FISKERIDIREKTORATETS SKRIFTER
SERIE TEKNOLOGISKE UNDERSØKELSER
VOL. 5 NO. 9

Reports on Technological Research concerning
Norwegian Fish Industry

THE NUTRITIVE VALUE OF THAI FISH PRODUCTS
II. AMINO ACID COMPOSITION

by

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BERGEN, NORWAY
1972

INTRODUCTION

The importance of protein, in particular animal protein, has been repeatedly stressed in connection with the food problems of the world. It is generally accepted that information as to the amino acid composition is needed for the nutritional evaluation of the different protein sources. The amino acid composition of fish muscle was reviewed by LOVE et al. (1959) and recently the Nutrition Division of F.A.O. compiled extensive tables on the amino acid contents of food and biological data on proteins (F.A.O. 1968).

Relatively few values are reported for the amino acid composition and nutritive value of fish and fish products consumed in different parts of the world. Considering the many different species of fish, and the varieties of methods of preservation and preparation, special studies may be needed for each area. In the Far East with its hot climate freezing of food is still very limited, and canning is not common. Fish may be sold fresh, but is usually cured by drying, salting, fermenting or smoking, or by combining such methods. With the exception of qualitative data for fermented fish sauce (DEVAKUL, 1963; SAKORNMONKOL et al., 1966) no data are available for the amino acid composition of fish and fish products marketed in Thailand.

The present study reports on the amino acid composition of the edible portion of 19 Thai fish products, all common and popular foods on the market in Bangkok.

METHODS

The products analyzed were described previously (SORASUCHART, 1971). They were with minor modification treated as described by BRÆKKAN and BOGE (1962).

The edible portions were ground in a meat grinder, with the exception of leatherskin and squid, which were products so tough that they had to be chopped in a chopper and subsequently ground in a hammer mill. The ground samples were extracted three times with acetone. The amount of acetone varied depending on the moisture content of the samples. After the extraction the residues were spred out and dried in the air, then ground in a hammer mill, and stored in closed jars at room temperature.

Studies in this laboratory (unpublished) have shown that there are negligible losses of amino acids during the acetone treatment.

No extraction was needed for the fish sauce (nam pla), but to eliminate variations in the microbiological assays due to the high salt content, sodium chloride was precipitated by the addition of 8.1 ml concentrated HCl per 25 g of the sample before hydrolysis. The precipitated salt was was filtered off.

PROTEIN

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

AMINO ACID ASSAYS

The assays were carried out microbiologically in hydrolysates which were prepared in dublicate as described by Brækkan and Boge (1962). The lactic acid produced after a 72 hours incubation period was measured by potentiometric titration. Each sample was run at six different levels with a total volume of 2.0 ml per tube. An automatic dispenser and titrator was used in the dispensing and titration processes. The methods are summarized in Table 1. The test organisms were obtained from the American Type Culture Collection and maintained as described by Barton-Wright (1952).

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

Each amino acid was assayed in two separate hydrolysates analysed at different times. L-amino acid standards were used, except for tryptophan, where the DL-form was employed.

For 13 of the samples amino acids were also determined by ion exchange chromatography as described by Njaa et al. (1968), tryptophan was not determined chemically.

RESULTS AND DISCUSSION

The amino acid contents in the samples analyzed are given in Tables 2—5. The results are presented as gram amino acid per 16 g nitrogen (or per 100 g protein). They should be referred to the original products by use of Table 2 in the previous communication (Sorasuchart 1971). The results obtained chromatographically are presented together with the values obtained microbiologically. For many amino acids the agreement between the values from both methods is good, but there are obvious exceptions (e. g. isoleucine). The reasons for these discrepancies are under investigation and will be reported on (NJAA and BRAEKKAN, to be published).

The nutritive value of a protein source is mainly dependent on its content of essential amino acids. The contents of isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine in the Thai fish products investigated were compared to the values reported for teleost fish muscle by BRÆKKAN and BOGE (1962). These reference values were chosen because they were obtained by use of the same methods and with the same equipment as those used by me, and because they showed little variation about the means. Thus, it was arbitrarily chosen to consider a value less than 90 % of the reported mean value of a particular amino acid as being lower than this. By this criterion salted dried pla tu, salted dried pla slid, salted dried leatherskin, seasoned dried catfish and pla ra had essential amino acid patterns equivalent to fish muscle protein, and eight products were somewhat low in only one of the essential amino acids. Of these products six were low in threonine (dried snake-head, salted dried mackerel, salted dried carp, salted dried anchovy, salted dried mullet and kapi) one in lysine (salted dried threadfin) and one in tryptophan (smoked catfish). Three products were somewhat low in two essential amino acids, namely shrimp (threonine and valine), mussel (leucine and methionine) and featherback (methionine and tryptophan). Salted dried ray was low in four of the essential amino acids (methionine, threonine, tryptophan and valine), dried squid in five (lysine, phenylalanine, threonine, tryptophan and valine) and nam pla was low in six (isoleucine, leucine, methionine, phenylalanine, threonine and tryptophan). Of the other amino acids often considered together with the essential arginine was extremely low in the two fermented products nam pla and kapi. Histidine was high in salted dried pla tu, salted dried mackerel and nam pla, all of scombroid or clupeid origin, but not in salted dried anchovy which is of similar raw material as nam pla. It is well known that scombroid and clupeid fish may be very high in free histidine and that the level show seasonal variations (HUGHES 1959, SAKAGUCHI and SHIMUZU 1965). Cystine was very high in mussel and high in shrimps, but not in fermented crustaceans (kapi), low in salted dried carp and seasoned dried catfish and very low in nam pla. The high cystine content in shellfish is in agreement with literature data (ALTMAN and DITTMER 1968), nam pla was also very low in tyrosine.

As sources for the essential and for the nutritionally most important of the non-essential amino acids the Thai fish products generally rank high as compared with fish muscle protein. The only exception to this seems to be nam pla, but even this may be a nutritionally valuable product because it is rather high in lysine and because it is mainly used as an additive to rice which is low in lysine. It may be of importance that so many of the products are somewhat low in threonine (10 out of 19) because threonine is among the amino acids contained in relatively low amounts in rice and other cereals.

The other non-essential amino acids showed greatly varying values between products. Compared to fish muscle protein they were generally lower in alanine and higher in glycine and proline. This is probably due to the fact that most of the Thai products included skin and scales, and sometimes bones, thus the high collagen and gelatin content may account for high glycine and proline values (NEUMAN 1949). By and large the results obtained with the Thai fish products showed that there was a great similarity between the products in the essential amino acid pattern. This is in agreement with the findings of KONUSU et al. (1956, 1958) who analyzed fish muscle, cuttle fish muscle and crustacean muscle protein, with those of SIGIMURA et al. (1954) who analysed fish muscle and squid muscle protein and with those of CABBAT and STANDAL (1964) who determined essential amino acids in Hawaiian fish and octopus. The obvious exception to this are the results obtained for nam pla. It is difficult to make a meaningful comparison of the results with this product with other published amino acid data. AMANO (1962) gave the amino acid content of ordinary quality Nuoc-mam, but took the reservation that for superior quality sauce the figures should be multiplied by 1.6—2.0. The results for nam pla showed values relative to the data given by AMANO (1962) ranging from 1.0—3.0 for 12 of the amino acids, histidine showed a ratio of 7.0 whereas tyrosine, isoleucine and leucine showed ratios ranging from 0.6 to 0.8 and arginine had a ratio of 0.3. The very low value for arginine in nam pla, and also in kapi indicate that this amino acid is especially liable to destruction in some fermented products. ITO (1959) showed that free arginine fell rapidly in spoiling shellfish muscle, this may well be a parallel to the assumed destruction of arginine in some fermented products. Arginase was found in the muscle of fish by MATSUMA et al. (1963). SAISITHI et al. (1966) suggested that the flavour of nam pla can arise in part from glutamic acid, histidine and proline, in agreement with the conclusion of JONES (1961). It is probably significant in this connection that the three amino acids mentioned are those showing the highest ratios relative to data given for Nuoc-mam by AMANO (1962). Dried snake-head and pla ra, as well as salted dried anchovy and nam pla and probably shrimp and kapi may be considered to be of similar raw materials. Thus it should be possible to get general indications of the effects of fermentation on the amino acid pattern. The indications are, however, conflicting: Dried snake-head and pla ra showed approximately identical patterns; between shrimps and kapi the differences were small except for the fact that kapi was very low in arginine, and between salted dried anchovy and nam pla several amino acids besides arginine and tyrosine already mentioned showed considerable differences. PROCTOR and LAHIRY (1956) showed that methods of processing and preservation of fish do not affect the amino acid composition of the product. This generalization obviously do not hold for fermented products.

As might have been expected the unfermented products which showed greatest differences from the amino acid pattern in teleost fish muscle were those derived from species other than teleost fish. Ray and squid which were relatively low in four and five essential amino acids respectively, neverthless showed fairly good essential amino acid patterns. Selachian muscle protein may, however, be somewhat less efficiently utilized than teleost muscle protein (NJAA et al. 1968) whereas squid muscle protein may be well utilized provided it is carefully treated (NJAA et al. 1966): commercial squid meal was poorly digested whereas laboratory dried squid was well digested and utilized.

It will be of obvious interest to study products of the type described here in animal feeding experiments to obtain information on the point of whether the processes used affect the utilization of the protein.

SUMMARY

Amino acid analyses are presented for 19 samples of Thai fish products bought at the market in Bangkok. All samples were analysed by microbiological techniques, 13 samples were also analysed by column chromatographic technique.

The essential amino acid patterns were compared with that in teleost fish muscle. Most of the products showed fairly good essential amino acid patterns. Two products were very low in arginine.

ACKNOWLEDGMENT

The author carried out this study on a fellowship from the Norwegian Agency for International Development (NORAD). I am very grateful Dr. L. R. Njaa, Dr. O. R. Brækkan and Mr. G. Boge for help and encouragement.

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Table 1. Microbiological methods employed for 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*	0—30	37
Arginine***	*Streptococcus faecalis* (8043)	0—15	30
Threonine	» » »	0—15	37
Tryptophan	» » »	0—3	37

* Medium supplemented with 0.01 µg biotin/ml (double strength).
** Medium adjusted to pH 6.0.
*** Medium supplemented with 40 mg tryptophan/100 ml (double strength).

Table 2. Amino acid content (g/16 gN) in dried and boiled-dried products
(a: microbiological determination, b: chromatographic determination)

Protein content of edible portion (%) / Amino acid	Dried products				Boiled-dried products			
	Snake-head 60.0		Squid 65.6		Shrimp 61.9		Mussel 39.5	
	a	b	a	b	a	b	a	b
Arginine	7,21	6,63	8,04	6.56	9.25	8.93	7.90	6.74
Histidine	2.11	2.48	1.72	1.77	2.04	1.93	1.81	1.87
Isoleucine	7.19	4.63	5.72	3.83	6.94	4.24	6.41	4.08
Leucine	9.37	8.20	7.85	6.73	7.95	7.16	7.38	4.94
Lysine	10.41	9.39	7.38	6.95	10.45	7.89	8.26	7.20
Methionine	3.34	2.80	2.88	2.49	3.08	2.74	2.42	2.23
Phenylalanine	4.38	4.81	3.34	3.58	4.12	4.27	3.72	4.13
Threonine	4.03	4.45	3.64	3.64	3.76	3.77	4.24	4.35
Tryptophan	0.94	—	0.82	—	0.88	—	0.97	—
Valine	6.40	4.92	4.77	3.57	5.30	4.21	5.86	4.35
Tyrosine	3.22	3.46	2.67	2.97	3.56	3.66	3.59	3.78
Cystine	1.20	0.86	1.16	0.63	1.40	1.00	1.93	1.08
Alanine	7.36	6.73	6.42	5.37	6.18	5.36	5.53	4.62
Aspartic acid	10.29	10.38	9.64	8.21	10.57	9.35	9.09	9.05
Glutamic acid	16.00	15.94	13.63	12.03	14.84	14.74	11.80	11.31
Glycine	6.82	6.63	7.36	6.15	7.90	6.67	6.50	5.57
Proline	4.28	5.01	4.16	4.01	3.37	2.95	4.24	2.84
Serine	5.72	3.74	5.57	3.41	5.89	3.75	6.18	4.26

Table 3. Amino acid content (g/16 g N) in salted dried products
(a: microbiological determination, b: chromatographic determination)

Protein content of edible portion (%)	Pla tu 25.6		Mackerel 31.3		Threadfin 32.6		Pla slid 41.8		Leatherskin 42.8	
Amino acid	a	b	a	b	a	b	a	b	a	b
Arginine	6.58	6.19	4.81	4.38	5.31	4.56	6.72	5.90	7.17	6.48
Histidine	3.70	3.87	3.27	3.09	2.38	2.30	1.84	1.92	2.16	2.35
Isoleucine	7.95	5.37	7.43	4.86	7.49	4.83	6.99	4.32	7.03	4.48
Leucine	9.32	8.45	9.51	7.65	9.90	7.93	9.38	7.66	8.88	7.88
Lysine	8.90	8.93	7.19	6.51	7.56	7.16	8.37	8.59	8.69	8.34
Methionine	3.31	3.09	3.28	2.86	3.47	3.01	2.82	2.70	3.11	2.96
Phenylalanine	3.60	4.62	4.07	3.62	3.67	4.23	3.83	4.24	3.77	3.78
Threonine	4.93	4.91	3.92	3.64	4.34	3.94	4.15	4.06	4.21	4.53
Tryptophan	1.21	—	1.15	—	1.08	—	0.88	—	0.97	—
Valine	7.24	5.80	7.50	5.22	6.66	4.98	6.41	4.68	6.61	5.03
Tyrosine	3.87	4.05	3.52	3.62	3.47	3.51	3.10	3.17	3.19	3.47
Cystine	1.03	0.75	1.17	0.49	1.12	0.82	1.20	0.77	1.15	0.85
Alanine	7.29	6.37	8.12	6.58	8.35	7.32	7.52	6.33	7.01	6.69
Aspartic acid	10.33	10.30	8.76	7.59	9.15	8.18	10.11	9.45	10.47	9.49
Glutamic acid	13.95	14.85	13.93	12.74	14.19	14.25	14.28	14.04	13.99	14.51
Glycine	6.06	5.55	5.58	4.83	6.32	5.62	7.82	6.76	7.37	6.65
Proline	4.18	—	3.77	3.69	4.16	4.19	4.54	4.76	5.01	4.75
Serine	5.06	3.91	4.50	2.91	4.79	3.19	5.81	3.86	5.65	4.04

Table 4. Amino acid content (g/16 g N) in salted dried and seasoned dried products (a: microbiological determination, b: chromatographic determination)

	Salted dried products					Seasoned dried fish
Protein content of edible portion (%)	Carp 43.4		Ray 58.9	Anchovy 45.9	Mullet 28.4	Catfish 28.8
Amino acid	a	b	a	b	a	b
Arginine	6.73	8.00	6.57	6.10	5.51	5.97
Histidine	1.81	2.40	2.06	2.02	1.85	2.22
Isoleucine	5.89	3.82	5.58	6.63	6.13	6.64
Leucine	7.98	6.94	7.85	8.93	8.14	9.11
Lysine	9.52	9.05	8.30	9.48	8.86	9.80
Methionine	2.71	2.30	2.41	3.16	2.74	2.92
Phenylalanine	3.95	4.03	3.63	4.24	3.96	3.99
Threonine	3.58	4.45	3.90	2.82	4.02	4.52
Tryptophan	0.76	—	0.86	1.04	0.98	1.03
Valine	5.96	4.22	5.26	6.20	5.96	6.20
Tyrosine	2.99	2.87	2.76	3.54	3.16	3.34
Cystine	0.78	0.68	1.04	1.38	1.15	0.58
Alanine	6.76	7.22	6.37	6.96	6.29	6.78
Aspartic acid	11.07	9.37	9.08	10.91	10.15	10.03
Glutamic acid	13.65	14.33	12.85	15.85	13.32	14.71
Glycine	10.32	8.84	8.44	6.13	7.65	5.55
Proline	5.84	8.68	4.87	4.18	4.75	4.08
Serine	5.80	3.69	5.61	6.10	5.35	5.70

Table 5. Amino acid content (g/16 g N) in smoked and fermented products (a: microbiological determination, b: chromatographic determination)

	Smoked products			Fermented products				
	Catfish		Feat-her-back	Pla ra		Nam pla	Kapi	
Protein content of edible portion (%)	71.3		67.8	20.8		11.6	22.6	
Amino acid	a	b	a	a	b	a	a	b
Arginine	6.93	5.62	6.77	6.41	5.40	0.89	2.50	1.72
Histidine	2.00	2.14	2.06	2.12	2.24	2.81	1.63	1.47
Isoleucine	6.82	4.16	6.08	8.59	5.44	4.35	7.87	4.96
Leucine	8.91	7.28	8.39	10.04	8.39	4.87	9.68	8.30
Lysine	9.83	7.85	9.80	8.60	8.13	8.89	10.08	7.48
Methionine	2.74	2.50	2.63	3.49	3.05	2.11	3.29	2.78
Phenylalanine	3.78	3.85	4.22	4.68	4.70	2.47	4.67	4.51
Threonine	4.19	4.29	4.14	4.73	4.63	3.60	4.02	3.72
Tryptophan	0.78	—	0.75	1.22	—	0.86	1.48	—
Valine	6.06	4.42	5.68	7.37	5.43	5.71	6.61	5.16
Tyrosine	2.90	3.06	3.01	3.71	4.21	0.69	4.38	4.39
Cystine	0.97	0.73	0.89	0.94	0.53	0.36	0.97	0.87
Alanine	6.80	6.17	6.28	7.49	6.70	7.12	6.84	6.91
Aspartic acid	10.59	9.42	11.01	10.65	9.07	8.18	11.62	8.92
Glutamic acid	14.26	14.13	14.51	14.27	13.40	16.17	16.45	15.57
Glycine	7.11	6.05	9.23	7.46	6.71	4.74	6.74	6.14
Proline	4.40	3.76	5.32	5.06	4.49	2.49	3.48	—
Serine	5.72	3.66	5.86	5.89	4.00	2.63	3.66	2.65