A method for the reduction of methionine sulphoxide to methionine using titanium trichloride is described. This method, coupled with the gas chromatographic determination of methionine after reaction with CNBr, measures methionine sulphoxide by the increase in methionine values after reduction with titanium trichloride. Results obtained for total methionine by gas chromatography after reduction and CNBr reaction, showed close agreement with those obtained by ion-exchange chromatography after performic acid oxidation. This method was applied to pure proteins which had been subjected to hydrogen peroxide oxidation, and to a wide range of food proteins. An evaluation of other, but less effective, reducing agents is briefly discussed.

1. Introduction

Analysis by ion-exchange chromatography of total methionine content as methionine sulphone in acid hydrolysates of performic acid-oxidised proteins has been regarded as the most reliable method for this amino acid. This method, however, has some disadvantages in that it does not distinguish between methionine, methionine sulphoxide or pre-existing methionine sulphone, and does not account for the interconversion of these components that occurs during acid hydrolysis. This is particularly important in view of the current interest in the effects of oxidation on the nutritional value of proteins. There are several existing methods for the determination of methionine and methionine sulphoxide, but the simplicity of the gas chromatographic determination of unmodified methionine prompted the authors to combine this technique with a chemical reduction procedure. The objective, therefore, was to reduce methionine sulphoxide to methionine without including any methionine sulphone that might be present. Preliminary experiments using titanium trichloride as a reducing agent were promising, and this paper describes the development of a quantitative assay together with its application to a variety of food proteins.

2. Experimental

2.1. Materials

2.1.1. Reagents for reduction

Hydrochloric acid (Analar) and titanium trichloride solution (150 g litre⁻¹), which was low in iron and contained zinc chloride, were purchased from BDH Chemicals, Poole, Dorset.

2.1.2. Reagents for methionine determinations

The reagents were prepared as described previously. The gas chromatography column packing material was changed from Porapak P-S to Chromosorb 101, 80–100 mesh (Perkin Elmer), as modification in the production process of Porapak reduced its effectiveness under the experimental conditions of the method.
2.1.3. Sample preparation

Samples were ground to pass through a 60 mesh sieve and dried to constant weight.

2.1.4. Preparation of oxidised proteins

Oxidised lactalbumin was prepared by treating lactalbumin (Sigma) with hydrogen peroxide (Analar) under conditions which favoured oxidation of methionyl residues to the sulphoxide. Lactalbumin (100 g) was suspended in 650 ml 0.5M hydrochloric acid : methanol (3:2 v/v). A volume of hydrogen peroxide (300 g litre\(^{-1}\) H\(_2\)O\(_2\)) equivalent to a 20% excess was added, and the mixture stirred vigorously for 3 h at room temperature. After settling overnight, the lactalbumin was washed until free of peroxide and then dried. The absence of methionine sulphone was established by ion-exchange chromatography after direct hydrolysis.

Oxidised casein was prepared from commercial casein (Glaxo) as described for lactalbumin, but with an 80% excess of hydrogen peroxide.

2.2. Method

Samples, containing approximately 5 \(\mu\)mol of methionine, were dispersed in 15% (1M) titanium trichloride : conc. hydrochloric acid (20:1 v/v). The volume used for reduction was dependent on the amount necessary to wet the samples: for pure proteins and fish-meals, 100 \(\mu\)l was sufficient, whereas cereals and legumes required up to 400 \(\mu\)l. To improve penetration of the reagent, samples were subjected to ultrasonic treatment for 15 min at room temperature, and were then heated for 100 min at 70°C. Concentrated formic acid was added to give maximum swelling and therefore easier access to the CNBr reagent. The CNBr was added in aqueous formic acid to give a final reaction volume of 3 ml 70% formic acid. The weight of CNBr used in the reaction for all fish products was decreased from 65 mg to 20 mg to prevent the formation of a product which interfered at the gas chromatographic stage.

3. Results and discussion

Several reducing agents were evaluated under various conditions of pH and using different organic solvents. These included mercaptooethanol, dithioerythritol and thioglycollic acid, but only the latter gave substantial recoveries of reduced methionine. However, during the subsequent gas chromatographic stage using the method of Ellinger and Duncan, thioglycollic acid produced interference which proved time-consuming to remove by extraction procedures.

Dichloroborane, a specific reagent for the reduction of sulphoxides, was synthesised and conditions were established to give substantial but variable recoveries (80–90%) of methionine. The hazardous nature of the reagent required precautions for the exclusion of air and moisture from the reaction, and the extraction of the reagent before gas chromatographic analysis. The method was therefore complicated and unsuitable for routine analysis.

Titanium trichloride was not originally considered because the reaction was initially reported to require concentrated acetic acid which was known to interfere with the subsequent gas chromatography. Reduction with titanium trichloride acidified with hydrochloric acid was, however, found to be a viable alternative and this forms the basis of the method described.

Results for the oxidised samples of casein and lactalbumin (Table 1) show that peptide-bound methionine sulphoxide is quantitatively reduced by acidified titanium trichloride. By contrast, free methionine sulphoxide was found to be only 20% reduced under these conditions. Low recoveries of reduced methionine were obtained when the titanium trichloride had oxidised on storage. This was prevented by maintaining the level of the solution in the neck of the bottle by the addition of glass beads. Provided that the titanium trichloride had not oxidised, a more dilute solution (0.1M) also gave 100% recoveries of methionine. Optical absorption characteristics changed with oxidation of the reagent, and the decrease in absorbance at 500 nm compared with that of the freshly opened bottle, was used to monitor the oxidation.

The method was applied to a wide range of food proteins including fish-meals, leaf proteins,
Table 1. Results for methionine content of some food proteins (g kg⁻¹ protein). Values are the means ± s.e. (mean) for the numbers of determinations given in parentheses.

<table>
<thead>
<tr>
<th>Sample</th>
<th>g.l.c. after CNBr reaction</th>
<th>Ion-exchange of methionine sulphone after performic acid oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unreduced</td>
<td>Reduced with TiCl₃</td>
</tr>
<tr>
<td>Casein</td>
<td>30.7±0.4 (6)</td>
<td>29.1±0.3 (5)</td>
</tr>
<tr>
<td>Oxidised casein</td>
<td>0.0</td>
<td>25.9±0.3 (7)</td>
</tr>
<tr>
<td>Lactalbumin</td>
<td>24.4±0.3 (5)</td>
<td>24.7±0.3 (4)</td>
</tr>
<tr>
<td>Oxidised lactalbumin</td>
<td>8.2±0.1 (5)</td>
<td>23.7±0.3 (7)</td>
</tr>
<tr>
<td>Pilchard meal</td>
<td>19.2±0.2 (4)</td>
<td>26.0±0.4 (4)</td>
</tr>
<tr>
<td>Stabilised pilchard meal</td>
<td>24.0±0.1 (4)</td>
<td>25.0±0.3 (4)</td>
</tr>
<tr>
<td>Lucerne leaf protein (after 4 years storage)</td>
<td>18.8±0.2 (6)</td>
<td>21.4±0.5 (4)</td>
</tr>
<tr>
<td>Lucerne leaf protein</td>
<td>15.4±0.2 (2)</td>
<td>21.2±0.1 (2)</td>
</tr>
<tr>
<td>(after 4 years storage)</td>
<td></td>
<td>21.2±0.6 (2)</td>
</tr>
<tr>
<td>Blood-meal—ring dried</td>
<td>15.9±0.2 (3)</td>
<td>13.8±0.0 (3)</td>
</tr>
<tr>
<td>—vat dried</td>
<td>14.3±0.2 (4)</td>
<td>9.8±0.1 (5)</td>
</tr>
<tr>
<td>—commercial</td>
<td>10.8±0.2 (3)</td>
<td>8.4±0.2 (2)</td>
</tr>
<tr>
<td>Soya bean meal</td>
<td>13.2±0.2 (6)</td>
<td>13.7±0.2 (3)</td>
</tr>
<tr>
<td>Hydrocarbon yeast</td>
<td>14.1±0.1 (4)</td>
<td>17.2±0.1 (6)</td>
</tr>
<tr>
<td>Milk powder</td>
<td>29.4±0.2 (5)</td>
<td>27.8±0.5 (4)</td>
</tr>
</tbody>
</table>


Blood-meals, milk powders, soya bean meal and yeast, and the results were compared with those obtained by ion-exchange chromatography (Table 1) after performic acid oxidation. Agreement between the two methods was generally good, except in cases where samples had been subjected to specific chemical or physical pre-treatments. Methionine values for the oxidised samples of casein and lactalbumin by ion-exchange chromatography after performic acid oxidation were low; this was probably due to acid denaturation, which prevented complete access of the performic acid, to give approximately 90% recoveries of methionine. Incomplete oxidation was confirmed by the presence of methionine in addition to methionine sulphone. Normally chromatograms for methionine sulphone are not continued to the emergence of the methionine peak, a factor that may account for some low methionine values being reported in the literature. Combination of the methionine and methionine sulphone gave values which agreed with those obtained by gas chromatography after titanium trichloride reduction and CNBr reaction, and with values for unoxidised lactalbumin.

Methionine values for blood-meals by ion-exchange chromatography after performic acid oxidation depended on their manufacturing process. Vat-dried blood-meal, for example, gave exceptionally low methionine values (9.8 g kg⁻¹ protein) when determined as the sulphone. Methionine values, when determined as methionine and methionine sulphone after direct hydrolysis and omission of the performic acid oxidation step, were much higher (12.8 g kg⁻¹ protein) and in better agreement with those obtained by gas chromatography after reduction with titanium trichloride and CNBr reaction (Table 1). This suggests that the performic acid reagent gains only limited access to methionine residues in such meals. For blood-meals in particular, analysis of total methionine by gas chromatography after reduction and CNBr reaction, is considered more reliable than by ion-exchange chromatography after performic acid oxidation.

4. Conclusions

Total methionine analyses by gas chromatography after reduction of methionine sulphone and CNBr reaction, were carried out on isolated proteins and a wide range of food proteins. Results generally agreed with the ion-exchange method after performic acid oxidation. The reduction method, however, involved no transfers and was less time-consuming. With certain products, total methionine values were low when determined as the sulphone because of specific chemical or physical pre-treatments, which reduced access of the performic acid to the protein. Examples were
casein treated with acidified hydrogen peroxide and heat treated blood-meals. The reduction method for determining total methionine was therefore preferred for routine analysis. By omitting the reduction step with titanium trichloride, the method effectively differentiated between methionine and methionine sulphoxide; hence oxidation of food proteins may now be assessed and the degree of oxidation related to their nutritional value.

Acknowledgement

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References


Note: Results of the following comparable investigation were published during preparation of this paper.