A RAPID DETERMINATION OF METHIONINE IN CRUDE PROTEINS

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The determination of the methionine content of proteins ordinarily is a time-consuming procedure fraught with the danger of methionine destruction unless the protein is highly purified. The acid hydrolysis of proteins preliminary to amino acid determination usually requires 8 to 48 hours or longer. Hess and Sullivan (1945) have determined the methionine content of a group of proteins employing a 2-hour, alkaline hydrolysis. In these cases the proteins were highly purified. McCarthy (1940) had previously found that hydrochloric acid hydrolysis of casein for 1 hour was as satisfactory as a 24-hour hydrolysis for methionine determination.

The present investigation was undertaken as a result of several unsuccessful attempts to apply the McCarthy-Sullivan (1941) procedure for methionine determination to impure commercial protein samples. Using 6 N hydrochloric acid as the hydrolyzing agent excessive destruction of methionine was encountered in some samples. Even when the time of hydrolysis was decreased to 1 hour more than 30 per cent of the methionine in gluten was destroyed. One-hour hydrolysis failed to dissolve completely some of the other proteins used. When the alkaline hydrolysis procedure of Hess and Sullivan (1945) was employed with these impure samples some proteins gave excellent analytical data compared with standard procedures. Other protein samples could not be analyzed satisfactorily by this procedure because of heavy precipitates formed

in the final step of color development. As a result of the present investigation we have found that 1/2 hour, alkaline hydrolysates may be used successfully in conjunction with the McCarthy-Sullivan reaction for methionine when the procedure is modified and the final acidification in the procedure is done with n-heptafluorobutyric acid instead of the hydrochloric acid-phosphoric acid mixture originally employed.

MATERIALS

Five commercial samples of proteins were taken from stock and pulverized to pass through a 100-mesh sieve. The nitrogen content ranged from 12.2% for the egg albumin to 16.0% for the edestin.

The n-heptafluorobutyric acid was purchased from Matheson, Coleman and Bell and used without further purification.

EXPERIMENTAL

Hydrolysis of Proteins. The partial hydrolysis of proteins was accomplished by adding 40 mg. of protein to a screw-capped test tube, 20 x 110 mm. Two ml. of water and 6 ml. of 10% sodium hydroxide were added to the test tube. The tube was sealed tightly with the rubber-lined cap, vigorously shaken, and submerged in an oil bath at 115°C for 30 minutes. The tube was inverted several times during the period to ensure solution. The test tube was cooled to room temperature and the color-forming reaction was run directly in this hydrolysis tube.

Analysis of Hydrolysates. To the hydrolysate were added the following reagents, shaking the capped tubes vigorously after each addition and waiting 10 minutes after each addition:

0.3 ml. 10% sodium nitroprusside

2.0 ml. 3% glycine

4.0 ml. n-heptafluorobutyric acid

The tubes were kept in a large water bath at room temperature during this color development period, and were shaken occasionally. Ten minutes after the addition of the n-heptafluorobutyric acid the red color was read at 510 mμ against a standard methionine solution containing 0.8 mg./2ml. Six ml. of 10% sodium hydroxide were added to the standard and the methionine color was developed by the above analytical procedure along with the hydrolysate.

RESULTS

Table I compares the results of methionine analysis by the shortened procedure described above with those obtained by the standard 8-hour hydrolysis with 20% hydrochloric acid using the modification of the McCarthy-Sullivan reaction described by Horn et al (1946). The acid hydrolysis value for gluten A is an average value of 15 8-hour hydrolyzed samples, 3 1-hour hydrolyzed samples and 3 24-hour samples.

Table I.

Grams of Methionine per 16 Grams of Protein Nitrogen

Protein	1/2 hr. NaOH hydrolysis	8 Hr. HCl hydrolysis	Literature values (Aver.) Block and Weiss (1956)
egg albumin	4.31	4.43	4.9
casein	3.23	3.27	3.4
edestin	1.88	1.78	1.9
zein	1.84	1.80	1.7
gluten A	1.61	0.94	1.6
gluten A(Purified)	1.57	1.52	1.8

DISCUSSION

The first four proteins in the table gave clear, red solutions and readily reproducible results which compare favorably with the 8-hour acid hydrolysates.

The final colored solution in the gluten determination had a

slight opalescence. Centrifugation of this solution gave a completely satisfactory spectrophotometric sample. The results were in excellent agreement with literature values. Results of methionine determination of gluten using acid hydrolysis for periods of 1 hour, 8 hours or 24 hours showed considerable destruction of methionine. Hydrolyses which were protected with nitrogen or carbon dioxide during heating, or using hydrochloric-formic acid mixtures, failed to prevent destruction of methionine. Even the 1-hour acid hydrolysate had more than 30% destruction. Only after purification of the gluten sample were acceptable results obtained by the acid hydrolysis procedure. The successful analysis of the impure commercial sample of gluten suggests the possible wide application of this modified procedure to a wide variety of impure protein samples.

The modified colorimetric procedure was thoroughly tested for recovery of methionine under hydrolysis conditions; recovery of methionine where cystine was present in amounts up to 7.5 times the methionine concentration; and recovery where the tryptophane and histidine concentrations were equal to the methionine concentration.

SUMMARY

A procedure for the determination of the methionine content of proteins in 1 hour, including hydrolysis time, is described. The procedure also permits the analysis of relatively impure samples in this brief period giving good results in agreement with the literature and with samples analyzed by standard procedures.

BIBLIOGRAPHY

Block, R. J. and Weiss, K., Amino Acid Handbook, Charles C. Springfield, Illinois (1956)