Food Chemistry **41** (1991) 227–236

Effect of Local Processing Methods (Cooking, Frying and Smoking) on Three Fish Species from Ghana: Part 2— Amino Acids and Protein Quality

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(Received 12 February, 1990; revised version received and accepted 24 May 1990)

ABSTRACT

The effect of processing methods (cooking, frying and smoking) on the amino acid composition of Sardinella *sp.,* Dentex *sp. and* Tilapia *sp. was studied. Protein quality, as expressed by AD, TD, NPU and Bal% was also investigated.*

All the processed fish had good quality protein; as expressed by their high AD, TD and NPU. The good quality of the processed fish was further supported by the amino acid composition. Cooked fish was better absorbed than fried and smoked.

INTRODUCTION

Fish is particularly widely used in diets in many parts of the world. Along coastal regions and lakes, fish is a regular constituent of the diet.

Fish smoking and frying are popular in many parts of West Africa. There are various grades of quality depending on the degree of smoking or frying. Long periods and high temperatures are used. Few data are, however, available on the amino acid and protein quality of these products. This paper presents the effect of processing (cooking, frying and smoking) on the

Food Chemistry 0308-8146/91/$03·50

amino acid profile and protein quality of three commonly consumed fish species—*Sardinella* sp., *Dentex* sp. and *Tilapia* sp.—from Ghana (West Africa).

MATERIALS AND METHODS

The collection of samples and their preparation prior to analysis have been described (Steiner-Asiedu *et al.*, 1990).

After hydrolysing the sample with 6M HCl, the amino acids were separated and analysed with a Waters HPLC system (a modification of the Pico-tag method, 1987). Norleucine was used as an internal standard. Methionine was determined after alkaline hydrolysis both directly and after reduction by titanium chloride. Small quantities of cadmium acetate were added to the sample before hydrolysis to eliminate cysteine–cystine interference. The direct method gives the methionine content while the reduction method indicates the sum of methionine and methionine sulphoxide. Methionine was determined spectrophotometrically as described by Njaa (1980). Tryptophan was determined after Sachse (1981).

The quality of the fish proteins was tested in balance experiments using Young Wistar-Møll male rats imported from Møllegård, Denmark. The metabolic cages employed comprise an upper living area with feeding system and below a device for the collection of urine and faeces (Eggum, 1973). The rats were assigned randomly to nine experimental groups of five. They weighed between 61·1 and 69·0 g at the start of the experiment. A preliminary period of 4 days and a balance period of 5 days were employed. Each rat received 10 g feed containing 8% protein from the fish samples daily. The composition of the basal diet is shown in Table 1. The fat contents of the fish meals were taken into account so that the fat content for each group was 5%. Soya bean oil was used to level the fat contents of the diets.

TABLE 1
Composition of the Basal Diet

Item	*Amount (%)*
Fat	5
Vitamins	1
Minerals	4
Sugar	20
Cellulose	1
Protein	8
Partially dextrinized potato starch made to 100	

The rats were weighed at the beginning of the experiment and at the end of the preliminary and balance periods. Access to feed and water were closed 3 h before weighing.

AD, TD, NPU, and Balance were calculated.† Endogenous faecal nitrogen was calculated as 2·02 mg faecal nitrogen per g feed and endogenous urinary nitrogen was calculated as $W^{0.75} \times K$; where W is the average body weight (g) of the animal during the 5 days experimental period and K is 0·645, after Njaa (1963).

Analysis of results

Analyses of variance were used to assess any differences in the processing methods and between fishes as judged by the balance experiments. Differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

The amino acid profiles of the fishes are given in Table 2. The amino acid content of the marine fishes, sea bream and flat sardine, is similar to the findings of Naja and Utne (1982) and Haaland *et al.* (1988). Within the marine species there are no variations in the amino acid content except histidine. Flat sardine contained higher amounts of histidine (34·5 ± 1·3 mg/g protein). This value is higher than what was observed in mackerel by Njaa and Utne (1982). Mackerel has high histidine levels (Brækkan & Boge, 1962; Sakaguchi & Shimizu, 1965; Kjosbakken & Larsen, 1981). High contents of histidine in herring meals have been reported by Power *et al.* (1969); and flat sardine belongs to this species. Species with high levels of free histidine should be stored with care as adverse storing conditions may result in the formation of histamine (Takagi *et al.*, 1969). Histamine is toxic (Arnold & Brown, 1978).

Tilapia contains more hydroxyproline, glycine, and proline than the marine fishes. This indicates a greater proportion of connective tissue in these species. The levels of the essential amino acids and the E/T ratio (Table 2) were similar between tilapia and the other fishes. It is not likely, therefore, that the higher content of connective tissue in tilapia would have any significant effect on NPU.

The effects of cooking, frying and smoking on the amino acid composition

† AD = Apparent Digestibility = $(I-F)/I \times 100\%$, TD = True Digestibility = $[I-(F-F')]/I \times 100\%$, Bal = Nitrogen Balance = $(I-F-U)/I \times 100\%$, NPU = Net Protein Utilization = Bal% + 100% $(F'/I + U'/I)$ where: I = Nitrogen intake, F = faecal nitrogen, U = Urinary nitrogen, and U' and F' = Obligatory loss of nitrogen in urine and faeces, respectively.

TABLE 2
Amino Acid Composition (mg/g protein)[a]

Sample	*Flat sardine*				*Sea bream*				*Tilapia*			
Amino acid (AA)	*Fresh*	*Cooked*	*Fried*	*Smoked*	*Fresh*	*Cooked*	*Fried*	*Smoked*	*Fresh*	*Cooked*	*Fried*	*Smoked*
Asp	80	84	93	88	85	87	96	92	88	94	94	87
Glu	117	123	138	130	123	125	140	133	133	139	137	132
Hpy	7	5	7	4	7	4	6	7	18	19	18	21
Ser	36	37	41	40	38	38	42	40	38	41	40	39
Gly	54	51	61	49	48	46	54	54	75	80	78	84
His	34	36	35	33	21	25	21	24	17	21	21	20
Arg	52	61	69	61	56	58	65	61	65	68	66	65
Thr	37	40	44	42	40	41	44	42	41	43	42	41
Ala	57	57	65	59	53	54	60	57	63	67	65	65
Pro	36	35	41	35	32	33	38	37	44	47	44	48
Tyr	25	28	30	29	25	27	30	28	25	27	27	25
Val	39	39	43	40	37	38	42	40	37	39	38	37
Met	30	31	34	32	30	31	33	32	29	30	31	32
Ile	36	37	40	37	34	35	39	37	35	37	37	36
Leu	68	69	77	73	67	69	77	72	68	73	71	69
Phe	36	35	39	36	33	33	37	35	35	38	37	36
Lys	74	77	83	79	77	75	84	78	73	79	79	75
Try	9	11	12	11	10	11	11	10	8	9	9	9
Total AA	827	856	952	878	816	830	919	879	892	951	934	921
Total essential AA	329	339	372	350	328	333	367	346	326	348	344	335
Ratio E/T[b]	2·05	2·12	2·32	2·18	2·05	2·08	2·29	2·16	2·04	2·18	2·15	2·09

[a] Analyses were run in duplicates and 5% deviations between parallels were accepted.
[b] Sum essential AA/total N.

of the three fishes were studied. The results indicate that there is no effect of these processing methods on the amino acid content as compared with the fresh fish. Studies on the amino acid content of raw, precooked and processed meat and fish products have been carried out by Dunn *et al.* (1949) and Neilands *et al.* (1949). The amino acids were determined by microbiological methods. No differences were found in any of the individual amino acids as a result of precooking or processing.

Earlier workers like Proctor and Lahiry (1956) tested the effect of dehydration on the amino acid content of shad and haddock. There were no significant differences in the amino acid content between raw and dehydrated fish, but valine, phenylalanine and tryptophan tended to be lower in the dehydrated relative to the raw fish. Hoffman *et al.* (1977) also found no consistent effect of smoking on the amino acid content of tilapia species from Africa.

In this study, the fish samples were cooked for 30 min. No effects on the amino acid content were observed. Seet and Brown (1983), however, reported that cooking of frozen stored tuna at 100°C for 3 h reduced the amount of lysine, histidine and cystine but had no effects on other amino acids. Opstvedt *et al.* (1984) cooked minced pollock and mackerel for 20 min after the internal temperature was 95°C. They found that cooking had no effect on the amino acid contents but there was a reduction of the amino acids digestibility in pollock but no effect in mackerel. It can therefore be inferred that cooking or processing *per se* does not affect the amino acid content but the contributing factor might be the type of species in question.

The extent of methionine oxidation in the fish samples was determined in alkaline hydrolysates. Table 3 shows the total methionine and the ratio of unoxidized (U) to total methionine. The analyses were run in duplicates and 5% deviation between the parallels was accepted. The results indicate that fish, whether it is fresh or processed, has 10 to 15% of its methionine in the form of methionine sulphoxide. The range of methionine compares well with the findings (2·7–3·2 g/16 N) of Olley *et al.*, (1968) on fish meal.

The results of the balance experiment with the nine samples of fish protein sources are given in Table 4. The statistical comparisons were based on AD and Bal% as the other items (TD and NPU) contain estimated quantities besides the measured quantities. Thus AD is taken to show the variability in TD and Bal% is taken to show the variability in NPU. As expected there were high degrees of correlation between AD and TD ($r = 0{\cdot}99$) and between Bal% and NPU ($r = 0{\cdot}99$). Furthermore, NPU which is an index inclusive of digestibility correlates well with TD ($r = 0{\cdot}86$).

The differences observed in relation to the various species could be due to individual species variation. The rats given fried tilapia grew slightly better and had lower urinary nitrogen excretions than rats given other tilapia

TABLE 3
Total Methionine (*T*) and the Ratio of Unoxidized Methionine (*U*) to Total Methionine (*U/T*)

Fish	*Total methionine (mg/g protein)*	*U/T %*
Flat sardine		
Fresh	29·8	88·2
Cooked	30·9	89·2
Fried	34·4	88·8
Smoked	32·1	85·7
Sea bream		
Fresh	30·3	83·7
Cooked	31·4	82·4
Fried	32·7	94·3
Smoked	32·3	90·4
Tilapia		
Fresh	29·0	87·1
Cooked	29·8	91·0
Fried	30·8	90·4
Smoked	31·8	88·9

TABLE 4
AD, TD, NPU and Bal. % of Fish Protein[a]
(five rats/sol group)

Sample	*AD*	*TD*	*NPU*	*Bal%*
Flat sardine				
Cooked	82·1 ± 2·9	88·4 ± 2·9	89·8 ± 3·5	70·2 ± 4·0
Fried	76·2 ± 1·3	82·5 ± 1·2	82·8 ± 3·4	62·7 ± 3·8
Smoked	79·4 ± 3·0	85·7 ± 3·0	90·1 ± 2·1	70·4 ± 2·4
Sea bream				
Cooked	84·6 ± 4·0	91·0 ± 4·0	91·2 ± 6·6	71·8 ± 7·3
Fried	81·8 ± 2·5	88·1 ± 2·5	90·4 ± 3·4	70·8 ± 3·8
Smoked	81·1 ± 1·5	87·4 ± 1·5	92·8 ± 2·0	73·3 ± 2·0
Tilapia				
Cooked	77·1 ± 3·5	83·4 ± 3·5	82·5 ± 6·4	62·7 ± 7·0
Fried	80·9 ± 3·5	87·2 ± 3·5	91·5 ± 2·5	71·6 ± 2·6
Smoked	74·4 ± 0·6	80·7 ± 0·6	83·3 ± 2·3	63·8 ± 2·6

[a] Means ± standard deviation.

samples. This resulted in a higher NPU in the fornmer, but it is difficult to explain this from the amino acid analyses.

The digestibility values are in good agreement with the findings of De Groot (1963); Yanes *et al.* (1970) and Herborg *et al.* (1974) on fish samples treated in different ways. AD in the range of 71–84 for herring meal and other protein concentrates of marine origin have been reported (Njaa *et al.*, 1966). Rats fed unsalted, air dried coal fish had AD of 85·2 (Njaa *et al.*, 1968). These values compare favourably with the results of this work.

Statistical analysis of the AD values obtained showed significant differences between fishes ($P < 0{\cdot}001$) and between treatments ($P < 0{\cdot}05$). Treatment and fishes' sum of squares were partitioned into single degrees of freedom for further analysis. Between fishes, there was significant difference ($P < 0{\cdot}01$) between the mean of sea bream taken over treatments and that of sardine and tilapia taken together. This implies that sea bream protein was better absorbed than the other two fish proteins. Further analyses with respect to treatments indicated significant differences between the mean of cooked taken over fishes and that of fried and smoked ($P < 0{\cdot}001$) taken together and the means of fried taken over fishes and smoked ($P < 0{\cdot}05$).

Cooking of fish causes solubilization of proteins and hence lead to loss of protein from the final product (March, 1984). The results of this study showed that cooked fish had as high protein content as the raw fish and was better absorbed than fried and smoked. It therefore implies that there is leaching of the less utilizable protein on cooking. In most developing countries fish is cooked directly in soups and stews. So that even if these proteins leach, they are taken together in the diet. The *in vitro* digestibilities of different species have been reported by Krauze *et al.*, (1970). Digestibility decreased in the following order: fish fried in oil, boiled fish and raw fish.

NPU was high for both species and treatments. These values are in good agreement with the results from De Groot (1963), Njaa *et al.* (1968) and Olley *et al.* (1968) on marine fish samples. Freshwater fish, fresh and salted dried, both dried at 105°C had NPU values of 73·2 and 69·5, respectively (El-Wakeil *et al.*, 1975). These values are slightly lower than what was observed in this study for both species and treatments. The balance % for both fishes and treatments were high. The values are higher than literature data on fish meals. Njaa *et al.* (1966) and Gjøen and Njaa (1977) have reported balance % in the range of 30–40% and 59–60%, respectively. The higher balance values may indicate that the proteins (N) were used by the rats for synthetic purposes not associated with maintenance. It would have been important to carry out a growth experiment to justify the above statement but due to shortage of fish samples this was not possible.

Nevertheless, statistical analysis of Bal % showed statistically significant differences between fishes ($P < 0{\cdot}01$). To find out where the differences lie

segregation of the sum of squares into single degrees of freedom was carried out. This showed that the mean of sea bream was greater than that of tilapia and sardine; in other words, there were differences between sea bream and tilapia and sardine taken together. There were no significant differences between treatments.

Orraca-Tetteh (1961) found that sun-dried carp from Ghana had a lower protein value (NPU of 57%) than dried stock fish from Norway. In the same report it was mentioned that smoked fish from Ghana had a relatively low protein value. The present work contradicts this as the smoked fish had comparably high protein values as cooked and fried fish. The findings of Munro and Morrison (1965) further suggests that smoked fish has good quality protein as judged by rat feeding experiments.

The protein contents of the fried fish were lower than that of the cooked and smoked fish. The fried fish had values above 70% for AD and NPU. On a weight basis much more of the fried fish would be required to meet the protein requirement. The smoked fish were smoked once. It would be of interest to know what happens to the composition of fish due to resmoking normally carried out to maintain edibility and prolong storage.

There is a need to make comparisons with the raw sample of the same species and batch. In the biological procedures, it was not considered necessary to include the raw fish. This is because fish (with particular reference to Ghana) are not eaten raw. If the raw fish had been included, however, it might have given an indication of any damage as well as improvement in protein digestibility and utilization.

REFERENCES

Arnold, S. H. & Brown, W. D. (1978). Histamine toxicity from fish products, *Adv. Food Res.*, **24**, 113–47.

Brækkan, O. R. & Boge, G. (1962). A comparative study of amino acids in the muscles of different species of fish. *Fisk. Dir. Skr., Ser. Tekn. unders.*, **4**(3), 5–19.

De Groot, A. P. (1963). The influence of dehydration of foods on the digestibility and biological value of the protein. *Food Technol.*, **17**, 103–7.

Dunn, M. S., Camien, M. N., Eidson, S. & Malin, R. B. (1949). The nutritive value of canned foods. XXXIII. I. amino acid content of fish and meat products. *J. Nutr.*, **39**, 177.

Eggum, B. O. (1973). A study of certain factors influencing protein utilization in rats and pigs. 406. Beretning fra forsøgslaboratoriet, Denmark.

El-Wakeil, F. A., KHairy, M., Morsi, S. & Abo-Raya, S. H. (1975). Chemical and biological evaluation of fresh and preserved Lake Nasser's fish. II Salt-dried Keshr-bayad (*Lates niloticus*), *Egypt. J. Food Sci.*, **3**, 65–72.

Gjøen, A. U. and Njaa, L. R. (1977). Methionine sulphoxide as a source of sulphur-containing amino acid for the young rat. *Br. J. Nutr.*, **37**, 93–105.

Haaland, H., Arnersen, E. & Njaa, L. R. (1988). Technical note: Amino acid composition of whole herring over the spawning season and during anaerobic storage at 2°C. *International J. Fd. Sc. Tech.*, **23**, 521–4.

Herborg, L., Vilian, F., Bruan, A. & Eggum, B. (1974). Roller-dried fish protein. In *Fishery Products*, ed. R. Kreuzer, FAO, Rome, pp. 309–11.

Hoffman, A., Barranco, A., Francis, B. J. & Disney, J. G. (1977). The effect of processing and storage upon the nutritive value of smoked fish from Africa. *Tropical Sci.*, **19**, 41–53.

Kjosbakken, J. & Larsen, H. (1981). The non-protein nitrogenous compounds of fish used in meal production, with special reference to Capelin (*Mallotus villosus*). *Fisk. Dir. Skr. Ernæring*, **2**(1), 7–24.

Krauze, S., Oledska, R. & Buczynska, Z. (1970). Enzymatic digestion *in vitro* of animal proteins. II. Investigation of digestibility of raw fish as well as fish subjected to thermal treatment. *Roczniki Panstwowego Zakladu Hig.*, **21**, 287–92.

Liquid chromatographic analyses of amino acids in feed and foods using a modification of the Pico-Tag method. Millipore Corp., 1987.

March, B. E. (1984). Effect of processing on the nutritive value of food. Fish. In *Handbook of Nutritive Value of Processed Food.* Vol. 1. ed. Miloslave Recheigl Jr, pp. 363–81.

Munro, I. C. & Morrison, A. B. (1965). Effect of salting and smoking on protein quality of cod. *J. Fisheries Board Can.*, **22**, 13–16.

Neilands, J. B., Sirny, R. J., Sohljell, I., Strong, F. M. & Elvehjem, C. A. (1949). The nutritive value of canned foods. XXXIII. II. Amino acid content of fish and meat products. *J. Nutr.*, **39**, p. 187.

Njaa, L. R. (1963). A study on the Mitchell method for determination of the biological value of protein. *Fisk. Dir. skr. Ser. Tek. unders.*, **4**(5).

Njaa, L. R., Utne, F. & Brækkan, O. R. (1966). Protein value of herring meal and other protein concentrates of marine origin for the young rat. *Fisk. Dir. technol. Unders.*, **5**(1), 3–25.

Njaa, L. R., Utne, F. & Brækkan, O. R. (1968). Protein value of cod and coalfish and some products for the young rat. *Fisk. Dir. Skr. Technol. Unders*, **5**(4), 1–13.

Njaa, L. R. (1980). A method for determination of unoxidized and total methionine in protein concentrates, with special reference to fish meals. *Brit. J. Nutr.*, **43**, 339.

Njaa, L. R. & Utne, F. (1982). A comparison of the amino acid composition of fifteen species of whole fish. *Fisk. Dir. Skr. Ernæring*, **II**(2), 25–33.

Olley, J., Ford, J. E. & Williams, A. P. (1968). Nutritional value of fish visceral meals. *J. Sci. Fd. Agr.*, **19**, 282–5.

Opstvedt, J., Miller, R. W. & Spirelli, J. (1984). Heat induced changes in sulphydryl groups and disulphide bonds in fish protein and their effects on protein and amino acid digestibility in rainbow trout. *J. Agr. Food Chem.*, **32**, 920–35.

Orraca-Tetteh, R. (1961). Problems and some solutions: Ghana. *Proc. Nutr. Soc.*, **20**, 109–11.

Power, H. E., Savagaon, K. A., March, B. E. & Biely, J. (1969). *Fish. Res. Bd. Can. Techn. Rep., No.* 114, 14.

Proctor, B. E. & Lahiry, N. L. (1956). Evaluation of amino acids in fish processed by various methods. *Food Res.*, **21**, p. 91.

Sakaguchi, M. & Shimizu, W. (1965). Studies on the muscle of aquatic animals-XXXXV. Variation with season and growth in nitrogenous extractives of

mackerel muscle. *Bulletin of the Japanese Society of Scientific Fisheries.* **31**, 72–5, after *Chem. Abstr.*, **66**, 62915y (1967).

Sachse, J. (1981). Ein beitrag Zur bestimmung von tryptophan in mais und futterepflanzen. *Zeitschrift für Lebensmitteluntersuchung und Forschung*, **172**, 272–7.

Seet, S. T. & Brown, W. D. (1983). Nutritional quality of raw, precooked and canned aibacore tuna (*Thunnus alalunga*). *J. Food Sci.*, **48**, 288–9.

Steiner-Asiedu, M., Julshamn, K. & Lie, Ø. (1990). Effect of local processing methods (cooking, frying and smoking) on three fish species from Ghana. I—Proximate composition, fatty acids, minerals, trac elements and vitamins. *Fd. Chem.* (submitted).

Takagi, M., Jida, A. & Murayama, H. (1969). On the formation of histamine during loss of freshness and putrefaction of various marine products. *Bull. Fac. Fisheries. Hokkaido Univ.*, **20**, 227–34.

Yanes, E., Ballester, D. & Donoso, G. (1970). Effect of drying temperature on quality of fish protein. *J. Sci. Food Agr.*, **21**, 426–8.