J. mar. biol. Ass. India, 1976, 18 (3) : 516-524

BIOCHEMICAL STUDIES ON THE FISHES OF THE ORDER CLUPEIFORMES*

V. RAMAIYAN AND AL. PAUL PANDIAN

CAS in Marine Biology, Marine Biological Station, Porto Novo, India

ABSTRACT

The chemical composition of 11 species of commercially important fishes belonging to the order Clupeiformes was investigated. The protein, carbohydrate, fat and the water content of the muscle tissue of the fresh fish were estimated. The water content varied from 65 to 75% of the wet weight. The protein accounted for 15 to 25% whereas the total carbohydrate was found to be not more than 0.3%. 3 to 5% of the wet tissue was found to be extractable fat. The effect of long storage under deep-freeze condition on the composition of the muscle tissue was also investigated. The dark muscle present in certain fishes had a higher carbohydrate content than the white muscle. The salted and sun-dried fish muscle revealed 25 to 35% loss in protein and carbohydrate content whereas the fat content showed only a minimum loss.

INTRODUCTION

FISHES form the major food source for human beings. Nearly 30 million metric tons of fish are landed every year. The Clupeids contribute the largest share which is more than 12 million metric tons. Because of this the chemical composition of Clupeids has been studied extensively (Bramsnaes, 1962). The proximate composition of fish meal and the influence of various methods of processing on the proximate composition has been studied in many species of fishes (Stansby, 1962 ; Cutting, 1962 ; Bramsnaes, 1962). However only very few studies have been made on the chemical composition and the effect of processing on chemical composition in Clupeid fishes occurring in Indian waters. (Anon., 1962 ; Basu and Hee, 1938-1939 ; Chari, 1948 ; Antony Raja, 1969 ; Chari and Pai, 1948 ; Kamasastri, 1961 ; Kamasastri and Rao, 1965 ; Kamasastri *et al.*, 1965 ; and Nair, 1965). Hence an attempt is made to study the proximate composition of eleven species of Clupeids. The effect of deep-freezing and oven drying on the protein, and carbohydrate content has also been investigated. Further the composition of sun dried Clupeid fishes available in the local market is also determined.

We wish to thank Dr. R. Natarajan, Director, Centre of Advanced Study in Marine Biology for his keen interest and guidance in this work. Our thanks are due to Dr. V. K. Venugopalan for his helpful suggestions and for going through the manuscript, one of us (V. R.) thank the University Grants Commission, for awarding Junior Research Fellowship.

---

* Presented at the 'Symposium on Indian Ocean and Adjacent Seas - Their Origin, Science and Resources' held by the Marine Biological Association of India at Cochin from January 12 to 18, 1971.

Material and Methods

30 species of Clupeids are commonly found in the inshore waters of Porto Novo. Of these, eleven species were used in the present study. They are *Megalops cyprinoides, Dussumieria acuta, Sardinella fimbriata, Kowala coval, Opisthopterus tardoore, Ilisha elongata, Nematalosa nasus, Anodontostoma chacunda, Thryssa hamiltonii, Thryssa malabarica,* and *Setipinna taty.* The fishes were procured from the commercial catch immediately after landing. They were brought to the laboratory and analysis carried out within 30 minutes. The duration between the time of catch and the time of analysis did not exceed 2 hrs. In all the fishes used the tissue was in good condition. For the proximate analysis only muscle tissue, taken just below the dorsal fin and above the lateral line was used.

After removing a portion of the muscle tissue, the fishes were deep-frozen in a freezer maintained at – 20°C. Another portion of the muscle tissue was weighed and kept in a hot-air oven maintained at a temperature of 70°C.

Protein

For total protein estimation, about 30 mg of fresh or frozen muscle tissue was homogenized with 1 ml of distilled water in a hand homogenizer and the total protein was estimated colorimetrically by biuret method (Raymont *et al.*, 1964). In the case of oven-dried and sun-dried tissue only 7 mg of tissue was used.

Carbohydrate

For total carbohydrate estimation the phenol-sulphuric acid method of Dubois *et al.* (1956) was adopted. About 20 mg of fresh or frozen tissue or 5 mg of oven-dried and sun-dried tissue was taken for carbohydrate analysis.

Lipid

For the extraction of lipids chloroform methanol mixture (3 : 1) was used (Folch *et al.*, 1956). Preliminary analyses carried out on fresh tissues showed that the chloroform-methanol mixture extracted not only the lipids but also a considerable amount of salts. To avoid the extraction of salts in fresh tissue, a known amount of wet tissue was dried in hot air oven (70°C) and then the lipid was extracted with chloroform methanol mixture. For oven-dried and sun-dried fish tissues, 400 mg of powdered tissue was used. The lipid content was estimated gravimetrically.

Moisture

The moisture content of frozen and fresh tissue was estimated gravimetrically. 2 gms of muscle tissue was dried in a hot-air oven (70°C) for 48 hrs and reweighed. The difference in the weight was taken as the moisture content of the tissue.

Ash

For the determination of ash 2 gms of fresh muscle tissue was ashed in a muffle furnace at 500°C for 5 hrs.

RESULTS

Fresh tissue

The moisture, protein, fat, carbohydrate and ash content of fresh muscle tissue of the eleven species of fishes is given in Table 1. Each value represents the mean of 6 samples for each species. The values are expressed as per cent wet weight.

TABLE 1. *Proximate composition of fresh muscle tissue. Values are expressed in per cent of wet weight*

Fresh Tissue

Species	Moisture	Protein	Fat	Carbohydrate	Ash
Megalops cyprinoides ..	75.2	22.1	1.81	0.23	1.57
Dussumieria acuta ..	75.1	20.7	2.47	0.31	1.49
Sardinella fimbriata ..	75.3	22.1	2.40	0.29	1.97
Kowala coval ..	76.5	18.9	1.81	0.30	1.37
Opisthopterus tardoore ..	77.6	22.3	1.92	0.21	1.61
Ilisha elongata ..	78.2	22.2	1.90	0.25	1.55
Nematalosa nasus ..	74.2	20.8	2.70	0.22	1.89
Anodontostoma chacunda ..	78.9	21.1	1.73	0.27	1.52
Thryssa hamiltoni ..	76.2	22.0	1.94	0.19	1.68
Thryssa malabarica ..	76.4	24.3	2.32	0.20	1.62
Setipinna taty ..	74.5	26.6	3.62	0.23	1.61

The moisture content in all the fishes is above 74%. The maximum moisture content is in *Anodontostoma chacunda* (78.9%) and minimum is found in *Nematalosa nasus* (74.2%). The protein content varies from 18% to 27%. The fat content varies from 1.7 to 3.6%. In *Setipinna taty* only, the fat content is very high (3.6%). The carbohydrate content of these fishes varies from 0.22 to 0.3%. Significant variation among these fishes in the carbohydrate content is not evident. The ash content varies from 1.3% to 1.9%. Here also no significant variation is noted.

Frozen tissue (– 20°C)

The mean values for moisture, protein, and carbohydrate contents of the frozen fish muscle tissue are given in Table 2. No significant difference is observed in the moisture content of the frozen tissue when compared with that of the fresh tissue. In all the species the moisture content ranges from 71 to 79%. More than 2% reduction in the moisture content has been found in *N. nasus, D. acuta, M. cyprinoides, T. hamiltoni, T. malabarica* and *S. taty*. In other species the difference in moisture content is less than 1%. The mean protein value for each species of fish, either decreases or increases by not more than 2% when compared to that of the fresh tissue except in the case of *S. taty* and *O. tardoore*. The mean carbohydrate

value of the frozen tissue shows a slight decrease in *S. fimbriata, I. elongata* and *S. taty*. In all other species the mean value for carbohydrate shows slight increase, over that of the fresh tissue.

TABLE 2. *The moisture, Protein and Carbohydrate content of the frozen* (−20°C) *muscle tissue. Values are expressed in per cent of wet weight*

Frozen Tissue

Species	Moisture	Protein	Carbohydrate
Megalops cyprinoides ..	77.2	22.7	0.29
Dissumieria acuta ..	72.3	20.5	0.32
Sardinella fimbriata ..	76.6	20.4	0.27
Kowala coval ..	76.8	20.3	0.31
Opisthopterus tardoore ..	78.8	19.7	0.23
Ilisha elongata ..	78.6	21.3	0.24
Nematalosa nasus ..	71.9	20.1	0.28
Anodontostoma chacunda ..	77.2	19.7	0.28
Thryssa hamiltoni ..	71.8	22.7	0.28
Thryssa malabarica ..	73.7	22.2	0.27
Setipinna taty ..	72.4	23.3	0.18

Oven-dried tissue (70°C)

The oven dried muscle tissue shows considerable variation in the protein content among the eleven species of fishes (Table 3). In *I. elongata* the protein content is only 60% of the dryweight whereas in the case of *N. nasus* and *T. malabarica* it is more than 80%. The carbohydrate content varies from 0.62% to 0.96% of the dryweight. In *O. tardoore* and *T. hamiltoni*, it is less than 0.6% and in *S. fimbriata* and in *D. acuta* it is more than 0.8%.

TABLE 3. *The Protein, Fat and Carbohydrate content of the oven-dried* (70°C) *muscle tissue. Values are expressed in per cent of dry weight*

Oven-dried Tissue

Species	Protein	Fat	Carbohydrate
Megalops cyprinoides ..	73.1	6.62	0.68
Dussumieria acuta ..	66.6	9.28	0.96
Sardinella fimbriata ..	76.9	8.96	0.88
Kowala coval ..	72.1	7.20	0.77
Opisthopterus tardoore ..	68.1	7.78	0.62
Ilisha elongata ..	59.9	8.08	0.71
Nematalosa nasus ..	84.3	10.01	0.74
Anodontostoma chacunda ..	70.9	6.72	0.76
Thryssa hamiltoni ..	69.0	7.62	0.62
Thryssa malabarica ..	82.6	9.19	0.75
Setipinna taty ..	75.8	13.69	0.75

The fat content of the dried tissue varies from 6.5% to 13.5% of the dry-weight. The lowest fat content is seen in *A. chacunda* and in *M. cyprinoides*. The fat content of *S. fimbriata, N. nasus, D. acuta, T. malabarica* and *S. taty* is a little over 9%. The highest fat content is observed in *S. taty* (13.7% of dry-weight).

Sun-dried fish tissue

Of the eleven species of fish investigated, only seven species have been available in the market as sun-dried fish. The moisture content of the sun-dried fish varies greatly, not only between species but within the species also. This is mainly dependent upon the variations in processing, namely the duration of sun-drying, method of storage, and the moisture content of the atmosphere.

The protein content of sun-dried muscle tissue is between 63% and 82% of dry weight in all the species (Table 4). In *K. coval* and *T. malabarica* it is between 70-77%. The fat content also varies greatly in the sun-dried fishes. In *N. nasus* only 5.2% of the dry-weight is constituted by fat where as in *I. elongata* and *S. taty*, it is more than 12% of the dry-weight. Like the fat, the carbohydrate content also varies greatly. As before *N. nasus* shows very low (0.3%) carbohydrate content. In the case of *K. coval* and *O. tardoore* the carbohydrate is 0.8%.

TABLE 4. *The Protein, Fat and Carbohydrate content of the sun-dried fish muscle tissue. Values are expressed in per cent of dry weight*

Species		Sun-dried Fish Tissue		
		Protein	Fat	Carbohydrate
Sardinella fimbriata	..	63.71	9.72	0.37
Kowala coval	..	81.85	6.8	0.81
Opisthopterus tardoore	..	76.1	8.05	0.81
Ilisha elongata	..	74.2	14.92	0.58
Nematalosa nasus	..	69.4	5.2	0.29
Thryssa malabarica	..	81.6	9.82	0.46
Satipinna taty	..	77.5	12.4	0.60

It is evident from the above data that the proximate composition of the fish varies in any one species of fish according to the type of processing namely whether it is frozen, or oven-dried. In order to find out the apparent variation in the protein, and carbohydrate, content of the fresh tissue and that of frozen tissue and fresh tissue and oven-dried tissue, the following experiments have been conducted. One or two fishes belonging to each species is taken and the protein and carbohydrate content of the tissue is estimated (a) in fresh condition and (b) after 30 days of storage at −20°C. In another experiment, carbohydrate and protein have been estimated in tissues of fresh fishes and fishes dried at 70°C. The results are shown in Table 5—a comparison of the moisture, protein, and carbohydrate content of the fresh and frozen muscle tissues, of the same fish for different species. In seven of the eleven species there is not much variation in the moisture content between the fresh and

TABLE 5. *Comparison of moisture, Protein and Carbohydrate content of fresh and frozen muscle tissue of the same fish. Values are expressed in per cent of wet weight*

Species	Fresh tissue			Frozen tissue		
	Moisture	Protein	Carbo-hydrate	Moisture	Protein	Carbo-hydrate
Megalops cyprinoides ..	75.2	22.08	0.23	77.19	22.73	0.29
Dussumieria acuta ..	75.3	23.59	0.36	72.32	20.46	0.32
Sardinella fimbriata ..	76.0	23.04	0.29	76.57	21.80	0.27
Kowala coval ..	76.3	22.73	0.34	76.0	20.26	0.31
Opisthopterus tardoore ..	77.6	19.00	0.19	78.77	19.70	0.23
Ilisha elongata ..	77.9	22.15	0.21	78.6	21.26	0.24
Nematalosa nasus ..	71.0	20.78	0.31	71.93	20.13	0.28
Anodontostoma chacunda ..	74.9	21.14	0.26	77.17	19.70	0.28
Thryssa hamiltoni ..	75.0	21.88	0.19	74.8	21.73	0.28
Thryssa malabarica ..	75.1	27.78	0.21	73.7	22.23	0.27
Setipinna taty ..	72.6	26.57	0.22	72.45	23.33	0.18

frozen tissues of the same fish. In *T. malabarica* and *D. acuta* the loss in moisture due to freezing is more than 2%. Whereas in the case of *A. chacunda* and *M. cyprinoides*, more than 2% increase in moisture content is evident.

The protein content decreases by 2 to 4% in the case of *T. malabarica*, *S. taty*. *D. acuta*, *A. chacunda* and *K. coval*. In all other species there is no apparent loss in protein content due to freezing.

The carbohydrate content shows a slight increase in *T. hamiltoni*, *T. malabarica*, *O. tardoore*, *A. chacunda*, *M. cyprinoides* and *I. elongata*. In the other 5 species the carbohydrate content decreases slightly in the frozen tissue.

Table 6 gives the protein and carbohydrate content of fresh and dried tissue, for the same fish.

Due to drying there is a loss of 3 to 30% in protein. However, the values obtained are not consistant. For instance in *O. tardoore* itself the individual variations range from 4.6% to 22% as far as loss in protein is considered. Similar results have been obtained for carbohydrate also, the range being 0.01 to 0.8%.

DISCUSSION

On the basis of protein and oil content of fresh tissue, Stansby (1962) has classified the proximate composition of fish into 5 categories. According to this classification the Clupeid fishes investigated belong to category D in which the oil content is below 5% and the protein content is over 20%. Anchovies, herring, and sardines with only medium oil (5-15%) and high protein (15-20%) are grouped under category B (Stansby, 1962).

TABLE 6. *Comparison of Protein and Carbohydrate content of fresh and oven-dried* (70°C) *muscle tissue of the same fish. Values are expressed in per cent of dry weight.*
(In the case of fresh tissue the values are converted to dry weight basis taking into account, the moisture content)

Species	Sample	Protein			Carbohydrate		
		Fresh tissue	Dried tissue	Difference	Fresh tissue	Dried tissue	Difference
Megalops cyprinoides ..	1	89.0	73.1	15.9	0.93	0.68	0.25
Dussumieria acuta ..	1	95.4	65.9	29.5	1.41	0.88	0.53
	2	93.3	67.3	26.0	1.21	1.04	0.17
Sardinella fimbriata ..	1	89.1	71.0	18.1	1.44	0.94	0.5
	2	89.0	82.9	6.1	0.83	0.82	0.01
Kowala coval ..	1	84.5	67.9	16.6	1.34	0.79	0.55
	2	79.7	76.3	3.4	1.43	0.75	0.68
Opisthopterus tardoore ..	1	77.8	73.2	4.6	1.10	0.66	0.44
	2	84.9	63.1	21.8	1.04	0.58	0.82
Ilisha elongata ..	1	86.4	63.1	23.3	0.99	0.73	0.26
	2	87.0	56.6	30.4	1.02	0.70	0.32
Nematalosa nasus ..	1	89.4	84.7	4.7	1.13	0.74	0.39
	2	88.7	83.8	4.9	0.88	0.75	0.13
Anodontostoma chacunda ..	1	84.2	70.9	13.3	1.06	0.76	0.30
Thryssa hamiltoni ..	1	82.6	65.3	17.3	0.87	0.62	0.25
	2	80.9	72.8	8.1	0.79	0.62	0.17
Thryssa malabarica ..	1	89.6	86.6	3.0	0.83	0.81	0.02
	2	82.6	78.7	3.9	0.86	0.71	0.15
Setipinna taty ..	1	86.5	75.8	10.7	1.11	0.7	0.41

The apparent inverse relationship between the oil and moisture content of the fish is not obvious in the Clupeids. The summation of oil and water ranges from 77-80%. Generally when the oil content is high the moisture content is low as in the case of *Setipinna taty*. The reverse is true in the case of *Anodontostoma chacunda*.

The ash content of all the species investigated is similar to that of other species of fishes. Carbohydrate content of the muscle tissue is the least of all the three and thereby indicates that the muscle tissue does not store carbohydrates to any appreciable extent.

In frozen tissue, loss of moisture is not considerable. The slight loss or increase in moisture content as shown by the mean values may be due to loss during thawing (Bramsnaes, 1962). When compared with the wet tissue, the protein content of frozen tissue shows only a slight decrease. In cod muscle even at $-17°C$ enzymes have been found to be active (Bramstedt, 1962). This is mainly because about 6.5% of the total water of fish is in liquid form at $-18°C$ which may sustain enzymatic activity (Dyer *et al.*, 1957) and consequently reduces the protein content. In the present investigation also there is a possibility of protein disintegration by enzymatic activity, before the tissue attains $-20°C$.

In the oven-dried tissue considerable loss in protein and carbohydrate is evident. During the initial stages of drying the enzymes may have been highly active upto a critical temperature, after which, the enzyme breakdown as well as protein dénaturation would have caused an appreciable loss in protein content. The same may be true for carbohydrate also. However individual variations are very high as shown in Table 6. Therefore further work is necessary to determine the proportionate loss due to oven-drying. Compared to the oven-dried tissue the sun-dried tissues seems to have not lost much of the proteins. It has been pointed out by Cutting (1962) that the effect of traditional sun-drying on the nutritive value is probably very slight.

REFERENCES

ANON. 1962. The Wealth of India. Raw materials Volume-IV supplement. Fish and Fisheries. *CSIR Bulletin. New Delhi*. Pp. 1-132.

ANTONY RAJA, B. T. 1969. The Indian oil sardine. *Bull. Cent. mar. Fish. Res. Inst.*, **16** : 1-128.

BASU, K. P. AND H. N. HEE 1938-39. Nutritional investigation of some species of Bengal fish. Biological value of the proteins of Rohu (*Labeo rohita*) and Hilsa (*Clupea ilisha*) by the nitrogen balance and growth methods, and supplementary effect of fish on pulse proteins. *Indian J. med. Res.* **26** : 177-89.

BRAMSNAES, F. 1962. The influence of refrigeration and canning on the Nutritive value of fish. In : E. Heen and R. Kreuzer (Ed.). *Fish in Nutrition*. Fishing News (Books) Ltd., London, Pp. 153-160.

BRAMSTEDT, A. L. F. 1962. Amino acid composition of fresh fish and influence of storage and processing. In : E. Heen and R. Kreuzer (Ed.). *Fish in Nutrition*. Fishing News (Books) Ltd., London, Pp. 61-67.

CHARI, S. T. AND P. ANANTHA PAI 1948. Fish meal from shoaling fishes of the Madras Presidency and their role in animal Nutrition. *Indian Farming*, **9** : 358-363.

——— 1948. Nutritive value of some of the west coast marine food fishes of the Madras province. *Indian. J. med. Res.*, **36** : 253-259.

CUTTING, C. L. 1962. The influence of drying, salting and smoking on the Nutritive value of fish. In : E. Heen and R. Kreuzer (Ed.). *Fish in Nutrition*. Fishing News (Book) Ltd., London, Pp. 161-179.

DUBOIS, M., K. A. GILLS, J. K. HAMILTON, P. A. REBERS AND F. SMITH 1956. Colorimetric method for determination of sugars and related substances. *Analyt. Chem.*, **28** : 350-356.

DYER, W. J., D. I. FRASER, D. G. ELLIS AND W. A. MACCALLUM 1957. Influence of intermittent short storage periods at 15°F as encountered during refrigerator car transportation, on the quality of frozen cod stored at 0°F. *J. Fish. Res. Bd. Canada.*, 14 (4) : 627-635.

FOLCH, J., M. LEES AND G. H. SOLANE STANLEY 1956. A simple method for the isolation and purifications of total lipids from animal tissues. *J. Biol. Chem.*, 226 : 497-509.

KAMASASTRI, P. U. 1961. Studies on Indian sardine oil. *Indian J. Fish.*, 7 : 443-447.

——— AND D. R. RAO 1965. Studies on Indian fish meal part I. Chemical composition and storage characteristic of fish meals prepared from different types of fishes. *Ibid.*, 9 B (2) : 108-117.

———, P. U. PRABHU AND D. R. RAO 1965. Further studies on Indian sardine oil. *Ibid.*, 9 B (1) : 84.

NAIR, M. R. 1965. A preliminary study of the changes associated with lipid breakdown in oil sardine (*Sardinella longiceps*) stored at refrigerated temperature. *Ibid.*, 9 B (2) : 126-132.

RAYMONT, J. E. G., J. AUSTIN AND E. LINFORD 1964. Biochemical studies on marine Zooplankton I. The biochemical composition of *Neomysis integer*. *J. cons. perm. Explor. Mer.*, 28 : 354-363.