# Possible Interference of Fats, Carbohydrates, and Salts in Amino Acid Determinations in Fish Meals, Fish Protein Concentrates, and Mixed Animal Feeds

PRESTON SMITH, Jr., M. E. AMBROSE, and G. M. KNOBL, Jr.

Bureau of Commercial Fisheries Technological Laboratory, College Park, Md.

In the preparation of protein hydrolyzates for amino acid determination, the presence of nonprotein components may result in the destruction of some amino acids. This study was to determine if destruction would be caused by the fats, carbohydrates, and salts present in fish meals, fish protein concentrates, or mixed animal feeds. In general, lipids, salts, and most levels of carbohydrate had no effect. High levels of carbohydrate, however, interfered in the case of some amino acids, particularly proline and tyrosine.

ACCURATE determination of amino acids in protein by automatic chromatography is important in our nutrition research because the data so obtained can be utilized as a rapid, additive tool for evaluating the quality of the protein in our fishery products.

Potential conditions affecting the destruction of amino acids for preparative acid hydrolysis include the purity and quantity of the acid used and the length of time of hydrolysis. There, however, has been little information on the optimum conditions for hydrolyzing the protein of fishery products with hydrochloric acid.

In the hydrolysis of proteins, the nature of both the protein molecule and the nonprotein components, either naturally occurring or added, may also affect the destruction of some amino acids—even under so-called optimum conditions (*2–7*). Interfering materials such as fats, carbohydrates, and salts have been studied in conjunction with proteins of some types of foods, but very little work has been done with the protein of fishery products.

Before the optimum conditions of hydrolysis can be ascertained for any protein or possible interfering factors can be studied, the precision and accuracy of the chemical method used for the determination of amino acids after hydrolysis of the protein must be determined.

The objectives of the work were to determine: the precision and accuracy of the amino acid analysis by means of an automatic amino acid analyzer, using a standard hydrochloric acid hydrolysis procedure; the optimum conditions of hydrochloric acid hydrolysis (in this case of the proteins of fish meal and fish protein concentrate, FPC); and the effect of fats, carbohydrates, and salts on the accuracy of the hydrolyses of proteins of fish meal and fish protein concentrate and of these fishery products in mixed animal feeds.

## *Precision and Accuracy Using Standard Hydrolysis Procedures*

**Precision.** The experimental work was conducted, with one exception, on samples of a fish protein concentrate containing about 90% protein and 0.04% fat and of a fish meal containing 61% protein and 17.1% fat. (The 17.1 is an unusually high percentage of fat to be present in fish meal; this meal was chosen, however, because it represents the most difficult conditions of hydrolysis.) Cystine and tryptophan were not determined, since independent analyses are required for them.

Hydrolyzates of six samples of the fish meal were prepared by refluxing a 0.5-gram sample with 250 ml. of 6*N* HCl for 24 hours. The acid was removed by rotary evaporation, and the hydrolyzate was dissolved in distilled $H_2O$. These samples were then analyzed, using a Beckman-Spinco Model 120 automatic amino acid analyzer.

Table I presents the mean values expressed in gram per cent for concentrations of the amino acids of the six replicate hydrolyzates. The maximum standard deviation from the mean was ± 0.21.

**Table I. Reproducibility of Analysis**

| Amino Acid | Mean, G. % | Std. Dev. | Std. Error |
|---|---|---|---|
| LYS | 7.3 | 0.12 | 0.05 |
| HIS | 2.3 | 0.05 | 0.02 |
| ARG | 5.6 | 0.17 | 0.07 |
| ASP | 8.8 | 0.17 | 0.07 |
| THR | 4.0 | 0.10 | 0.04 |
| SER | 3.6 | 0.10 | 0.04 |
| GLU | 12.9 | 0.19 | 0.08 |
| PRO | 4.8 | 0.10 | 0.04 |
| GLY | 7.1 | 0.21 | 0.09 |
| ALA | 6.1 | 0.18 | 0.07 |
| VAL | 4.9 | 0.09 | 0.04 |
| MET | 2.7 | 0.09 | 0.04 |
| ILEU | 4.3 | 0.09 | 0.04 |
| LEU | 7.0 | 0.15 | 0.06 |
| TYR | 3.1 | 0.12 | 0.05 |
| PHE | 3.9 | 0.10 | 0.04 |

**Accuracy.** Recoveries were made by the addition of amino acids to separate samples of fish meal and of fish protein concentrate before and after hydrolysis. on aliquots equal to 0.2 μmole of amino acids added plus the concentration of the amino acids normally used for analyses. As is shown in Table II, the recovery of added amino acids was within experimental error of the precision of our procedure in the case of both the fish meal and fish protein concentrate samples, the widest variation in fish meal being 5% for both serine and proline, and in the fish protein concentrate being 4% for threonine.

## *Optimum Conditions of Hydrolysis*

To determine the optimum conditions for hydrolysis, we considered the use of constant-boiling HCl or of 6*N* HCl for hydrolysis, use of excess acid, and length of time of hydrolysis.

**Use of Constant-Boiling HCl *vs.* 6*N* HCl.** For pure proteins, glass-distilled constant-boiling HCl is recommended. Crude proteins can be analyzed, however, after hydrolysis with 6*N* HCl

**Table II. Recovery of Added Amino Acids**

| Amino Acid | Fish Meal, % | | FPC, % | |
|---|---|---|---|---|
| | Before | After | Before | After |
| LYS | 99 | 100 | 98 | 100 |
| HIS | 100 | 100 | 97 | 100 |
| ARG | 99 | 100 | 98 | 100 |
| ASP | 98 | 100 | 100 | 100 |
| THR | 99 | 100 | 96 | 100 |
| SER | 95 | 100 | 99 | 100 |
| GLU | 98 | 100 | 100 | 100 |
| PRO | 105 | 100 | 100 | 100 |
| GLY | 101 | 100 | 100 | 100 |
| ALA | 101 | 100 | 98 | 100 |
| VAL | 104 | 100 | 97 | 100 |
| MET | 103 | 100 | 99 | 100 |
| ILEU | 101 | 100 | 98 | 100 |
| LEU | 101 | 100 | 98 | 100 |
| TYR | 99 | 100 | 97 | 100 |
| PHE | 99 | 100 | 97 | 100 |

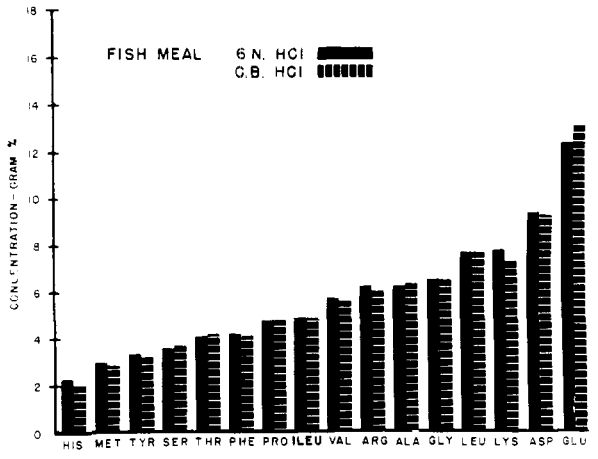


Figure 1. Effect of purity of acid

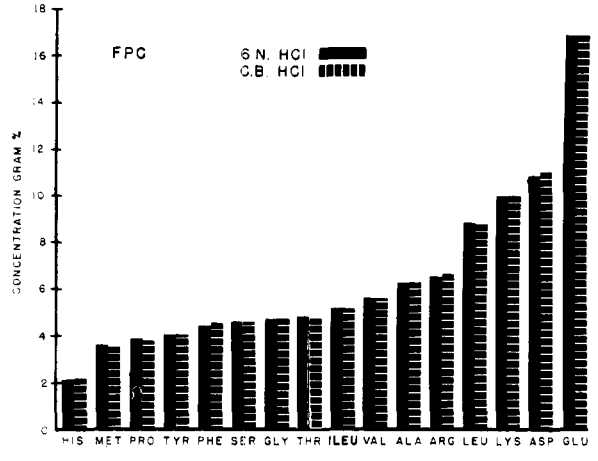


Figure 2. Effect of purity of acid

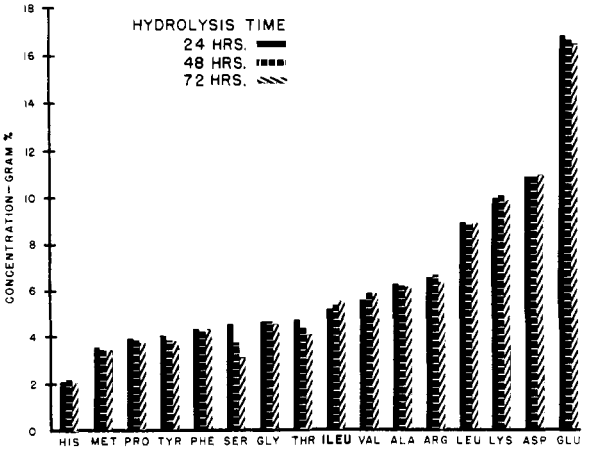


Figure 3. Effect of hydrolysis time

Fish meal

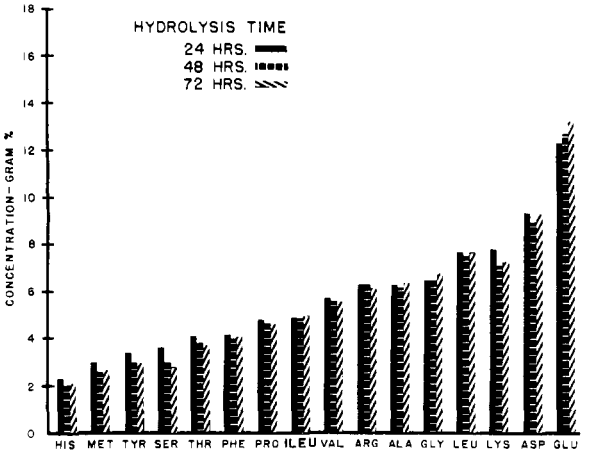


Figure 4. Effect of hydrolysis time

Fish protein concentrate

prepared from reagent grade commercial acid.

Figure 1 represents the data obtained from hydrolysis of samples of the fish meal with constant-boiling HCl and with 6*N* HCl. No appreciable differences were noted in the amino acid analyses.

Figure 2 represents the data obtained from similar hydrolyses of the fish protein concentrate. All differences were within experimental error.

**Use of Excess Acid.** The use of a large excess of acid has been observed to increase the accuracy of the amino acid determination (*3*). The following variations in the sample-acid ratio were studied:

| *Sample, G.* | *6N HCl, Ml.* |
|---|---|
| 0.5 | 250 |
| 0.5 | 500 |
| 0.25 | 500 |

The variations under these conditions were within experimental error.

**Time of Hydrolysis.** For the most accurate work, it is advisable to hydrolyze proteins for different lengths of time, since the rate of liberation of amino acids varies (*7*). Comparative studies were made on 24-, 48-, and 72-hour hydrolyzates of a fish meal sample and a fish protein concentrate.

As is shown in Figure 3, the amino acid content in the fish meal sample decreased with time of hydrolysis in the case of histidine (10%), threonine (10%), serine (22%), methionine (10%), and tyrosine (13%); and increased in the case of glutamic acid (8%).

Figure 4 presents the data obtained from the fish protein concentrate sample. Threonine and serine decreased 14.5 and 30%, respectively, with time; isoleucine increased 8%.

### *Effect of Nonprotein Components*

**Fats.** It is sometimes advisable to remove fat before hydrolysis of proteins for amino acid determination. Some fat (or lipid) solvents, however, are also good solvents for nonlipid components. A comparative study was therefore made on the variations in the amino acid analysis of fat-extracted samples of a fish meal and a fish protein concentrate. A fish protein concentrate prepared experimentally by a biological digestion procedure was used because it could contain more than the normal concentrations of free amino acids, which might be removed more readily by solvents. Three solvents were used: ethyl ether, a mixture of ethyl alcohol and ethyl ether (3 to 1), and petroleum ether. Separate samples of fish meal and of fish protein concentrate were extracted with the solvents for 2 hours, using a Soxhlet and 0.5-gram samples.

In Figure 5, the data on the fish protein concentrate sample extracted by the three solvents are compared with the data for an unextracted sample. Slight differences were noted in the amino acid concentration with both ethyl ether and ethyl alcohol–ethyl ether (3 to 1) as fat solvents. Most variation occurred in the values for proline, methionine, tyrosine, and phenylalanine. With petroleum ether, no appreciable variations were noted between defatted and nondefatted samples, and no appreciable increases in amino acid concentration

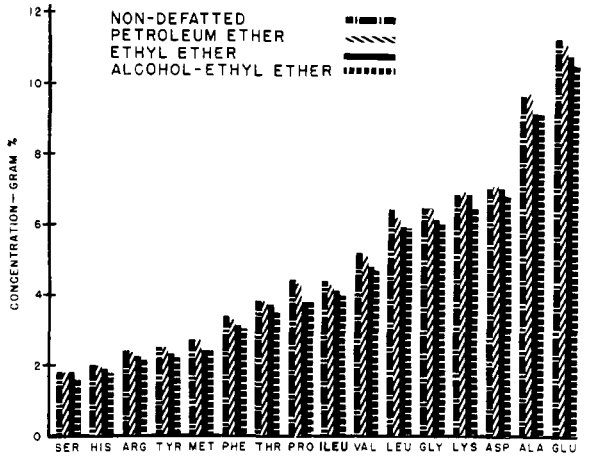


Figure 5. Comparison of fat solvents

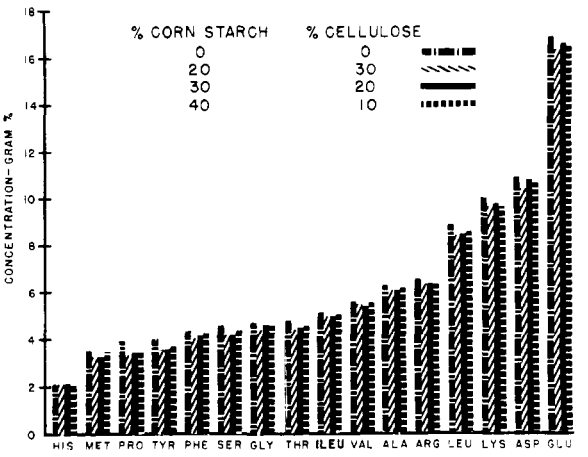


Figure 6. Effect of carbohydrates

**Table III. Amino Acid–Carbohydrate Mix**

| | Recovery from Hydrolysis, % | | |
|---|---|---|---|
| Amino Acid | A.A. only | Mix-before | Mix-after |
| LYS | 100 | 102 | 102 |
| HIS | 100 | 100 | 104 |
| ARG | 100 | 98 | 98 |
| ASP | 100 | 102 | 97 |
| THR | 100 | *92* | 95 |
| SER | 100 | *82* | 95 |
| GLU | 100 | 99 | 96 |
| PRO | 100 | 96 | 97 |
| GLY | 100 | 99 | 96 |
| ALA | 100 | 101 | 95 |
| VAL | 100 | 96 | 95 |
| MET[a] | 100 | *83* | *93* |
| ILEU | 100 | 98 | 96 |
| LEU | 100 | 97 | 96 |
| TYR | 100 | 91 | 96 |
| PHE | 100 | 95 | 96 |

[a] No correction for methionine sulfoxides.

occurred upon defatting. The changes in the fish meal samples were all within experimental error for all solvents tested.

**Carbohydrate Occurring Naturally.** The presence of carbohydrate may cause some destruction of certain amino acids (*1*). We added D-ribose, a component of most cells, to a fish meal and to a fish protein concentrate at the 2% level. These products normally contain very little carbohydrate, and 2% approximates more than one would expect in fishery products. Within experimental error, no differences were noted between the samples with and without D-ribose.

**Added Amounts of Carbohydrate.** It is advisable to study the determination of amino acids from proteins when the hydrolysis takes place in the presence of large amounts of added carbohydrates and to determine whether carbohydrates have any greater effect on amino acids in protein and peptide combination than on free amino acids.

In our laboratory, mixed diets containing protein, cornstarch, and purified cellulose are used in animal-feeding studies. To ascertain the effect of these components on the amino acid analysis, we analyzed mixed diets containing 45% protein from fish protein concentrate with 20, 30, and 40% cornstarch and 30, 20, and 10% purified cellulose, respectively. As is shown in Figure 6, differences were noted in the concentrations of proline and tyrosine with all combinations of cornstarch and purified cellulose and in methionine in the two higher concentrations of cellulose.

To differentiate between the effect of destruction and possible change by absorption of amino acids on the column, we analyzed mixtures of 0.5 μmole of each amino acid with 100 mg. of cornstarch and 150 mg. of purified cellulose added before and after hydrolysis. Lower values were obtained for threonine, serine, methionine, and tyrosine in the presence of 100 mg. of cornstarch and 150 mg. of purified cellulose (Table III). The addition of the same concentration of amino acids after hydrolysis of the carbohydrate alone resulted in a lower value for methionine only. The loss of methionine may be due to the oxidation of this particular amino acid.

A fourfold increase in the ratio of acid to sample resulted in no appreciable change in the amino acid analysis in samples containing 20% cornstarch plus 30% purified cellulose during hydrolysis.

**Salts.** The effect of inorganic salts added to fish meal and fish protein concentrates on the chromatographic analysis was studied by the addition of 10% tricalcium phosphate, a component of bone. Within experimental error, no differences in values were observed in either the fish meal or fish protein concentrate samples. The amino acid chromatogram obtained on an automatic analyzer showed no distortion of peaks.

### Literature Cited

(1) Block, R. J., Bolling, D., "Amino Acid Composition of Proteins and Foods," 2nd ed., p. 576, Charles C Thomas, Springfield, Ill., 1956.
(2) Block, R. J., Weiss, D. W., "Amino Acid Handbook," p. 11, Charles C Thomas, Springfield, Ill., 1956.
(3) Dustin, J. P., Czaikowska, C., Moore, S., Bigwood, E. J., *Anal. Chim. Acta* **9,** 256 (1953b).
(4) Hamilton, P. B., Anderson, R. A., *Federation Proc.* **13,** 224 (1954a).
(5) Lieben, F., *J. Biol. Chem.* **151,** 117 (1943).
(6) Lugg, G. W. H., *Biochem. J.* **32,** 775 (1938).
(7) Tristram, G. R., *Ibid.*, **33,** 1271 (1939).

*Received for review May 1, 1964. Accepted January 13, 1965. Division of Biological Chemistry, 145th Meeting, ACS, New York, N. Y., September 1963.*