·06

[a] Values given are

$$\frac{\text{Total amino nitrogen content of sample (assessed after acid hydrolysis)}}{\text{Unsubstituted amino nitrogen content of the sample (assessed before acid hydrolysis)}}$$

fractions were obtained one was comprised of large-sized peptides of approximately 5 to 6 aa residues on average (designated as P_1 fraction) while the other had relatively smaller peptides of 2 to 3 aa residues (P_2 fraction). The four proteins that gave only one peptide fraction (P) were found to contain peptides of around 3 to 5 aa residues on average. In general, there was about a two- or three-fold increase in the AN content of the enzymatic digests upon acid hydrolysis. However, there were no marked differences between different enzymatic digests in this parameter. Approximate sizes of the peptides observed in this study correspond well with those reported by Crampton *et al.* (1971) in their digestion study *in vitro* and also to those of peptides present in the intestinal lumen of

experimental animals and humans following protein ingestion (Chen *et al.*, 1962; Adibi & Mercer, 1973).

It was observed that the distribution (actual values as well as percentages) of total AN of the enzymatic digests between peptide and free amino-acid fractions was similar in both animal and plant protein digests. In general, about 70–75% of the total AN was in the peptide fraction and the rest in the free amino-acid fraction (Table 1). Here again the values obtained in the present study agreed well with those reported for digestion *in vitro* (Crampton *et al.*, 1971; Amiot *et al.*, 1981) and for intestinal lumen aspirates (Chen *et al.*, 1962) indicating that digestion *in vitro* can be a useful model for digestion *in vivo*.

However, differences between plant and animal proteins were apparent, in that animal protein digests had a significantly higher proportion of the total AN as small peptides (P_2) than plant protein digests, which had a large peptide (P_1) fraction. Essentially similar observations were made regarding the release of amino acids from proteins (percent of total aa of the protein released) as peptides and free aa and their relative distribution. It was surprising to observe that the meat lyophilisate behaved more like a plant protein in having a larger proportion of P_1 than P_2 peptide fraction. The reasons for this are not clear at present although another such observation has been reported by Satterlee *et al.* (1981) during digestion of beef protein. We assume that this may be due to the presence in meat lyophilisate of certain factors affecting the susceptibility of meat to digestion by different enzymes. This, however, needs further investigation.

The quality of the different fractions obtained during digestion *in vitro* was judged by their essential amino acids/non-essential amino acids (EAA/NEAA) ratio. These values are presented in Tables 3 and 4. In general, the EAA/NEAA ratio was highest for the free amino-acid fractions. This was higher than that of the protein itself while those of P_2 and P_1 were usually lower. Among the peptide fractions, P_2 had a higher ratio than P_1 in both animal and plant proteins, except in the case of zein where the ratio of P_1 was higher than that of P_2. Although not statistically significant, the ratios of P_2 of animal protein digests and P_1 of plant protein digests were higher than those of P_2 and P_1 of plant and animal proteins, respectively.

The amino-acid composition (expressed as mmol of aa/mol of total AN in the sample under study) of the proteins and the various fractions obtained during their sequential digestion *in vitro* are also given in Tables

TABLE 3

Amino Acid Composition of the Protein Isolates (Animal Proteins) and the Peptide and Free Amino Acid Fractions Obtained During Their Sequential Enzymatic Digestion *in vitro*[a]

Amino acid	*Bovine serum albumin*				*Casein*				*α-Lactalbumin*			
	Protein	P_1	P_2	*aa*	*Protein*	P_1	P_2	*aa*	*Protein*	P_1	P_2	*aa*
EAA												
Phe	32·9	11·2	32·6	35·8	34·0	17·0	40·3	119	36·9	10·2	23·5	59·8
Val	45·3	104	72·0	4·4	27·4	94·1	96·1	41·7	58·7	61·0	64·9	57·8
Ile	17·1	20·3	20·2	5·3	34·6	65·2	68·4	22·4	48·4	50·2	64·1	43·6
Leu	76·1	62·8	86·9	44·2	68·7	63·0	92·2	119	102	100	118	200
Lys	93·1	40·2	144	56·3	118	37·3	72·6	226	85·9	46·0	86·1	217
Met	0	4·7	0	1·4	4·6	1·9	5·3	0	16·1	19·0	15·0	6·4
Thr	38·9	60·9	70·9	3·2	59·9	83·5	57·8	18·3	51·0	78·6	85·0	19·7
Tyr	20·1	10·1	27·7	17·0	31·5	2·9	14·9	69·4	19·6	5·8	0	39·9
Mean ± SEM	46·2 ± 10·8	39·2 ± 12·2	64·9 ± 16·4	20·9 ± 8·14	47·3 ± 12·2	45·6 ± 12·8	56·0 ± 11·9	99·4 ± 32·0	52·3 ± 10·6	46·4 ± 11·9	65·2 ± 13·7	70·5 ± 24·1
NEAA												
His	22·9	0	0	3·3	21·9	7·5	24·9	23·5	13·5	12·6	33·5	35·2
Arg	22·1	0	0	36·7	20·4	0	4·9	85·2	21·9	6·9	15·4	20·9
Asp	97·6	135	87·9	2·0	53·6	123	81·4	19·7	101	172	145	11·7
Ala	64·0	84·3	77·9	7·1	37·3	62·2	67·7	26·2	61·7	90·9	110	34·8
Glu	115	180	132	2·0	170	316	201	63·0	130	249	170	39·8
Pro	0	0	0	0	0	0	0	0	0	0	0	0
Gly	22·4	35·5	71·7	7·5	22·1	41·0	55·0	20·6	30·9	53·1	57·3	11·7
Ser	33·1	41·4	78·2	3·2	56·1	124	79·9	19·6	47·6	87·7	89·2	13·5
Mean ± SEM	53·9 ± 14·8	95·3 ± 27·7	89·5 ± 10·9	8·8 ± 4·72	54·5 ± 20·1	112·2 ± 44·8	73·5 ± 23·8	36·8 ± 9·96	58·0 ± 16·3	96·0 ± 33·1	88·6 ± 21·6	23·9 ± 4·66
Mean EAA / Mean NEAA	0·857	0·412	0·725	2·37	0·867	0·407	0·762	2·70	0·903	0·483	0·736	2·95

[a] Values are expressed as mmol of each amino acid per mol of total amino nitrogen in the sample under study.

TABLE 3—*contd.*

Amino acid	*Meat lyophilisate*				*Egg albumen*			*Blank digest*		
	Protein	P_1	P_2	*aa*	*Protein*	*P*	*aa*	*Enzymic digest*	*P*	*aa*
EAA										
Phe	15·8	4·2	5·1	16·0	40·8	4·9	54·6	19·5	10·6	22·0
Val	57·0	37·4	33·9	28·7	66·4	24·6	34·8	66·6	43·1	46·5
Ile	24·5	27·6	24·9	145	51·1	19·5	34·3	31·8	29·1	35·0
Leu	48·6	26·1	22·4	25·0	68·8	14·9	103	76·5	36·0	66·8
Lys	50·4	20·8	35·8	94·3	189	11·5	65·2	78·2	54·3	86·7
Met	7·4	0	0	0	52·3	0	20·2	6·2	0	0
Thr	28·0	47·6	22·1	18·3	35·9	17·1	27·8	43·7	148	37·3
Tyr	14·3	6·5	8·1	43·8	20·7	2·5	21·6	32·6	6·2	25·2
Mean ± SEM	30·7 6·65	24·3 ± 5·90	21·8 ± 4·42	53·0 ± 18·4	65·6 ± 18·5	13·6 ± 2·99	45·2 ± 9·93	44·4 ± 9·49	46·7 ± 18·0	45·6 ± 8·86
NEAA										
His	8·6	6·9	15·1	9·5	12·8	3·0	6·9	12·7	9·8	3·1
Arg	26·7	5·9	8·9	71·8	27·4	2·2	37·6	23·9	9·6	14·1
Asp	26·8	88·3	25·2	17·5	81·0	39·2	10·9	77·1	139	14·2
Ala	59·4	81·6	67·5	42·5	63·9	39·6	41·3	125·9	60·8	90·6
Glu	25·8	138	27·2	49·7	11·3	51·9	5·7	112	153	65·8
Pro	25·1	70·4	19·9	0	0	0	0	60·9	60·6	27·3
Gly	39·0	143	84·6	27·8	43·3	24·9	13·9	229	165	29·0
Ser	19·6	35·0	26·3	12·5	71·8	45·9	26·2	55·3	102	31·9
Mean ± SEM	28·9 ± 5·28	71·1 ± 20·5	35·6 ± 9·44	33·0 ± 8·61	58·8 ± 22·2	29·5 ± 7·61	20·4 ± 5·56	86·9 ± 24·5	87·4 ± 21·8	34·5 ± 10·4
Mean EAA Mean NEAA	1·07	0·342	0·612	1·61	1·12	0·459	2·22	0·510	0·535	1·32

TABLE 4

Amino Acid Composition of the Protein Isolates (Plant Proteins) and the Peptide and Free Amino Acid Fractions Obtained During Their Sequential Enzymatic Digestion *in vitro*[a]

Amino acid	*Rice protein isolate*				*Redgram dhal protein isolate*				*Corn zein*			
	Protein	P_1	P_2	*aa*	*Protein*	P_1	P_2	*aa*	*Protein*	P_1	P_2	*aa*
EAA												
Phe	48·0	24·9	23·5	14·9	26·9	24·6	31·6	139	51·3	26·0	18·1	23·1
Val	605	61·2	77·4	45·6	43·5	40·9	32·5	25·6	27·2	41·7	40·9	20·7
Ile	32·0	41·4	40·5	102	38·8	54·6	19·7	38·7	37·4	46·6	109	645
Leu	91·0	50·3	52·0	75·6	103	42·1	60·5	137	183	139	15·2	47·9
Lys	42·6	19·3	30·7	63·5	80·2	30·1	53·2	116	0	15·6	9·3	16·6
Met	5·2	4·6	0	14·0	0	0	0	0	0	0	0	0
Thr	41·4	44·4	40·5	13·5	53·1	47·3	14·4	16·3	26·7	37·9	14·7	7·4
Tyr	36·5	18·2	11·4	0	24·5	15·2	8·3	29·4	32·3	17·9	31·6	146
Mean ± SEM	113 ± 70·9	33·0 ± 6·78	39·4 ± 8·05	47·0 ± 13·2	52·8 ± 10·9	33·5 ± 4·21	31·5 ± 7·37	71·6 ± 21·1	59·7 ± 25·0	46·4 ± 16·0	34·1 ± 13·1	130 ± 87·7
NEAA												
His	15·0	12·7	26·7	14·7	43·4	17·8	21·4	13·2	5·4	6·2	12·4	5·5
Arg	76·8	11·0	32·3	105·5	51·5	10·7	19·7	97·9	0	0	0	34·8
Asp	88·8	138	90·4	13·6	90·1	112	32·7	29·1	43·4	51·7	45·2	12·4
Ala	87·5	75·1	117	42·2	63·0	57·4	52·4	27·8	132	142	167	48·6
Glu	238	192	157	36·8	185	207	54·1	40·4	140	238	178	42·4
Pro	35·7	54·2	45·6	0	35·3	56·0	20·4	0	0	110	41·0	7·6
Gly	69·2	93·8	106	16·1	66·1	59·5	42·0	12·0	18·8	24·8	15·8	8·3
Ser	57·9	56·5	88·3	14·8	85·7	57·4	14·3	14·8	69·5	31·0	61·6	11·7
Mean ± SEM	83·6 ± 23·8	79·1 ± 21·8	82·9 ± 16·0	34·8 ± 12·6	77·5 ± 16·8	72·2 ± 22·1	32·1 ± 5·54	33·6 ± 11·4	68·2 ± 23·3	86·1 ± 31·3	74·4 ± 26·1	21·4 ± 6·20
Mean EAA	1·35	0·418	0·476	1·35	0·681	0·464	0·979	2·13	0·875	0·538	0·458	6·04
Mean NEAA												

[a] Values are expressed as mmol of each amino acid per mol of total amino nitrogen in the sample under study.

TABLE 4—*contd.*

Amino acid	*Wheat gluten*			*Soya protein isolate*			*Groundnut protein isolate*		
	Protein	*P*	*aa*	*Protein*	*P*	*aa*	*Protein*	*P*	*aa*
EAA									
Phe	33·6	24·9	62·1	31·6	15·3	97·4	15·2	91·3	26·1
Val	31·5	58·7	48·0	29·5	38·8	44·7	6·4	40·1	75·5
Ile	29·1	28·0	47·1	34·6	35·1	47·7	10·2	42·1	54·6
Leu	59·0	34·7	133	59·6	39·1	149	20·2	144	54·4
Lys	11·3	5·6	0	36·3	32·2	73·3	29·7	87·4	116
Met	0	3·1	12·0	0	5·0	3·1	5·1	4·6	4·9
Thr	22·5	19·0	60·9	27·8	49·7	28·1	20·2	20·0	50·7
Tyr	18·3	13·4	40·7	18·0	3·5	59·6	7·7	34·9	119
Mean ± SEM	29·3 ± 5·77	23·4 ± 6·33	43·4 ± 6·54	33·9 ± 4·84	27·3 ± 6·07	62·8 ± 15·9	14·3 ± 3·03	58·0 ± 16·2	62·7 ± 14·2
NEAA									
His	10·4	6·0	8·6	4·0	10·5	16·9	10·6	17·5	17·8
Arg	11·3	3·3	0	32·4	12·6	68·4	48·7	134	20·1
Asp	23·3	17·0	22·2	77·6	137	55·8	85·2	36·7	252
Ala	31·4	45·3	42·8	46·8	68·6	48·0	19·1	47·9	103
Glu	265	225	192	117	193	88·2	125	90·4	342
Pro	0	0	0	0	0	0	0	0	0
Gly	49·3	70·4	18·6	50·1	88·5	18·5	20·4	21·4	154
Ser	55·3	27·6	36·2	53·7	82·2	27·8	48·8	27·5	127
Mean ± SEM	63·7 ± 34·1	56·3 ± 29·4	53·4 ± 28·1	54·5 ± 13·4	84·5 ± 24·6	46·2 ± 10·1	51·1 ± 15·6	53·6 ± 16·3	145 ± 44·8
Mean EAA Mean NEAA	0·461	0·416	0·813	0·623	0·323	1·36	0·280	1·08	0·433

3 and 4. It is evident that there are differences between proteins, peptides and free amino-acid fractions from both plant and animal protein, as far as the content of individual aa (EAA or NEAA) is concerned. A comparison of the means of EAA and NEAA compositions of the proteins, peptides and free aa fractions (with all its inherent lacunae, in view of the high values of standard errors observed) suggests, in general, for animal proteins that: (a) EAA content of aa > P_2 > protein > P_1 > P, and (b) NEAA content of P_1 > P_2 > protein > aa > P, while in the case of plant proteins: (a) EAA content of aa > protein > P_1(P) > P_2, and (b) NEAA content of aa > protein > P_1 > P_2 > P. It also appears that P_2 values for animal protein digests are better than P_2 for plant protein digests as far as their EAA composition is concerned. Since P_2 is the major peptide fraction of the animal protein digests, it can be inferred that a greater proportion of the EAA of the animal proteins are released as smaller peptides compared to plant proteins. Thus if one considers the ratio as a parameter for quality, the observations presented here indicate P_2 to be as good as or slightly better than the protein itself in its quality. P_1 is definitely poorer than the protein while the free amino-acid fraction is the best.

The dipeptide content of the various peptide fractions was estimated by the Biuret method (Raghunath & Narasinga Rao, 1983) and expressed as dipeptide total AN as a percentage of total AN in the fraction or enzymatic digest. Results presented in Table 5 indicate that, as one would expect, the smaller peptide fraction (P_2) contained a significantly higher quantity of dipeptides than the large peptide fractions (P_1). It was observed that the total dipeptide content of the peptide fractions obtained from animal protein digests or of the animal protein digests themselves was significantly higher than that of plant protein digests or their peptide fractions. This observation indicates that animal proteins, known to be of better nutritional quality, in general, than plant proteins, yield greater quantities of dipeptides than do the plant proteins during sequential enzymatic digestion *in vitro*, simulating conditions *in vivo*. It was interesting to observe a positive correlation between dipeptide content of the enzymatic digests on the one hand and the quality parameters of these proteins, viz. PER (protein efficiency ratios) and BV (biological values) (literature values), on the other, thereby indicating that the quantity of dipeptides released during enzymatic digestion of proteins increases with the nutritional quality of the protein. These observations agree well with the recent findings of Amiot *et al.* (1981). Thus it would appear that the

TABLE 5

Dipeptide Content of Sequential Enzymatic Digests of Some Proteins and the Peptide Fractions Obtained During Their Sequential Enzymatic Digestion *in vitro*

Protein	*PER*[a]	*BV*[a]	*Peptide 1*		*Peptide 2*		*Peptide P or $P_1 + P_2$*[b]		*Enzymatic digest*[c]	
			Amount	*% of total*	*Amount*	*% of total*	*Amount*	*% of total*	*Amount*	*% of total*
Animal proteins										
Egg albumen	3·9	93·7	—	—	—	—	0·46[d] (0·58)	78·3[e]	0·46 (0·985)	46·7
Bovine serum albumin	3·6	86·5	0·190 (1·38)	13·9	1·19 (1·82)	65·4	1·38 (3·20)	43·1	1·38 (5·34)	25·9
α-Lactalbumin	3·4	82·0	0·263 (1·31)	20·0	0·995 (1·63)	61·0	1·26 (2·94)	42·7	1·26 (3·70)	34·0
Casein	2·9	79·7	0·157 (2·08)	7·5	1·59 (2·82)	56·3	1·75 (4·90)	35·6	1·75 (6·04)	28·9
Meat lyophilisate	2·3	72·0	0·439 (3·55)	12·4	0·476 (1·00)	47·6	0·92 (4·55)	20·1	0·92 (6·12)	15·0
Mean ± SEM (with meat)				13·4 ± 2·58		57·6 ± 3·80		43·97 ± 9·54		30·1 ± 5·19[g]
Mean ± SEM (without meat)				—		—		49·9 ± 9·61[f]		33·9 ± 4·59[h]
Plant proteins										
Soya protein isolate	2·3	72·8	—	—	—	—	0·74 (3·15)	23·4	0·74 (4·27)	17·3
Rice protein isolate	2·2	64·0	0·301 (4·64)	6·5	1·08 (1·58)	68·6	1·38 (6·22)	22·2	1·38 (7·72)	17·9
Groundnut protein isolate	1·6	58·4	—	—	—	—	0·83 (2·10)	39·5	0·83 (3·21)	25·9
Redgram protein isolate	1·5	57·1	0·153 (4·58)	3·3	0·955 (1·32)	72·3	1·11 (5·90)	18·8	1·11 (7·88)	14·1
Zein	1·3	59·4	0·304 (4·83)	6·3	0·652 (0·744)	87·6	0·96 (5·57)	17·1	0·96 (6·18)	15·5
Wheat gluten	1·1	58·2	—	—	—	—	1·17 (4·08)	28·7	1·17 (5·83)	20·1
Mean ± SEM				5·4 ± 1·035		76·2 ± 5·82		24·9 ± 3·34		18·5 ± 1·71

[a] Values of PER and BV are from the literature.

[b] Simple correlation coefficient with PER $r = 0{\cdot}7366$, $P < 0{\cdot}02$; simple correlation coefficient with BV $r = 0{\cdot}7515$, $P < 0{\cdot}01$.

[c] Simple correlation coefficient with PER $r = 0{\cdot}7588$, $P < 0{\cdot}01$; simple correlation coefficient with BV $r = 0{\cdot}7654$, $P < 0{\cdot}01$.

[d] Values given are total amino nitrogen content of the dipeptides in the fraction obtained, expressed as mg.

[e] (Total amino nitrogen content of the dipeptides in the fraction/Total amino nitrogen content of the fraction) × 100.

[f] $P < 0{\cdot}05$.

[g] $P < 0{\cdot}05$ by modified 't' test.

[h] $P < 0{\cdot}01$ by Student's 't' test between plant and animal proteins.

quantity of dipeptides (and small peptides) released during enzymatic digestion may play a role, at least partially, in determining the quality of protein.

The results presented in this paper demonstrate that differences do exist between plant and animal proteins regarding their susceptibility to digestion by different enzymes, and differences also exist between quantities of small peptides (especially the dipeptides) that they yield during enzymatic digestion. It would appear, in general, that, animal proteins yield higher quantities of small peptides (especially dipeptides) during sequential enzymatic digestion.

ACKNOWLEDGEMENTS

The authors wish to thank Dr P. G. Tulpule, ex-Director, for his encouragement during this study and Sri S. Narasimha Murthy for his excellent assistance in the amino-acid analysis of the various samples.

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