FISKERIDIREKTORATETS SKRIFTER
Serie Teknologiske Undersøkelser
Reports on Technological Research concerning Norwegian Fish Industry
Vol. IV. No. 3
Published by the Director of Fisheries

A Comparative Study of Amino Acids in the Muscle of Different Species of Fish

By

OLAF R. BRÆKKAN and GJERMUND BOGE

Governmental Vitamin Laboratory
Norwegian Fisheries Research Institute,
Bergen, Norway

1962

A.S JOHN GRIEGS BOKTRYKKERI, BERGEN

CONTENTS

Introduction 5
Methods 6
Results 8
Discussion 9
Summary 17
References 18

INTRODUCTION

The importance of exact information with regard to the amino acid composition of food proteins is today generally accepted. LOVE et al. (1959) have given the most complete survey of figures reported from studies of amino acids in fish muscle. They pointed out that with the exception of one study (ÅGREN, 1944), the figures reported by different authors are roughly comparable. If, however, the values for the different amino acids reported in these studies are scrutinized, several cases of considerable variations may be pointed out. When ÅGREN's figures are excluded, the following variations for some amino acids are reported: Glycine 1.8 to 5.6, histidine 1.9 to 5.7, tryptophan 0.1 to 1.4, tyrosine 0.4 to 4.6 and valine 4.0 to 9.4 per cent of protein (N x 6.25).

If the amino acid pattern is repeated throughout the animal kingdom, as claimed by BEACH et al. (1943), such great variations should not be observed. The uniformity should be even greater as only values for a closely related group, fishes, are considered at present. The variations must thus express either actual conditions or unreliable data in the literature. The latter possibility may be related to lack of precision in the analytical procedure. The analytical methods have been improved several times in the progress of application, and especially the introduction of specific microbiological methods has been of importance. It should, however, be pointed out that most of the methods for estimation of amino acids are dependent on exact methods of hydrolysis. The best way of checking the possible correctness of the concept of uniformity in the pattern of amino acids in fish, is to carry out simultaneous determinations for a large number of species. The microbiological methods offer an excellent opportunity for such comparative studies. Many samples may conveniently be extracted and tested at the same time, and exact relative values should thus be ensured. Such values for the amino acid composition of fish muscle protein is of interest from a biochemical point of view,

but of the greatest importance with regard to nutrional evaluation of fish.

In the present study the complete amino acid composition of the muscle from ten different teleosts have been determined under strictly uniform conditions with regard to simultaneous extractions and assays.

METHODS

Samples.

Fresh frozen fillets were obtained from the freezing plants for the species cod (*Gadus morrhua*), coalfish (*Gadus virens*), haddock (*Gadus aeglefinus*), redfish (*Sebastes marinus*), catfish (*Anarrhichas lupus*), plaice (*Pleuronectes platessa*) and halibut (*Hippoglossus hippoglossus*). Three samples were collected at different times and mixed. The species ling (*Molva molva*), torsk (*Brosme brosme*) and mackerel (*Scomber scombrus*) were obtained fresh at the fish market. The fillets from respectively 3, 4 and 6 fishes were mixed.

All samples were ground in a meat grinder and subsequently extracted three times with acetone.: To 100 g sample was added 750 ml acetone. After 5 min. blending of the mixture in a multi-mixer, it was filtered with suction on paper. The residue was treated twice in the same way, each time with 400 ml acetone. The powder was dried in the air, ground in a hammer-mill, and stored in closed jars at room temperature.

Protein.

Nitrogen was determined by the micro-Kjeldahl method, and protein calculated as N x 6.25.

Dry matter.

Dry matter was determined by drying in an oven for four hours at 105°C.

Preparation of hydrolyzates.

Tryptophan was released by alkaline hydrolysis. To 1 g samples was added 16 ml 4 N NaOH, and the mixture autoclaved in nickel crucibles for 15 hrs at 120°C. After cooling, the pH was adjusted to 4.5, the mixture made up to a volume of 100 ml and filtered through a Schleicher & Schüll filter No 589[2] (Weissband).

Cystine was extracted by acid hydrolysis as described by ALEXANDER et al. (1953). To 250 mg sample was added 10 ml 3 N HCl. The mixture was autoclaved for 2 hrs at 120°C. After cooling, 1 ml 2.5 M sodium

acetate was added to the mixture, which was adjusted to pH 4.5, made up to volume 100 ml and filtered.

The remaining 16 amino acids assayed were all determined in an extract prepared by acid hydrolysis as described by BARTON-WRIGHT (1953). To 2 g sample was added 50 ml 2.5 N HCl. The mixture was autoclaved for 10 hrs at 120°C and cooled. 4 ml 2.5 M sodium acetate was added and the pH adjusted to 4.5. The volume was made up to 200 ml and the extract filtered.

All samples were extracted in duplicate at different times.

All extracts were stored under toluene in a coldroom, and aliquots taken for analysis.

Amino acid assays.

All amino acids were determined microbiologically by acidimetric methods. The lactic acid produced after incubation for 72 hrs was measured by potentiometric titration. Each sample was examined at six different levels. An automatic dispenser-titrator was employed, and all

Table 1. *Microbiological methods employed for the amino acid assays.*

Amino acid	Test organism	Standard range µg	Incubation temperature °C
Glutamic acid	*Lactobacillus plantarum* (8014)	0—35	37
Isoleucine	« « «	0—20	30
Leucine	« « «	0—20	30
Valine	« « «	0—20	30
Aspartic acid	*Leuconostoc mesenteroides* (8042)	0—40	37
Cystine	« « «	0—10	37
Glycine	« « «	0—20	37
Histidine	« « «	0— 5	37
Lysine	« « «	0—40	37
Methionine*	« « «	0—10	37
Phenylalanine	« « «	0—10	37
Proline**	« « «	0—20	37
Serine	« « «	0—15	37
Tyrosine*	« « «	0—12	30
Alanine	*Leuconostoc citrovorum* (8081 &8082)	0—50	37
Arginine	*Streptococcus faecalis* (8043)	0—15	37
Threonine	« « «	0—15	37
Tryptophan***	« « «	0— 3	37

*Medium supplemented with 0.01 µg biotin/ml (double strength).
**pH of medium adjusted to 6.0.
***Method of Kuiken et al. (1947).

assays carried out with an experimental volume of 2.0 ml per tube. The methods employed for the different amino acids were, except for tryptophan, as described by BARTON-WRIGHT (1953). Minor modifications have been introduced as a result of preliminary experiments which showed the present conditions to give better standard curves. The methods are summarized in Table 1, which report on test organisms, standard range and incubation temperatures. The test organisms were obtained from the American Type Culture Collection and maintained as described by BARTON-WRIGHT (1953).

Tryptophan was assayed according to the method of KUIKEN et al. (1947).

Each amino acid was assayed in two separate hydrolyzates analyzed at different times. For standards were used pure L-amino-acids, except for tryptophan, where the DL-form was employed.

RESULTS

In Table 2 are reported the percentage contents of dry matter, nitrogen and protein (N x 6.25). The fish muscle preparations showed slight variations, with the following values: Dry matter 87.0—92.5%, nitrogen 13.82—14.70% and protein 86.3—91.9%.

Table 2. *Dry matter, nitrogen and protein in the defatted samples.*

Sample of	Dry matter %	Nitrogen %	Protein (N×6.25) %
Cod (*Gadus morrhua*)	87.0	13.96	87.3
Coalfish (*Gadus virens*)	87.7	13.96	87.3
Haddock (*Gadus aeglefinus*).....	87.6	14.15	88.4
Redfish (*Sebastus marinus*)	87.5	13.82	86.4
Catfish (*Anarrhichas lupus*)	87.3	13.81	86.3
Plaice (*Pleuronectes platessa*)	87,7	14.04	87.8
Halibut (*Hippoglossus hippoglossus*)	88.1	13.82	86.4
Ling (*Molva molva*)	90.9	14.52	90.8
Torsk (*Brosme brosme*)	92.6	14.70	91.9
Mackerel (*Scomber scombrus*)	92.5	14.65	91.6
Ave	88.9	14.14	88.42

In Table 3 and 4 are reported the amino composition of the ten species investigated. The amino acid has been calculated as percentage of the protein, and the corresponding amino acid-N in mg per g sample has been recorded.

In Table 5 are summarized the amino acid percentages from the present study compared with summarized values from the literature. For each amino acid has been reported min — max, mean and standard error of the mean.

DISCUSSION

The first problem which had to be considered in the present study, was the preparation of suitable samples. The fat content varies much in fish muscles from different species. As fatty materials may interfere markedly with the microbiological assays (SNELL, 1950), they had to be removed. It was found most convenient to accomplish this by direct extraction of the samples. After preliminary experiments, acetone extraction of the homogenized muscles was decided on. POTTINGER and BALDWIN (1946) also used acetone dried products in their study of amino acids in fish. It may be objected that the acetone treated samples still contain nitrogenous extractives. Such substances, however, do not interefere in the microbiological assays. CONNEL and HOWGATE (1959) studied the amino acid composition of the flesh of four common food fishes. They extracted the minced fillets with absolute ethanol and re-extracted the precipitate with 80% (v/v) aquous ethanol. The precipitated protein was then extracted continuously with ether, air-dried and ground. They claimed that this process removed nitrogenous extractives as well as fat. Amino acids are, however, fairly soluble in water-ethanol mixtures. Thus free amino acids would be removed, and the results would be valid only for the protein powder, not the original muscle or flesh. If the values for the total dry matter of the present samples are considered in relation to the corresponding values for protein calculated as N x 6.25 (Table 2), a close relation can be observed. The slight variations between the samples from different species may be due to differences in the temperature and humidity during the final drying of the samples in open air. On an average the protein content was found 99.4% of the total dry matter. For a few samples, like cod, haddock and plaice, the calculated protein was even more than 100% of the dry matter. Fresh fish muscle contains usually about 1% ash, corresponding to 5% in the dried samples. We here have a clear indication that the conversion factor 6.25 for the calculation of protein is too high. This aspect will be discussed further below, as the amino acid values offer a better approach to this problem.

If the values for each of the eighteen amino acids found in the ten species studied are compared, a high degree of uniformity can be observed (Tables 3 and 4). The only exception is the value for histidine in mackerel, which is twice as high as the values found in the remaining

Table 3. *Amino acid composition of cod, coalfish, haddock, redfish and catfish.*

Amino acid	Cod (*G. morrhua*)		Coalfish (*G. virens*)		Haddock (*G. aeglefinus*)		Redfish (*S. marinus*)		Catfish (*A. lupus*)	
	Amino acid % of protein	Amino acid—N mg per g	Amino acid % of protein	Amino acid—N mg per g	Amino acid % of protein	Amino acid—N mg per g	Amino acid % of protein	Amino acid—N mg per g	Amino acid % of protein	Amino acid—N mg per g
Arginine	5,79	16,24	5,99	16,81	5,65	16,08	6,05	16,80	5,76	15,98
Histidine	1,84	4,34	2,01	4,76	2,03	4,87	2,01	4,70	2,07	4,83
Isoleucine	5,47	5,10	6,23	5,80	6,09	5,75	6,31	5,82	6,06	5,59
Leucine	8,10	7,55	8,14	7,58	8,20	7,74	8,55	7,89	7,83	7,21
Lysine	8,45	14,62	9,09	15,20	8,52	14,43	9,15	15,15	8,88	14,69
Methionine	3,06	2,51	3,03	2,48	2,84	2,36	3,01	2,44	2,86	2,31
Phenylalanine	3,70	2,74	3,79	2,80	4,00	3,00	3,97	2,91	3,98	2,91
Threonine	4,52	4,63	4,40	4,51	4,36	4,53	4,77	4,85	5,01	5,08
Tryptophan	0,87	0,11	0,98	0,12	1,00	1,21	0,95	1,13	0,94	1,12
Valine	5,61	5,86	6,06	6,33	6,04	6,39	5,93	6,13	5,78	5,97
Tyrosine	3,28	2,21	3,27	2,20	3,35	2,29	3,20	2,14	3,11	2,07
Cystine	0,92	0,94	1,00	1,02	1,01	1,04	1,14	1,15	1,08	1,09
Alanine	7,87	10,79	7,94	10,89	8,04	11,17	8,49	11,54	7,75	10,51
Aspartic acid	10.06	9,23	10,22	9,38	10,19	9,48	10,61	9,65	10,38	9,42
Glutamic acid	14,76	12,26	14,50	12,04	14,83	12,48	15,36	12,64	14,62	12,00
Glycine	4,60	7,49	4,30	7,00	4,57	7,54	4,64	7,48	4,96	7,98
Proline	3,57	3,80	3,38	3,59	3,46	3,72	3,42	3,60	3,68	3,85
Serine	4,65	5,40	4,63	5,38	5,04	5,93	5,16	5,95	5,30	6,09
Sum	97,12	115,82	98,96	117,89	99,22	120,01	102,72	121,97	100,05	118,70
Total N (see Table 1)		139,6		139,6		141,5		138,2		138,1
Amino acid—N as % of total N.		82,95		84,4		84,8		88,3		85,95

Table 4. *Amino acid composition of plaice, halibut, ling, torsk and mackerel.*

Amino acid	Plaice (*P. platessa*)		Halibut (*H. hippoglossus*)		Ling (*M. molva*)		Torsk (*B. brosme*)		Mackerel (*S. scombrus*)	
	Amino acid % of protein	Amino acid—N mg per g	Amino acid % of protein	Amino acid—N mg per g	Amino acid % of protein	Amino acid—N mg per g	Amino acid % of protein	Amino acid—N mg per g	Amino acid % of protein	Amino acid—N mg per g
Arginine	6,32	17,83	6,02	16,72	5,91	17,25	6,25	18,45	5,73	16,87
Histidine	2,08	4,93	2,17	5,09	1,94	4,78	1,97	4,91	4,50	11,16
Isoleucine	5,92	5,55	5,88	5,42	6,11	5,92	6,27	6,15	5,96	5,83
Leucine	8,28	7,78	8,09	7,46	9,02	8,74	9,06	8,89	8,78	8,58
Lysine	8,73	14,67	7,91	13,08	9,48	16,48	9,39	16,54	8,50	14,91
Methionine	2.80	2,30	2,79	2,26	3,06	2,61	3,16	2,73	3,12	2,69
Phenylalanine	3,87	2,88	3,80	2,78	4,11	3,17	4,12	3,21	3,90	3,03
Threonine	4,59	4,73	4,61	4,69	4,62	4,93	4,57	4,93	4,71	5,08
Tryptophan	0,98	1,18	0,96	1,13	0,92	1,15	1,01	1,27	0,94	1,18
Valine	5,69	5,97	5,98	6,18	6,10	6,62	6,20	6,82	6,13	6,72
Tyrosine	3,23	2,19	3,27	2,18	3,32	2,33	3,37	2,40	3,34	2,37
Cystine	1,05	1,07	1,06	1,07	1,08	1,14	1,04	1,12	1,02	1,09
Alanine	8,13	11,21	7,68	10,43	7,71	10,99	7,77	11,23	7,75	11,15
Aspartic acid	9,92	9,15	10,11	9,18	10,30	9,84	10,76	10,40	10,86	10,49
Glutamic acid	15,13	12,64	14,44	11,88	15,36	13,27	15,83	13,85	14,30	12,47
Glycine	5,38	8,81	4,84	7,79	4,16	7,04	4,27	7,33	4,24	7,24
Proline	3,72	3,97	3,65	3,83	3,28	3,62	3,50	3,92	3,50	3,90
Serine	5,99	7,00	5,67	6,53	5,05	6,11	4,98	6,11	4,89	5,97
Sum	101,81	123,87	98,93	117,70	101,53	125,99	103,52	130,26	102,17	130,73
Total N (see Table 1)		140,4		138,2		145,20		147,0		146,5
Amino acid—N as % of total N.		88,2		85,2		86,8		88,6		89,2

Table 5. *Amino acid composition of fish muscle. A comparison of results from the present study and values found in the literature.*

Amino acid	Present study			Literature*		
	Number	Percentage		Number	Percentage	
		Min.—max.	M ± s_m		Min.—max.	M ± s_m
Arginine	10	5.7— 6.3	5.95 ± 0.07	24	4.3— 7.6	6.11 ± 0.16
Histidine** ...	9	1.8— 2.2	2.01 ± 0.06	24	1.6— 5.7	2.75 ± 0.23
Isoleucine	10	5.5— 6.3	6.03 ± 0.08	32	4.4— 7.7	5.44 ± 0.18
Leucine	10	7.8— 9.1	8.41 ± 0.13	32	6.7—11.4	7.99 ± 0.18
Lysine	10	7.9— 9.5	8.81 ± 0.15	33	7.8—14.4	10.11 ± 0.30
Methionine ...	10	2.8— 3.2	2.97 ± 0.04	32	2.2— 3.7	2.98 ± 0.07
Phenylalanine .	10	3.7— 4.1	3.92 ± 0.04	33	2.9— 5.0	4.01 ± 0.10
Threonine	10	4.4— 5.0	4.62 ± 0.06	33	3.7— 6.2	4.85 ± 0.12
Tryptophan ..	10	0.9— 1.0	0.96 ± 0.01	24	0.1— 1.4	1.03 ± 0.06
Valine	10	5.6— 6.2	5.95 ± 0.06	32	4.0— 7.4	5.61 ± 0.52
Tyrosine	10	3.1— 3.4	3.27 ± 0.02	12	2.2— 4.6	3.67 ± 0.35
Cystine*** ...	10	0.9— 1.1	1.04 ± 0.02	12	1.2— 1.5	1.26 ± 0.03
Alanine	10	7.7— 8.5	7.91 ± 0.08	9	5.2— 7.3	6.49 ± 0.23
Aspartic acid .	10	9.9—10.9	10.34 ± 0.10	9	6.2—11.2	9.34 ± 0.75
Glutamic acid .	10	14.3—15.4	14.91 ± 0.15	9	14.5—16.6	15.48 ± 0.24
Glycine	10	4.2— 5.4	4.60 ± 0.12	9	3.5— 5.6	4.61 ± 0.24
Proline	10	3.3— 3.7	3.52 ± 0.04	9	3.1— 4.3	3.78 ± 0.11
Serine	10	4.6— 6.0	5.14 ± 0.14	10	4.8— 5.4	5.11 ± 0.08

* Literature for values except cystine: Beach et al. (1943), Deas & Tarr (1949), Ingalls et al. (1950), Konosu et al. (1956), Lahiry & Proctor (1956), Landgraff (1953), Neilands et al. (1949).

** Values for mackerel (*Scomber scombrus*) omitted in the average of the present study (see text).

*** The literature values refer to Pottinger and Baldwin (1946).

nine species. This one exception called for a control of correctness. Three additional samples of mackerel were prepared and analyzed. They gave values from 4.37 to 4.60, with an average of 4.50 per cent of the protein, which incidently was the same value as originally found. Regardless of the absolute accuracy of the values reported in Tables 3 and 4, their high degree of uniformity allows for the conclusion that the amino acid composition is essentially the same in the muscle from different fishes.

In Table 5 are reported average values from the present study compared with average values reported in representative studies in the literature. The latter averages refer to values for different species, although studies including many species occasionally may dominate the figures. For the amino acids arginine, leucine, phenylalanine, threonine, valine, glutamic acid, glycine, proline and serine, the present values and the averages from the literature agree very well, and the figures may be con-

sidered as valid for fish muscle. The values for methionine and tryptophan also agree closely, but deserve some comments. MATSUURA et al. (1952) investigated methionine in 20 Japanese species, including 12 teleosts. For the latter they found an average of 3.79 per cent methionine of the protein. They applied the chemical method of BAERNSTEIN (1932), which usually results in higher values than other methods. CONNEL and HOWGATE (1959) found poor agreement for the methionine content in cod, haddock, lemon sole and herring by chromatographic analysis, while application of the colorimetric method of HORN et al. (1946) gave values ranging for 3.1 to 3.6 per cent of the protein. The present authors consider 3.0 per cent to be a valid figure for fish muscles.

Tryptophan showed an average of 0.97 in the present study, with a standard error of the mean of only $\pm$ 0.01. This should allow the conclusion that the tryptophan content of fish muscle is essentially the same in different species. Although the average from the data in the literature is almost the same, the variation from one study to another is extreme. Thus DEAS and TARR (1949) reported values as low as 0.1 per cent in *Sebastodes sp.* when alkali hydrolysis was applied, while enzyme hydrolysis gave 1.4 per cent tryptophan in the same species. POTTINGER and BALDWIN (1946) applied the method of FOLIN and CIOCALTEU (1927) on 24 species and found a content of 1.22 $\pm$ 0.044 ($M \pm s_M$). Finally, it may be pointed out that CONNEL and HOWGATE (1959) in their study of the amino acid composition of fishes, applied the method of SPIES and CHAMBERS (1949) for the determination of tryptophan, and reported values from 1.32 to 1.56 per cent of the protein. The choice of a valid value is further confused by the requirement studies, a problem which will be returned to below. In the authors' opinion, a final value for tryptophan in fish, as in most foods, has to await the establishment of a reliable method for the estimation of this amino acid.

Tyrosine showed an average value of 3.3 per cent of the protein in the present study. Only two extensive studies are reported in the literature. KONOSU et al. (1956) found an average of 3.7 per cent tyrosine in their investigation of ten species, while CONNEL and HOWGATE (1959) reported values from 4.0—4.3 per cent in four species. The quantitative liberation of tyrosine from the proteins is still a controversial question. Thus acid as well as alkaline hydrolysis are employed. In studies in our laboratory, the acid hydrolysis has given higher values than the alkali-digestion described for tryptophan. In the authors' opinion more studies are needed for final conclusions to be drawn with regard to a valid value for tyrosine in fish.

Cystine showed an average value of 1.0 per cent of the protein in the present study. Also for this amino acid the liberation and determination

are disputed problems. POTTINGER and BALDWIN (1946) analyzed cystine in twelve teleosts, applying the chemical method of SULLIVAN and HESS (1930). They found an average value of 1.26 per cent, but pointed out that their hydrolyzates were highly colored, which made the colorimetric comparisons difficult. CONNEL and HOWGATE (1959) liberated cysteine + cystine by performic acid oxydation and hydrolysis as described by SCHRAM et al. (1954), and determined the cysteic acid formed by column chromatography. They found values ranging from 1.38—1.44 per cent for four species studied.

The values for isoleucine, lysine, alanine and aspartic acid found in the present study agree generally with the average values reported in the literature. It may, however, be pointed out that the values from the literature show a greater variation than the values for the ten species reported in this paper.

The values for histidine in the nine species apart from mackerel, show a lower average than the values reported in the literature. If, however, the literature data are scrutinized, many of the higher values reported are found to derive from analysis of scombroid fishes. Thus the two averages are not directly comparable. CONNEL and HOWGATE (1959) found values from 3.5 to 3.7 for cod, haddock, lemon sole and herring. Compared with the average from the present study, as well as the average from the literature, their values seem to be too high. The high value found for histidine in mackerel, 4.50 per cent of the protein, is very well established. NEILANDS et al. (1949) found 4.0 per cent for *Scomber sp.* and 5.7 per cent for *Thunnus sp.* These high values for histidine in scombroid fishes may be seen in relation to the frequently reported accumulation of toxic substances during "spoilage". Toxic poisoning from eating of tunny has been reported by STRØM and LINDBERG (1945) and LEGROUX et al. (1946). Other cases of poisoning after consumption of spoiled fish have been observed by Van VEEN and LATUASAN (1950) and HALSTEAD (1954). In all these cases it was suggested that histamine was responsible for the poisoning. GEIGER (1956) studied critically the role of histamine in poisoning with spoiled fish. He found that large amounts of histamine was produced during spoilage of fish. Animal experiments did not support the contention that this histamine was the cause of fish poisoning, but probably other toxins produced. In all cases, however, the rapid formation of histamine in amounts up to 200 mg per 100 g fresh substance, can only be derived from histidine available for bacterial decarboxylation. This points towards a possible special position of histidine in the metabolism of some fishes, a problem which is under investigation.

In connection with protein studies in foods, we are in the situation that the amino acids can be determined with greater accuracy than the

protein. MARTIN and SYNGE (1945) have reviewed the analytical chemistry of proteins, and pointed out that it would be a great advantage if data for each amino acid were expressed in the form of nitrogen as percentage of the total protein nitrogen. In Tables 3 and 4 are calculated amino acid-N in mg per g sample. The sum of these values for each fish has been given. Based on these sums the percentage amino acid-N of the total-N have been calculated and recorded. For the ten species studied these values vary from 83.0 to 89.2, with an average of 86.4 per cent. Thus 13.6% of the total-N could not be accounted for by the amino acid-N, and had to be derived from other sources. When the different reports on non-protein N in the literature were considered, calculation showed that this percentage could be ascribed to the presence of nitrogenous compounds such as creatine, trimethylamine oxide, betaine and ammonia (LOVE et al. 1959, SHEMAN 1951). The conversion factor 6.25 refers to proteins with 16% nitrogen. Only in a few cases has this factor been obtained exactly. Proteins with high contents of basic amino acids contain usually more than 16% nitrogen, which should demand a lower conversion factor. The fairly high content of lysine makes it probable that a factor lower than 6.25 should be expected for fish proteins. The factor 6.25 has, however, been generally accepted and applied in food analysis. The presence of non-protein nitrogen in many products should call for consideration before its application. Fish muscle is very much a case of such a product. In the present study the calculated amino acid as percentages of protein (N x 6.25) give an average sum of 100.6 for the ten species investigated. If the protein is assumed to be built up with peptide linkages, the sum of the split products should yield 100% of the protein, while the sum calculated as amino acids should be larger, and yield about 118% of the protein. (BRAND and EDSALL, 1947). This difference expresses the water taken up on hydrolysis, usually between 17 and 18 g per 100 g of protein. As the calculated amino acid percentages are inversely related to the conversion factor applied, it is apparent that the factor 6.25 is too high for application on the present samples. The amino acids expressed as percentages of the total-N offer a possibility for the calculation of more exact conversion factors for the present samples of fish. The amino acid-N was found to amount to 86.4 (83.0—89.2) per cent of the total-N, and the correct conversion factor for the calculation of protein should be 5.40 (5.19—5.58).LÜCKE (1954) claimed that a conversion factor of 6.0 should be applied for the calculation of protein in fish, and of 5.55 for the flesh of mammals. BRANDES and DIETRICH (1954) found an average conversion factor of 5.72 for the calculation of protein in herring. Their value was based on analysis of net-protein in 127 samples. Calculations based on CONNEL and HOWGATE's (1959) data gave an

average conversion factor of 6.07. It may, however, be emphasized that this factor applies to a purified fish muscle protein. Their amino acid percentages are thus higher than in most other studies. The present study refers to total-N and gives values for fish flesh, where a factor af about 5.40 should give a proper estimate of the net-protein content.

Table 6. *Essential amino acids in fish compared with requirements.*

Amino acid	Provisional pattern (FAO*)	Target value (Bender**)	Present values for fish
Histidine		1.8	2.0
Isoleucine	4.2	4.3	6.0
Leucine	4.8	7.5	8.4
Lysine	4.2	5.2	8.8
Phenylalanine	2.8	3.8	3.9
Tyrosine	2.8		3.3
Tryptophan	1.4	0.7	1.0
Valine	4.2	5.0	6.0
Methionine	2.2	2.7	3.0
Cystine		2.0	1.0
Total sulphur containing a.a.	4.2	4.7	4.0

*FAO Nutritional Studies (1957).
**Bender (1958, 1960).

Fish has always been considered a valuable protein source. The data arrived at in the present study for the essential amino acids in fish muscle, should allow a check on the value of this protein source. In Table 6 are recorded assumed requirements compared with the average values found for fish by the authors. The "provisional pattern" of FAO (1957) and the "target values" of BENDER (1958, 1960) have been used for the comparison. These requirement recommendations disagree on several points. Comparison with the FAO-pattern showed that all amino acids except tryptophan and cystine were in excess. If this pattern is correct, tryptophan should be the first limiting amino acid (71% of the claimed requirement), then the sulphur containing amino acids would enter into the picture (95% of the claimed requirement). If BENDER's target values are correct, all amino acids would meet the requirement except the sulphur containing amino acids (85% of the requirement). The extremely different requirement proposed for tryptophan in the FAO and in BENDER proposals, respectively 1.4 and 0.7, may be pointed out. BENDER's pattern is the only one which predicts the S-amino acids as the sole limiting factor in fish. Biological tests have given support to this claim, thus

MILLER (1956) found them limiting in fish meal, and NJAA (1961) in herring and herring meal. This finding indicates that the desirable pattern of essential amino acids proposed by FAO (1957) may need a revision with regard to tryptophan. If the sulphur containing amino acids are accepted as the only limiting factors, it may be seen that methionine meets the requirement of both proposals if cystine is present in the diet in required amounts.

SUMMARY

1. The complete amino acid composition in the muscles of ten common food fishes has been investigated by microbiological methods. Each amino acid was determined simultaneously in all samples, thus making a direct comparison of the contents in the different species possible.
2. The amino acid percentages of the protein (N x 6.25) were essentially the same in the muscle of the different fishes. The only exception was the value for histidine in mackerel, which was twice as high as the contents found in the remaining nine species.
3. The present values were generally of the same order as the averages of the data in the literature.
4. The total amino acid nitrogen compared with the total nitrogen of the samples, made it possible to calculate more exact conversion factors for the estimation of protein in fish muscle. This problem has been discussed.
5. Compared with FAO's "provisional pattern" for the nutritional requirement, tryptophan was the first limiting amino acid followed by the sulphur containing amino acid. Compared with BENDER's "target values", the sulphur containing amino acids were the only limiting factors.

REFERENCES

ALEXANDER, J. C., C. W. BECKNER and C. A. ELVEHJEM (1953): J. Nutr. *51*, 319.

BAERNSTEIN, H. D. (1932): J. biol. Chem. *97*, 663.

BARTON-WRIGHT, E. C. (1953): „Microbiological Assay of the Vitamin B-Complex and Amino Acids", Pittman & Son, London.

BEACH, E. F., B. MUNKS and A. ROBINSON (1943): J. biol. Chem. *148*, 431.

BENDER, A. E. (1958): Proc. Nutrition Soc., *17*, xxxix.

— (1960): Clin. Chim. Acta, *5*, 1.

BRAND, E. and J. T. EDSAL (1947): Ann. Rev. Biochem. *16*, 223.

BRANDES, C. H. and R. DIETRICH (1954): Fett u. Seifen *56*, 499.

CONNEL, J. J. and P. F. HOWGATE (1959): J. Sci. Fd. Agric. *10*, 241.

DEAS, C. P. and H. L. A. TARR (1949): J. Fish. Res. Bd. Can. *7*, 513.

FAO Nutritional Studies No. 16 (1957): Protein Requirements, Rome.

FOLIN, O. and V. CIOCALTEU (1927): J. biol. Chem. *73*, 627.

GEIGER, E. (1956): „Symposium on Nutritive Aspects of Preserved Food", SIK-Publication No. 115, Göteborg.

HALSTEAD, B. W. (1954): California Fish and Game *40*, 61.

HORN, M. J., D. B. JONES and A. E. BLUM (1946): J. biol. Chem. *166*, 313.

INGALLS, R. L., J. F. KLOCKE, J. P. RAFFERTY, R. E. GREENSMITH, M. L. CHANG, P. I. TACK and M. A. OHLSON (1950): Mich. Agric. Exp. Sta. Tech. Bull. No. 219.

KUIKEN, K. A., C. M. LYMAN and F. HALE (1947): J. biol. Chem. *171*, 551.

KONOSU, S., S. KATORI, R. OTA, S. EGUCHI and T. MORI (1956): Bull. Jap. Soc. sci. Fish. *21*, 1163.

LAHIRY, N. L. and B. E. PROCTOR (1956): Food. Res. *21*, 87.

LANDGRAF, R. G. (1953): Comm. Fish. Rev. *15* (7), 20.

LEGROUX, R., J. LEFADITI, G. BONDIN and D. BOVET (1946): Presse med. *53*, 743.

LOVE, R. M., J. A. LOVERN and N. R. JONES (1959): Food Investigation, Sp. Rep. No. 69, London.

LÜCKE, F. (1954): „Fischindustrielles Taschenbuch", Sergel & Hempel, Braunschweig.

MARTIN, A. J. P. and R. L. M. Synge (1945): Adv. Prot. Chem. *2*, 1—83.

MATSUURA, F., T. KOGURE, and G. FUKUI (1952): Bull. Jap. Soc. sci. Fish. *17*, 359.

MILLER, D. S. (1956): J. Sci. Fd. Agric. *7*, 337.

NEILANDS, J. B., R. J. SIRNY, I. SOLHJELL, F. M. STRONG and C. A. ELVEHJEM (1949): J. Nutr. *39*, 187.

NJAA, L. R. (1961): J. Sci. Fd. Agric. *12*, 757.

POTTINGER, S. R. and W. H. BALDWIN (1946): Comm. Fish. Rev. *8*, No. 8, 5.

SCHRAM, E., S. MOORE and E. J. BIGWOOD (1954): Biochem. J. *57*, 33.
SHEWAN, J. M. (1951): Biochem. Soc. Symposia (Cambridge, Engl.) No. *6*, 28.
SNELL, E. E. (1950): In „Vitamin Methods" edited by P. GYÖRGY, p. 327, Academic Press, New York.
SPIES, J. R. and D. C. CHAMBERS (1949): Analyt. Chem. *21*, 1249.
STRØM, A. and W. LINDBERG (1945): Nord. Med. *26*, 903.
SULLIVAN, M. X. and W. C. HESS (1930): „Studies on the Biochemistry of Sulfur. VII. The Cystine Content of Purified Proteins". Supplement No. 86 to Public Health Reports.
VAN VEEN, A. G. and H. E. LATUASAN (1950): Documenta Neerlandica et Indonesica de Morbis Tropicis *2*, 18.
ÅGREN, G. (1944): Acta physiol. Scand. *7*, 134.