Impact of exocrine pancreatic adaptation on *in vitro* protein digestibility

BY P. VALETTE*, H. MALOUIN, T. CORRING* AND L. SAVOIE

Département de Nutrition humaine et consommation, FSAA, Université Laval, Québec GIK7P4, Canada

(*Received 17 July 1991 – Accepted 25 March 1992*)

An *in vitro* enzymic method was used to study the kinetics of digestion of casein and rapeseed proteins. After a predigestion step with pepsin (*EC* 3.4.23.1), the protein substrates were submitted to a 24 h hydrolysis either with pancreatin or pancreatic juices of pigs adapted either to casein or rapeseed diets and whose enzyme activities were different. After 3, 6 and 24 h of *in vitro* digestion, dialysates were collected and analysed for content of nitrogen, amino acids and low-molecular-weight peptides. For a long-term hydrolysis (24 h), overall digestibility of both substrates was not affected by the composition of pancreatic enzyme mixtures. However, at the beginning of hydrolysis a significant effect of pancreatic juices was observed, i.e. individual amino acid digestibility was generally higher when casein pancreatic juice was used for hydrolysis and their relative pattern of release was modified. For both substrates the proportion of amino acids released as low-molecular-weight peptides was not affected by the enzyme mixture used and made up about two-thirds of the total digested material. It is concluded that exocrine pancreatic adaptation to protein sources does not affect the total capacity of protein digestion. However, the changes in initial kinetics of release of amino acids are more dependent on the nature of the protein tested than on the composition of pancreatic enzyme mixtures.

In vitro **protein digestibility: Casein: Rapeseed: Pancreatic juice**

Hydrolysis of food proteins is performed by a mixture of proteolytic enzymes mainly secreted by the exocrine pancreas. Some of these enzymes, such as trypsin (*EC* 3.4.21.4), are highly specific while others, such as elastase (*EC* 3.4.21.36), have broader specificity (Kakade, 1974). The proportion of each in the mixture modulates the nature and kinetics of release of digestion products.

Various *in vitro* methods to simulate proteolysis have been developed. These range from one- or two-step processes using single and pure enzymes (Akeson & Stahmann, 1964; Maga *et al.* 1973) to multi-enzyme systems using pure enzymes or pancreatic extracts (Hsu *et al.* 1977). Using a two-step hydrolysis (in which, after a predigestion with pepsin (*EC* 3.4.23.1), substrates are hydrolysed by pancreatin within a dialysis tube with specific molecular weight cut-off and continuous collection of digestion products (Savoie & Gauthier, 1986)), Savoie *et al.* (1988) showed that each amino acid (AA) was released according to specific patterns. These kinetics of hydrolysis were partly related to the specificity of enzymes for target amino acids, but also to the nature of the protein source. A recent *in vitro* study (Savoie & Charbonneau, 1990) showed that the pattern of release of AA of casein was not affected by the content of trypsin or chymotrypsin (*EC* 3.4.21.1) in pancreatin. However, with rapeseed protein a 2-fold increase in trypsic activity of the preparation mixture markedly favoured the release of arginine and lysine at the expense

* Present address: LEPSD, Bât. 405, INRA–CRJ, 78352 Jouy-en-Josas cedex, France.

https://doi.org/10.1079/BJN19930039 Published online by Cambridge University Press

of target AA of chymotrypsin and elastase. In addition, increasing the chymotrypsin activity of the enzymic preparation, which favoured protein digestibility, was without effect on AA release patterns.

It is known that the protein content of a meal modifies quantitatively and qualitatively the exocrine pancreatic secretion (Corring, 1980). In the pig fitted with a permanent cannula in the pancreatic duct, the feeding of a meal whose protein component was either casein or rapeseed concentrate markedly modified the composition of pancreatic enzyme secretion (Valette *et al.* 1992). Total activity of elastase and particularly carboxypeptidase A (*EC* 3.4.17.1) was higher in the casein group, whereas total activity of chymotrypsin, carboxypeptidase B (*EC* 3.4.17.2) and amylase (*EC* 3.2.1.1) was greater with the rapeseed diet. Trypsin was not affected by the type of protein ingested.

The purpose of the present work was to study the impact of this pancreatic response on the release of AA of the two proteins casein and rapeseed concentrate. After a predigestion with pepsin, casein and rapeseed proteins were hydrolysed *in vitro* with the digestion cell technique (Savoie & Gauthier, 1986) in the presence of the pancreatic juices of pigs adapted either to casein or to rapeseed diets. The hydrolysis kinetics as well as the form of release of digestion products were compared with standard digestion with pancreatin.

MATERIALS AND METHODS

Material

Casein (sodium caseinate; Union des Caséineries Coopératives de Charente Poitou, France; 863 g protein/kg dry matter) and rapeseed concentrate (protein concentrate 00; Tandem, Cetiom, France; 592 g protein/kg dry matter) were used for the different *in vitro* assays. Pepsin (hog stomach mucosa pepsin 1:60000, specific activity 3152 units/mg) and trypsin were purchased from Sigma Co, St Louis, MO, USA. Pancreatin (hog pancreas 5 X) and thimerosal were obtained from ICN Pharmaceutical, Life Science Group, Cleveland, OH, USA. Pancreatic enzymes (freeze-dried) were obtained from pigs with a permanent cannula in the pancreatic duct, and adapted for 15 d to a diet containing either casein or rapeseed concentrate as the only protein source (120 g/kg dry matter diet) (Valette *et al.* 1992). Before use the enzyme mixture (50 mg protein) was activated with 1 ml of trypsin in Tris–Ca^{2+} buffer (10 ml), pH 7·9, for 24 h at 4°. Pancreatin was used as such. Specific enzyme activities of proteolytic enzymes in these preparations are shown in Table 1.

Protein-N (N × 6·25; 40 mg) was suspended with 15 ml 0·1 M-HCl (50 μg thimerosal/l) in a flat-bottomed glass tube and stirred magnetically for 7 min at 37°. The pH was adjusted to 1·9 and 1 ml pepsin solution (1 mg/ml 0·1 M-HCl) was added to obtain an enzyme:substrate (E:S) ratio of 1:250. After 30 min the digestion was stopped by raising the pH to 7·5.

The mixture was poured into the dialysis tube (1000 dalton MWCO, Spectra Por®6; Spectrum Medical Industries, Inc., Los Angeles, CA, USA) of the digestion cell (Savoie & Gauthier, 1986). Digestion was started by adding 1 ml pancreatin (10 mg/ml phosphate buffer) or 2 ml pre-activated pancreatic juices (5 mg/ml). Digested material (free AA and low-molecular-weight peptides) diffusing through the membrane was collected by a circulating (1·6 ml/min) phosphate buffer (0·01 M, pH 7·5). Digestion lasted for 24 h with sampling after 1, 2, 3, 4, 6, 8, 10, 12 and 24 h. All experiments were repeated four times.

Nitrogen determination

The N content of the protein sources was measured on a Kjel Foss apparatus (model 16210; Foss Co., Hillerod, Denmark). Total dialysates were analysed for N using an

https://doi.org/10.1079/BJN19930039 Published online by Cambridge University Press

https://doi.org/10.1079/BJN19930039 Published online by Cambridge University Press

Table 1. *Specific activity (μmol hydrolysed substrate/min per mg protein) of proteolytic enzymes in pancreatin (P), casein (C) and rapeseed (R) pancreatic juices of pigs adapted to either casein or rapeseed diets*

Enzymes*	Enzymic sources		
	P	C	R
Trypsin (*EC* 3.4.21.4)	7·3	4·8	4·9
Chymotrypsin (*EC* 3.4.21.1)	27·0	36·1	45·1
Carboxypeptidase A (*EC* 3.4.17.1)	15·6	23·2	15·2
Carboxypeptidase B (*EC* 3.4.17.2)	1·80	1·07	1·46
Elastase (*EC* 3.4.21.36)	8·2	2·2	1·7

* Trypsin, units BAEE (Reboud *et al.* 1962); Chymotrypsin, units ATEE (Reboud *et al.* 1962); Carboxypeptidase A, units HPLA (Yamasaki *et al.* 1963); Carboxypeptidase B, units BGA (Folk *et al.* 1960); Elastase, units $A(A)_3E$ (Gertler & Hofmann, 1970).

AutoAnalyzer method no. 329/74 WB of Technicon (Technicon Industrial Systems, Tarrytown, New York, NY, USA). Protein digestibility was calculated as follows:

$$\text{protein digestibility} = \frac{\text{N in dialysate (mg)}}{\text{N in protein source (mg)}}.$$

Characterization of digestion products

Three pools of dialysates were constituted corresponding to digestion products collected during the intervals 0–3, 0–6 and 0–24 h.

A portion of each sample was concentrated to 0·32 mg N/ml and loaded (1 ml) onto Copper–Sephadex G-25 columns (15 × 60 mm) to separate peptides from free AA (Savoie & Parent, 1987). Peptides collected were further analysed for N and AA content.

AA determination

AA composition of protein sources (4 mg protein, N × 6·25), total dialysate and peptide fractions (1 mg protein, N × 6·25) was determined after acid-hydrolysis in 2 ml 6 M-HCl carried out under vacuum for 24 h at 100° in vacuum hydrolysis tubes (19 mm × 100 mm, Pierce Chemical, Rockford, IL, USA) with norleucine (10 μmol/ml) as an internal standard. After hydrolysis, samples were evaporated, suspended in 2 ml sample dilution buffer (Beckman, Palo Alto, CA, USA) and filtered through 0·22 μm Millipore filters (Millipore Corporation, Bedford, MA, USA). For determination of cystine, protein sources and dialysates were first subjected to performic acid oxidation followed by acid-hydrolysis. An internal standard, norvaline (10 μmol/ml), was added to samples before performic acid oxidation. AA were measured using an AA analyser (Model 6300; Beckman). For each individual AA, digestibility was calculated as follows:

$$\text{AA digestibility} = \frac{\text{AA in dialysate (mg)}}{\text{AA in protein sample (mg)}}.$$

Statistical analysis

Results of digestibility were analysed by a 2 × 3 factorial analysis of variance with protein and enzyme sources being the main effects (Steel & Torrie, 1980). Values for each time of digestion or each AA were treated separately. Following this analysis the simple effects

Table 2. *In* vitro *protein digestibility of protein sources with pancreatin (P), casein (C) or rapeseed (R) pancreatic juices of pigs adapted to either casein or rapeseed diets**

Period of digestion (h)	Casein			Rapeseed				Statistical significance of factors: $P=$		
	P	C	R	P	C	R	SED	Protein	Enzyme	Protein × enzyme
1	0·09	0·12	0·09	0·05	0·08	0·06	0·010	0·001	0·001	0·586
2	0·19	0·23	0·19	0·12	0·18	0·15	0·018	0·001	0·003	0·399
3	0·28	0·32	0·28	0·19	0·27	0·24	0·027	0·001	0·008	0·306
4	0·37	0·41	0·36	0·26	0·35	0·32	0·028	0·001	0·013	0·247
6	0·51	0·55	0·49	0·39	0·50	0·44	0·032	0·004	0·015	0·236
8	0·61	0·64	0·58	0·51	0·61	0·55	0·036	0·030	0·040	0·374
10	0·69	0·72	0·65	0·60	0·68	0·63	0·038	0·066	0·092	0·483
12	0·75	0·77	0·71	0·67	0·73	0·68	0·039	0·093	0·180	0·664
24	0·91	0·90	0·85	0·86	0·85	0·82	0·041	0·153	0·267	0·899

SED, standard error of difference.
* For details of procedures, see pp. 360–361.

(analysis of one treatment within one level of the other treatment) were measured and, when appropriate, comparison between means were performed using the Duncan's multiple-range analysis. Values of $P < 0{\cdot}05$ were accepted as statistically significant. All calculations were made using the GLM-procedure (SAS Institute Inc., 1982).

RESULTS

Protein and AA digestibilities

Table 2 shows the *in vitro* protein digestibility of casein and rapeseed concentrate with various pancreatic mixtures. Analysis of variance showed protein and enzyme effects. During the first 12 h, casein digestibility with commercial pancreatin was significantly higher than that of rapeseed concentrate. After 24 h the differences levelled. With casein as a substrate, in comparison with the kinetics obtained with pancreatin, the use of pancreatic juice from pigs adapted to the casein diet (casein pancreatic juice) produced a significant increase in protein digestibility only during the first 2 h. At first the pancreatic juice of pigs adapted to the rapeseed diet (rapeseed pancreatic juice) gave rise to the same kinetics as those obtained with commercial pancreatin, but values were lower in the last interval. With rapeseed concentrate as a substrate the use of rapeseed pancreatic juice produced a small but non-significant rise in protein digestibility during the first intervals of digestion compared with pancreatin. However, rapeseed digestibility significantly increased with the casein pancreatic juice during the 6 h of digestion, values being equivalent to those obtained with casein hydrolysed by rapeseed pancreatic juice.

As shown in Table 3, after 3 h of *in vitro* digestion, analysis of variance showed significant protein and enzyme effects on the digestibility of some AA with an interaction in the case of arginine and lysine. The small increase in casein digestibility elicited by casein pancreatic juice was due to a larger release ($P < 0{\cdot}05$) of arginine, histidine, isoleucine, leucine, phenylalanine, tyrosine and valine. The rapeseed pancreatic juice did not produce significantly different values from the pancreatin. Rapeseed AA digestibilities were generally enhanced by pancreatic juices. Compared with values obtained with pancreatin, casein pancreatic juice increased the digestibility of all AA, significant differences being observed particularly for arginine (+110%), cystine (+100%), histidine (+48%), leucine (+36%), lysine (+94%) and methionine (+38%). When the rapeseed pancreatic juice was used the AA digestibility also increased, but less significant effects were noted. As

https://doi.org/10.1079/BJN19930039 Published online by Cambridge University Press

Table 3. In vitro *amino acid digestibility of protein sources after 3 h of digestion with pancreatin (P), casein (C) or rapeseed (R) pancreatic juices of pigs adapted to either casein or rapeseed diets**

Amino acids	Casein			Rapeseed				Statistical significance of factors: *P* =		
	P	C	R	P	C	R	SED	Protein	Enzyme	Protein × enzyme
ARG	0·52	0·59	0·45	0·19	0·40	0·34	0·032	0·001	0·001	0·001
CYS	0·24	0·25	0·24	0·05	0·10	0·08	0·032	0·001	0·243	0·642
HIS	0·28	0·35	0·30	0·21	0·31	0·27	0·028	0·024	0·003	0·582
ILE	0·23	0·28	0·24	0·21	0·26	0·25	0·026	0·690	0·031	0·652
LEU	0·35	0·41	0·36	0·22	0·30	0·27	0·025	0·001	0·005	0·570
LYS	0·43	0·49	0·44	0·18	0·35	0·30	0·030	0·001	0·001	0·028
MET	0·30	0·34	0·29	0·21	0·29	0·27	0·025	0·004	0·016	0·241
PHE	0·37	0·43	0·37	0·31	0·37	0·34	0·027	0·008	0·021	0·618
THR	0·25	0·28	0·24	0·18	0·24	0·22	0·025	0·011	0·098	0·422
TYR	0·42	0·49	0·44	0·38	0·44	0·42	0·032	0·074	0·063	0·710
VAL	0·22	0·27	0·22	0·19	0·24	0·23	0·025	0·387	0·036	0·662
ALA	0·29	0·32	0·28	0·21	0·28	0·24	0·026	0·007	0·043	0·558
ASP	0·22	0·26	0·22	0·19	0·23	0·22	0·025	0·228	0·126	0·728
GLU	0·21	0·25	0·22	0·15	0·22	0·19	0·025	0·028	0·019	0·407
GLY	0·30	0·33	0·29	0·16	0·23	0·21	0·025	0·001	0·050	0·205
PRO	0·22	0·23	0·18	0·11	0·16	0·14	0·022	0·001	0·085	0·244
SER	0·20	0·24	0·20	0·18	0·25	0·23	0·025	0·591	0·036	0·380

SED, standard error of difference.
* For details of procedures, see pp. 360–361.

Table 4. In vitro *amino acid digestibility of protein sources after 6 h of digestion with pancreatin (P), casein (C) or rapeseed (R) pancreatic juices of pigs adapted to either casein or rapeseed diets**

Amino acids	Casein			Rapeseed				Statistical significance of factors: *P* =		
	P	C	R	P	C	R	SED	Protein	Enzyme	Protein × enzyme
ARG	0·76	0·77	0·67	0·37	0·62	0·54	0·070	0·001	0·017	0·017
CYS	0·44	0·51	0·45	0·13	0·24	0·21	0·030	0·001	0·003	0·330
HIS	0·54	0·60	0·51	0·48	0·60	0·52	0·047	0·681	0·023	0·496
ILE	0·45	0·50	0·45	0·44	0·53	0·48	0·036	0·430	0·015	0·607
LEU	0·60	0·66	0·59	0·44	0·55	0·49	0·039	0·001	0·011	0·410
LYS	0·72	0·75	0·70	0·42	0·62	0·55	0·037	0·001	0·001	0·011
MET	0·51	0·55	0·51	0·44	0·54	0·50	0·041	0·190	0·048	0·490
PHE	0·61	0·67	0·60	0·57	0·62	0·58	0·035	0·130	0·070	0·932
THR	0·49	0·54	0·45	0·36	0·46	0·42	0·040	0·003	0·013	0·206
TYR	0·70	0·71	0·69	0·68	0·72	0·57	0·058	0·310	0·156	0·336
VAL	0·42	0·47	0·42	0·42	0·50	0·44	0·032	0·277	0·022	0·855
ALA	0·50	0·52	0·48	0·44	0·50	0·45	0·042	0·133	0·154	0·657
ASP	0·44	0·46	0·42	0·38	0·45	0·41	0·038	0·172	0·122	0·463
GLU	0·41	0·44	0·40	0·34	0·45	0·40	0·042	0·488	0·056	0·309
GLY	0·57	0·59	0·52	0·36	0·46	0·42	0·034	0·001	0·016	0·072
PRO	0·37	0·51	0·36	0·25	0·34	0·29	0·040	0·001	0·001	0·177
SER	0·37	0·37	0·36	0·39	0·47	0·43	0·040	0·011	0·270	0·422

SED, standard error of difference.
* For details of procedures, see pp. 360–361.

https://doi.org/10.1079/BJN19930039 Published online by Cambridge University Press

digestion proceeded (after 6 h, Table 4) the higher casein-AA digestibilities observed previously with casein pancreatic juice were now virtually eliminated. With rapeseed as a substrate, the increase elicited by rapeseed pancreatic juice was still significant for arginine, cystine and lysine. The increase produced by casein pancreatic juice was still perceptible, but more equally distributed among AA. After 24 h (Table 5), when the protein sources were almost completely digested, the enzyme effect disappeared completely, and the difference between proteins was negligible and only relative to cystine and proline digestibility.

These results showed that the digestibility of some AA not only depended on the enzyme source, but also on the substrate itself. Thus, digestibilities of arginine, cystine, leucine, lysine and glycine were significantly higher for casein than for rapeseed, especially at the beginning of hydrolysis kinetics. Moreover, it was obvious that within a protein AA showed different patterns of hydrolysis kinetics. In the casein substrate more than 40% of arginine, lysine and tyrosine were released after only 3 h of digestion. After 6 h these AA were still favourably released, followed by leucine and phenylalanine. By contrast, serine and glutamic acid were slowly released and their digestibility reached only 80% at the end of *in vitro* digestion. Cystine was poorly released during the first hours of digestion, but its digestibility increased in the latter interval and reached more than 95% at the end of the 24 h hydrolysis. With rapeseed concentration fewer differences were observed between digestibilities of individual AA. Tyrosine was released rapidly during the first hours of digestion, followed by phenylalanine and histidine, but proline and especially cystine were poorly released compared with the other AA.

Total Nitrogen and AA in the peptide fraction

As shown in Tables 6 and 7, the proportion of N found as peptides in the digestion products of both proteins was not affected by the enzyme mixture used for hydrolysis. After 3 or 6 h of digestion the proportion ranged from 60 to 70% of total digested material with both protein substrates.

With casein as a substrate it was noticed after 3 h of digestion (Table 6) that isoleucine, threonine, valine and all non-essential AA were preferentially released as low-molecular-weight peptides. On the contrary, arginine, lysine and phenylalanine were mostly found as free AA. It was observed that the use of casein pancreatic juice significantly enhanced the proportion of leucine, lysine, alanine, glycine and serine in the peptide fraction, compared with the values obtained with rapeseed pancreatic juice (+35%, +35%, +22%, +14% and +18% respectively; $P < 0.05$). For rapeseed concentrate (Table 6) the nature of enzyme source used for *in vitro* digestion also slightly modified the AA distribution in the peptide fraction. Release of isoleucine and valine as peptides showed a significant increase when pancreatic juices were used in comparison with values obtained with pancreatin (+31% and +35% respectively; $P < 0.05$). After 6 h of digestion, with either pancreatin or casein pancreatic juice, casein-AA distribution in the peptide fraction showed an equivalent profile (Table 7). Except for arginine, the proportion of AA recovered as low-molecular-weight peptides was enhanced when the digestion was pursued with rapeseed pancreatic juice compared with the other pancreatic mixtures. For instance, histidine was mostly released as free AA with casein pancreatic juice or pancreatin; conversely, with rapeseed pancreatic juice it was present in the peptide fraction at nearly 70%. For the rapeseed concentrate (Table 7), AA were mostly released as peptides when pancreatic juices were used, except for arginine: compared with standard digestion with pancreatin, the proportion of histidine, isoleucine, phenylalanine, valine and alanine in the peptide fraction significantly increased with both pancreatic juices, whereas proportion of leucine, tyrosine

https://doi.org/10.1079/BJN19930039 Published online by Cambridge University Press

Table 5. In vitro *amino acid digestibility of protein sources after 24 h of digestion with pancreatin (P), casein (C) or rapeseed (R) pancreatic juices of pigs adapted to either casein or rapeseed diets**

Amino acids	Casein			Rapeseed			SED	Statistical significance of factors: $P =$		
	P	C	R	P	C	R		Protein	Enzyme	Protein × enzyme
CYS	0·97	> 0·98	> 0·98	0·57	0·63	0·63	0·026	0·001	0·137	0·624
HIS	0·93	0·93	0·93	0·94	0·95	> 0·98	0·040	0·342	0·778	0·860
ILE	0·92	0·89	0·89	0·84	0·85	0·87	0·062	0·779	0·946	0·203
LEU	0·91	0·90	0·93	0·82	0·85	0·86	0·045	0·009	0·610	0·714
LYS	> 0·98	> 0·98	> 0·98	> 0·98	> 0·98	> 0·98	0·005	0·394	0·616	0·167
MET	0·96	0·94	0·95	> 0·98	0·89	> 0·98	0·043	0·933	0·108	0·256
PHE	0·97	> 0·98	> 0·98	> 0·98	0·95	0·97	0·026	0·814	0·784	0·269
THR	0·87	0·88	0·88	0·82	0·80	0·81	0·060	0·056	0·981	0·889
TYR	> 0·98	0·89	0·95	0·96	> 0·98	> 0·98	0·059	0·274	0·788	0·385
VAL	0·88	0·86	0·83	0·80	0·82	0·85	0·058	0·444	0·993	0·535
ALA	0·86	0·83	0·80	0·79	0·76	0·77	0·061	0·128	0·567	0·801
ASP	0·92	0·89	0·87	0·88	0·81	0·90	0·050	0·329	0·382	0·380
GLU	0·79	0·78	0·77	0·80	0·77	0·75	0·059	0·833	0·768	0·936
GLY	> 0·98	> 0·98	> 0·98	0·91	0·86	0·94	0·064	0·022	0·693	0·560
PRO	0·82	0·95	0·89	0·64	0·67	0·71	0·083	0·001	0·429	0·569
SER	0·72	0·67	0·66	0·75	0·78	0·77	0·072	0·049	0·890	0·671

SED, standard error of difference.
* For details of procedures, see pp. 360–361.

https://doi.org/10.1079/BJN19930039 Published online by Cambridge University Press

Table 6. *Distribution (% of total digested material) of nitrogen and amino acids in the peptide fraction after 3 h of* in vitro *digestion with pancreatin (P), casein (C) or rapeseed (R) pancreatic juices of pigs adapted to either casein or rapeseed diets**

Amino acids	Casein			Rapeseed				Statistical significance of factors: $P =$		
	P	C	R	P	C	R	SED	Protein	Enzyme	Protein × enzyme
ARG	27	16	20	34	29	27	6·606	0·046	0·211	0·022
HIS	56	39	48	59	57	59	9·570	0·126	0·535	0·526
ILE	79	93	69	49	64	64	8·384	0·001	0·072	0·085
LEU	49	54	40	36	41	43	4·740	0·015	0·084	0·111
LYS	30	35	26	45	31	36	3·990	0·010	0·018	0·093
MET	68	48	60	55	68	54	11·871	0·514	0·094	0·506
PHE	39	37	30	26	28	29	3·695	0·006	0·355	0·228
THR	84	97	83	75	81	89	7·151	0·151	0·045	0·780
TYR	45	59	43	33	36	31	7·044	0·009	0·675	0·224
VAL	88	95	78	62	84	84	8·297	0·062	0·100	0·085
ALA	90	99	81	67	80	86	7·063	0·016	0·040	0·141
ASP	92	100	97	86	86	98	5·185	0·060	0·046	0·537
GLU	85	97	82	78	76	80	8·361	0·061	0·316	0·631
GLY	89	100	88	85	87	92	5·360	0·217	0·090	0·697
PRO	97	98	95	92	100	94	5·660	0·689	0·764	0·452
SER	90	99	84	77	88	92	6·154	0·172	0·062	0·201
N	72	77	63	61	64	65	6·205	0·063	0·333	0·201

SED, standard error of difference.

* For details of procedures, see pp. 360–361.

Table 7. *Distribution (% of total digested material) of nitrogen and amino acids in the peptide fraction after 6 h of* in vitro *digestion with pancreatin (P), casein (C) or rapeseed (R) pancreatic juices of pigs adapted to either casein or rapeseed diets**

Amino acids	Casein			Rapeseed				Statistical significance of factors: $P =$		
	P	C	R	P	C	R	SED	Protein	Enzyme	Protein × enzyme
ARG	30	17	25	58	19	25	8·257	0·138	0·020	0·097
HIS	45	44	68	42	70	75	7·295	0·021	0·006	0·025
ILE	68	66	76	51	71	73	6·391	0·208	0·038	0·081
LEU	44	37	45	39	51	56	4·333	0·036	0·155	0·014
LYS	26	24	29	41	35	47	4·644	0·001	0·332	0·100
MET	66	44	53	52	76	61	15·787	0·367	0·719	0·409
PHE	37	30	37	26	38	40	3·210	0·956	0·098	0·005
THR	78	73	93	83	94	99	7·486	0·038	0·115	0·087
TYR	42	38	49	35	55	61	6·367	0·163	0·046	0·041
VAL	84	74	82	66	91	91	6·610	0·489	0·112	0·010
ALA	80	78	91	67	90	95	7·354	0·838	0·019	0·041
ASP	94	86	100	90	99	99	7·224	0·455	0·282	0·242
GLU	80	75	88	75	88	98	7·030	0·180	0·105	0·035
GLY	82	80	96	82	91	98	6·934	0·342	0·115	0·116
PRO	100	60	93	96	98	100	3·376	0·001	0·001	0·001
SER	88	90	94	85	97	100	6·653	0·475	0·164	0·435
N	66	58	70	67	75	81	5·945	0·022	0·066	0·179

SED, standard error of difference.

* For details of procedures, see pp. 360–361.

https://doi.org/10.1079/BJN19930039 Published online by Cambridge University Press

and glutamic acid found as peptides increased only with rapeseed pancreatic juice. The same overall pattern was found after 24 h of digestion (results not shown).

DISCUSSION

In the present study we observed that protein digestibility was not significantly different after 24 h of an *in vitro* digestion (1) between values obtained with various enzyme mixtures for the same protein source, (2) between protein substrates whatever the enzyme sources used for hydrolysis. However, the nature of enzyme sources and protein substrates led to significant differences during the first hours of digestion reflecting the kinetics of release of AA. With casein as a substrate the digestibility of all AA was slightly improved by the use of casein pancreatic juice during the first hours of digestion. Rapeseed AA release was larger with pancreatic juices than with pancreatin and the casein pancreatic juice had the best potency. Moreover, it was noticed that the use of pancreatic juices modified the relative proportion of some AA in the dialysate; for instance, arginine and lysine proportions measured after 3 or 6 h of digestion were clearly enhanced. The relative digestibility index (RDI; AA digestibility: protein digestibility), which reflects the release of individual AA in comparison with the sum of AA, illustrates this phenomenon. Indeed, after 3 h of digestion RDI values of lysine and arginine were 0·97 and 1·02 respectively when using pancreatin, 1·25 and 1·50 with rapeseed pancreatic juice and finally 1·31 and 1·42 with casein pancreatic juice. When the digestion was pursued to 6 h the same ratio was obtained. Consequently, arginine and lysine were released more slowly with pancreatin than with pancreatic juices. The better efficiency of casein pancreatic juice remains without explanation since the plausible hypothesis suggesting a direct relationship between the hydrolysis potency and the proteolytic enzyme composition of enzyme mixtures has not been proven. When interpreting these results one must take into account variable reactions with either independent or subsequent actions, i.e. the nature of the substrate, the overall nature of the enzyme sources, their specific composition in the proteolytic enzymes and the *in vitro* conditions of hydrolysis.

Concerning the nature of the protein substrate, the kinetics of hydrolysis of casein was markedly higher than that of rapeseed substrate during the first 12 h of digestion when hydrolysed with the pancreatin. The same results were obtained by Savoie *et al.* (1988) and these differences may be due to the predigestion with pepsin. The main focus of this digestion step is protein denaturation, thus opening its chain and offering more accessible sites for enzymic action, and also to solubilize the protease substrate (Adler-Nissen, 1986). Pepsin might destabilize the micellar structure of casein by a specific breakdown of its kappa fraction (Pélissier, 1984). It may not have the same effect on the more complex and chemically stable structure of rapeseed globulins (Gray & Cooper, 1971). The release of digestion products by pancreatic enzymes was, thus, delayed with this latter protein compared with the casein substrate. This observation showed that the protein nature itself was an essential factor in digestibility. The purity of the protein was also expected to be a factor liable to modulate digestibility since the non-protein components of a foodstuff are known to interfere with protein digestion (Silano, 1977). In the experiment reported here, the protein content (per kg dry matter diet) was lower in the rapeseed concentrate than in casein (592 g and 863 g respectively) since rapeseed contained some non-protein components such as fibres or tannins.

With regard to the release of individual AA by pancreatin, arginine, cystine, glycine and lysine were less readily released from rapeseed than from casein, while isoleucine, phenylalanine, tyrosine, aspartic acid and serine were relatively more easily released. This is only partly a consequence of enzyme specificity (Savoie *et al.* 1988). For instance, increased release of rapeseed lysine and arginine when using pancreatic juices instead of

https://doi.org/10.1079/BJN19930039 Published online by Cambridge University Press

pancreatin might suggest a larger trypsin activity in these enzyme mixtures. However, specific activity of trypsin was higher in pancreatin than in both pancreatic juices. In the same way, the greater specific activities of carboxypeptidase A and chymotrypsin in the casein pancreatic juice compared with pancreatin did not result in a larger release of target AA of these enzymes (tyrosine and phenylalanine). The capacity of a proteolytic enzyme to act specifically on target AA is greatly influenced by the structure (primary, secondary or tertiary) of the protein (Kakade, 1974; Savoie *et al.* 1989). Finally, it is difficult to anticipate as regards the specific contribution of each enzyme. According to Gertler *et al.* (1980), hydrolysis efficiency of one enzyme is greatly dependent on the simultaneous action of the other peptidases. The nature of digestion products could be more dependent on the relative proportion of various proteases rather than on their individual amounts.

Using the digestion cell technique it was possible to collect low-molecular-weight peptides (two to five AA residues) corresponding to the end-products of luminal digestion (Nixon & Mawer, 1970), larger peptides diffusing less rapidly and being further hydrolysed by exopeptidases. Results showed that the overall proportion of N being released in the peptide fraction was not influenced by the nature of enzyme source (approximately two-thirds of total N) and was relatively constant whatever the digestion time. The same results were obtained by Savoie *et al.* (1988) with hydrolysis of casein or rapeseed concentrate by pancreatin. In man, Adibi & Mercer (1973) showed that in the proximal jejunum contents one-third of the total N was represented by free AA, the other two-thirds being released as low-molecular-weight peptides. The use of different enzyme sources involved some more or less pronounced modifications, depending on the protein substrate, in the distribution of AA in the peptide fractions. For casein, differences observed were few; only at the 6th hour and thereafter did pancreatin or rapeseed pancreatic juice lead to an increased proportion of AA in the peptide fraction. With rapeseed protein, differences were mostly observed during the first digestion intervals; so, pancreatic juices favoured overall AA release in the peptide fraction, this effect being more marked after 6 h of digestion. An important factor affecting this distribution could be the action of exopeptidases (carboxypeptidases A and B). The *in vitro* hydrolysis of casein or wheat gluten with pancreatic juice released 20% of total N as free AA, whereas only 1% was obtained with trypsin, chymotrypsin or both enzymes (Camus & Laporte, 1980). This difference is probably due to the fact that the specific contribution of exopeptidases will be strongly dependent on the simultaneous activity of endopeptidases, as shown by Gertler *et al.* (1980). Decreased AA distribution in the peptide fraction should suggest a larger contribution of exopeptidases in the hydrolysis. However, these results did not show this kind of relationship.

One could argue that the adaptation of the exocrine pancreatic secretion to the nature of protein occurs in order to facilitate or accelerate the hydrolysis process. According to that assumption the pancreas should adapt so as to optimize the kinetics of release of some key AA in order to approach an optimum pattern of absorbable AA, or to compensate for some AA deficiency or imbalance in dietary proteins. This could be achieved by modifying the proportions of various proteolytic enzymes. These results did not show such a relationship since casein pancreatic juice revealed a higher capacity for release of specific AA (i.e. arginine and lysine) in rapeseed than did the rapeseed pancreatic juice.

In the present study it was observed that the nature of the enzyme mixture can influence casein and rapeseed hydrolysis in terms of N digestibility as well as in the sequence and form of release of their AA. However, these results showed that variations due to the enzyme source were only observed during the first hours of *in vitro* digestion, the results observed after a long-term proteolysis being independent of the enzyme mixture. The physiological meaning of these results cannot be exactly defined and some other *in vivo* experimental studies would be interesting (1) to confirm these *in vitro* results, (2) to specify the physiological effects of digestion products of these dietary proteins. Dietary AA are

https://doi.org/10.1079/BJN19930039 Published online by Cambridge University Press

absorbed and metabolized differently according to their sequence and form of release. Then, any effects on digestion kinetics due to the protein nature would affect the AA bioavailability. However, the optimum distribution of digestion end-products between free AA and low-molecular-weight peptides for the subsequent transport and utilization in the organism is still unknown.

The authors wish to thank Ms France Pitre for her skilful technical assistance. This work was supported by the Ministère de l'éducation du Québec, Grant FCAR EQ 1188 and France-Québec exchange programme on Biotechnology.

REFERENCES

Adibi, S. A. & Mercer, D. W. (1973). Protein digestion in human intestine as reflected in luminal, mucosal and plasma amino acid concentrations after meals. *Journal of Clinical Investigation* **52**, 1586–1594.

Adler-Nissen, J. (1986). *Enzymatic Hydrolysis of Food Proteins*. London: Elsevier, Applied Science Publishers.

Akeson, W. R. & Stahmann, M. A. (1964). A pepsin pancreatin digest index of protein quality evaluation. *Journal of Nutrition* **83**, 257–261.

Camus, M. C. & Laporte, J. C. (1980). Protéolyse *in vitro* de la caséine et du gluten par les enzymes pancréatiques (*In vitro* proteolysis of casein and gluten by pancreatic enzymes). *Reproduction, Nutrition, Développement* **20**, 1025–1039.

Corring, T. (1980). The adaptation of digestive enzymes to the diet: its physiological significance. *Reproduction, Nutrition, Développement* **20**, 1217–1235.

Folk, J. E., Piez, K. A., Carroll, W. R. & Gladner, J. A. (1960). Carboxypeptidase B. VI. Purification and characterization of the porcine enzyme. *Journal of Biological Chemistry* **235**, 2272–2277.

Gertler, A., Abrahami, B. & Bondi, A. (1980). Specific contribution of porcine pancreatic proteinases to *in vitro* digestion of animal and plant proteins. *Nutrition Reports International* **22**, 771–779.

Gertler, A. & Hofmann, T. H. (1970). Acetyl-L-alanyl-L-alanyl-L-alanine methyl ester: a new highly specific elastase substrate. *Canadian Journal of Biochemistry* **48**, 384–386.

Gray, G. M. & Cooper, H. L. (1971). Protein digestion and absorption. *Gastroenterology* **61**, 535–544.

Hsu, H. W., Vavak, D. L., Satterlee, L. D. & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science* **42**, 1269–1273.

Kakade, M. S. (1974). Biochemical basis for the differences in plant protein utilization. *Journal of Agricultural Food Chemistry* **22**, 550–555.

Maga, J. A., Lorentz, K. & Onayemi, O. (1973). Digestive acceptability of proteins as measured by the initial rate on *in vitro* proteolysis. *Journal of Food Science* **38**, 173–174.

Nixon, S. E. & Mawer, G. E. (1970). The digestion and absorption of protein in man. 2. The form in which digested protein is absorbed. *British Journal of Nutrition* **24**, 241–258.

Pelissier, J. P. (1984). Protéolyse des caséines (Proteolysis of casein). *Sciences des Aliments* **4**, 1–35.

Reboud, J. P., Ben Abdeljlil, A. & Desnuelle, P. (1962). Variations de la teneur en enzymes du pancréas de rat en fonction de la composition des régimes (Variation of the enzymic content of rat pancreas as a function of the composition of the diet). *Biochimica et Biophysica Acta* **58**, 326–337.

SAS Institute Inc. (1982). SAS User's Guide: Basics. Cary, North Carolina: SAS Institute Inc.

Savoie, L. & Charbonneau, R. (1990). Specific role of endopeptidases on modulating the nature of proteins digestion products. *Plant Food for Human Nutrition* **40**, 233–242.

Savoie, L., Charbonneau, R. & Parent, G. (1989). *In vitro* amino acid digestibility of various protein sources. *Plant Food for Human Nutrition* **30**, 93–107.

Savoie, L., Galibois, I., Parent, G. & Charbonneau, R. (1988). Sequential release of amino acids and peptides during *in vitro* digestion of casein and rapeseed proteins. *Nutrition Research* **8**, 1319–1326.

Savoie, L. & Gauthier, S. (1986). Dialysis cell for the *in vitro* measurement of protein digestibility. *Journal of Food Science* **51**, 494–498.

Savoie, L. & Parent, G. (1987). Characterization of protein *in vitro* digestion products by copper-Sephadex chromatography. *Nutrition Reports International* **35**, 783–791.

Silano, V. (1977). Factors affecting digestibility and availability of proteins in cereals. In *Nutritional Evaluation of Cereal Mutants*, pp. 13–46. Vienna: International Atomic Energy Agency.

Steel, R. C. D. & Torrie, J. H. (1980). *Principles and Procedures of Statistics*. 2nd ed. New York: McGraw-Hill.

Valette, P., Malouin, H., Corring, T., Savoie, L., Gueugneau, A. M. & Berot, S. (1992). Effects of diets containing casein and rapeseed on enzyme secretion from the exocrine pancreas in the pig. *British Journal of Nutrition* **67**, 215–222.

Yamasaki, M., Brown, J. R., Cox, D. I., Greenshields, R. N., Wade, R. D. & Neurath, H. (1963). Procarboxypeptidase A-S6. Further studies of its isolation and properties. *Biochemistry* **2**, 859–866.

Printed in Great Britain

https://doi.org/10.1079/BJN19930039 Published online by Cambridge University Press