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A PRELIMINARY STUDY OF THE DISTRIBUTION OF NON-PROTEIN NITROGEN IN SOME MARINE FISHES AND INVERTEBRATES*

BY N. K. VELANKAR AND T. K. GOVINDAN
(Central Marine Fisheries Research Station, Mandapam Camp)

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THE non-protein nitrogen of fish muscle constitutes a small fraction of the total nitrogen content of the fish. However, the nitrogenous extractives of fish muscle which consist of ammonia and trimethylammonium bases, guanidine and imidazole derivatives, and miscellaneous substances such as urea, amino acids, purines and pyrimidines are of significance from the aspect of preservation and processing of fish. These substances probably influence the potential keeping quality and appear to contribute to the flavour of the fish. The nature and concentration of their decomposition products are of primary importance in deciding the edibility of the fish. The changes in the concentration of some of these constituents reflect the early changes occurring in fish muscle prior to the onset of organoleptically detectable spoilage and hence are of value as tests for the quality of fish.

The distribution of the nitrogenous components varies in different fishes and also factors such as environment, spawning cycle, season, size and age probably influence this distribution. Available information on these aspects relating to fishes of the Atlantic, Pacific and Arctic oceans, the North Sea etc., has been critically reviewed by Shewan.[1] The study of non-protein nitrogenous extractives in Indian marine fishes is therefore of interest from the comparative aspect besides being of value in fish processsing.

Preliminary to a comprehensive investigation, the authors surveyed the distribution of non-protein nitrogen in a number of marine fishes and some invertebrates. The trimethylamine oxide, free α-amino acid nitrogen, total volatile basic nitrogen, etc., were also determined in some cases. The results of these investigations are reported in this paper.

MATERIALS AND METHODS

The fishes used in this study were obtained mainly from shore seine catches at Dhanushkodi and brought to the laboratory in crushed ice. Some

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were also obtained from nearby fishing villages and from the 'Kellong' fishing operations carried out by this Station at Mandapam Camp in the Gulf of Mannar. The oysters were collected from the coral rocks near the shore and the clams from the sandy bottom of the shallow regions in the Gulf of Mannar.

(*a*) *Total nitrogen.*—Total nitrogen of the fish muscle was determined by the Kjeldahl method.

(*b*) *Trimethylamine oxide nitrogen.*—A known weight (ca. 5 g.) of the muscle was ground with acid washed sand in a mortar and 5 ml. of distilled water added followed by 10 ml. of 10% trichloracetic acid.[2] It was mixed well and left for 2 hrs. after which it was filtered and the precipitate washed repeatedly with 1% trichloracetic acid. The filtrate and washings were made up to a known volume (25 ml.) and 5 ml. of this was treated with 1 ml. of N hydrochloric acid and 0·5 g. of Devarda's alloy and kept on the boiling water-bath for 15 minutes. It was then cooled, filtered, washed with distilled water and the filtrate and washings made up to 50 ml. The trimethylamine-oxide was then determined by the difference in the volatile base before and after reduction.[3]

(*c*) *Total water-soluble nitrogen.*—A known weight of the muscle was taken in a Waring Blendor, enough distilled water added to cover the blades and minced for two minutes. The pasty mass was then transferred quantitatively to a conical flask with the help of distilled water so that the final volume came to about five times in ml. of the weight of muscle taken. 2 ml. of toluene were added, mixed well by swirling and left overnight in the refrigerator with occasional swirling. It was then made up to the nearest convenient volume and filtered or centrifuged. The total water-soluble nitrogen was then determined by the micro-Kjeldahl procedure.

(*d*) *Non-protein nitrogen.*—To a known volume (10 ml.) of the filtrate from (*c*) were added 5 ml. of 20% trichloracetic acid, the precipitated protein was filtered and washed repeatedly with 1% trichloracetic acid and the filtrate and washings made up to 25 ml. The nitrogen in this filtrate was determined by the micro-Kjeldahl method.

(*e*) *Free α-amino acid nitrogen.*—From 20 ml. of the filtrate from (*c*), the proteins were removed as described in (*d*) above and the filtrate was neutralised to thymolphthalein with N sodium hydroxide. The free α-amino acid nitrogen was then determined by the method of Pope and Stevens.[4]

(*f*) *Volatile base nitrogen.*—This was determined using 1 ml. of the filtrate obtained in (*c*) by the Conway micro diffusion method.

(*g*) *Glutamine amide nitrogen.*—This was determined according to the procedure described by Kermack *et al.* (*loc. cit.*)

RESULTS AND DISCUSSION

The results are shown in Tables I and II. The total nitrogen content varied from 2·663 to 3·628% in the teleosts, the usual value being about 3·4% of the wet weight of the muscle (Table I). In the elasmobranchs the total nitrogen content is higher than that in the teleosts, the values ranging from 3·77 to 4·41% of the muscle. Elasmobranch muscle is known to contain urea in fairly large concentrations, up to 2%, unlike in the teleosts and this presumably explains the higher total nitrogen content of the former. The total nitrogen content of the invertebrates is of the same level as that of the teleosts.

The total water-soluble nitrogen is also high in the elasmobranchs, *viz.*, 1,342 to 1,994 mg. nitrogen per 100 g. of wet muscle, while in the teleosts it ranges from 594·6 to 1,042 mg. nitrogen per 100 g. of wet muscle. The high values of water-soluble nitrogen in the invertebrates is accounted for by the presence of considerable quantities of free amino acids in their muscle.

The non-protein nitrogen (N.P.N.) content of the teleosts is about 10% of the total nitrogen. Shewan (*loc. cit.*) has stated the N.P.N. to be about 9 to 14% of the total nitrogen in the case of gaddoids, flat fishes, etc., and 16 to 18% in the herring group. The N.P.N. content of the Indian marine fishes examined is comparatively low. The N.P.N. constitutes about $\frac{1}{3}$ to $\frac{1}{2}$ of the total water-soluble nitrogen in the teleosts, about $\frac{3}{4}$ in the elasmobranchs, about 3/5 in the crustaceans and 4/5 in the molluscs.

The free α-amino acid nitrogen in the teleosts is only about 6% of the N.P.N. while in the invertebrates it accounts for over 40% of the N.P.N. The presence of free amino acids in significant quantities in the crustacean muscle probably renders it more susceptible to bacterial invasion resulting in the lower potential keeping quality of the crustaceans compared with teleost fishes. The amount of amino acid nitrogen in extractives and its ratio to the total extractive nitrogen was found to be related to the flavour of the tuna meat and relatively higher proportion of diamino acids was associated with good flavour.[5] An examination of the different amino acids present in the free condition in the muscle appears to be necessary.

High levels of free amino acids are found in the molluscs as well as in the crustaceans (Table I). On the other hand in the sea turtle, a representative of a different order of vertebrates besides the fishes, the level of free amino acids is of the same order as in the fishes. The data adduced here supports the view expressed by the authors in an earlier communication[6] that high levels of free amino acids are probably characteristic of the invertebrate muscle. The free amino acid nitrogen content of the elasmobranchs is about 2 to 3 times as much as in the teleosts and this accords well with the intermediate position of the elasmobranchs as between the teleosts and invertebrates.

While the urine of fishes contains only a small quantity of amino acid nitrogen, 14% of the nitrogen excreted by invertebrates has been found to be amino acid nitrogen.[7] Presumably the prevalence of high levels of amino acid nitrogen in their muscle is related to the high levels of the amino acid nitrogen in the excretions of the invertebrates. The observations recorded here lend support to Baldwin's view[7] that the invertebrate is less efficient than the vertebrate in metabolising amino acids. Whether the free amino acids present in the invertebrate muscle play any significant role in its physiology needs to be examined.

The volatile base nitrogen is below 20 mg. per 100 g. of the wet muscle in the fishes, molluscs and in the crustaceans excepting the prawns. The quantity of volatile base nitrogen ranges from 9·7 to 23·6 mg. per 100 g. of wet muscle. The volatile base nitrogen in fresh fish muscle is mainly ammonia which has its origin in the deamination of the muscle adenylic acid, a reaction which is associated with muscular work and also with processes leading to the denaturation of the muscle proteins. Hence though the total volatile nitrogen content is not considered to be a sensitive index of fish spoilage unlike the trimethylamine, mainly on account of the wide variation in its concentration in the fresh fish muscle, its significance needs a critical examination.

The trimethylamine oxide content varies widely in the different fishes (Table II). The values are low compared with those of the related species from the west coast described by Venkataraman and Chari,[8] the difference being particularly noticeable in the elasmobranchs and in the prawns. Since the species examined in their investigations were different from those used in our studies the observed differences are either specific or/and are related to other factors such as the environmental differences, etc. Shewan (*loc. cit.*) has cited considerable differences in the trimethylamine oxide content of identical species from the Atlantic and Pacific oceans respectively. The trimethylamine oxide content of different fishes is of special significance

TABLE I

Distribution of nitrogen in the fresh muscle of some marine fauna

(The figures in the table are expressed in mg. nitrogen per 100 g. of wet muscle)

Sl. No.	Name of Fish	Total nitrogen	Total water-soluble nitrogen	Non-protein nitrogen	α-amino nitrogen	Volatile base nitrogen	Glutamine amide nitrogen
	Elasmobranchs						
1	*Scoliodon* sp.	3996·0	1587·0	1096·0	71·69	..	..
2	*Sphyrna malleus*	4184·0	1520·0	1174·0	58·75	11·49	18·61
3	*Chiloscyllium griseum*	3770·0	1485·0	1095·0	52·16	12·42	23·82
4	*Trygon microps*	3845·0	1342·0	973·4	22·80	12·83	15·33
5	*Trygon urnak*	4063·0	1879·0	1369·0	42·13	18·23	23·69
6	*Trygon imbricata*	4411·0	1581·0	1229·0	22·60	..	..
7	*Myliobatis maculata*	4257·0	1876·0	1320·0	71·55	13·75	28·50
8	*Rhynchobatus djeddensis*	4097·0	1994·0	1487·0	129·80	29·76	26·15
	Teleosts						
1	*Hilsa toli*	3593·0	662·7	311·1	17·86	..	..
2	*Chirocentrus dorab*	3253·0	791·1	272·1	16·81	17·89	20·31
3	*Tylosurus lieurus*	3628·0	804·6	374·1	24·29	..	..
4	*Athlennes hians*	3416·0	893·4	399·0	38·93	23·62	27·82
5	*Scomberomorus commersonii*	3316·0	594·6	335·6	24·84	18·81	14·68
6	*Pampus argenteus*	3248·0	653·3	353·0	20·32	18·96	17·07
7	*Decapterus russellii*	3529·0	690·4	301·4	20·77	..	..
8	*Caranx hippos*	3402·0	807·4	332·6	14·02	17·64	21·40
9	*Drepane punctata*	2663·0	722·6	236·7	9·10	..	..
10	*Scatophagus argus*	3273·0	700·0	296·1	23·81	..	..
11	*Scatophagus argus*	3294·0	709·1	350·6	11·55	12·38	18·13
12	*Mugil* sp.	3291·0	730·3	251·9	59·73	19·89	23·16

13	*Cypsilurus* sp.	3394·0	831·4	363·4	56·67	10·56	30·48
14	*Sardinella albella*	3206·0	802·8	375·0	..	20·99	24·49
15	*Chorinemus* sp.	3041·0	861·4	318·5	25·80	..	..
16	*Lethrinus nebulosus*	3583·0	910·7	313·3	12·95	16·47	..
17	*Lutjanus rivulatus*	2997·0	1042·0	388·5	42·42	22·03	..
18	*Cynoglossus bengalensis*	3262·0	691·6	350·0	14·45	21·65	17·31
	Crustacea						
1	*Panulirus dasypus*	3271·0	1134·0	683·0	241·30	16·17	34·73
2	*Neptunus pelagicus*	3214·0	1148·0	914·3	356·20	..	..
3	*Neptunus pelagicus*	2531·0	733·8	473·9	210·30	19·93	27·17
4	*Charybdis quadrimaculata*	..	..	447·1	197·80	..	..
5	*Thalamita crenata*	..	..	660·4	304·90	..	..
6	*Penæus indicus*	3513·0	1548·0	772·9	326·10	31·27	46·93
7	*Penæus monodon*	3415·0	1231·0	756·5	394·20	64·37	33·78
8	*Metapenæus affinis*	3599·0	1423·0	794·7	365·90	38·44	46·87
	Mollusca						
1	*Sepioteuthis arctipinnis*	3271·0	989·5	789·8	349·40	16·93	13·87
2	*Mesodesma glabratum* (muscle alone)	2423·0	870·8	428·0	124·40	19·18	9·86
3	*Ostrea cucullata* (whole contents of shell)	2445·0	687·1	188·7	41·46	9·70	23·81
	Reptilia						
1	*Chelone imbricata*	2855·0	848·4	402·4	68·79	10·42	23·10

TABLE II

Trimethylamine oxide content of the fresh muscle of some marine animals

(The values are expressed as mg. nitrogen per 100 g. of wet muscle)

Sl. No.	Name of Fish	Trimethyl amine oxide nitrogen	Trimethyl amine oxide values as reported for allied species on the west coast (see Ref. No. 8)
	Elasmobranchs		
1	*Scoliodon* sp.	72·26	239·4, *Carcharias laticaudus.* (for 1 and 2)
2	*Chiloscyllium griseum*	82·94	
3	*Sphyrna malleus*	107·10	245·0, *Zygæna blochii.*
4	*Trygon microps*	49·00	
5	*Trygon urnak*	103·90	
6	*Trygon imbricata*	46·43	
7	*Myliobatis maculata*	28·73	
8	*Rhynchobatus djeddensis*	87·31	159·6, *Rhinoptera* sp.
	Teleosts		
1	*Pampus argenteus*	54·99	65·8, *Stromateus cinereus* 68·2, *Stromateus niger.*
2	*Mugil* sp.	24·04	38·5, *Mugil œur.*
3	*Chirocentrus dorab*	36·46	65·8, *Chirocentrus dorab*
4	*Cypsilurus* sp.	25·26	
5	*Sardinella albella*	54·40	30·2, *Sardinella longiceps* 43·4, *Clupea lile* 49·0, *Sardinella fimbriata* 39·2, *Hilsa kanagurta*
6	*Athlennes hians*	43·06	
7	*Scomberomorus commersonii*	28·15	
8	*Platophrys pantherina*	36·43	
9	*Cynoglossus bengalensis*	36·11	38·6, *Cynoglossus semifasciatus*
10	*Saurida tumbil*	54·59	
11	*Sillago sihama*	20·57	
12	*Synagris japonicus*	40·00	
13	*Lethrinus cinereus*	54·84	77·4, *Serranus diacanthus*
14	*Caranx leptolepis*	53·92	
15	*Gerres* sp.	39·82	
16	*Mulloides flavolineatus*	51·04	
17	*Arius sona*	35·75	17·8, *Arius dussumieri*
18	*Scatophagus argus*	33·11	
	Crustacea		
1	*Panulirus dasypus*	111·30	
2	*Penæus indicus*	19·60	74·2, *Trachypenæus aspar* 114·8, *Parapenæopsis stylifera* (for 2–4)
3	*Penæus monodon*	18·20	
4	*Metapenæus affinis*	23·80	
5	*Neptunus pelagicus*	54·13	
6.	*Neptunus pelagicus*	56·91	
	Mollusca		
1	*Sepioteuthis arctipinnis*	82·94	
2	*Mesodesma glabratum* (muscle only)	55·51	
3	*Ostrea cucullata* (whole contents		

in the application of the trimethylamine test for assessing the extent of spoilage in sea fish.

The sea turtle, the only reptile examined in the present study, has the lowest trimethylamine oxide nitrogen content among the marine animals included in the present investigation.

SUMMARY

The distribution of non-protein nitrogen in some marine fishes, crustacea and molluscs was studied. The non-protein nitrogen constituted about 10% of the total nitrogen in the teleosts, 20% in the crustaceans and molluscs and over 30% in the elasmobranchs. High levels of free α-amino acid nitrogen (300 mg. N/100 g. of wet muscle) were found in the crustaceans and molluscs unlike in the fishes (20 to 40 mg. N/100 g. of wet muscle). The trimethylamine oxide content varied greatly in the fishes, etc. The significance of these results from the comparative aspect and in fish processing is discussed.

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