*Br. J. Nutr.* (1980), **43**, 339



# A method for determination of unoxidized and total methionine in protein concentrates, with special reference to fish meals

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(*Received* 12 *June* 1979 – *Accepted* 11 *September* 1979)

1. An automated colorimetric method for determination of methionine using an iodoplatinate reagent is described. Methionine sulphoxide does not react under the chosen conditions.

2. The method may be used to distinguish between unoxidized and total methionine by doing one determination without and one determination with previous reduction of a portion of the sample with titanium trichloride. Methionine sulphoxide is then obtained by difference.

3. The method has been used with protein concentrates, mainly fish meals, after hydrolysis with barium hydroxide. Interference from cysteine–cystine is eliminated by adding a small amount of cadmium acetate to the sample before hydrolysis.

4. Results obtained for total methionine and for methionine sulphoxide by independent methods show good agreement with results obtained with the iodoplatinate method.

Methionine determined after conventional hydrolysis of proteins in 6 M-hydrochloric acid is the sum of the unoxidized methionine and that formed by reduction of methionine sulphoxide (Njaa, 1962). It has been tacitly assumed either that the amounts of sulphoxide in proteins are negligible, or that L-methionine and its sulphoxide(s) are equally well utilized by experimental animals. Gjøen & Njaa (1977) indicated that up to 30% of the methionine in fish meal could be in the form of sulphoxide, and Ellinger (1978) found approximately 30% in unstabilized fish meal and in grass meal. Values ranging from 4 to 10% were reported for rapeseed flour, fish meal and casein (Slump & Schreuder, 1973; Anderson *et al.* 1975; Sjöberg & Boström, 1977). The biological utilization of protein-bound methionine sulphoxide has been discussed by several authors (Ellinger & Palmer, 1969; Slump & Schreuder, 1973; Gjøen & Njaa, 1977; Sjöberg & Boström, 1977; Ellinger, 1978; Cuq *et al.* 1978).

The chemical methods used in these publications are complicated and time-consuming, and are not well suited for routine analysis of large numbers of samples. This paper describes a relatively simple colorimetric method for determination of methionine in barium hydroxide hydrolysates using an iodoplatinate reagent (Sease *et al.* 1948). Methionine is determined directly as well as after reduction of the methionine sulphoxide present with titanium trichloride (Gawargious, 1971). The former determination gives the amount of unoxidized methionine, the latter gives total methionine not including methionine sulphone. Methionine sulphoxide is obtained by difference between total and unoxidized methionine. Interference from cysteine–cystine (Awwad & Adelstein, 1966) is eliminated by addition of a small amount of cadmium acetate before hydrolysis. The method may be useful for assessment of the biological availability of protein-bound methionine sulphoxide.

## MATERIALS AND METHODS

### *Protein sources*

*Oxidized fish meal.* 100 g commercial fish meal were stirred with 400 ml water, pH was adjusted to 5·5 with 6 M-HCl. The slurry was heated on a water-bath kept at 80–85°. After 30 min 6·7 ml Perhydrol 30% $H_2O_2$ (Merck 7209) were added, heating and stirring were

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continued for 2 h. After cooling the liquid was decanted, the insoluble residue was washed five times with 50 ml portions of water and five times with 50 ml portions of acetone. Between washings the residue was separated by centrifugation. The washed meal was then air-dried.

*Peroxidized fish meal.* To 15 g commercial fish meal were added 20 ml Perhydrol 30% $H_2O_2$ and 200 ml formic acid 98–100%. The slurry was shaken overnight and evaporated in a rotary evaporator. The meal was washed with water several times and then washed with ethanol (960 ml/l) and air-dried.

*Dried eggs.* Whole eggs, egg-yolks and egg-whites were freeze-dried in the laboratory. After drying, whole eggs and egg-yolks were extracted with hexane to remove most of the fat. All samples were then ground to fine meals.

*Fish meals and other protein sources.* The samples analysed were commercial products.

## *The iodoplatinate method*

### (*a*) *Reagents*

*Stock solutions.* L-methionine, L-methionine sulphoxide and L-methionine sulphone were used as 50 mM solutions in 1 M-HCl.

*Working standard methionine.* Stock solution diluted to 1 mM.

*Reagent solutions.* The following solutions were used: 0·15 M-phosphate buffer, pH 7, 0·2 M-acetate buffer, pH 5, 0·53 M-barium chloride, 0·56 M-sodium sulphate, 0·1 M-cadmium acetate, 0·1 M-lead acetate, 0·5 M-sodium hydroxide; titanium trichloride solution contained 5 ml of approximately 150 g $TiCl_3$ in HCl (approximately 100 ml/l) (Merck 808307) diluted to 25 ml with acetate buffer. Iodoplatinate reagent: 0·5 ml hexachloroplatinic IV acid solution (100 ml/l) (Merck 7341), 20 ml 0·66 M-potassium iodide and 20 ml water were mixed and left overnight, then diluted to 500 ml with 1 M-acetic acid.

### (*b*) *Procedures*

*Hydrolysis.* Samples containing approximately 400 mg protein were weighed into polypropylene weighing bottles (60 ml capacity) and mixed with 8·4 g $Ba(OH)_2 . 8H_2O$ using a glass rod. Water (10 ml) and 1 ml 0·1 M-cadmium acetate were added and the glass rod was rinsed with 5 ml water. The bottles were heated at 15 psi for 10 h at 120°. After cooling, the hydrolysates were adjusted to pH 3–4, diluted to 50 ml through a Selecta folding filter No. 597½ or No. 595½ (Schleicher & Schüll).

*Dilution for determination of unoxidized methionine.* Methionine working standards (1, 2, 3 and 4 ml) were pipetted into 50 ml volumetric flasks. $BaCl_2$ (0·53 M, 6 ml) and 6 ml 0·56 M-$Na_2SO_4$ were added and after dilution with phosphate buffer the mixture was filtered through a blue band filter No. 589³ (Schleicher & Schüll).

Depending on the methionine content of the samples 1–6 ml of the filtered hydrolysates were pipetted into 50 ml volumetric flasks. $BaCl_2$ (0·53 M) to a total volume of 6 ml, and 6 ml 0·56 M-$Na_2SO_4$ were added. After dilution with phosphate buffer the mixture was filtered as described previously.

*Reduction for determination of total methionine.* Methionine working standards (1, 2, 3 and 4 ml) were pipetted into screw-capped test tubes (26 mm outside diameter, capacity 30 ml). The volume was adjusted to 14 ml with water and 2 ml $TiCl_3$ solution was added. The tightly capped tubes were heated in a boiling water-bath for 30 min and then cooled in running tap water. NaOH (0·5 M, 5 ml), 6 ml 0·53 M-$BaCl_2$ and 6 ml 0·56 M-$Na_2SO_4$ were added. The mixture was diluted to 50 ml with phosphate buffer and filtered.

The samples were treated in the same way as the standards except that the amount of filtered hydrolysate taken and the amount of 0·53 M-$BaCl_2$ added totalled 6 ml.

*Colorimetric analysis.* The filtered standards and samples were analysed using the

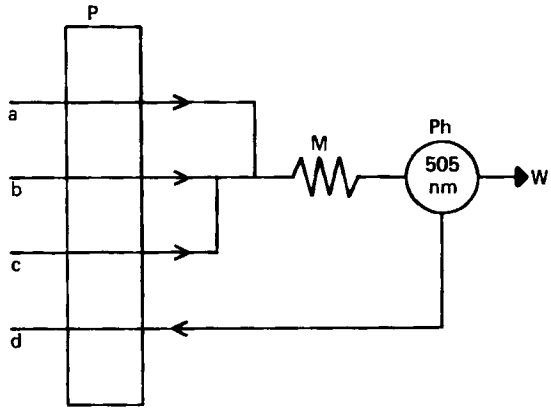


Fig. 1. Manifold used in the colorimetric methionine method. (a), Iodoplatinate reagent (0·32 ml/min); (b), air (0·32 ml/min); (c), sample (0·60 ml/min); (d), return (0·80 ml/min); (P), peristaltic pump; (M) mixing and reaction coil (fourteen turns); Ph, photometer (8 mm flow-cell); W, waste.

Technicon AutoAnalyzer system with the manifold shown in Fig. 1. The bleaching of the iodoplatinate reagent was monitored at 505 nm. The base line was established with water in the sample line.

Standards were run before and after the samples to account for drift. For calculations of the methionine concentrations in the samples regression equations obtained with the standard readings at four points were used. The standards used were in the concentration range 3–12 $\mu$g/ml (approximately 20–80 $\mu$M).

*Analysis of methionine by ion-exchange chromatography.* Cation exchange chromatography was performed for some fish-meal samples (Table 5) using a Technicon Amino Acid Analyzer (model NC-2P). The samples were prepared by two methods. In the first method methionine was oxidized to methionine sulphone essentially by the method described by Weidner & Eggum (1966) with some modifications suggested by B. T. Viuf (personal communication). The modifications involved the use of an amount of sodium pyrosulphite which is stoichiometrically equivalent to the amount of hydrogen peroxide used for oxidation, and evaporation to dryness before adding 6 M-HCl for hydrolysis. After hydrolysis methionine sulphoxide was added as an internal standard. Under the present conditions sulphoxide was eluted as a single peak before methionine sulphone. In no case have we found any methionine sulphoxide in hydrolysates treated as described when samples without added sulphoxide were chromatrographed. In the second method the sample was hydrolysed directly with 70 ml 6 M-HCl containing 2 ml $TiCl_3$ solution. In this case norleucine was used as internal standard.

*Analysis of methionine sulphoxide by the Lunder* (1972) *method.* The method used was essentially as described by Lunder (1972). We used a Parnas-Wagner apparatus for distilling the formaldehyde before the colorimetric determination with chromotropic acid.

## EXPERIMENTS AND RESULTS

### *Expt* 1. *Recovery of methionine from mixtures of methionine, methionine sulphoxide and methionine sulphone*

Appropriate portions of the stock solutions were mixed in 50 ml graduated flasks to give the amounts of methionine, methionine sulphoxide and methionine sulphone indicated in Table 1. The solutions were analysed for unoxidized and total methionine omitting the

Table 1. *Recovery of unoxidized (U) and total methionine (T) from mixtures of methionine, methionine sulphoxide and methionine sulphone*

(Theoretical quantities are given in parentheses)

| Amounts* present (mg) | | | Methionine found (mg) | |
|---|---|---|---|---|
| Methionine | Methionine sulphoxide | Methionine sulphone | U | T |
| 12·0 | 0 | 0 | 11·7 (12·0) | 11·7 (12·0) |
| 9·6 | 0 | 0 | 9·4 (9·6) | 9·5 (9·6) |
| 9·6 | 2·4 | 0 | 9·6 (9·6) | 12·2 (12·0) |
| 9·6 | 2·4 | 2·4 | 9·6 (9·6) | 12·2 (12·0) |
| 9·6 | 0 | 2·4 | 9·4 (9·6) | 9·6 (9·6) |
| 7·2 | 0 | 0 | 7·0 (7·2) | 7·6 (7·2) |
| 7·2 | 4·8 | 0 | 7·0 (7·2) | 11·6 (12·0) |
| 7·2 | 4·8 | 4·8 | 7·1 (7·2) | 12·2 (12·0) |
| 7·2 | 0 | 4·8 | 6·9 (7·2) | 7·5 (7·2) |
| 4·8 | 0 | 0 | 4·4 (4·8) | 4·9 (4·8) |
| 4·8 | 7·2 | 0 | 4·4 (4·8) | 12·1 (12·0) |
| 4·8 | 7·2 | 7·2 | 4·3 (4·8) | 12·1 (12·0) |
| 4·8 | 0 | 7·2 | 4·4 (4·8) | 4·6 (4·8) |
| 2·4 | 0 | 0 | 2·2 (2·4) | 2·9 (2·4) |
| 2·4 | 9·6 | 0 | 2·0 (2·4) | 12·5 (12·0) |
| 2·4 | 9·6 | 9·6 | 2·3 (2·4) | 12·1 (12·0) |
| 2·4 | 0 | 9·6 | 2·2 (2·4) | 2·1 (2·4) |
| 0 | 0 | 0 | tr (0) | tr (0) |
| 0 | 12 0 | 0 | tr (0) | 12·5 (12·0) |
| 0 | 12·0 | 12·0 | tr (0) | 12·1 (12·0) |
| 0 | 0 | 12·0 | tr (0) | tr (0) |

tr, trace.
* Methionine equivalents.

hydrolysis step. The results obtained are given in Table 1 together with the theoretical values. The mean (±SD) recovery of unoxidized methionine was 94·3±4·5% and of total methionine 101·3±6·2%. The recoveries of unoxidized methionine at the four levels 9·6, 7·2, 4·8 and 2·4 mg (or 80, 60, 40 and 20% of the total) were analysed further in an analysis of variance. There was no significant effect of the additions of methionine sulphoxide or of methionine sulphone, or of the two together. Between levels there were significant differences, the mean recoveries being 98·9, 97·2, 91·2 and 90·6% at the four levels respectively. The linear component of the sum of squares was significant ($P < 0{\cdot}01$). Thus, recovery of unoxidized methionine was very satisfactory at least down to a level of 60% of the total.

*Expt 2. Interference from cysteine–cystine and some other sulphur-containing compounds*

To 7·2 mg methionine was added 3 mg cysteine as cysteine HCl.$H_2O$ or equivalent amounts of lanthionine and cystathionine. Methionine with one of these additions as well as methionine without such addition were hydrolysed with barium hydroxide alone, or with either cadmium acetate or lead acetate. The results shown in Table 2 indicate that neither cysteine nor lanthionine interfered with the determination of unoxidized methionine irrespective of whether cadmium acetate or lead acetate were added. However, both interfered with the determination of total methionine if the salts were not added. Cystathionine interfered in either instance. This and similar experiments indicated that more consistent results were obtained with cadmium acetate than with lead acetate. Cadmium acetate addition was therefore included in the hydrolysis procedure.

With the method adopted the following S-containing compounds were found to interfere with the methionine analysis: homocysteine, homocystine, ethionine, cystathionine, methionine ethyl ester, N-acetylmethionine.

Table 2. *Analyses of unoxidized (U) and total methionine (T) in solutions containing 7·2 mg methionine alone or with additions of 3 mg cysteine as cysteine hydrochloride hydrate or equivalent amounts of lanthionine or cystathionine. (Solutions were hydrolysed in the presence or absence of cadmium acetate or lead acetate)*

| Form of acetate added --- S-amino acid added | None | Cd | Pb | None | Cd | Pb |
|---|---|---|---|---|---|---|
| | Methionine found (mg) | | | | | |
| | U | | | T | | |
| None | 7·1 | — | — | 7·6 | — | — |
| Cysteine | 7·3 | 7·3 | 7·6 | 8·9 | 7·3 | 7·6 |
| Lanthionine | 7·4 | 7·1 | 7·3 | 9·0 | 7·0 | 7·6 |
| Cystathionine | 11·3 | 11·4 | 11·3 | 11·2 | 11·4 | 11·4 |

The following did not interfere: methionine sulphone, S-methylcysteine, taurine, lanthionine, glutathione, cysteic acid, homocysteic acid, sodium sulphite, sodium thiosulphate, sodium hydrogen sulphite.

The common amino acids: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine did not interfere.

### *Expt 3. Recovery of methionine from mixtures of methionine and methionine sulphoxide added to a peroxidized fish meal, with and without added cysteine*

To portions of 500 mg peroxidized fish meal, in which all methionine and cysteine had been oxidized to methionine sulphone and cysteic acid, were added different amounts of methionine and methionine sulphoxide. The total amount added was equivalent to 15 mg methionine, the portion of methionine sulphoxide varying from 0 to 40% of the total. To one portion of each sample 5 mg cysteine were added and one portion had no such addition. After hydrolysis with cadmium acetate, unoxidized and total methionine were determined. The results obtained together with the theoretical results are given in Table 3.

The mean recoveries for unoxidized methionine were $99{\cdot}8 \pm 3{\cdot}0\%$ without added cysteine and $98{\cdot}4 \pm 1{\cdot}5\%$ with added cysteine. Corresponding recoveries were $100{\cdot}0 \pm 4{\cdot}6\%$ and $96{\cdot}8 \pm 2{\cdot}5\%$ for total methionine. The recoveries were slightly lower with added cysteine than without, the over-all difference of 2·5% being just significant ($P < 0{\cdot}05$).

### *Expt 4. Methionine in an oxidized fish meal, a peroxidized fish meal and casein, and in mixtures of these*

The three protein sources used in this experiment were analysed for unoxidized and total methionine separately and in 1:1 and 1:1:1 mixtures. The results obtained are given in Table 4 together with the expected values for the mixtures. The expected values were calculated from the values obtained for the separate protein sources; in these calculations traces were assumed to be zero. The general trend that analysed values were slightly higher than the calculated values may indicate that the traces were in fact greater than zero.

### *Expt 5. Comparison of the iodoplatinate method with two chromatographic methods*

Ten samples of commercial fish meals were analysed for total methionine by the iodoplatinate method, and by chromatography either after oxidation to methionine sulphone, or directly in a hydrolysate containing $TiCl_3$ to protect methionine against oxidation.

Table 3. *Recovery of unoxidized (U) and total methionine (T) from a total amount of 15 mg methionine added to 500 mg of a peroxidized fish meal; methionine sulphoxide accounted for from 0 to 40% of the total; each sulphoxide level was analysed with (+) and without (−) addition of 5 mg cysteine*

(Theoretical quantities are given in parentheses)

| Added methionine as sulphoxide (% of total) | Cysteine | Methionine found (mg) | |
|---|---|---|---|
| | | U | T |
| 0 | − | 14·6 (15·0) | 16·0 (15·0) |
| 0 | + | 14·4 (15·0) | 15·1 (15·0) |
| 10 | − | 13·2 (13·5) | 15·2 (15·0) |
| 10 | + | 13·2 (13·5) | 14·3 (15·0) |
| 20 | − | 11·9 (12·0) | 14·4 (15·0) |
| 20 | + | 11·9 (12·0) | 14·7 (15·0) |
| 30 | − | 11·0 (10·5) | 15·1 (15·0) |
| 30 | + | 10·4 (10·5) | 14·3 (15·0) |
| 40 | − | 9·0 (9·0) | 14·3 (15·0) |
| 40 | + | 9·0 (9·0) | 14·2 (15·0) |

Table 4. *Unoxidized (U) and total methionine (T) in an oxidized fish meal (1), in a peroxidized fish meal (2) and in casein (3), and in 1:1 and 1:1:1 mixtures*

(Expected values calculated from 1, 2, 3 are given in parentheses)

| Sample | Methionine found (g/kg sample) | |
|---|---|---|
| | U | T |
| 1 | tr | 29 |
| 2 | tr | tr |
| 3 | 25 | 27 |
| 1+2 | tr | 15 (14·5) |
| 1+3 | 14 (12·5) | 28 (28) |
| 2+3 | 13 (12·5) | 15 (13·5) |
| 1+2+3 | 9 (8·3) | 19 (18·7) |

tr, trace.

Table 5. *Total methionine in ten fish meals determined by the iodoplatinate method (A) and cation-exchange chromatography after oxidation of methionine to methionine sulphone (B) or after hydrolysis with titanium trichloride present (C)*

| Fish meal no. | Total methionine (g/kg protein) | | |
|---|---|---|---|
| | A | B | C |
| 1 | 31 | 35 | 29 |
| 2 | 33 | 25 | 34 |
| 3 | 32 | 31 | 32 |
| 4 | 29 | 31 | 30 |
| 5 | 29 | 25 | 28 |
| 6 | 33 | 31 | 28 |
| 7 | 31 | 29 | 29 |
| 8 | 29 | 28 | 26 |
| 9 | 32 | 28 | 34 |
| 10 | 36 | 36 | 36 |
| Mean ± SD | 31·5 ± 2·2 | 29·7 ± 3·4 | 30·6 ± 3·2 |

Table 6. *Unoxidized (U) and total methionine (T) (g/kg protein) in two fish meals (16/78 and 28/78) analysed by two persons (A.S. and E.H.) on four different days and with five new hydrolysates of each meal on each day*

(Mean values and standard deviations)

| | Fish meal 16/78 | | | | | | Fish meal 28/78 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | U | | T | | U/T | | U | | T | | U/T | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Day 1 (A.S.) | 24·1 | 0·3 | 31·5 | 0·5 | 0·765 | 0·017 | 24·2 | 0·8 | 30·6 | 1·2 | 0·792 | 0·016 |
| Day 2 (E.H.) | 24·1 | 0·4 | 33·6 | 2·3 | 0·720 | 0·060 | 24·6 | 0·3 | 32·1 | 2·2 | 0·769 | 0·053 |
| Day 3 (A.S.) | 24·4 | 0·4 | 33·9 | 0·6 | 0·721 | 0·016 | 24·4 | 0·4 | 32·1 | 0·4 | 0·761 | 0·009 |
| Day 4 (E.H.) | 23·8 | 0·3 | 31·0 | 0·6 | 0·768 | 0·018 | 24·4 | 0·3 | 30·1 | 0·5 | 0·821 | 0·019 |

The results are given in Table 5. Analysis of variance showed no significant difference between methods. The coefficients of variations were between 7 and 11 for the three methods, being lowest for the iodoplatinate method. With such small variations between samples good correlations between methods could not be expected. Only the correlation between the iodoplatinate method and the direct chromatographic method was significant ($P < 0{\cdot}02$).

### *Expt 6. Comparison of results obtained for the methionine sulphoxide content in ten fish meals obtained by Lunder's (1972) method, and by difference between total and unoxidized methionine with the iodoplatinate method*

Ten commercial fish meals were analysed by Lunder's (1972) direct method based on the measurement of formaldehyde formed by treating methionine sulphoxide in intact proteins with acetic anhydride. The first batches of acetic acid and acetic anhydride used were satisfactory but later there were some difficulties with the method because of high 'blank' values with new batches. The results presented in Fig. 2 are mean values. For comparison the results obtained by difference between total and unoxidized methionine by the iodoplatinate method are given. The agreement between methods is fairly good and lies well within the errors inherent in both methods. Because of the high 'blank' values due to acetic acid and acetic anhydride Lunder's (1972) method was found to be difficult to use.

### *Expt 7. Reproducibility of results*

In Table 6 are given the results obtained with two commercial fish meals which were analysed for unoxidized and total methionine on four different days. Two technicians performed analyses on 2 d each. For each day's analyses five hydrolysates of each fish meal were prepared, thus in all twenty hydrolysates of each fish meal were analysed. The results show good agreement both between days and between technicians for unoxidized methionine for both meals. For total methionine both technicians found mean values which were 5–8% higher on one of the days than on the other. This is probably within the error of the method but it may to some extent be compensated if a standard protein is run each time.

### *Expt 8. Methionine in various protein sources analysed with the iodoplatinate method*

Whereas our main concern has been analyses of fish and fish products, the iodoplatinate method has also been applied for analyses of some other protein sources. In Table 7 results obtained with some of these are presented. The results indicate that methionine sulphoxide was present in all the samples tested, but mostly in small amounts. The outstanding exceptions were gelatin and Promine D, in which practically no unoxidized methionine was found. These analyses were repeated several times with the same result. Cod sample No. 23

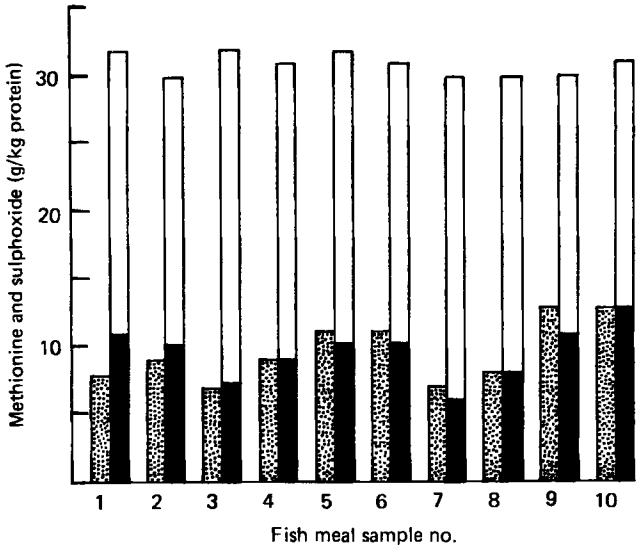


Fig. 2. Methionine sulphoxide in ten fish meals determined by Lunder's (1972) method (▦) and by difference between total methionine (▬) and unoxidized methionine (□). The results are given as methionine equivalents.

Table 7. *Unoxidized (U) and total methionine (T) (g/kg protein) in some protein sources determined with the iodoplatinate method*

| | U | T |
|---|---|---|
| Casein | 29 | 34 |
| Egg (whole) | 34 | 38 |
| Egg (white) | 40 | 44 |
| Egg (yolk) | 27 | 29 |
| Gelatin | tr | 10 |
| Fish (cod sample no. 23)* | 31 | 33 |
| Milk (dried skimmed) | 32 | 36 |
| Beans (brown) | 11 | 13 |
| Gluten (maize) | 18 | 28 |
| Gluten (wheat) | 16 | 19 |
| Groundnut meal | 10 | 15 |
| Peas (green) | 9 | 11 |
| Promine D | tr | 15 |
| Rapeseed meal | 11 | 16 |
| Soya-bean meal | 14 | 19 |
| Soyamin | 12 | 16 |
| Toprina (BP) | 20 | 27 |

tr, trace.

* The sample was obtained from Dr K. J. Carpenter (School of Agriculture, University of Cambridge) in 1967 and analysed in 1967 by cation-exchange chromatography. Results from several other laboratories were published by Miller *et al.* (1965).

was of special interest because it was over 10 years old when it was analysed. It showed practically no oxidation of methionine despite the fact that no special precautions were taken during the storage time since it was analysed first by cation-exchange chromatography in 1967.

## DISCUSSION

The present method distinguishes satisfactorily between unoxidized and total methionine when methionine and methionine sulphoxide are mixed and analysed without hydrolysis (Table 1), when they are added to a peroxidized fish meal and analysed after hydrolysis (Table 3), and when they are constituents of proteins which are analysed alone or mixed together (Table 4). Methionine sulphone did not interfere, and the interference by cysteine–cystine could be eliminated by adding cadmium acetate to the sample before hydrolysis (Tables 1–4). Results for total methionine agreed well with results obtained chromatographically with fish meals which had been treated with performic acid (Weidner & Eggum, 1966) or which had been hydrolysed with $TiCl_3$ present to protect methionine against oxidation (Moorhouse *et al.* 1977).

Pieniązek *et al* (1975) distinguished between available and total methionine. Available methionine in their work is identical with unoxidized methionine in the present work, whereas total methionine in their work may have included methionine sulphone. Before it has been conclusively established to what extent methionine sulphoxide may be utilized by experimental animals (Gjøen & Njaa, 1977) it seems premature to use the term 'available' only for unoxidized methionine.

In the interpretation of experiments to establish the biological availability of methionine and methionine sulphoxide, when they occur together in proteins, the present method offers the advantage that unoxidized and total methionine are determined in the same hydrolysate by use of the same colorimetric method. Most other chemical methods require two or three hydrolysates (Slump & Schreuder, 1973; Anderson *et al.* 1975) or different techniques (Lunder, 1972; Pieniązek *et al.* 1975; Ellinger, 1978) to differentiate between methionine and methionine sulphoxide. An alternative is to determine methionine and its oxidation products in alkali hydrolysates by ion-exchange chromatography (Cuq *et al.* 1978).

The following points are of significance in judging the usefulness of the present method.

*Hydrolysis.* Both methionine and methionine sulphoxide seem to be stable under the conditions chosen. In contrast, the use of NaOH (5 M) instead of barium hydroxide showed poor recoveries. Also, there was less discoloration of the hydrolysates with barium hydroxide than with sodium hydroxide. Discoloration is often a problem with the McCarthy & Sullivan (1941) method so that a decoloration step may be necessary (Smith & Elmayergi, 1971). The use of $Ba(OH)_2$ has the disadvantage that the barium ion must be removed before the colorimetric reading because reaction with carbon dioxide in the air may cause turbidity from barium carbonate. To compensate for the effect of the precipitated barium sulphate in the analysis, the Ba concentration in samples and standards was kept constant in the final dilution.

*Sensitivity.* The sensitivity of the iodoplatinate method was high, the lowest standard used was 20 $\mu$M. In contrast, the lowest standard used by Ussary & Gehrke (1970) in an automated version of the McCarthy & Sullivan (1941) method was 300 $\mu$M; whereas the lowest standard in the manual method of Smith & Elmayergi (1971) was 100 $\mu$M. Thus, because of the high dilution, discoloration of the hydrolysates is of minor importance in the present method.

With the method described 5 g methionine/kg protein may be determined when approximately 400 mg protein are taken for hydrolysis. The method was not used with samples containing less than 10 g/kg protein, but values lower than 5 g unoxidized methionine/kg protein were found in a few samples (Table 7). So far the method has not been found to be satisfactory with samples containing less than 200 g protein/kg sample on a dry-matter basis.

*Interference.* There was no interference from the common amino acids, the known reaction between cysteine–cystine and iodoplatinate (Awwad & Adelstein, 1966) was eliminated by

adding cadmium acetate in the hydrolysis step. In the McCarthy & Sullivan (1941) method there is interference from histidine and tryptophan (Ussary & Gehrke, 1970). They eliminated this interference by adding an excess of glycine as suggested by McCarthy & Sullivan (1941) but this does not always seem to be effective (Smith & Elmayergi, 1971).

In the analysis of total methionine it was found that titanium must be removed after reduction because it interferes with the iodoplatinate reagent. This was accomplished by adjusting to pH 7 (addition of 5 ml 0·5 M-NaOH, diluting with phosphate buffer). Titanium was then quantitatively precipitated and removed by filtration.

*Iodoplatinate reagent.* It is important that the molar ratio, potassium iodide:chloroplatinate is high. In the present method the value was approximately 100. Awwad & Adelstein (1966), Fowler & Robins (1972) and Moorhouse *et al.* (1977) used values of approximately 70, 90 and 75 respectively. The value 2·5 first used by Barber (1967) was totally unsatisfactory in a flow system (Fowler & Robins, 1972).

The concentration of 1·0 M-acetic acid in the reagent was found to be satisfactory. In contrast Awwad & Adelstein (1966) and Fowler & Robins (1972) used approximately 7 M-acetic acid, whereas Moorhouse *et al.* (1977) used 6 M-HCl. It is an advantage to be able to work with the weaker acid solution in the flow system, and it is also more convenient for those handling the reagent.

*Routine analysis.* The present method is sufficiently simple to be used in routine analyses of many samples. In our experience twenty hydrolysates which have been neutralized and diluted may be handled by one person within a working day of 8 h.

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*Printed in Great Britain*