Estimation of available methionine and cysteine in proteins of food products by in vivo and in vitro methods

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(*Received* 19 *April* 1974 – *Accepted* 21 *March* 1975)

1. The available methionine and cysteine contents of proteins were determined by chemical methods after preliminary enzymic hydrolysis.

2. The values for the available methionine and cysteine contents of pure proteins (casein and bovine serum albumin) estimated by chemical methods were similar to those for the total content determined by the method of Moore, Spackman & Stein (1958).

3. Reductions of 15 and 11 % respectively, when compared with unprocessed samples, were found in the available methionine contents of sweetened and unsweetened, condensed milks; of roller-dried milk and whey powders, and of mackerel sterilized at 126°, the reductions were 22, 14 and 19 % respectively.

4. The available cysteine content of sweetened, condensed milk was reduced by about 32 %, whereas for mackerel sterilized at 115 and 126° it was reduced by 64 and 75 % respectively.

5. The contents of total sulphur amino acids for these food products did not differ from those for the unprocessed samples.

6. Values obtained for available S amino acid contents by rat bioassay confirmed the results of the in vitro estimations.

Amino acids containing sulphur most commonly limit the nutritive values of proteins in the human diet (Autret, Perisse, Sizaret & Cresta, 1968), because of the low methionine and cysteine contents of proteins. The S amino acid contents may be considerably reduced as the result of their partial destruction during processing, which consequently may increase the deficiency of these amino acids in foodstuffs and fodders. During processing, protein fragments resistant to the action of proteolytic enzymes are produced also and these may contain S amino acids (Donoso, Lewis, Miller & Payne, 1962; Ford & Shorrock, 1969; Bjarnason & Carpenter, 1970). In this form they cannot be used by an organism.

The results of in vitro studies of the effect of drastic heating of cod fillets on the level of available amino acids indicated that the greatest decrease, next to that for lysine, was in methionine content (Ford & Salter, 1966). Similar results were obtained by Miller, Hartley & Thomas (1965) in studies using animals. Rakowska & Zielinska (1972) reported that long-term sterilization of vegetable and meat mixtures results in a 29 % reduction in the amount of available methionine; no reduction in total methionine content was reported.

The differences between values for total and available methionine content are the result of the use of the acid-hydrolysis procedure for the estimation of the total amino acid content as this disrupts all peptide bonds, including those resistant to proteolytic enzymes. Moreover, the estimation of S amino acid contents involves oxidation of proteins by performic acid to convert methionine into methionine sulphone and cysteine into cysteic acid. This process prevents the destruction of

labile S amino acids during the subsequent acid-hydrolysis but makes it impossible to distinguish the proportion of methionine and cysteine which may have been oxidized during processing.

The oxidized forms of S amino acids (methionine sulphone, cysteic acid) are not used by higher organisms (Njaa, 1962; Miller & Samuel, 1968; Ellinger & Palmer, 1969).

Biological assays must be the ultimate methods of reference for the evaluation of available amino acids. However, bioassays for determination of available methionine are both laborious and inaccurate. Therefore quick, accurate chemical methods for the determination of available forms of S amino acids in proteins are required. This need has been stressed by Mauron (1971), Bender (1972) and other workers.

Our results suggest that available methionine and cysteine in proteins may be determined after enzymic hydrolysis, by selective chemical methods. These methods allow the quantitative estimation of both intact methionine and cysteine in the products of thermal processing of proteins.

They may be useful in the assessment of biological value of proteins in which methionine and cysteine are limiting.

In the present work the content of available methionine and cysteine of proteins was studied using in vivo and in vitro procedures.

MATERIALS AND METHODS

Materials. The following chemicals were used: (1) pure amino acids: DL-methionine (Reanal, Budapest, Hungary), L-cysteine (RCB, Belgium), L-cystine (Calbiochem, California, USA); (2) pure proteins: casein (BDH, Poole, England), bovine serum albumin (Serva Feinbiochemica, Heidelberg, Germany); (3) food proteins: rye, wheat, rye flour, wheat flour, isolated rapeseed protein, pork, beef, potato, fresh milk, sweetened and unsweetened, condensed milk, spray-dried and roller-dried milk, fresh whey, spray-dried and roller-dried whey, fresh mackerel and mackerel sterilized at 115 and 126°.

Proteolytic enzymes used in enzymic hydrolysis were: papain (*EC* 3.4.4.10) (Koch-Light Laboratories Ltd, Colnbrook, Bucks.); pancreatopeptidase E (*EC* 3.4.4.7) (K & K Laboratories Inc., California, USA); pepsin (*EC* 3.4.4.1), triple recrystallized from porcine stomach mucosa, B grade (Calbiochem AG, Lucerne, Switzerland).

Analytical procedures

Preparation of test materials. Products requiring defatting (pork, beef and mackerel) were defatted by the method of Brieskorn & Sheida (1963). The samples were then dried at room temperature and ground, using a mortar, to pass a 60-mesh sieve.

Methods of enzymic hydrolysis. The proteins were hydrolysed either with pepsin and then with pancreatopeptidase E, or with pancreatopeptidase E alone.

A weighed portion of test protein corresponding to 4 mg pure protein nitrogen was dissolved in 5 ml 0·05 M-HCl, pH 2. Then 0·1 ml of a solution containing 10 mg pepsin/ml was added and the mixture was incubated at 40° for 24 h with continuous

Table 1. *Composition (g/kg) of the basal casein diet fed with or without methionine supplementation to rats*

Ingredient		Ingredient	
Casein*	130	Cod-liver oil	20
Wheat starch	450	Mineral mixture†	40
Potato starch	50	Vitamin B mixture‡	10
Sucrose	200	Methionine (mg/kg)	0, 340, 500, 800, 1000, 1500 or 2000
Soya-bean oil	120		

* Contained (g/kg crude protein (nitrogen × 6·25)): methionine 31·8, cysteine 4·5.
† Hawk, Oser & Summerson (1947), modified as described by Kunachowicz (1970).
‡ Prepared as described by El-Maraghi, Platt & Stewart (1965).

Table 2. *Composition (g/kg) of diets containing casein and processed products of milk, whey and mackerel as protein sources for rats*

	Diet							
Ingredient	1	2	3	4	5	6	7	8
Spray-dried milk	120	—	—	—	—	—	—	—
Roller-dried milk	—	120	—	—	—	—	120	—
Spray-dried whey	—	—	60	—	—	—	—	—
Roller-dried whey	—	—	—	60	—	—	—	60
Fresh mackerel*	—	—	—	—	25	—	—	—
Sterilized mackerel (126°)*	—	—	—	—	—	25	—	—
Casein	80	80	90	90	100	100	80	90
Wheat starch	360	360	410	410	435	435	360	410
Potato starch	50	50	50	50	50	50	50	50
Sucrose	200	200	200	200	200	200	200	200
Soya-bean oil	120	120	120	120	120	120	120	120
Cod-liver oil	20	20	20	20	20	20	20	20
Mineral mixture†	40	40	40	40	40	40	40	40
Vitamin B mixture‡	10	10	10	10	10	10	10	10
Methionine (mg/kg)	—	—	—	—	—	—	950	700
Nitrogen content§	16·2	16·4	16·1	16·2	16·3	16·2	16·1	16·6

* Added in the form of a defatted powder.
† Hawk, Oser & Summerson (1947), modified as described by Kunachowicz (1970).
‡ Prepared as described by El-Maraghi, Platt & Stewart (1965).
§ Determined by the Kjeldahl method (Person, 1973).

shaking. The pH of the hydrolysate was adjusted to 8·2 using 1 M-Na_2HPO_4 and, after adding 0·1 ml toluene and 0·1 ml of a solution containing 10 mg pancreatopeptidase E/ml, the incubation was continued for a further 24 h.

For pancreatopeptidase E alone, a weighed portion containing 4 mg pure protein N or 6 mg food protein N was dissolved in 5 ml 0·05 M-phosphate buffer (Na_2HPO_4–HCl, pH 8·2), 0·1 ml toluene and 0·1 ml of a solution containing 10 mg pancreatopeptidase E/ml were then added. The mixture was incubated at 40° for 24 h with continuous shaking. An incubation mixture without the test protein was used as a control. The reaction was stopped by addition of 0·2 ml 1·8 M-$HClO_4$/ml hydrolysate and after 30 min the hydrolysates were centrifuged at 3000 *g* for 10 min. The super-

Table 3. *The differences in methionine contents of proteins when these were determined by acid-hydrolysis after preliminary oxidation of the protein (Moore, Spackman & Stein, 1958) or without preliminary oxidation (Schram, Moore & Bigwood, 1954)*

(Mean values for two determinations)

Test protein	Methionine (g/kg CP)		Difference in methionine content (%)
	Without oxidation	With oxidation	
Pure proteins			
Casein	15·3	31·4	50
Ovalbumin	3·2	36·2	91
Food proteins			
Wheat flour	8·8	20·1	56
Skim-milk powder	10·2	28·1	64
Potato	3·9	9·9	61
Rapeseed protein	3·5	30·7	89

CP, crude protein (nitrogen × 6·25).

natant fraction was collected and for pepsin–pancreatopeptidase E hydrolysates was adjusted to pH 7 with $KHCO_3$.

Determination of available methionine in enzymic hydrolysates. After partial enzymic hydrolysis of the protein, the hydrolysate was reacted with sodium nitroprusside as described by McCarthy & Sullivan (1941). The extinction at 520 nm of the coloured complex formed was measured using a spectrophotometer (Beckman DB; Beckman Instruments Inc., Fullerton, California, USA). As the $-S-CH_3$ group of methionine takes part in the reaction, the amount of the amino acid can be determined in peptides, if this group is free to react with sodium nitroprusside. Ussuary & Gehrke (1969) suggested that of twenty amino acids studied, only tryptophan and histidine interfered in this reaction. This interference was prevented by adding glycine (30 g/l). It should be emphasized that determination of methionine content by this method is selective, as oxidized forms of methionine (sulphone, sulphoxide) do not participate in this reaction. The results obtained are the absolute values for the methionine content of the proteins for both the raw materials and the products obtained after technological processing.

Determination of available cysteine in enzymic hydrolysates. The cysteine content of proteins was determined by the Zahler–Cleland method (Zahler & Cleland, 1968) after enzymic hydrolysis of protein to release cystine which was then reduced by dithiothreitol (DTT, Cleland's reagent; Sigma Chemical Corp., St Louis, USA) to cysteine. The thiol group of cysteine formed a coloured complex with 5,5′-dithiobis-2-nitrobenzoic acid (Sigma Chemical Corp.) and the extinction at 412 nm was measured spectrophotometrically. By this method the intact cysteine content of both raw materials and their products which had been processed on an industrial scale could be estimated quantitatively, as only the free thiol groups of cysteine take part in the reaction.

Table 4. *Total methionine content of rye, wheat, rye flour and wheat flour determined microbiologically (Ford, 1964) (acid-hydrolysis before methionine determination) and by the method of Moore, Spackman & Stein (1958) (preliminary oxidation then acid-hydrolysis)*

(Mean values for two determinations)

	Methionine (g/kg CP)	
Protein source	Microbiological method	Moore *et al.* (1958) method
Rye	13·9	19·7
Wheat	14·5	20·5
Rye flour	16·3	18·7
Wheat flour	16·5	19·8

CP, crude protein (nitrogen × 6·25).

Total contents of methionine and cysteine in proteins. These were determined after oxidation of the protein followed by acid-hydrolysis (Moore, Spackman & Stein, 1958) or after acid-hydrolysis only, the method of Schram, Moore & Bigwood (1954). The amino acid contents of the acid-hydrolysates were estimated using an amino acid analyser (TSM; Technicon Instrument Corporation, Tarry Town, New York, USA).

The total methionine content was also determined by the microbiological method of Ford (1964) using *Streptococcus zymogenes.*

Preparation of a standard curve for the utilization of S amino acids by rats. Rats (Wistar strain, 25 d old) were divided into groups of five (one rat from each litter). The weights of the groups did not differ by more than 2 g. The rats were fed *ad lib.* for 14 d on a basal casein diet with or without methionine supplementation (Table 1). The food intake and the body-weight gains were recorded daily. After 14 d the average food conversion efficiency for each rat was calculated.

Determination of available S amino acid (methionine + cysteine) content of proteins of milk and fish products. Groups of five rats were given *ad lib.* a diet in which part of the casein was replaced by one of the test products. The composition of the diets is given in Table 2. The food intake and body-weight of the rats were recorded daily. After 14 d the food conversion efficiency of the animals was determined, and the S amino acid content of the test product was calculated using the equation: $a = c - a_1$, where a, c and a_1 are the S amino acid contents (mg/kg diet) of the test product, the diet containing the test product and the basal casein diet respectively. The S amino acid content of the test product was then expressed in terms of crude protein (N × 6·25) content.

The effect of dietary methionine supplementation on growth rate. Groups of five rats were given diets containing spray- or roller-dried milk or whey (Table 2) for 14 d. The food intake and body-weights were recorded daily and the food conversion efficiency was calculated.

Net protein utilization (NPU). The NPU of test products was determined by the method of Miller & Bender (1955).

Table 5. *Available methionine and cysteine contents of casein determined by chemical methods, after enzymic hydrolysis with pepsin* (EC 3.4.4.1) *and pancreatopeptidase E* (EC 3.4.4.7), *or pancreatopeptidase E alone. For comparison, sulphur amino acid contents of acid-hydrolysates of casein were determined also by the standard method of Moore, Spackman & Stein* (1958)

(Mean values with their standard errors for ten determinations; coefficient of variation ranged from 3·7 to 5·6 for methionine and from 3·1 to 6·0 for cysteine)

	Methionine (g/kg CP)		Cysteine (g/kg CP)	
Analytical procedure*	Mean	SE	Mean	SE
Enzymic hydrolysis				
Pepsin + pancreatopeptidase E	32·3	1·8	4·60	0·15
Pancreatopeptidase E	32·1	1·2	4·58	0·14
Acid-hydrolysis, 6 M-HCl	32·0	1·8	4·60	0·18

CP, crude protein (nitrogen × 6·25).
* For details of procedures, see pp. 176–9.

RESULTS

Reduction in methionine content during acid-hydrolysis. To study the reduction in methionine content during acid-hydrolysis, the methionine contents of various foods were determined after acid-hydrolysis with or without preliminary oxidation (Table 3). The values for the methionine contents of proteins which were acid-hydrolysed without preliminary oxidation were 50–90% lower than those which were acid-hydrolysed after oxidation; the value obtained was dependent on the type of protein.

These results were of significance in the interpretation of the results for the total methionine content when this was determined by the microbiological method. In the latter method, proteins were acid-hydrolysed for 6 h before methionine determination using the micro-organisms. Table 4 shows the results for the total methionine contents of food proteins determined by the microbiological method and by the method of Moore *et al.* (1958), which included a preliminary oxidation of S amino acids. In all instances, the values for the methionine content obtained by the microbiological method were lower than those obtained by the method of Moore *et al.* (1958). The differences in the methionine contents were undoubtedly associated with partial decomposition of this amino acid in the preliminary acid-hydrolysis of the proteins in the microbiological method.

Estimation of cysteine by the Zahler–Cleland method. Cysteine contains a very reactive thiol group which is readily oxidized both in the free amino acid and in the amino acid when it is bound in a polypeptide chain. As a result of dehydrogenation cystine is produced from two molecules of cysteine. In the Zahler–Cleland method, cystine is converted to cysteine by DTT, and the accuracy of the method depends, to a great extent, on the efficiency of this conversion.

It was found that the amount of cysteine in the test solution after reaction with DTT for 30, 45 and 60 min was the same. A conversion efficiency of 100% was obtained for cystine after reaction with DTT for 30 min; therefore, this reaction time was used subsequently.

Table 6. *Available methionine and cysteine contents of bovine serum albumin determined by chemical methods after enzymic hydrolysis with pepsin* (EC 3.4.4.1) *and pancreatopeptidase E* (EC 3.4.4.7), *pancreatopeptidase E alone or papain* (EC 3.4.4.10). *For comparison, sulphur amino acid contents of acid-hydrolysates of bovine serum albumin were determined also by the method of Moore, Spackman & Stein* (1958)

(Mean values with their standard errors for ten determinations; coefficient of variation ranged from 5·6 to 9 for both methionine and cysteine)

Analytical procedure*	Methionine (g/kg CP) Mean	SE	Cysteine (g/kg CP) Mean	SE	Nitrogen recovery after enzymic hydrolysis (%)
Enzymic hydrolysis					
Pepsin + pancreatopeptidase E	9·34	0·6	32·5	1·6	98
Pancreatopeptidase E	9·50	0·5	32·6	1·8	99
Papain	4·40	0·8	15·0	1·2	46
Acid-hydrolysis, 6 M-HCl	9·40	0·8	33·1	1·7	—

CP, crude protein (N × 6·25).
* For details of procedures, see pp. 176–9.

Available methionine and cysteine contents of enzymic hydrolysates of pure proteins. The method for the determination of available methionine and cysteine contents was based on the measurement of both free and peptide-bound methionine and cysteine. For the estimation of these amino acids for enzymic hydrolysates it was therefore necessary to find a procedure which would produce peptides completely soluble in the perchloric acid which was used for stopping the enzyme action.

Values for methionine and cysteine contents of a 'linear' protein (casein), which was initially hydrolysed with pepsin and pancreatopeptidase E, or with pancreatopeptidase E alone, are shown in Table 5. The values obtained for methionine and cysteine contents of casein by these procedures were similar to those for these amino acids determined as methionine sulphone and cysteic acid after acid-hydrolysis of casein by the method of Moore *et al.* (1958).

Values for methionine and cysteine contents of a 'globular' protein (bovine serum albumin) obtained by enzymic hydrolysis using various proteolytic enzymes are shown in Table 6. With pepsin and pancreatopeptidase E or with pancreatopeptidase E alone for the initial enzymic hydrolysis of bovine serum albumin, the methionine and cysteine contents were similar to those obtained by the method of Moore *et al.* (1958) but those obtained by enzymic hydrolysis with papain were 50% lower. This was associated with the release, after enzymic hydrolysis, of peptides which were insoluble in perchloric acid. This was confirmed by the 46% recovery of N in the perchloric acid-insoluble residue.

As there were no differences in the levels of methionine and cysteine for enzymic hydrolysates of 'linear' (casein) and 'globular' (bovine serum albumin) proteins which were hydrolysed with pepsin and pancreatopeptidase E or with pancreatopeptidase E alone and values were comparable with those determined by the method of Moore

Table 7. *Available (determined by enzymic hydrolysis*) and total (determined by acid-hydrolysis*) methionine and cysteine contents of food proteins*

(Mean values for at least two determinations)

	Nitrogen content (mg)			Methionine (g/kg CP)		Cysteine (g/kg CP)	
Test protein	Before hydrolysis	After hydrolysis	N recovery (%)	Available	Total	Available	Total
Pork	0·850	0·781	92	25·9	25·6	10·8	11·3
Beef	0·876	0·866	99	33·3	31·0	9·98	10·1
Fresh milk	0·495	0·489	99	29·0	29·7	9·2	9·4
Fresh whey	0·510	0·506	99	24·4	23·0	29·5	30·0

CP, crude protein (N × 6·25).
* For details of procedures, see pp. 176–9.

Table 8. *Body-wt gain, food dry-matter* (DM) *intake and food conversion efficiency for rats given a basal casein diet* supplemented with different amounts of methionine*

(Mean values for five rats/group)

Dietary methionine supplement (mg/kg)	Body-wt gain (g/rat per 14 d)	Food DM intake (g/rat per 14 d)	Food conversion efficiency (g body-wt gain/g food DM intake)
0	16	63·8	0·25
	21	70·7	0·30
	15	66·6	0·22
340	19	72·0	0·26
500	22	66·4	0·33
800	29	78·5	0·37
1000	35	83·3	0·42
	36	87·8	0·41
1500	31	80·7	0·38
2000	42	90·2	0·47
	46	97·4	0·47

* For details of diet, see Table 1.

et al. (1958), enzymic hydrolysis with pancreatopeptidase E was therefore used in further studies.

Recovery of methionine and cysteine after enzymic hydrolysis. The effect of enzymic hydrolysis on DL-methionine and L-cysteine standards was studied. The hydrolysis conditions did not affect free methionine and cysteine as the recovery of both methionine and cysteine was high (98 and 94% respectively). Values for the recovery of methionine and cysteine in the presence of casein were 95–100 and 89–95% respectively.

Available methionine and cysteine contents of enzymic hydrolysates of food proteins. The available methionine and cysteine contents of food proteins were determined after preliminary enzymic hydrolysis with pancreatopeptidase E. The values obtained were compared with those obtained by the method of Moore *et al.* (1958) (Table 7). There were no differences in values obtained using these two methods. The N

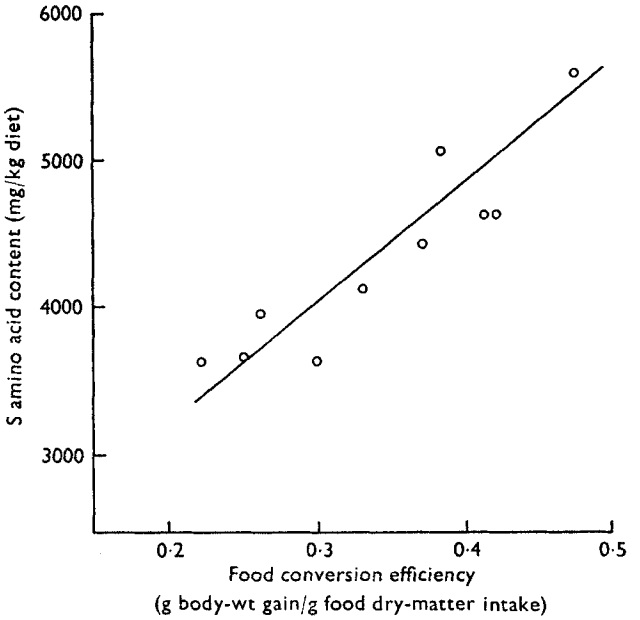

Fig. 1. Standard curve for the utilization of sulphur amino acids by rats given *ad lib.* a basal casein diet, with or without methionine supplementation, for 14 d, showing the relationship between dietary S amino acid content and food conversion efficiency. For details of experimental procedures, see p. 179.

recovery for the test samples (N content of samples before and after enzymic hydrolysis) was calculated; values were high and ranged from 92 to 100% (Table 7). This suggested that after enzymic hydrolysis peptides were produced which were completely soluble in perchloric acid and therefore values given for methionine and cysteine contents were absolute values for these amino acids for food proteins.

In vivo studies. The content of available methionine and cysteine in processed and unprocessed foodstuffs was calculated from a standard curve for the utilization of S amino acids by rats.

Body-weight gain, food intake and food conversion efficiency values are given in Table 8. The correlation between S amino acid content of the diet and the food conversion efficiency was high (r 0·96) (Fig. 1).

The S amino acid content of the diet (c; mg/kg) was calculated from the equation:

$$c = 147 + 856W,$$

where W is the food conversion efficiency (determined experimentally). The above equation is valid in the range 3670–5670 mg S amino acids/kg diet.

S amino acid contents of milk and mackerel products. The available (measured chemically) and total methionine and cysteine contents of proteins in fresh milk, whey and mackerel and their respective processed forms are shown in Table 9. The values for available and total methionine and cysteine contents of the proteins in the fresh materials were similar. Processing did not affect the total methionine and

Table 9. *The total and available methionine and cysteine contents (g/kg crude protein (nitrogen × 6·25)) of fresh milk, whey and mackerel, and their respective processed products, determined by chemical methods**

(Mean values with their standard errors)

Test products	Total: No. of determinations	Total: Methionine Mean	Total: Methionine SE	Total: Methionine Decrease† (%)	Total: Cysteine Mean	Total: Cysteine SE	Total: Cysteine Decrease† (%)	Available: No. of determinations	Available: Methionine Mean	Available: Methionine SE	Available: Methionine Decrease† (%)	Available: Cysteine Mean	Available: Cysteine SE	Available: Cysteine Decrease† (%)
Milk														
Fresh	4	29·7	1·0	—	9·20	0·8	—	7	29·0	1·0	—	9·16	0·1	—
Sweetened	4	28·2	1·0	3	9·00	0·9	2	4	24·7	0·8	15	6·28	0·1	32
Unsweetened	4	28·2	1·2	3	8·80	0·8	4	4	25·8	0·5	11	8·79	0·1	4
Spray-dried	34	29·7	1·5	0	8·39	1·0	9	28	29·3	1·4	0	8·29	0·5	9
Roller-dried	28	29·0	1·4	0	8·51	0·7	8	28	22·6	1·0	22	8·28	0·4	9
Whey														
Fresh	4	23·0	1·2	—	30·0	1·5	—	4	24·4	1·3	—	29·5	1·5	—
Spray-dried	4	23·7	1·5	0	30·3	1·2	0	18	23·7	1·7	3	29·0	1·5	0
Roller-dried	3	25·3	1·5	0	29·9	1·3	0	8	21·0	0·9	14	28·7	1·0	3
Mackerel														
Fresh	3	29·2	1·2	—	10·6	0·9	—	6	28·6	1·0	—	12·4	1·0	—
Steamed	3	31·0	1·2	0	11·5	0·9	0	8	28·1	1·0	0	12·6	1·0	0
Sterilized at 115°	3	30·2	1·2	0	11·2	1·9	0	6	29·5	1·9	0	4·43	0·4	65
Sterilized at 126°	3	29·6	1·2	0	12·9	1·0	0	7	23·1	1·2	19	3·05	0·4	75
Steamed and sterilized at 115°	3	31·1	1·2	0	10·3	0·9	0	6	29·2	1·0	0	4·37	0·4	65
Steamed and sterilized at 126°	3	31·6	1·2	0	11·2	1·0	0	7	23·1	1·0	19	3·15	0·4	75

* For details of methods, see pp. 176–9.

† Calculated as a percentage of values for the unprocessed material.

Table 10. *The sulphur amino acid contents of diets* containing casein and processed products of milk, whey and mackerel, determined by chemical methods†*

(Mean values for four determinations)

Test product	Nitrogen (g/kg diet)			Casein		Test product					
						Methionine (mg/kg)		Cysteine (mg/kg)		Methionine + cysteine (mg/kg)	
	Casein	Test product	Total	Methionine (mg/kg)	Cysteine (mg/kg)	Total	Available	Total	Available	Total	Available
Milk											
Spray-dried	9·96	6·25	16·21	1980	280	1150	1150	340	340	3750	3750
Roller-dried	9·96	6·49	16·45	1980	280	1140	930	330	330	3730	3520
Whey											
Spray-dried	11·21	4·89	16·10	2230	320	660	660	960	950	4170	4160
Roller-dried	11·21	5·01	16·21	2230	320	800	640	960	950	4290	4130
Mackerel											
Fresh	12·81	3·47	16·27	2540	360	620	620	270	270	3790	3790
Sterilized at 126°	12·81	3·42	16·23	2540	360	630	490	280	60	3810	3810

* For details of diets, see Table 2. † For details of methods, see pp. 176–9.

Table 11. *Body-wt gain, food dry-matter* (DM) *intake and food conversion efficiency for rats given diets* containing casein and processed products of milk, whey and mackerel, and sulphur amino acid contents of test products calculated from results obtained from rat bioassays*

(Mean values for five rats/group)

Test product	Body-wt gain (g/rat per 14 d)	Food DM intake (g/rat per 14 d)	Food conversion efficiency (g body-wt gain/ g food DM intake)	Calculated S amino acid content† (mg/kg diet)	Mean
Milk					
Spray-dried	24	85·4	0·27	3810	3740
	18	69·9	0·26	3690	
Roller-dried	16	67·3	0·24	3520	3490
	19	82·1	0·23	3460	
Whey					
Spray-dried	24	78·2	0·33	4280	4280
	24	78·2	0·33	4280	
Roller-dried	25	84·2	0·29	3990	3910
	21	74·7	0·28	3880	
	20	71·5	0·28	3880	
Mackerel					
Fresh	21	71·0	0·30	3990	3960
	21	72·8	0·29	3930	
Sterilized at 126°	21	88·9	0·24	3500	3480
	21	90·9	0·23	3460	

* For details of diets, see Table 2.

† Calculated S amino acid content of test product (mg/kg diet) $= c - a_1$, where c and a_1 are the S amino acid contents (mg/kg) of casein+test product and basal casein diets respectively, calculated from the equation: S amino acid content of diet (mg/kg) $= 147 + 856W$, where W is food conversion efficiency.

cysteine contents, with the exception of a slight decrease (8–9%) in the total cysteine content of spray- and roller-dried milks.

Percentage reductions in the available methionine content were found in the following processed products (relative to fresh form): sweetened, condensed milk, 15; unsweetened, condensed milk, 11; roller-dried milk and whey, 22 and 14 respectively, mackerel sterilized at 126°, 19. There was no decrease in the available methionine contents of spray-dried milk or whey, steamed mackerel or mackerel sterilized at 115°.

The available cysteine content (measured chemically) was 32% lower for sweetened, condensed milk and 9% lower for spray- and roller-dried milks, in which there was also a 9% decrease in total cysteine content. The reduction in the available cysteine content was probably due to destruction of this amino acid.

Decreases in the available cysteine content were also found in mackerel sterilized at 115 and 126° (64 and 75% respectively). There were no changes in available cysteine contents of unsweetened, condensed milk and steamed mackerel.

Generally the total S amino acid contents of the processed products tested were not different from those for the respective unprocessed materials. However, the availability of these amino acids was changed and the extent of the change was dependent on the type of protein and the type of processing.

Table 12. *Values for total and available sulphur amino acid contents of processed products of milk, whey and mackerel, determined by chemical (in vitro) and biological (in vivo) methods**

Test product	S amino acid content (g/kg CP)			Available S amino acid content (% total value)	
	Total	Available: In vitro (*a*)	Available: In vivo (*b*)	*a*	*b*
Milk					
Spray-dried	38·1	37·6	37·9	99	99
Roller-dried	37·5	30·9	31·3	82	82
Whey					
Spray-dried	54·1	52·8	56·6	97	105
Roller-dried	52·2	49·7	43·1	90	78
Mackerel					
Fresh	40·8	41·0	48·9	100	120
Sterilized at 126°	42·5	26·1	27·1	61	64

CP, crude protein (nitrogen × 6·25).
* For details of methods, see pp. 178 and 9.

Table 13. *Body-wt gain, food dry-matter* (DM) *intake and food conversion efficiency for rats given diets containing casein* and roller-dried milk or whey, with or without methionine supplementation*

(Mean values for five rats/group)

Test product	Body-wt gain (g/rat per 14 d)	Food DM intake (g/rat per 14 d)	Food conversion efficiency (g body-wt gain/g food DM intake): Mean	S amino acids (mg/kg diet), determined‡: In vitro	S amino acids (mg/kg diet), determined‡: In vivo
Milk					
Alone	17	74·7	0·23	3520	3490
+Methionine	26	73·6	0·35	4470	4300
	27	76·3	0·35 (mean 0·35)		
Whey†					
Alone	23	76·8	0·29	4130	3910
+Methionine	34	88·3	0·39	4830	4730
	33	89·1	0·37 (mean 0·38)		

* For details of diets, see Table 2. † Mean values from Table 11.
‡ For details of methods, see pp. 178 and 9.

Comparison of in vitro and in vivo values for the availability of S amino acids. To determine whether in vitro values for total and available S amino acid contents were physiologically significant, in vivo rat bioassays were done using spray- or roller-dried milk or whey, fresh mackerel or mackerel sterilized at 126°. The chemically determined total and available S amino acids content for the diet are given in Table 10.

The available S amino acid contents of the diets containing roller-dried milk or whey and that containing sterilized mackerel were lower than the total amount of these amino acids estimated after acid-hydrolysis. In diets containing spray-dried

Table 14. *Net protein utilization* (NPU) *values for spray- and roller-dried milk and whey determined by the method of Miller & Bender* (1955)

(Mean values with their standard errors for four determinations)

	NPU	
Test product	Mean	SE
Milk		
Spray-dried	0·71	0·051
Roller-dried	0·68	0·050
Whey		
Spray-dried	0·94	0·051
Roller-dried	0·78	0·092

products and that containing fresh mackerel, the total S amino acid content was the same as the available S amino acid content.

The body-weight gain, food intake, food conversion efficiency and the calculated S amino acid contents for the diets are shown in Table 11. The calculated S amino acid content was lower for diets containing roller-dried products than for those containing spray-dried products and lower for the diet containing sterilized mackerel than for that containing fresh mackerel. The total S amino acid contents of these products and the available S amino acid contents determined by chemical and biological methods are compared in Table 12. There was good agreement between 'biological' and 'chemical' values for the range of test products.

To determine whether the lower methionine content of roller-dried products was responsible for the poorer growth of test animals a methionine supplement was given. The body-weight gain, food intake, food conversion efficiency and available S amino acid contents are given in Table 13. Methionine supplementation resulted in an improved growth response, suggesting that the poorer growth response for rats given the diets containing roller-dried milk or whey was due to lower contents of available S amino acids. Values for NPU (Table 14) were also lower for roller-dried products compared with the corresponding spray-dried products.

DISCUSSION

Protein-quality evaluation is frequently done using chemical methods. These methods are based on the comparative changes in the amounts of specific amino acids in processed and unprocessed materials. These methods involve some alteration of the polypeptide chain to render individual amino acids accessible for selective chemical reaction, and should be specific for those amino acids which remain unaffected by processing.

Chemical methods have been developed for the estimation of lysine (Carpenter, 1960) and tryptophan (Skibinska & Kakowska-Lipinska, 1971), the two amino acids (other than S amino acids) which most frequently limit the nutritive value of proteins (Autret *et al.* 1968).

The present work describes a chemical method for the evaluation of the available

S amino acid content of proteins. The methods of McCarthy & Sullivan (1941) and Zahler & Cleland (1968) seemed appropriate for the estimation of the amounts of available methionine and cysteine, as oxidized forms of these amino acids do not interfere in these chemical reactions.

The estimation of the available methionine and cysteine content of pure proteins and food proteins using the above methods, after preliminary enzymic hydrolysis of the proteins, gave results consistent with those obtained by the method of Moore *et al.* (1958).

The results of these experiments were in agreement with the general opinion that there are no differences between the total and the available level of amino acids in protein which has not been exposed to technological processing.

In the present work the effect of processing on the content of available methionine and cysteine in selected milk and fish products was also studied.

Processing involved in the preparation of the following fish and milk products: unsweetened and sweetened, condensed milk, roller-dried milk and whey, and mackerel (sterilized at 126°) resulted in decreases in the available methionine contents of 11, 15, 22, 14 and 19% respectively. There was generally no measurable decrease in the available cysteine contents compared to those of the unprocessed materials, with the exceptions of sweetened, condensed milk and mackerel sterilized at 126°, in which decreases of 32 and 75% were found. A decrease of 64% in the amount of the available cysteine was found in mackerel sterilized at 115°.

The results reported here cannot be compared directly with those reported by other authors, as in general they have evaluated the quality of such products in terms of protein utilization.

R. Szklarska-Cyganska (personal communication) reported a 19% decrease in the availability of the methionine in roller-dried milk, determined microbiologically. This result is in agreement with the values obtained in the present work.

From the results for the availability of S amino acids determined using chemical methods, actual availability to the organism can be predicted. Rat bioassays were used to establish whether 'chemically determined' low levels of available S amino acids were significant physiologically, and consideration was given to the cysteine-sparing effect of methionine in the diet.

There was good agreement between results for the availability of S amino acids determined by chemical and biological methods for spray- and roller-dried milk and whey, and mackerel sterilized at 126°. Thus it must be assumed that the decrease in availability of S amino acids, determined chemically, is physiologically significant.

The good agreement between estimates obtained in vivo and in vitro suggests that the chemical methods may be applied as rapid indicator methods for determination of changes in the availability of methionine and cysteine associated with food processing.

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Printed in Great Britain