

Distribution of Non-Protein Nitrogenous Extractives in the Muscle of Indian Fish

Suseela Mathew, M.R. Raghunath and K. Devadasan
Central Institute of Fisheries Technology
Cochin - 682029, India

Major non-protein nitrogen (NPN) constituents like trimethyl amine oxide (TMAO), creatine and creatinine, α -amino nitrogen and peptides were estimated in 41 species of marine fish in sea-fresh condition. TMAO was found to be more in elasmobranchs and crustaceans than in teleosts. Euryhaline teleosts entering shallow waters had lower values of TMAO than benthic fish. Creatine and creatinine formed a major portion of NPN in fish, but in crustaceans and squid, only traces of these compounds could be detected. α -Amino nitrogen was found to be more in elasmobranchs and crustaceans. Highest level of peptides was noted in Indian mackerel, *Rastrelliger kanagurta*. Peptide levels were higher in elasmobranchs than in teleosts and lowest in crustaceans.

Key words : Non-protein nitrogen, fish, shellfish, α -amino nitrogen, trimethyl amine oxide, creatine, creatinine, peptides

The nitrogenous fractions other than proteins of fish muscle (non-protein nitrogenous extractives, NPN) comprise of a highly diverse group of compounds. Amino acids in free and combined forms, trimethyl amine oxide (TMAO), nucleotides, guanidine and imidazole derivatives, urea and betaine are commonly found in these fractions. The constituents of NPN fraction contribute to the flavour of the fish/shellfish. The sweet taste of prawn and squid is attributed to the high levels of glycine and free amino acids (Velankar & Govindan, 1958). As it provides a ready source of nutrients for the spoilage bacteria, the NPN also has a bearing on the keeping quality of fish. The distribution of these substances in fish varies with season, size and age (Hebard *et al.*, 1982). Extensive data are available on the concentration of the constituents of NPN fraction in fish from temperate waters (Konosu *et al.* 1974; Ikeda, 1980; Konosu & Watanabe, 1976; Suyama & Suzuki, 1975; Suyama & Yoshizawa, 1973; Jiang & Lee, 1985). Velankar & Govindan (1958) studied NPN fractions of a few Indian

fish. Venkataraman & Chari (1955) have reported the TMAO content of a few species of Indian fish. In either case landed or market samples of fish were used and post mortem changes in NPN could have occurred. An attempt has been made in the present study to estimate the major chemical constituents of the non-protein nitrogenous extractives of 41 species of marine fish, in sea-fresh condition.

Materials and Methods

Fish were caught by bottom and off bottom trawling on board FORV Sagar Sampada from the Bay of Bengal (between latitudes 18° 00' N to 21° 00' N and longitude 83° 00' E to 88° 00' E). Samples of fish were taken to the wet lab on board the vessel immediately after the catch and identified using FAO identification sheets (Fischer & Bianchi, 1984) and Day (1958). Muscle from 10 small sized or 2-3 medium sized fish were collected immediately and minced well. Ten gram portions of the minced muscle were thoroughly ground with 0.6 M perchloric

acid and kept at 4°C till landing. It was then brought to the main laboratory, filtered through Whatman No. 1 filter paper. The residue was washed with perchloric acid and made up the combined filtrate to the required volume with perchloric acid. Aliquots of the filtrate were used for the estimation of nitrogenous constituents. Trimethyl amine (TMA) content of the filtrate was estimated by colorimetry (Wekell & Barnett, 1991). TMAO was estimated after reduction to TMA using titanium chloride (Wekell & Barnett, 1991) and was computed as the difference between the TMA contents of the reduced and unreduced (assayed directly for TMA) samples. Creatinine was estimated by the Folin -Wu procedure (Oser, 1976) and creatine was estimated after conversion to creatinine by autoclaving with acid. α -Amino nitrogen was estimated by the ninhydrin method of EBC (1975) and peptide nitrogen by the Biuret reaction (Scopes, 1982). Regression analysis was done to find out the relationship between morphometric parameters of fish and the concentration of various NPN fractions. Analysis of variance for unequal number of observations was performed to analyse the difference between families of fish in the concentration of NPN constituents.

Results and Discussion

Common and scientific names of fish and their morphometric characteristics are given in Table 1. The concentration of the

major constituents of the NPN fractions in the various fish species are given in Table 2. In elasmobranchs, TMAO content was found to be quite high (175 mg N.100g⁻¹ muscle for shark, *Scoliodon laticaudus* and 102 mg N.100g⁻¹ muscle for eagle ray, *Myliobatus neivohofii*. Harada (1975) has reported similar high levels of TMAO in *Selachii* (190 mg N.100g⁻¹ muscle) and *Rajiida* (99 mg N.100g⁻¹ muscle). In elasmobranchs, TMAO is supposed to play a vital role in osmoregulation (Simidu, 1961). TMAO and urea together constitute 60-76% of total extractive nitrogen in elasmobranchs (Suyama & Suzuki, 1975).

In teleosts, TMAO content ranged from 9-68 mg N.100g⁻¹ muscle. TMAO is not believed to contribute to osmoregulation in teleosts, since the osmotic pressure of teleost blood is lower than that of sea water (Hebard *et al.*, 1982). However, Lange & Fugelli (1965) reported a system of intracellular osmotic regulation which exists in euryhaline teleosts and TMAO plays an active role in the volume regulation of muscle cells with a concomitant adjustment of the number of intracellular osmotically active particles. In the present study euryhaline teleosts which enter shallow waters and backwaters were found to have generally lower TMAO content than benthic fish. *Terapon jarbua*, *Valamugil cunnesius*, *Sardinella melanura*, *Leognathus equulus*, *Gerres filamentosus*, *Mene maculata* and *Sphyaena forsteri*, all of which are known to enter

Table 1. Scientific and Common names of Fish studied

Family	Scientific Name	Common Name	Length (mm)	Weight (g)
Teleosts				
1. Ariommidae	<i>Ariomma indica</i> (Day, 1870)	Indian drift fish	205	134
2. Belonidae	<i>Strongylura strongylura</i> (Van Hasselt 1823)	Spot tail needlefish	190	25
3. Bothidae	<i>Pseudorhombus sp</i>	Flounder	265	228
4. Carangidae	<i>Megalaspis cordyla</i> (Linnaeus 1758)	Torpedo scad	178	55

5. Carangidae	<i>Decapterus russelli</i> (Ruppell, 1830)	Indian scad	165	55
6. Carangidae	<i>Carangoides malabaricus</i> (Bloch & Schneider, 1801)	Malabar trevelly	280	375
7. Carangidae	<i>Parastromateus niger</i> (Bloch, 1795)	Black pomfret	480	409
8. Chirocentridae	<i>Chirocentrus nudus</i> (Swainson, 1839)	White fin wolf herring	250	115
9. Clupeidae	<i>Opisthopterus tardoore</i> (Cuvier, 1829)	Tardoore	160	36
10. Clupeidae	<i>Sardinella melanura</i> (Cuvier 1829)	Black tip sardinella	170	44
11. Clupeidae	<i>Dussumeiria acuta</i> (Valenciennes, 1847)	Rainbow sardine	150	35
12. Cynoglossidae	<i>Cynoglossus dubius</i> (Day 1873)	Carrot tongue sole	200	55
13. Engraulidae	<i>Thryssa malabaricus</i> (Bloch 1795)	Malabar thryssa	135	62
14. Ephippidae	<i>Ephippus orbis</i> (Bloch, 1787)	Spade fish	150	60
15. Gerridae	<i>Gerres filamentosa</i> (Cuvier, 1829)	Whipfin silver biddy	155	61
16. Haemulidae	<i>Pomadasys kaakan</i> (Curer 1830)	Javelin grunter	150	50
17. Lactaridae	<i>Lactarius lactarius</i> (Bloch & Schneider, 1801)	False trevally	210	170
18. Leiognathidae	<i>Leiognathus equulus</i> (Forsk, 1775)	Pony fish	180	134
19. Menidae	<i>Mene maculata</i> (Bloch & Schneider, 1801)	Moon fish	190	65
20. Mugilidae	<i>Valamugil cunnesius</i> (Valenciennes, 1836)	Long arm mullet	150	47
21. Mullidae	<i>Upeneus vittatus</i> (Forsskal, 1775)	Striped goat fish	135	33
22. Nemipteridae	<i>Nemipterus japonicus</i> (Bloch, 1791)	Japanese thread fin bream	200	88
23. Nemipteridae	<i>Nemipterus bleekeri</i> (Day, 1875)	Delagoa thread fin bream	180	72
24. Polynemidae	<i>Polynemus sextarius</i> (Bloch & Schnider, 1801)	Blackspot threadfin bream	210	120
25. Priacanthidae	<i>Priacanthus sp</i>	Bull eye	300	260
26. Scianidae	<i>Otolithus ruber</i> (Schneider 1801)	Tiger tooth croaker	675	1555
27. Scombridae	<i>Scomberomorus guttatus</i> (Lacepeda, 1801)	Narrow banded spanish seerfish	190	99
28. Scombridae	<i>Rastrelliger kanagurta</i> (Cuvier, 1817)	Indian mackerel	375	32
29. Sparidae	<i>Argyrops spinifer</i> (Forsk, 1775)	King soldier bream	201	174
30. Sphyraenidae	<i>Sphyraena forsteri</i> (Cuvier, 1829)	Big eye barracuda	305	140
31. Stromateidae	<i>Pampus argentius</i> (Epiphraes, 1788)	Silver pomfret	220	166
32. Synodontidae	<i>Saurida undosquamosis</i> (Richardson, 1840)	Brush tooth lizard fish	260	160
33. Teraponidae	<i>Terapon jarbua</i> (Forsskal, 1775)	Jarbua therapon	160	65

34. Trichiuridae	<i>Lepturacanthus savala</i> (Cuvier, 1817)	Savalai hair tail	475	92
Elasmobranchs				
1. Carcharinidae	<i>Scoliodon laticaudus</i> (Muller & Henle, 1838)	Spade nose shark	330	131
2. Torpentinidae	<i>Narcine sp</i>	Electric ray	200	94
3.	<i>Myliobatus neiwhofii</i>	Eagle ray	310	300
Crustaceans				
1. Penaidae	<i>Metapenaeus affinis</i> (H. Milne Edwards)	Jinga shrimp	165	30
2. Penaeidae	<i>Metapenaeopsis stridulens</i> (Alcock, 1905)	Fiddler shrimp	80	3
3. Scyllaridae	<i>Thenes orientalis</i> (Lund, 1793)	Flat head locust lobster	145	73
Cephalopods				
1. Loliginidae	<i>Loligo duvauceli</i> (Orbingy)	Squid	170	40

Size and weights are average of ten specimens of small and 2–3 specimens of large fish.

shallow waters, had TMAO content below 28 mg N.100g⁻¹ muscle (average 21 mg N.100g⁻¹). White fleshed (demersal) fish generally are reported to contain higher levels of TMAO than red fleshed (pelagic) fish (Horie & Sekine, 1956). In the present study, TMAO content was low in scombroids like mackerel and seer fish. Low values of TMAO have been reported by Konosu *et al.* (1974) for the common mackerel *Scomber japonicus*.

An enzymatic pathway leading to the demethylation of TMAO, producing dimethylamine and formaldehyde during frozen storage has been recognized in gadoid fish like cod, red hake etc (Hultin, 1992). Evidence of TMAO demethylase activity has been observed in lizard fish *Saurida undosquamosis* of Indian waters (Suseela Mathew & Devadasan, K. unpublished)

TMAO content was found to be quite high (63–78 mg N.100g⁻¹ muscle) in prawn, *Metapenaeus affinis* and *Metapenaeus stridulens* as compared to lobster, *Thenes orientalis* (48 mg N.100g⁻¹ muscle). TMA is produced by the action of bacteria on TMAO and it is responsible for the spoiled 'fishy' odour (Tarr, 1939). TMA was not detected in any of the samples analysed in the study. This is to be

expected as the extracts were prepared immediately after catch without any time lapse for bacterial spoilage.

Creatine and creatinine together accounted for the major part of the NPN fraction in most of the fish studied. In teleosts, the concentration ranged between 103–313 mg N.100g⁻¹. There was not much difference between elasmobranchs and teleosts in the average content of creatine and creatinine (mean concentrations in teleosts, 175 mg N.100g⁻¹ and in elasmobranchs, 198 mg N.100g⁻¹). Fish belonging to Clupeidae and Synodontidae families had higher creatine and creatinine levels than other teleost families. Lowest concentrations were found in *Narcine sp.* (63 mg N.100g⁻¹) and *Leiognathus equulus* (103 mg N.100g⁻¹). Konosu *et al.* (1974) have reported creatine and creatinine concentration of 117 to 273 mg N.100g⁻¹ muscle in eight species of fish, where it formed the major NPN constituent. In red sea bream, *Chrysophrys major*, the creatine and creatinine content was estimated to be 219 mg N.100g⁻¹ muscle (Konosu & Watanabe, 1976). Creatine and creatinine were present in traces only in crustaceans and molluscs. α -Amino constituents of NPN are important from the point of view of bacterial spoilage as they provide a source of nitrogen

Table 2. Trimethyl amine oxide, α - amino nitrogen, creatine + creatinine and peptide contents in marine fish & shellfish

Sl. No.	Scientific Name	TMAO mg N %	α - amino N mg N %	Creatine + Creatinine mg N %	Peptides mg / 100 g.
Teleosts					
1.	<i>Ariomma indica</i>	31	48	201	675
2.	<i>Pseudorhombus</i> sp	31	55	146	781
3.	<i>Megalaspis cordyla</i>	10	78	181	1125
4.	<i>Decapterus russelli</i>	30	66	221	1456
5.	<i>Carangoides malabaricus</i>	55	63	210	1150
6.	<i>Parastrumateus niger</i>	33	45	212	300
7.	<i>Chirocentrus nudus</i>	49	33	234	619
8.	<i>Opisthopterus tardoer</i>	53	40	172	800
9.	<i>Sardinella melanura</i>	16	47	270	1413
10.	<i>Dussumeiria acuta</i>	30	65	313	838
11.	<i>Cynoglossus dubius</i>	68	74	146	463
12.	<i>Thryssa malabaricus</i>	35	81	138	838
13.	<i>Ephippus orbis</i>	49	62	158	625
14.	<i>Gerres filamentosa</i>	23	60	182	1338
15.	<i>Pomadasys kaakan</i>	46	64	176	1269
16.	<i>Lactarius lactarius</i>	54	36	188	469
17.	<i>Leiognathus equulus</i>	21	29	103	519
18.	<i>Mene maculata</i>	21	29	103	519
19.	<i>Valamugil cunnesius</i>	12	58	166	681
20.	<i>Upeneus vittatus</i>	47	55	204	869
21.	<i>Nemipterus japonicus</i>	59	51	187	425
22.	<i>Nemipterus bleekeri</i>	48	37	204	1063
23.	<i>Polynemus sextarius</i>	60	64	164	788
24.	<i>Pricanthus</i> sp	52	42	213	906
25.	<i>Otolithus ruber</i>	53	17	180	750
26.	<i>Scomberomorus guttatus</i>	30	44	241	688
27.	<i>Rastrelliger kanagurta</i>	9	72	230	1775
28.	<i>Belone annulata</i>	60	27	177	856
29.	<i>Argyrops spinifer</i>	42	36	221	600
30.	<i>Sphyræna forsteri</i>	27	43	254	688
31.	<i>Pampus argentiis</i>	50	20	195	781
32.	<i>Saurida undosquamosis</i>	54	64	285	731
33.	<i>Therapon jarbua</i>	28	50	187	906
34.	<i>Lepturacanthus savala</i>	48	24	163	750
Elasmobranchs					
1.	<i>Scoliodon laticaudus</i>	175	86	209	406
2.	<i>Narcine</i> sp	7	74	64	1425
3.	<i>Myliobatus neiwhofii</i>	102	72	251	1406
Crustaceans					
1.	<i>Metapenaeus affinis</i>	78	220	Trace	363
2.	<i>Metapenaeopsis stridulens</i>	63	34	Trace	1613
3.	<i>Thenes orientalis</i>	48	281	Trace	313
Cephalopod					
1.	<i>Loligo duvaucelli</i>	64	—	8	513

that can be readily assimilated by the microflora associated with spoilage of fish. They contribute substantially to the flavour of the fish also. α -Amino nitrogen content varied from 17 mg N to 94 mg N.100g⁻¹ muscle in teleosts in fresh condition. Konosu & Watanabe (1976) have reported a range of 50-75 mg free amino N.100g⁻¹ in cultured and wild red sea breams. Elasmobranchs had higher levels of α -amino N (range 72-86 mg N.100g⁻¹ muscle) in comparison to teleosts. Among the free amino acids, histidine was the most abundant while taurine, lysine, glutamic acid and alanine were found in moderate amounts in yellow tail (Endo *et al.*, 1974). Arnold & Brown (1978) reported higher levels of free histidine in dark muscle of fish compared to the white muscle. In the present study the average amino nitrogen content of shell fish was 178 mg N.100g⁻¹ muscle, which was higher when compared to teleost fish (51 mg N.100g⁻¹ muscle). But the fiddler shrimp *M. stridulens* was an exception with an amino nitrogen of only 34 mg N.100g⁻¹ muscle. Shell fish contain large amounts of extractives and amino nitrogen which may contribute to the sweet taste of their flesh (Takagi & Simidu, 1962).

Concentration of peptides in different fish is given in Table 2. Peptide concentration in teleosts ranged from 300 mg to 1775 mg 100g⁻¹ muscle with the highest level observed in Indian mackerel (*Rastrelliger kanagurta*). This could be due to the high autolytic activity in the Indian mackerel (Jayan *et al.*, 1997; Leema & Raghunath, 1998). Other fish with high levels of peptides were *Megalaspis cordyla*, *Decapterus russelli*, *Carangoides malabaricus*, *S. melanura*, *Pomadasys kakaan*, *M. maculata*, *Nemipterus bleekeri*, all of which had more than 1000 mg peptides 100g⁻¹ muscle. Average peptide level in teleosts was 856 mg 100g⁻¹, which was much lower when compared to

elasmobranchs with an average peptide content of 1079 mg. 100g⁻¹. Elasmobranchs have high amount of urea in their muscle, which would cause interference in peptide estimation by the biuret reaction. Peptide levels in crustaceans were slightly lower than that of teleosts. *M. stridules* however had an unusually high content of peptides (1813 mg.100g⁻¹). Peptide contents observed in this study were higher than the previously reported values (Konosu *et al.*, 1974). This could be due to the fact that perchloric acid used to prepare the extracts may not be particularly effective in precipitating peptides (Oser, 1976).

Regression analysis showed that no significant relationship existed between morphometric parameters (length and weight) and the concentration of various NPN fractions. Although considerable differences appeared to exist between various families, Analysis of Variance for unequal number of observations showed that no significant differences existed between the families of fishes in the concentrations of NPN constituents.

The study showed that there is considerable variation in the concentration of various NPN constituents among fish and shellfish. Creatine and creatinine along with peptides constituted the major species of the non protein nitrogen fraction. The limited number of the species analysed in each family did not indicate the existence of any taxonomically related differences. Such data however can be useful as indicators of flavour and/or spoilage.

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References

- Arnold, S.H. & Brown, W.D. (1978) *Adv. Food Res.* **24**, 113
- Day, F. (1958) *The Fishes of India* Vol.II William Dawson & Sons Ltd., London
- EBC (1975) *European Brewing Convention Analytica*, 3rd edn., p.61. Zoeterwoude, The Netherlands
- Endo, K., Kishimoto, R., Yamamoto, Y. & Shimizu, Y. (1974) *Bull. Jap. Soc. Sci. Fish.* **40**, 67
- Fischer, W. & Bianchi, G. (1984) *FAO species identification sheets for fishery purposes. Western Indian Ocean* (Fishing area 51) FAO & DANIDA, Rome
- Harada, K. (1975) *J. Shimnaseki Univ. Fish.* **23**, 163
- Hebard, C.E., Flick, G.J. & Martin, R.E. (1982) In: *Chemistry and Biochemistry of Marine Food Products* (Martin, R.E., Flick, G.J., Hebard, C.E. & Ward, D.R. Eds) p.149, AVI Publishing Company, Westport, Connecticut
- Horie, S. & Sekine, Y. (1956) *J. Tokyo Univ. Fish.* **42**, 25
- Hultin, H.O. (1992) In: *Advances in Seafood Biochemistry* (Flick, G.J. & Martin, R.L., eds.) p.25, Technomic Publ. Co. Lancaster
- Ikeda, S. (1980) In: *Advances in Fish Science and Technology* (Connell, J.J. Ed.) p.111. Fishing News Books Limited, Farnham, Surrey, England
- Jayan, K., Leema, J., Raghunath, M.R. & Devadasan, K. (1997) In: *Technological Advancements in Fisheries* p.356 (Hamid, M.S. & Kurup, B.M. Eds.) Publication No.1 School of Industrial Fisheries, CUSAT
- Jiang, S.T. & Lee, T.C. (1985) *J. Agric. Food Chem.* **33**, 839
- Konosu, S., Watanabe, K. & Shimizu, T. (1974) *Bull. Jap. Soc. Sci. Fish.* **40**, 909
- Konosu, S. & Watanabe, K. (1976) *Bull. Jap. Soc. Sci. Fish.* **42**, 1263
- Lange, R. & Fugelli, K. (1965) *Comp. Biochem. Physiol.* **15**, 283
- Leema, J. & Raghunath, M.R. (1998) Paper presented at the Symposium on Advances and Priorities in Fisheries Technology, SOFT(I), Cochin, India 11-13 Feb. 1998
- Oser, B.L. (1976) *Hawk's Physiological Chemistry*, p.1030, Tata Mc Graw-Hill, New Delhi
- Scopes, R.K. (1982) *Protein Purification: Principles and Practice* Appendix B, Springer Verlag New York
- Simidu, W. (1961) In: *Fish as Food* Vol.1, (Borgstrom, G. ed) p.353, Academic Press, London
- Suyama, M. & Suzuki, H. (1975) *Bull. Jap. Soc. Sci. Fish.* **41**, 787
- Suyama, M. & Yoshizawa, Y. (1973) *Bull. Jap. Soc. Sci. Fish.* **39**, 1339
- Takagi, I. & Simidu, W. (1962) *Bull. Jap. Soc. Sci. Fish.* **28**, 1192
- Tarr, H.L.A. (1939) *J. Fish. Res. Bd. Can.* **4**, 367
- Velankar, N.K. & Govindan, T.K. (1958) *Proc. Ind. Acad. Sci.* **67**(4B), 202
- Venkataraman, R. & Chari, S.T. (1955) *Ind. J. Fish.* **2**, 37
- Wekell, J.C. & Barnett, H. (1991) *J. Food Sci.* **56**