CHAPTER - 6

STUDIES ON NUTRITIONAL VARIATIONS AND BIOCHEMICAL CHANGES DURING STORAGE UNDER VARIOUS CONDITIONS

INTRODUCTION

Fish is the main source of animal protein to the common man in Asia. Fish is also rich in fat, micro-nutrients and Vitamin contents. In comparison to other animal food materials fish is cheaper, abundantly available throughout the year.

According to Kandoran (1979) food material gets spoiled on storage and the type of spoilage depends on the composition, structures, types of micro-organisoms involved and the conditions of storage. The principal causes of spoilage in fish are the growth of micro-organisoms involved and the conditions of storage. The principal causes of spoilage in fish are the growth of micro-organisoms, the action of naturally occuring enzymes in the fishes, chemical reactions physical degradation and desication. The basic purpose of all fish preservation is to prevent to above type of spoilage and make the fish available at distant locations in acceptable condition. Storage at low-temperature has some adverse effect on many biological or indirectly for nearly all the undesirable side effects of low temperature of storage.

Freezing increases toughness and changes in physical qualities. The intrinsic quality is inherent in the materials and relates to species, size, sex and biochemical composition etc. Extrinsic quality is the sum of effects of all the treatments fish receives after catch till they reach consumer (Mathen, 1979). Various experiments were planned to study such effects, under experimental conditions for varied length of storage period.

Initially protein, lipid and moisture content has been estimated in different anatomical regions. Biochemical quality analyses of salt-treated and untreated fatty fish has been made. Loss of protein solubility, total volatile nitrogen trimethyl amine contents of fishes stored at different temperatures for varied period has been estimated to find out rate of spoilage. Experimental conditions were determined at different length of storage period by using different soaking media.

Variations of protein, lipid and moisture contents in different anatomical portions of edible muscle tissues of marine and inland fishes were determined. Studies on the influence of temperature on the keeping qualities were made.

Hess (1932) reported that the lowering of the temperatures becomes more effective in retarding bacterial decomposition. Experiment on the handling and storage of summer herring showed that temperature was the most important factor influencing preservation (Rea .y and She .wan, 1953). In herring kept without ice at 10°C to 15°C, the production of trimethyleamine, starting with about 1 mg. TMA-N per 100 ml of fish flesh was between 20-40 mg by the end of second day of storage. Sigurdsson (1947) reported short rise in TMA after only one day at 10°C. Castell and MacCallum (1950) stored fresh market cod and determined the keeping time. They also found that a reduction in storage temperature of 3 degrees immediately above freezing adds proportionally far more to the keeping time than a greater reduction at a higher temperature. The decrease in temperature doubled the keeping time as judged by the development of TMA. Similar results were obtained by Dyer and Dyer (1949). They also observed that fillets cut from fish still in regior became unacceptable after 3 days of storage at 5°C and after 8 days at 0°C. Slow spoilage takes place in case during freezing (Anonymous, 1961).

Deteriorative changes could be accurately and quantitatively measured throughout the course. The literature dealing with subjective grading of fish provides a variety of systems for observing and recording the visible changes that takes place in the fish as they deteriorate. Fazzina (1958) attempted to correlate the fish quality with the post-mortem changes occuring in the fish. Shewan et al, (1953) and Soudan et al (1956) gives an account of the appearence of the exterior of the fish as well as the odour flavour and texture of the muscles.

Various physical changes occur during spoilage and odour develops in the gills and gut during the time of spoilage. It was found that odour was very useful in determining the quality of the fish. There are many objective methods for assessing the quality of fish muscles. Total volatile nitrogen, trimethyle amine contents have given satisfactory results. Trimethyle amine and volatile nitrogen content determination was choosen for this work because of the long experiences we have had with it. It has proved to be satisfactory as a measure of spoilage.

The quality of the fish as indicated by the panel of graderes is correlated with the TMA values. A comparison was made using two different samples. The first set of experiment consisted of the nutritional quality and second set of experiment with dried soaked samples. TMA value might be used as an objective measurement of quality of dressed or gutted fish. Fatty fishes show marked differences in fat content according to various factors such as the age and size of the fish and the season of the year in which the fish was caught. As fish spoils various phy - sical changes becomes apparent (Keywood, 1957).

Fat hydrolysis is also considerable and the increases in the acid value of fat represents another possible measures of the degree of spoilage (Sigurdsson, 1947). A maximum acid value of 2.75 has been proposed for the grading of canned herrings (Charnley and Davies, 1944). Fresh water fish quality can also be assessed from fat rancidity values. In fresh water fish, volatile bases formed consist almost entirely of ammonia. Determination of the TVB is usually of little use for frozen fish but fat rancidity values are sometimes of value is assessing the quality of frozen fatty fish. The spoilage process are controlled by means of pH. A through knowledge of the degradation product of amines acid as well as the processes governing their appearence seems most desirable.

Impurities in the salt used in salt-curing appear to accelerate the oxidative deterioration of oils in fish flesh. The salt appears to act as a heterogenous catalyst of oxidation. In order to reduce the accessibility of oxygen fish must be completely covered by the brine (Borgstrom, 1965). The peroxide value of the oil present in the fish increase more rapidly. The acidity of the fish increase rapidly with the duration of storage whereas the value of extracted oil scarcely changes (Hashimato et al, 1946), when they are stored at room temperature. The oils in the flesh undergo rapid deterioration. The degree of change in the quality increases with the duration of storage (Lassen et al., 1951). In the refrigeration storage of fish the rate of deterioration of the oil decreases roughly with decreasing temperature. The oxidative deterioration of the oils proceeds at a some what greater rate than in the whole herrings (Banks, 1952).

Salt removes water from the fish body by osmosis. When the concentrations of salt within the fish is the same as that of brine in which the fish is inversed. If salting is heavy, the exterior will be leathery, while the interior will remain soft and flabby. Bad grade of salt retard penetration of the salt and also cause darkening of colour of the fishes. Micro-organisms sometimes develop on salted fish giving it a red color. These organisms come from the habitat of the fish. Mendelsohn (1974) reviewed the various rapid salting techniques for fish. In order to examine the effect of various processing parameters on the quality composition and storage life of salted fish products experiments were planned.

The results of experiments regarding the biochemical quality changes and distribution of nutritive substances of fishes were subjected to statistical analyses which may be represented by a linear regression equation. This findings of each set of experiments are shown in graphs.

MATERIALS AND METHODS

MATERIALS

Organoleptically fresh fish were procured from local market for the investigation. Average medium size fish species were collected and brought to the laboratory. The fish samples were washed with for removal of the adhering slime, blood etc. After washing the fish samples were cut into pieces (average thickness 2-3 cm in length) and kept in the refrigerator at 0°C, -10°C and 8°C temperatures. The whole fish samples were also kept at above mentioned lower temperatures for various experiments. Fish samples were taken out at different time intervals.

For nutritive quality studiesindifferent anatomical portions were chosen as follows : Dorsal-1 and Ventral-1 (just posterior of the head region). Dorsal-2 and Ventral-2 (Middle of the fish body), Dorsal-3 and Ventral-3 (just anterior of the tail region) muscle pieces were separated by sharp knife and properly washed for the removal of micro-organisms and adhering blood, viscera etc. Muscle tissue of particular portion were throughly macerated by mortar-pestle and bones were separated from the macerated muscle tissues.

For comparison of the nutritive values of different anatomical portions and whole body fish samples a separate test was carried out. Fish tissues were collected from different portions mentioned above were throughly macerated for the preparation of whole body fish samples. About twenty marine, estuarine and fresh water fish species were used for the present experiment.

Nutritive qualities were estimated at the time of purchase of the fresh fish before storage. The results of nutritive values are expressed as follows: The total protein contents as mg/100 gm of fish flesh. Total lipid contents mg/100 gm of fish flesh and moisture contents in terms of percentage.

PREPARATION OF FREEZING STORAGE DEHYDRATED FISH MUSCLE

The fish samples were collected from different storage temperature at different length of storage period. Then the samples were dried in open sun for three days. After drying the fish samples were wrapped in polythene and kept at room temperature. Now these samples were soaked in different soaking media in beaker. At different time intervals, the samples were taken out for determination of the reabsorption capacity of dehydrated fish muscle. Precautions were taken to prevent contamination.

PREPARATION OF THE SAMPLES

Average, medium sized fresh iced hilsa were procured from local market. For the experiment, fish samples were cut into 2-3 cm thick small pieces. The samples were properly washed with tap water. After washing, about sixty pieces of samples were mixed with 1:1 fish-salt ratio, another sixty pieces were mixed with 1:2 fish-salt ratio and remaining sixty pieces were kept as control. Common salt (Nacl) was used for this purpose. All the samples were kept in glass jar and stored at room temperature ± 30°C, pH values, total volatile nitrogen, trimethyle amine and iodine values were determined.

PREPARATION OF REHYDRATED SAMPLES

Fresh hilsa fish muscles were separated into ventral and dorsal portions and were kept at 8°C and 0°C temperatures for 1, 3, 5, 7, 9, 11 and 13 days of storage. Only tap water was used as a soaking media for this set of experiment. After definite period of storage the individual portions were soaked in tap water (pH 7.0) for 24, 48 and 72 hours to determine the rehydration ratio and rehydration percentage of ventral and dorsal muscles.

Fresh dorsal and ventral portions of hilsa fish muscles were dipped for 30 minutes in saturated and 10% salt solution. After that they were stored at room temperature for 1, 3, 5 and 7 days, these were then dried in sun for 3 days. These samples were then soaked in saturated and 10% salt solution for 24, 48 and 72 hours to determine the rehydration ratio and percentage.

Hilsa fish muscle pieces about 2-3 cm size were kept at ± 30°C (RT), 8°C, 0°C and -10°C for 1, 3, 5 & 7 days. They were then sun dried for three days. These samples were soaked in tap water for 24, 48 and 72 hours to determine the rehydration ratio.

Whole hilsa fish samples were stored at 0°C temperature for 1 to 7 days. They were then dried for 3 days. These samples were soaked in tap water for 24, 48 and 72 hours to determine rehydration ratio and percentage of water absorption.

Whole hilsa fish samples were stored at 0°C temperature for 1 to 13 days. They were then sun dried for three days. These samples were soaked in 5% $NaHCo_3$ solution for 48 and 72 hours. Rehydration ratio and percentage of water absorption were determined for different days of storage.

Small sized fresh hilsa fish were purchased from the market and without removing scales, viscera, gills, the whole fish was washed with common salt solution (fish:salt ratio 1:2). These fishes were stored at 8°C for 1, 2, 3, 4, 10, 12, 14 and 16 days for determination of the changes in moisture content of the dorsal and ventral muscles.

Fresh catla catla, Rastrelliger kanagurta, Scomberomonus guttatus guttatus were procured from local market. Fish muscle slices (average 3 cm in size) were stored at -10°C and 0°C temperature for 43 days Catla catla species and 39 days for Rastrelliger species and Scomberomonus species. Protein solubility and trimethyl amine values were determined for each of the samples at different time intervals.

Fresh medium sized Labeo rohita fish were procured from local fishermen, scales, skin and bones were removed, washed with tap water. Muscle pieces of about 2-3 cm in size were cut. Some pieces were kept at 0°C and others at -10°C for 14 days. Loss of protein solubility and total volatile nitrogen changes were determined.

Dorsal and ventral muscles pieces 10 cm long and 1-2 cm thick were cut from Labeo rohita following similar procedure as mentioned in previous experiment. These pieces were stored at 0°C and 8°C upto 14 days.

After that they were sun dried for 3 days and kept for a week in polythene bag at room temperatures for adjustment to ambient conditions. Now these muscles were dipped in water (pH 7.0) for 24, 48 and 72 hours to determine the reabsorption capacity. Results of rehydration percentage are expressed on wet weight basis.

IODINE VALUES

Add 10 ml of carbon tetrachloride to the oil and dissolve. Add 20 ml of wiys solution and allow to stand in the dark for thirty minutes. Add 15 ml of potassium iodite solution (10%) and 100 ml water mix and titrate with N/10 sodium thiosulfate solution using starch as indicator. Blank was carried out at the same time commencing with 10 ml of carbon tetra-chloride (Pearson, 1962).

TOTAL VOLATILE NITROGEN

The total volatile nitrogen value was estimated from TCA extract by Conway microdiffusion techniques (Conway and Byrne, 1933; modified by Pearson, 1962). The TVN values is expressed as mg.N/100 gm of fish flesh.

MOISTURE CONTENTS

Two grams of fish muscle was placed in a convention oven maintained at 100°C to 105°C and heated till the constant weight was reached. The weight loss was presumed to be entirely due to evaporation of water (Poulter *et al*., 1978).

TOTAL PROTEIN CONTENT

The estimation of protein was done following the Folin-Ciocatleau method of Lowry *et al*, (1951).

TOTAL LIPID CONTENT

Weighed tissue was ground separately with thoroughly washed sand and used for determination of total lipids. Total lipid was extracted using 2:1 chloroform: methanol mixtures as per the method of Folch *et al*, (1957). The lipid contents was calculated in terms of gm percentage of fresh tissue.

TRIMETHYLE AMINE

The extract was prepared by mixing 2 gm of the minced samples with 25 ml of 5% TCA in a morter. After 20 minutes the sample was filtered through a filter paper (Whatman - 42) and the filtrate was stored at

0°C temperature. Micro-diffusion technique of Conway and Byrne (1933); as modified by Pearson (1962) was used to determine the trimethyle amine values. In this method boric acid solution was used in the central compartment of the Conway dish which was titrated against 0.2 N H_2SO_4 solution.

WATER CONTENT

The water content was calculated as the difference between the initial weight and final weight of the wet samples after definite time intervals. This is expressed as percentage.

REHYDRATION PERCENTAGE

$$\frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

(Wet weight basis)

The rehydration ratio of the sample was determined by the method described by Vonloesecke (1955).

PROTEIN SOLUBILITY

PREPARATION OF THE SAMPLES

Two grams of the sample was taken. About 15 ml of chilled 5% NaCl solution (pH 7.3) was added. The homogenate was centrifuged for 30 minutes at 2000 rpm. The supernatent soluble protein fraction was decanted into a 100 ml volumetric flask. The residue was then stirred and centrifuged again. The supernatant was added to the previous supernatent solution and was made into 100 ml chilled 5% NaCl solution. Twenty ml of this solution was transferred into a 100 ml centrifuge tube. Protein from the supernatent was precipitated by adding 10 ml of 60% TCA solution. It was centrifuged for 30 minutes at 2000 rpm in the centrifuge, (Ironside and Love, 1958).

PROTEIN SOLUBILITY ESTIMATION

The estimation of the protein solubility from the above prepared sample was done by the folin-Ciocalteau method of Lowry *et al*, (1951). The volume of protein solubility is expressed as mg/gm of fish tissues.

pH VALUES

Two grams of fish muscle sample was homogenized in 10 ml distilled water. The pH was measured using a glass electrode. Each determination was performed in duplicate (Poulter *et al*, 1978).

RESULTS AND DISCUSSION

FISH NUTRITION

Moisture, lipid and protein contents in different anotomical regions viz. dorsal-1, 2, 3 and ventral-1, 2, 3 as well as in whole fish, both marine and freshwater species are shown in Table-9 and Figs. 1 to 20.

COMPARATIVE VARIATIONS OF NUTRITIVE VALUES

Among different marine and fresh water fish species of the present study, the highest and lowest moisture content were found in Harpodon nehereus and in Catla catla respectively. Highest and lowest lipid content were found in Tilapia species and Rita rita respectively. Highest and lowest protein values were found in red pomfret and in Catla catla fish species respectively. The nutritive values of other species were within the considerable range are shown in Table-9.

Table-9 : Distribution of total nutritive value among different anatomical portions in comparison with whole body fish samples of different inland and marine fishes

Species : Scientific name	Dorsal-1			Ventral-1			Dorsal-2			Ventral-2		
	M	L	P	M	L	P	M	L	P	M	L	P
Harpodon nehereus	84.77	2.75	25.0	83.87	2.25	28.33	83.76	2.75	28.13	83.87	2.25	40.0
Chirocentrus dorab	78.49	3.50	35.0	63.49	2.25	18.57	56.44	2.25	-	78.42	4.50	25.0
Red pomfret	77.68	3.25	13.33	78.08	1.78	16.25	76.60	3.9	30.0	77.27	5.5	24.55
Wallago attu	81.97	2.75	21.43	81.34	4.5	22.5	80.74	2.5	14.63	73.78	5.7	-
Rita rita	79.34	-	-	78.71	-	26.25	77.36	2.5	13.8	76.22	2.25	-
Lates calcarifer	76.61	-	28.57	69.84	-	20.83	75.39	-	-	74.89	-	18.0
Black pomfret	76.91	1.0	26.79	72.81	10.6	27.0	76.68	2.50	26.79	76.68	2.69	26.79
Mystus aor	79.61	1.25	36.25	82.02	1.31	16.25	78.54	1.56	17.86	79.38	1.79	36.46
Labeo rohita	82.08	3.25	23.33	83.5	1.25	27.27	83.51	1.25	27.27	82.08	1.0	17.14
Labeo bata	82.60	1.0	27.78	77.96	-	27.75	78.61	4.7	14.29	80.03	2.24	18.82
Mackerel	77.18	1.5	25.0	73.38	3.5	17.52	78.51	2.7	19.32	75.52	1.8	19.82
Catla catla	82.26	-	38.75	82.06	-	20.83	77.20	5.1	31.25	82.45	-	11.5
Red perch	82.04	3.75	20.83	80.21	-	20.45	82.71	2.0	32.85	70.35	-	-
Labeo calbasu	81.60	3.0	17.85	70.0	3.0	10.0	81.95	1.5	11.88	83.19	4.0	26.79
Tilapia Sp.	79.08	-	-	79.73	7.5	10.0	77.13	-	23.18	78.76	1.2	-
Serranus areolatus	79.54	1.83	28.57	78.55	-	18.75	78.62	1.0	14.29	77.24	3.2	17.85
Chironiemus Sp.	78.60	-	16.67	78.58	-	30.0	77.59	-	30.0	77.09	-	11.25

Unit: Protein - mg/100 gm fish tissue
Lipid - mg/100 gm fish tissue
Moisture - Percentage.

Contd. .....

Table-9 : (Continued)

Species : Scientific name	Dorsal-3			Ventral-3			Whole fish		
	M	L	P	M	L	P	M	L	P
Harpodon nehereus	73.73	3.0	15.0	74.10	1.0	-	88.47	1.63	12.15
Chirocentrus dorab	79.35	2.0	32.14	79.36	4.8	40.0	80.45	1.75	23.53
Red pomfret	79.35	3.5	30.25	77.38	2.5	24.38	80.99	2.25	28.57
Wallago attu	77.59	3.0	24.50	75.17	6.0	30.26	82.57	1.25	12.5
Rita rita	77.36	2.5	13.8	79.99	2.75	20.83	80.72	1.0	17.69
Lates calcarifer	74.41	-	35.0	76.97	1.0	25.0	79.92	1.75	19.53
Black pomfret	76.19	5.25	18.33	75.87	2.15	25.0	78.95	3.0	26.92
Mystus aor	80.19	1.37	26.0	76.90	3.25	30.0	82.68	2.01	12.50
Labeo rohita	76.76	3.0	21.88	83.58	1.0	37.50	83.46	1.5	14.37
Labeo bata	79.03	-	19.44	77.48	2.6	11.12	78.31	2.25	25.11
Mackerel	80.05	3.4	13.16	76.71	9.5	19.09	80.87	2.01	11.70
Catla catla	81.45		11.5	80.72	5.6	34.09	77.77	2.75	11.65
Red perch	79.63		30.56	80.33	10.9	12.61	-	-	-
Labeo calbasu	81.57	3.0	-	79.89	1.0	27.5	78.31	2.25	25.11
Tilapia Sp.	79.43	5.0	-	78.83	-	22.75	81.75	3.0	14.29
Serranus areolatus	79.28	1.0	17.50	81.03	1.83	28.57	80.50	1.5	20.0
Chironiemus Sp.	77.59	-	12.5	79.52	1.0	17.5	-	-	-

Unit: Protein - mg/100 gm fish tissue
Lipid - mg/100 gm fish tissue
Moisture - Percentage.

NUTRITIVE VALUE IN MARINE FISH AS COMPARED TO FRESH WATER FISHES

Nutritive quality data of marine and freshwater fish species is shown in table-9. The causes of differences were probably due to ecology of the fish, food habit, maturity and other environmental factors.

Moisture and protein content showed greater variations among fish samples but less variation was observed in lipid content. From average value the dorsal portion contained higher moisture and less fat, the ventral portions contained higher fat and less moisture. The protein content did not show any regular pattern of increase or decrease. The values of the proximate composition of individual species are shown in the same table-9. From this observation, it is concluded that nutritive values of fishes totally depends on particular fish species and its body constituents which in turn depends on food.

COMPARATIVE NUTRITIONAL STUDIES IN MARINE AND FRESH WATER FISH SPECIES

The differences in overall chemical composition of marine and fresh water species are shown in Table-9. The differences in major components of marine and fresh water fish species were wide. Correlation between the body composition and diet composition has been reported (Wood *et al.*, 1957b). Differences among species in the composition of the flesh itself are not responsible for many appreciable differences in the protein quality. The aminoacid composition of the fish muscle protein was relatively constant without distinction among species.

Differences in anatomical protein however, results in considerable variation among the nutritional quality among the different fish species. Spoilage would seem to be a possible factor affecting quality. There is very little information regarding the nutritive quality of fish which have undergone various degree of putrefaction. Lassen *et al.* (1951) reported that spoilage of the raw fish and little effect on the nutritive value. Oxidation was more extensive at the lower moisture contents. Tappel (1955) studied the interaction of protein with oxidizing fat. Insoluble dark brown copolymers of high oxygen and nitrogen content were formed from which the total amino acids recoverable even after acid hydrolysis was about 16% lower than would expected from their nitrogen content, indicating an appreciable destruction of amino acids. Lanham and Nilson (1947) found that spoilage of sardine meat with heat and moisture in which both chemical composition and chemical hydrolysis took place did not adversely affect the nutritive value of the protein.

When fish is improperly preserved, microbial decomposition may affect the amino acid content of fish in some cases which lower the value of fish protein. Partial destruction of some amino acid or the presence of toxic bacterial metabolic products are responsible for this apparent decrease in the nutritive value (Borgstrom, 1965). Autolysis due to the digestive action of tissue enzymes may also occur under such conditions. This may alter the texture, flavour and appearence of the fish product but seldom affects its nutritive value.

The dietary fat frequently is deposited in the fish tissue. Fat often undergo a series of changes that will affect their nutritive value. Westman et al.,(1969) compared growth and body composition of fish. The contents of water plus fat was relatively constant on the whole and there was something like an increase relationship between body water and body fat. Elevated level of body fat occurs not frequently as a consequence of feeding of high fat diets (Mann, 1959; Phillips et al., 1966). The composition of marine fish is influenced by a large number of environmental and physiological factors.

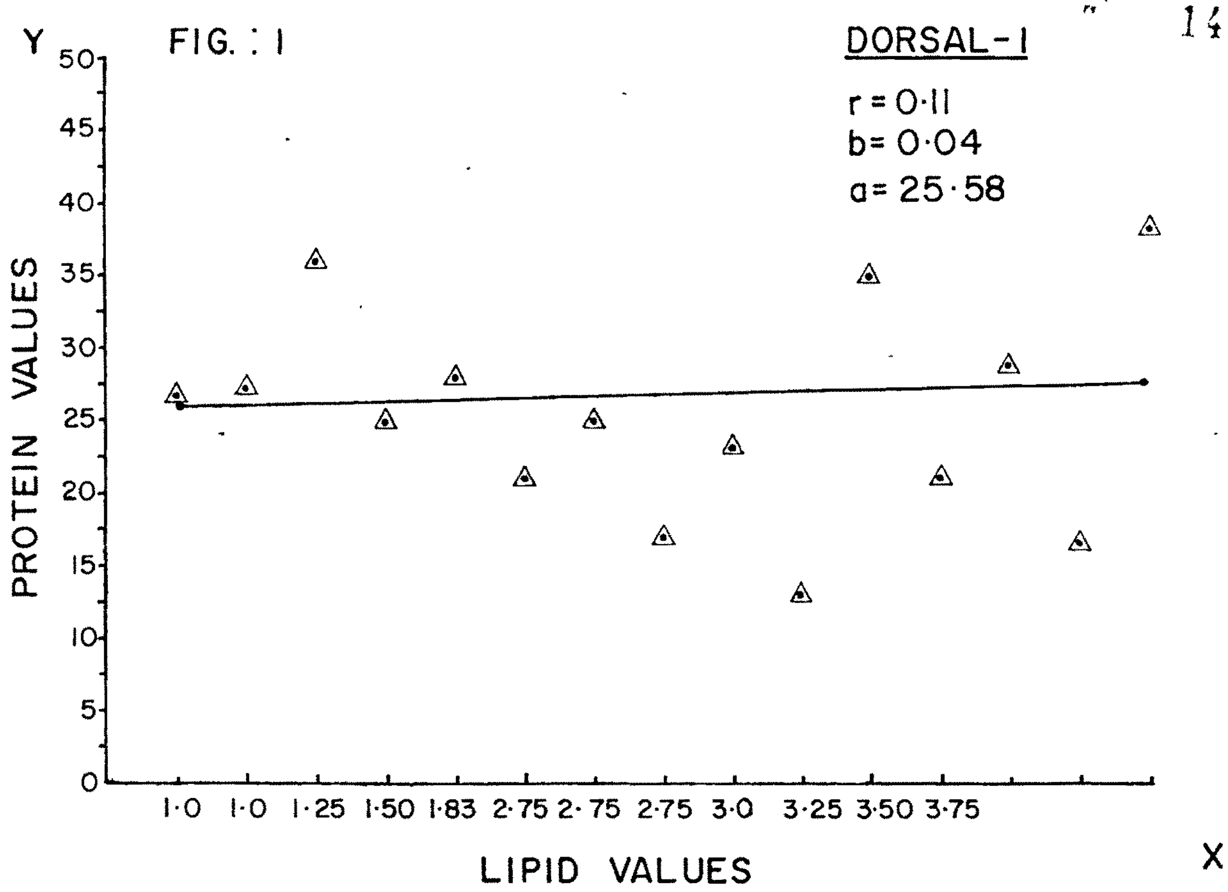
The changes in lipid contents could alter the fatty acid composition of a number of species. Body fat in fresh water species can be changed depending on dietary fat (Kelly et al, 1958b). Reiser et al., (1963) demonstrated that dietary linoleic and linolenic acids could be deposited in both fresh water fish and in salt water fish. Fish maintained on a low fat become deficient in polyenoic acids, which implies that fish can not synthesize these acids. The decrease in the environmental temperature induces an increase in degree of unsaturated in fish lipid (Hilditch and Williams, 1969; Johnston and Roots, 1964; Knipprath and Mead, 1968; Smith & Goldstein, 1967). The phenomenal lipid changed applies to fresh water, esturine and marine fish. The overall, lipid content of fresh water, esturine and marine fishes are relatively similar, particularly in marine species lipid fulfil certain functions, which do not occurs in fresh water fish. The natural diet of marine fish is very rich in protein. The aim of the nutritional research is the provision of a balanced diet, which will meet the requirement of food deficiency. This aim cannot be attained without knowing the nutritional composition of the species.

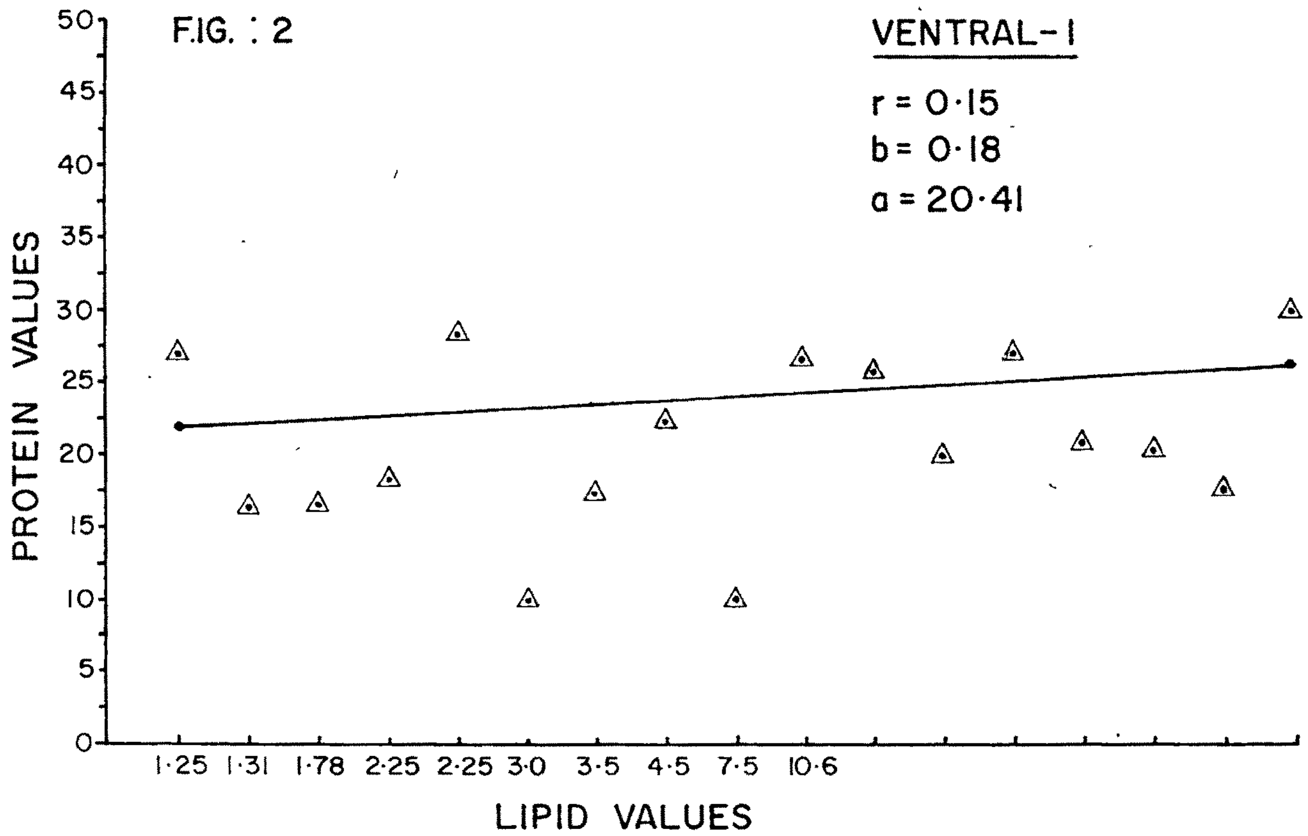
INTERRELATIONSHIP BETWEEN BODY COMPOSITION OF FISHES

The interrelationship between major body composition are shown in figures by plotting the regression lines.

PROTEIN AND LIPID RELATIONSHIP

The protein and lipid relationship within the species among the different marine and freshwater fishes are shown in figures by plotting the statistical regression analyses. Particular lipid values from different anatomical portions are plotted on x-axis. Corresponding protein values of y-axis for obtaining the relationship between the two nutritive variables. Linear regression equations were calculated for each of the particular portions. The diversification of dependent variables (Protein contents with corresponding lipid contents of each species) were estimated for each anatomical portion and also in whole body fish samples. Simultaneously, coefficient of correlation (r) values were plotted for each of the anatomical portion. The slope and intercept values (a and b) were given in each of the results for different anatomical portions and whole body fish samples. The pattern of interrelationship between lipid and protein contents and the values of the slope, intercept and correlation of coefficient (r) are presented in figures 1 to 7 (Relationship between protein and lipid values in different anatomical portions and in whole fish samples, (Regression lines y on x) in different fishes.





FIGS. : 1 TO 6 RELATIONSHIP BETWEEN LIPID & PROTEIN VALUES IN DIFFERENT ANATOMICAL PORTIONS OF DIFFERENT FISHES (REGRESSION LINES Y ON X)

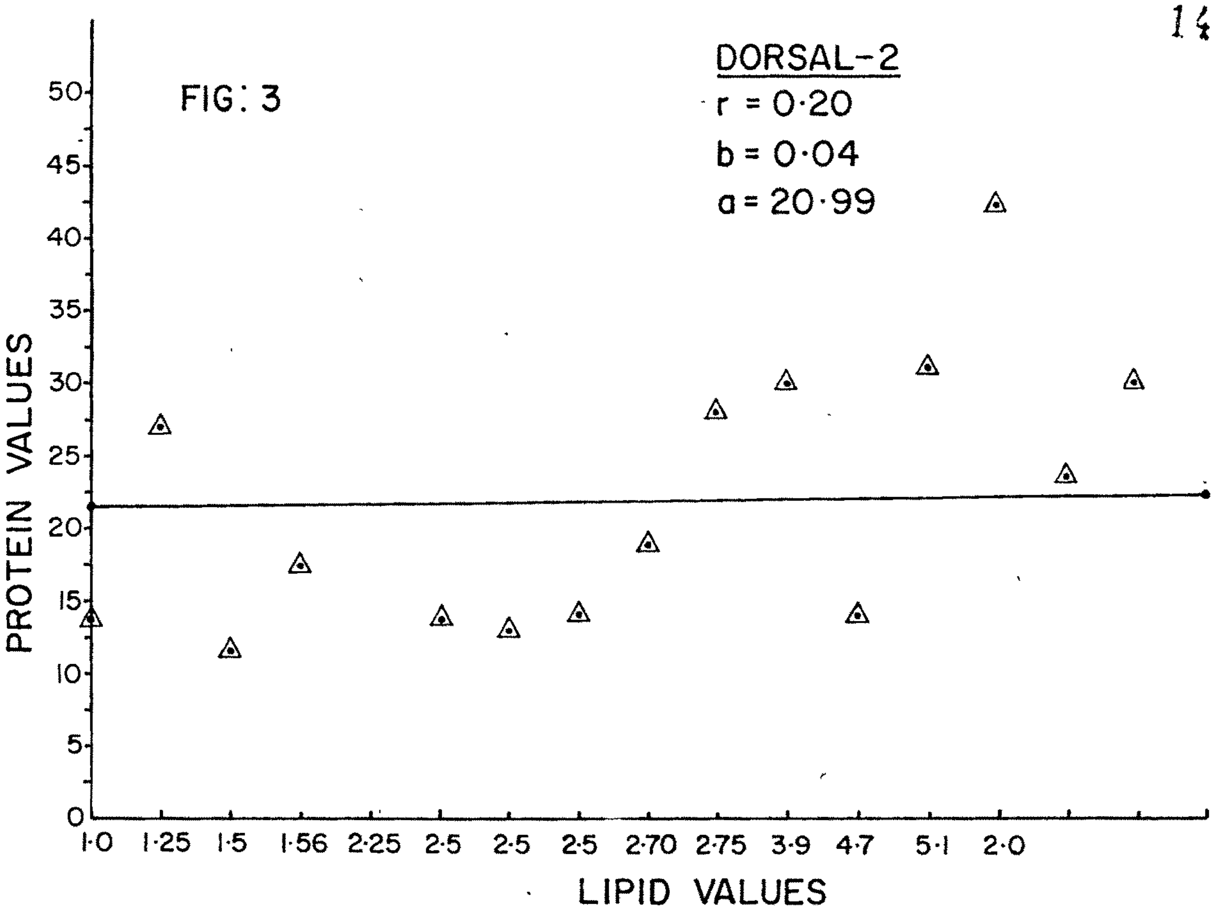

DORSAL-2
r = 0·20
b = 0·04
a = 20·99
PROTEIN VALUES
50
45
40
35
30
25
20
15
10
5
0
1·0 1·25 1·5 1·56 2·25 2·5 2·5 2·5 2·70 2·75 3·9 4·7 5·1 2·0
LIPID VALUES

FIG: 3

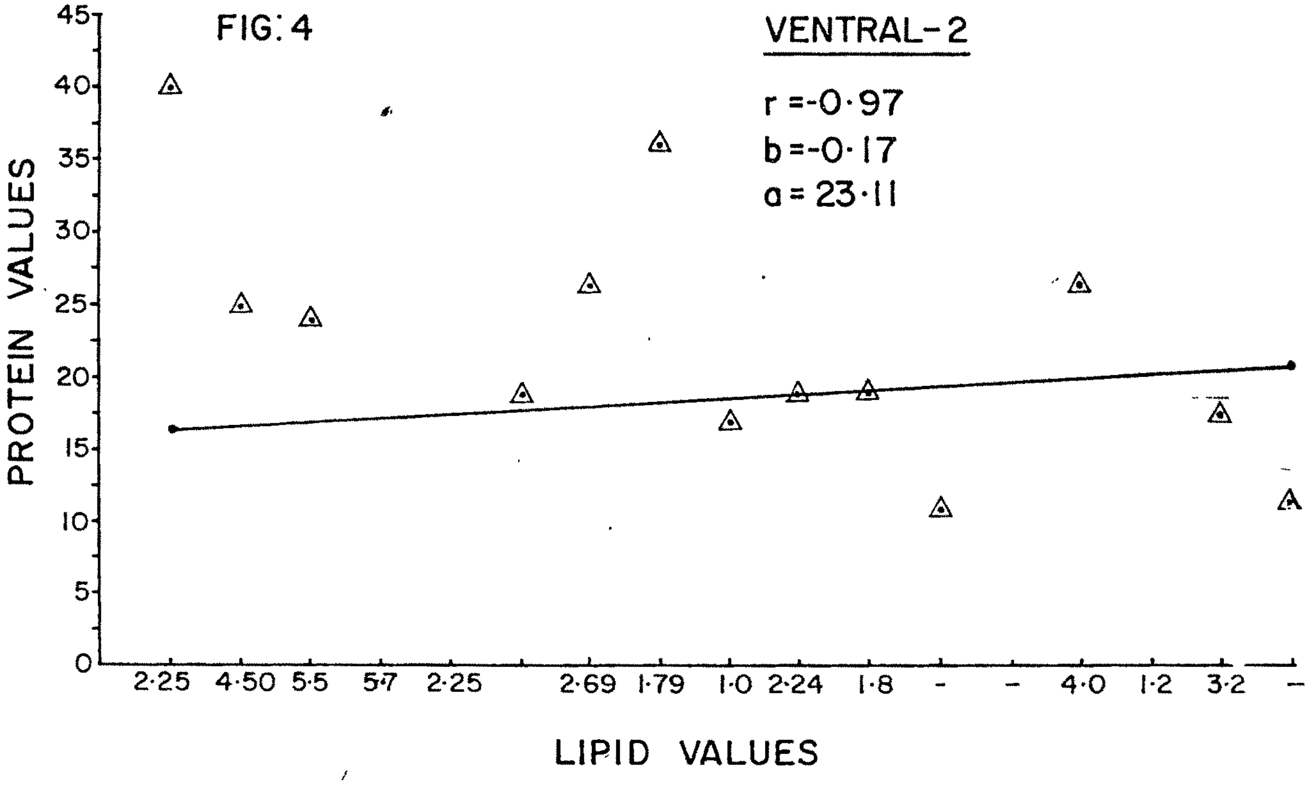

VENTRAL-2
r =-0·97
b =-0·17
a = 23·11
PROTEIN VALUES
45
40
35
30
25
20
15
10
5
0
2·25 4·50 5·5 5·7 2·25 2·69 1·79 1·0 2·24 1·8 - - 4·0 1·2 3·2 –
LIPID VALUES

FIG: 4

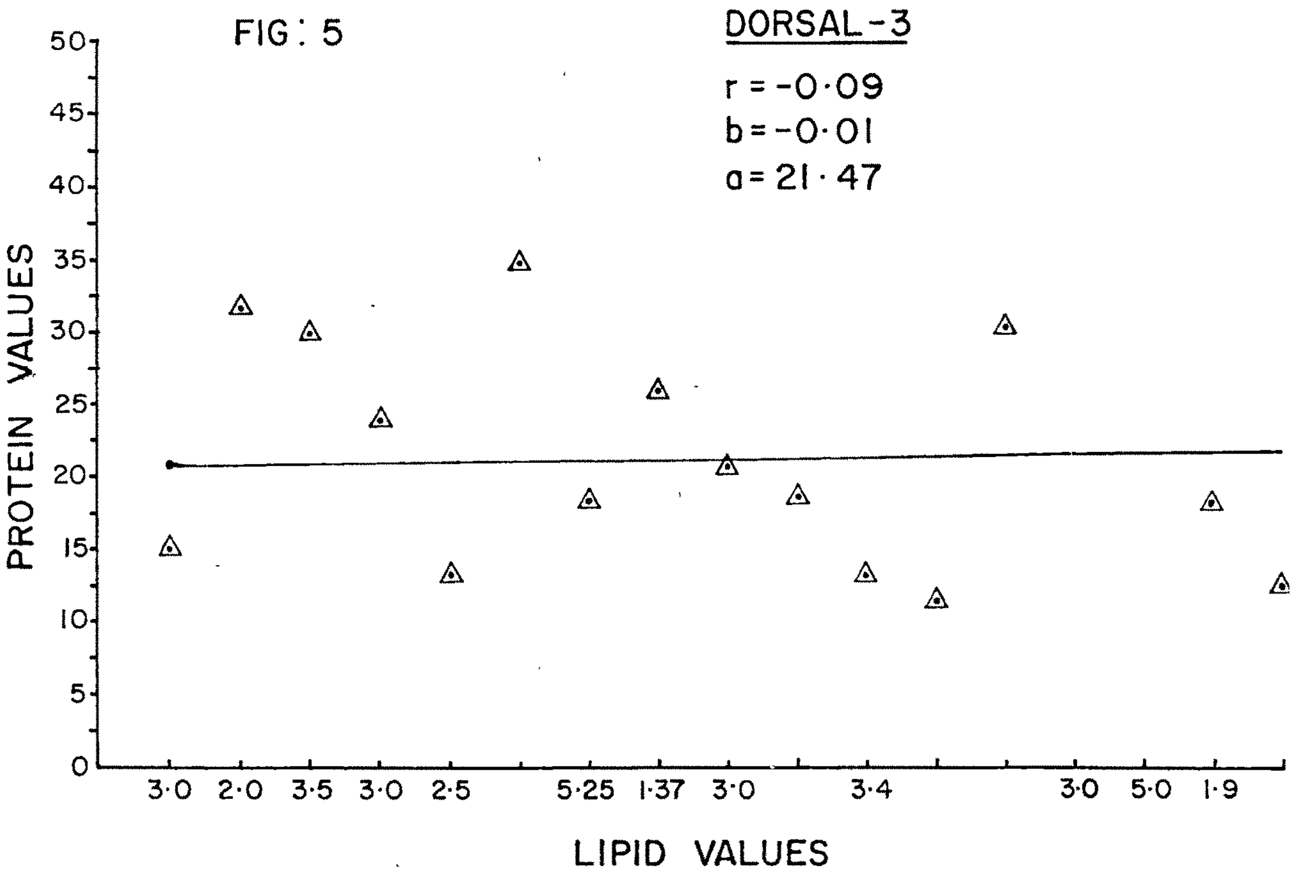


FIG: 5
DORSAL-3
r = -0·09
b = -0·01
a = 21·47
PROTEIN VALUES
50
45
40
35
30
25
20
15
10
5
0
3·0 2·0 3·5 3·0 2·5 5·25 1·37 3·0 3·4 3·0 5·0 1·9
LIPID VALUES

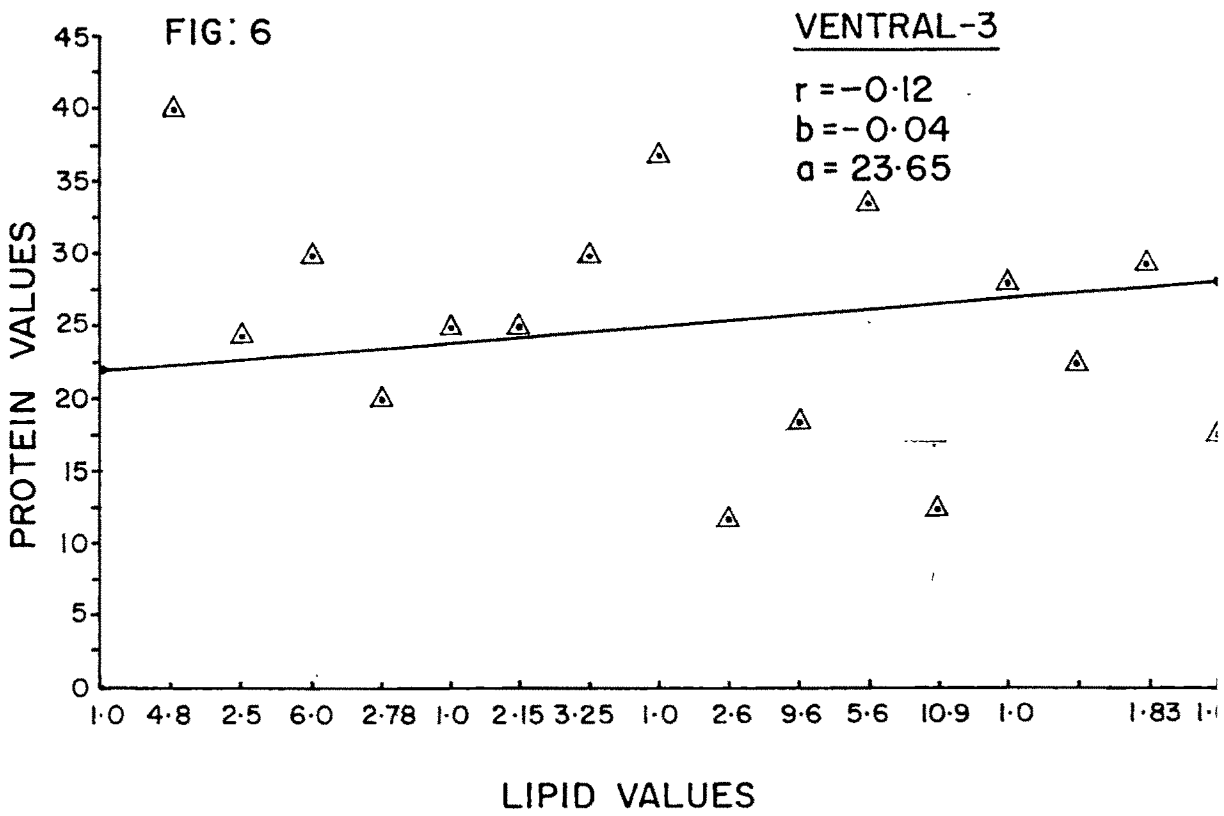


FIG: 6
VENTRAL-3
r = -0·12
b = -0·04
a = 23·65
PROTEIN VALUES
45
40
35
30
25
20
15
10
5
0
1·0 4·8 2·5 6·0 2·78 1·0 2·15 3·25 1·0 2·6 9·6 5·6 10·9 1·0 1·83
LIPID VALUES

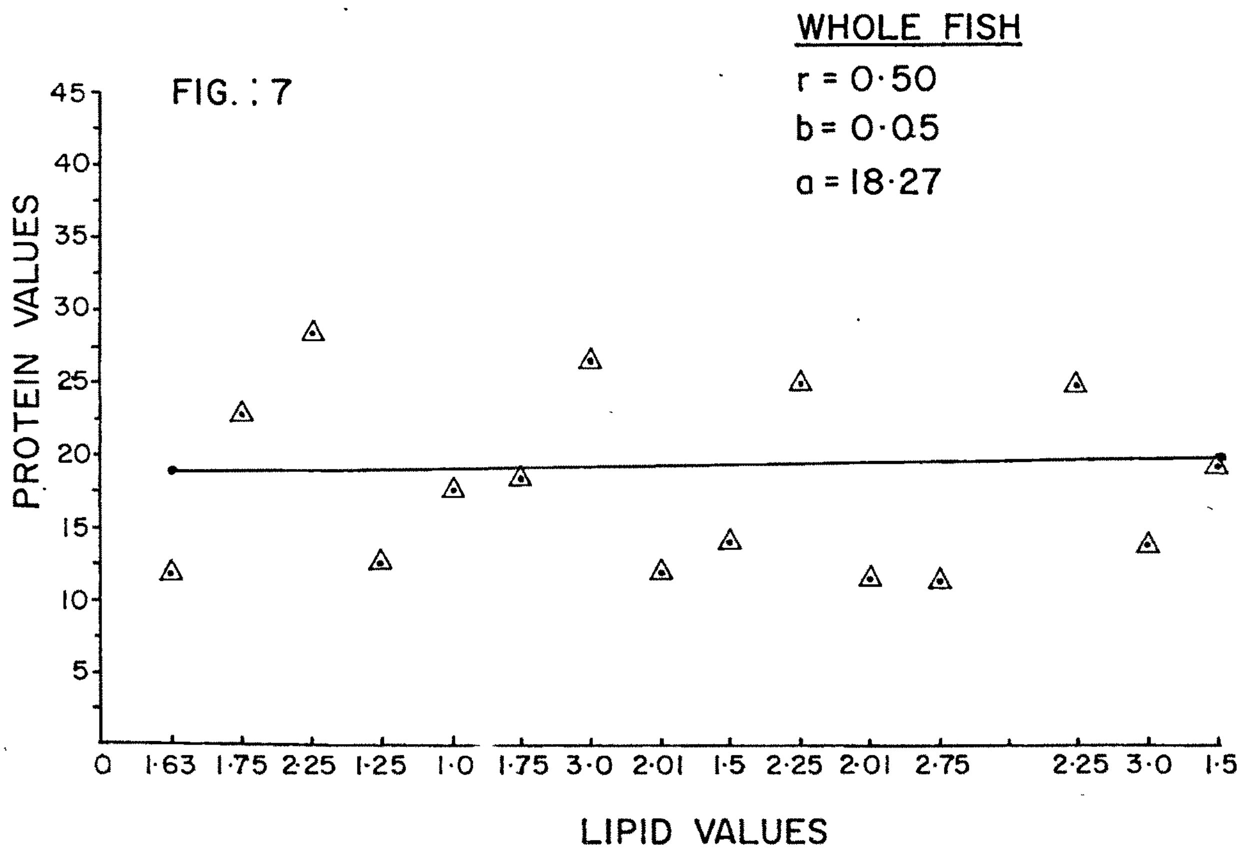


FIG. : 7. RELATIONSHIP BETWEEN LIPID & PROTEIN VALUES OF DIFFERENT FISHES (REGRESSION LINE Y ON X)

Nutritional values estimated from individual anatomical portions and whole body samples of marine and inland has been represented in figs. 1-7 (lipid and protein values), figs. 8-13 (protein and moisture contents) and figs. 17-20 (moisture and lipid values). The linear regression lines were plotted from the higher and lower values. Regression equation 'y axis on 'x-axis for measurement of the diversification of each nutritive value from the lines.

In the present study it has been found that there are considerable variation in the bio-chemical constituents of the fish body. Protein, lipid and moisture are the major constituents of the fish muscles. A brief account on the quantitative variations in water, protein and fat contents of the different fish species are shown in figures 1 to 20.

It was found from the results that lipid and water contents are inversely proportional. It was observed that protein content was higher in particular anatomical portion. The protein content of the dorsal muscle was higher as compared to the ventral muscles. The lipid value was low in dorsal muscle when compared with ventral muscle. The moisture content was less in ventral portion. An inverse relationship between water and other biochemical constituents in marine and fresh water fish were observed. The proportion of protein and lipid vary from species to species. The habitat of the species (marine and fresh water) does not play role in the interrelationship of the main constituents of the fish flesh.

In fatty fishes relationship was found between lipid and water, an increase in one value leading to a decrease in the other. Brandes and Dietrich (1953) showed similar results in Chupea herrengus.

The variations of main constituents are due to food supply, lipid loss due to starvation. A good fat water line was observed in various fish species in the present study. The lipid and water together make up about 80% of the constituents. The water content showed an inverse relationship with the lipid contents in the muscle of fatty fishes and with the protein in non-fatty fishes. The highest tissue water was noticed to be 88% in Harpodon nehereus (Table-9).

The water content also increased in fresh water fish as compared to marine fish. Average highest water content were observed in fresh-water fish. Few fresh water fish contained least water. Many workers suggested that in marine species different portions contained different proportions of water. According to Lavagna (1954) the ranges of water content was 38.5% to 56.3%; Gadus morhua (51.1%) of water which depends on particular portions and seasonal fluctuations and also types and nature of the species (Love, 1954), body lipid are markedly influenced by the diet. Starvation causes a progressive reduction in lipid value which reach a critical low level before protein begins to be utilized. The lipid values distribution in the bodies of different parts of species have been shown in table-9. It was observed that the water and lipid shows more or less similar pattern of values (Brandes and Dietrich, 1953). The fat content varies with certain anatomical portions of the fish. Large-sized fish contained more lipid than small sized fish, (Macpherson, 1933). The lipid content showed more variations in the ventral portion, as compared to the dorsal ones. In many species, the lipid values in the female fish was higher than that of the male (Templeman and Andrews, 1956).

Lipid changes which lead to lipid protein interaction and formation of formaldehyde for trimethyle amine in the tissue of fishes. Formaldehyde is known to be an important factor in protein changes in several species of fish (Sikorski et al., 1979). A definite relationship exists between the amount of protein and that of water (Dietrich, 1954). A similar regression line has in a corresponding way been computed from the relationship between protein and water contents (protein and water lines). In lean and semifatty fishes species the fat content generally increases from the head portion to the tail portion. In fatty fishes also the increase of value seems to prevail, the lowest amount of fat being in the tail region. This also seems to be valid for fatty fresh-water species when compared to other fatty fishes (Morawa, 1954). The close interrelationship between water content and the amount of fat was not distinct in non-fatty fishes (Kordyl, 1951). Nature of lipid is different in marine and fresh water fish species. In general, sea-water fish oils have a relatively more complex composition and fresh water fish lipid contained smaller amount of unsaturated fatty acids, than those in marine fish lipid. Such differences are due to differences in food, ecological conditions and seasonal variations (Lovern, 1952).

It was observed from table-9 that the lipid content in dorsal and ventral position may not be universal for all the species. Lipid in dorsal, ventral and posterior (tail) regions did not show consistent distribution pattern in various fish species. The distribution of lipid are reflected in those of water among the fatty species. Most anterior portion has the highest lipid and lowest water (Table-9), and the situation was reversed in the tail region. In non-fatty species lipid content was very low (Table-9), the water content was inversely related to the protein and presently showed different distribution (Figs. 1 to 20).

The amount of protein in fish was influenced by the fat and water content (Table-9). Protein content varies with size, age sexual maturity and season. There was an inverse relationship between fat and protein content of the edible part within the species of fishes. The fat content may vary between the species (Table-9). Generally, the lean fish has a higher protein content. The relationship between fat and protein of different anatomical portions,one species are shown by the regression lines. Even closely related species (same genus or group) may show basic differences in protein contents (Table-9). These differences do not seem to be directly connected with the fat contents (Dewberry, 1961). Different varieties of species (Marine and fresh water) contained widely varying amount of protein. Fish is also generally inexpensive in comparison of other protein food (Taylor, 1953).

Setna et al., (1944) analysed number of marine fishes and found protein content varying between 15 to 25%. Content may changes according to the lipid content but the proportions of the two did not vary. The water content of fishes never seems to rise above 80% except few species, especially Harpodon nehereus (88%). The variation of composition totally depends on species. Lovern (1938b) showed that there was a direct relationship between the size of the fish and their lipid contents.

There was a decline in protein nitrogen from head to tail region (Anon, 1966a), the concentration of lipid vary in different parts of the body. In fatty fish, there was usually a higher concentration of lipid immediately under the skin. Lovern (1958) showed that this lipid was rich in cholesterol than in lipid from other parts of the body. The higher concentration of lipid was present perhaps, because it cannt take an active part in swimming, so makes convenient lipid store house (Love et al, 1969). It has been found that lipid was more in thicker part of the body (Fraser Mannan and Dyer, 1961). Higher concentration of water was present in ventral part of the fish species as compared to the dorsal portion of the same region of the same species (Table-9). Lipid fluctuations depend on season, food and feeding habit of the species.

The overall, nutritive values of different fish species are presented in table and relationship are represented in several ways (Figs. 1 to 20), statistical analyses of variance (Table-16). The results showed that lesser concentration of protein was in ventral portion (average values) of the edible fish muscle, the variation of proteins contents in dorsal and ventral portion was not prominent. Increase of fat contents had been observed in ventral portions. Increase in lipid values have also been noticed in tail portions. Lipid content was found to be more in fresh water fish as compared to marine fish species. The quality of lipid was poor in dorsal portion and whole body as compared to ventral portions in marine and fresh water species (Table-9). The lipid content of fish varies widely, depending on season, latitute and sexual stage of fish.

Fish are found to deposit the maximum amount of fat just before the spawing season and to have a minimum fat content a few weeks afterward. The food supply also affect the composition of fish when they are forced away from the accustomed feeding ground by storm or by natural enemies, they are often encountered very lean condition (Borgstrom, 1965).

Fish as food can solve the great malnutrition problem, if it can be preserved and processed properly after knowing the actual food values.

STATISTICAL TEST (ANALYSES OF VARIANCE)

Statistical analyses of variance were carried

Table-16: Analysis of variance (from Table-9 while fish samples)

Variation	Sum of squares	Degree of freedom	Mean sum of squares	Observed 'F'	Tabulated value n=40 to 60 (Ave. values)
Between group	291250.95	2	145625.47		1% theoretical values of 'F' = 5.08
Within group	200598.70	42	4776.16	30.49	5% values of 'F' = 3.42 (Ave. values)
Total	491849.65	44			

Hypothesis = Nutritive quality of different fish species are not different.

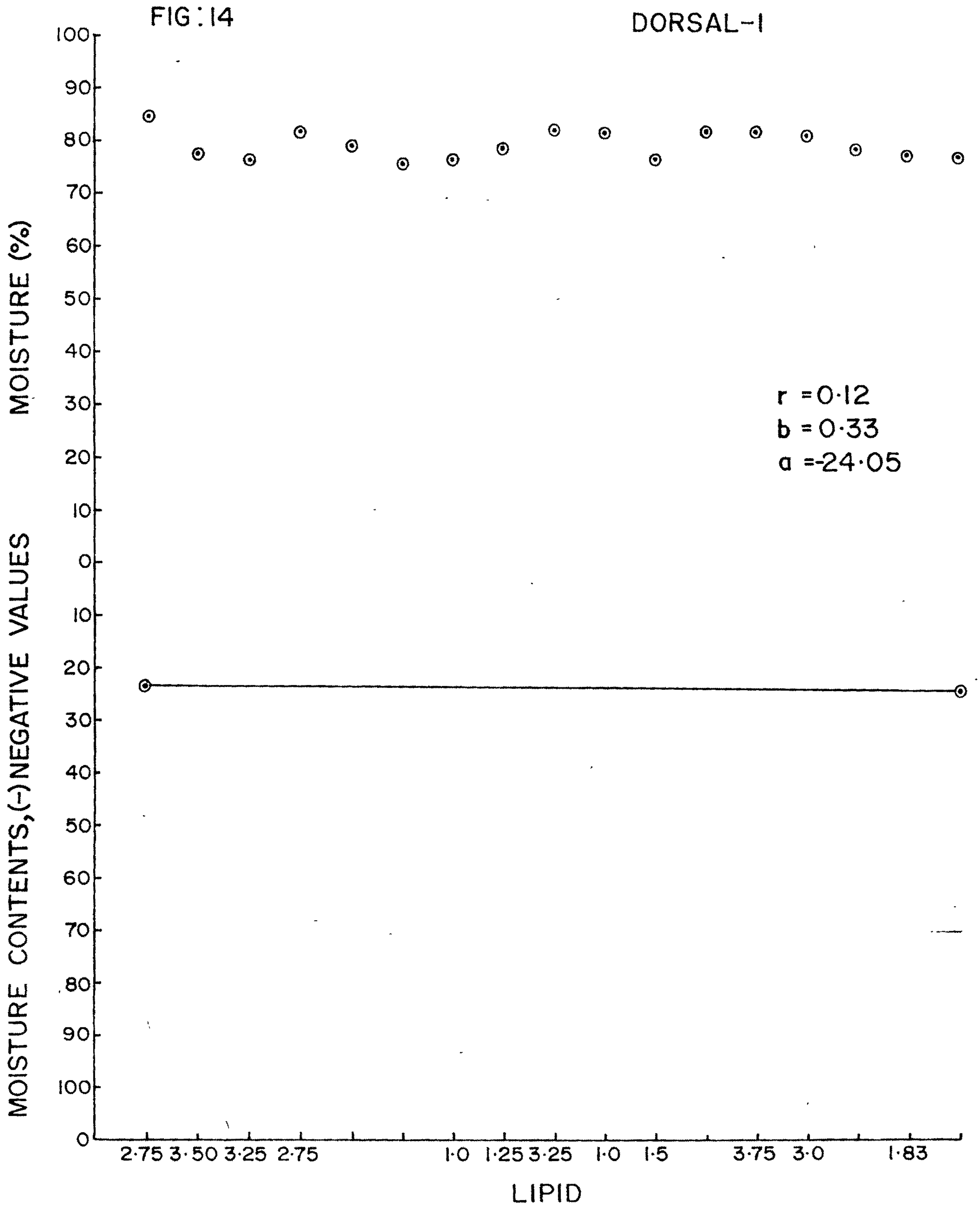
Conclusion: As the observed values 'F' is greater than the theoretical values of 'F' at 5% and 1% level of significance for 2 and 42 degree of freedom, the hypothesis is rejected, i.e., there is a significant difference in nutrative quality among different fish species of the present study.

out to find out whether the nutritive values of different species are same or not. It was found from the statistical findings that nutritive values of different fish species are different (Table-16). The observed values F (30.49) is greater than the theoretical value of F (5.08, 1% level of significance) and theoretical value of F 3.42 at 5% level of significance at 2 and 42 degree of freedom. The hypothesis is rejected. Therefore, it may be concluded that there was a significant difference in nutritive value among different fish species (Table-16) of the present study.

INTERRELATIONSHIP BETWEEN LIPID AND MOISTURE CONTENT IN DIFFERENT ANATOMICAL PORTIONS AND WHOLE FISH

The interrelationship of lipid and moisture in different anatomical portions of marine and freshwater fish species and shown in Figs. 15-20.

Moisture and lipid content in ventral-1 portion of different fishes are plotted in Fig. 15. The coefficient of correlation between two variables is $r = -0.13$ where slope and intercept were $a = 26.89$ and $b = -0.30$. Statistical regression equation was plotted from the highest and lowest values. The relationship between moisture and lipid content was found to be negative ($r = -0.13$).



FIGS.: 14 TO 19, RELATIONSHIP BETWEEN LIPID AND MOISTURE CONTENTS OF DIFFERENT ANATOMICAL PORTIONS OF DIFFERENT FISHES (REGRESSION LINES Y ON X)

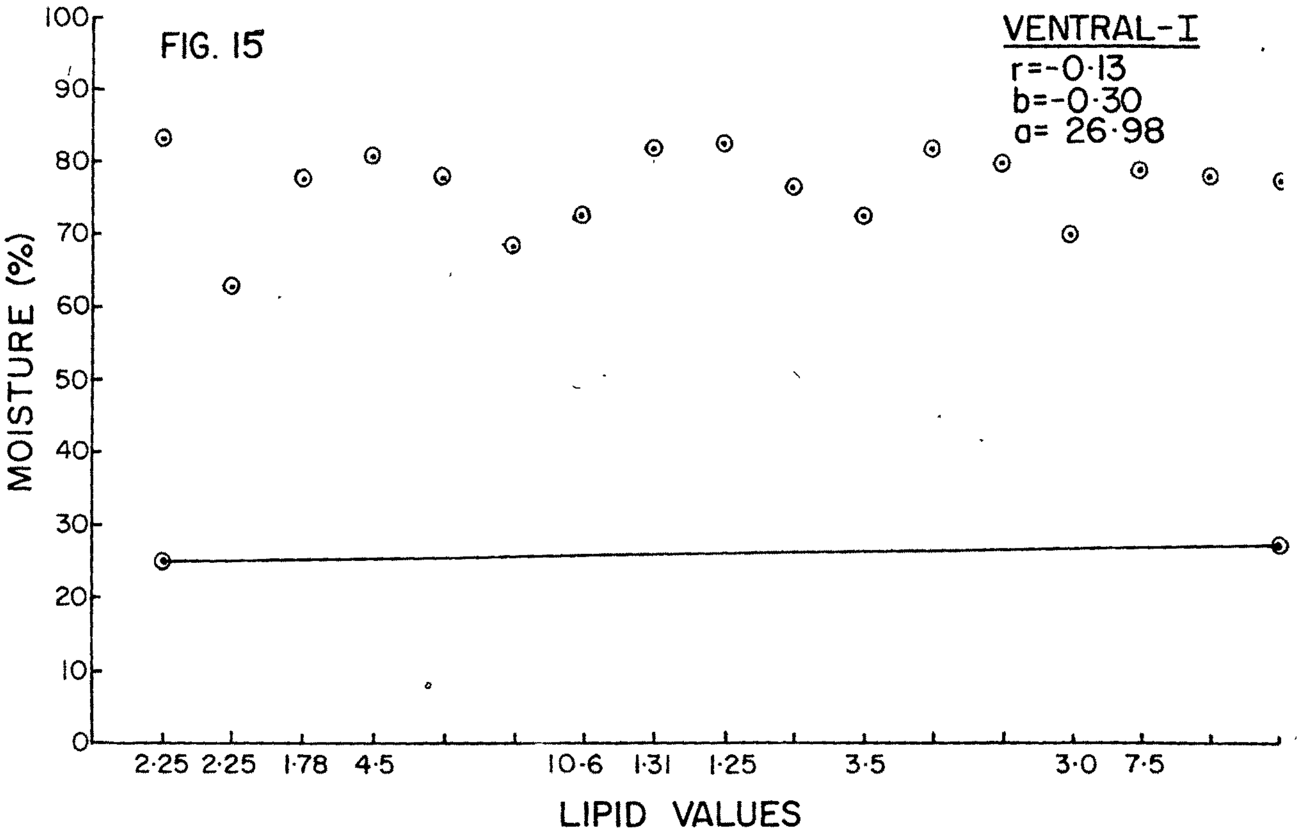


FIG. 15
VENTRAL-I
r=-0·13
b=-0·30
a= 26·98
MOISTURE (%)
100
90
80
70
60
50
40
30
20
10
0
2·25 2·25 1·78 4·5 10·6 1·31 1·25 3·5 3·0 7·5
LIPID VALUES

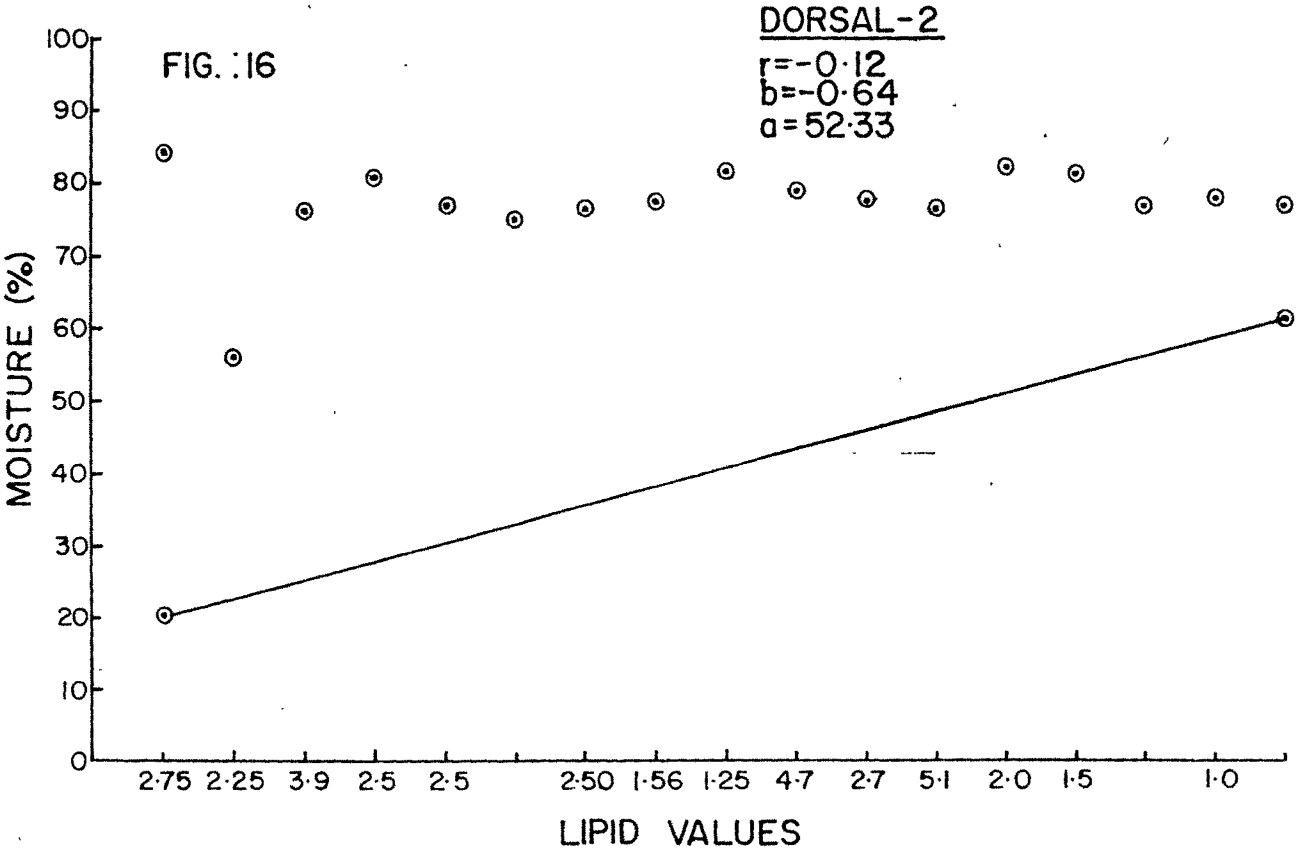


FIG. 16
DORSAL-2
r=-0·12
b=-0·64
a=52·33
MOISTURE (%)
100
90
80
70
60
50
40
30
20
10
0
2·75 2·25 3·9 2·5 2·5 2·50 1·56 1·25 4·7 2·7 5·1 2·0 1·5 1·0
LIPID VALUES

FIG : 17

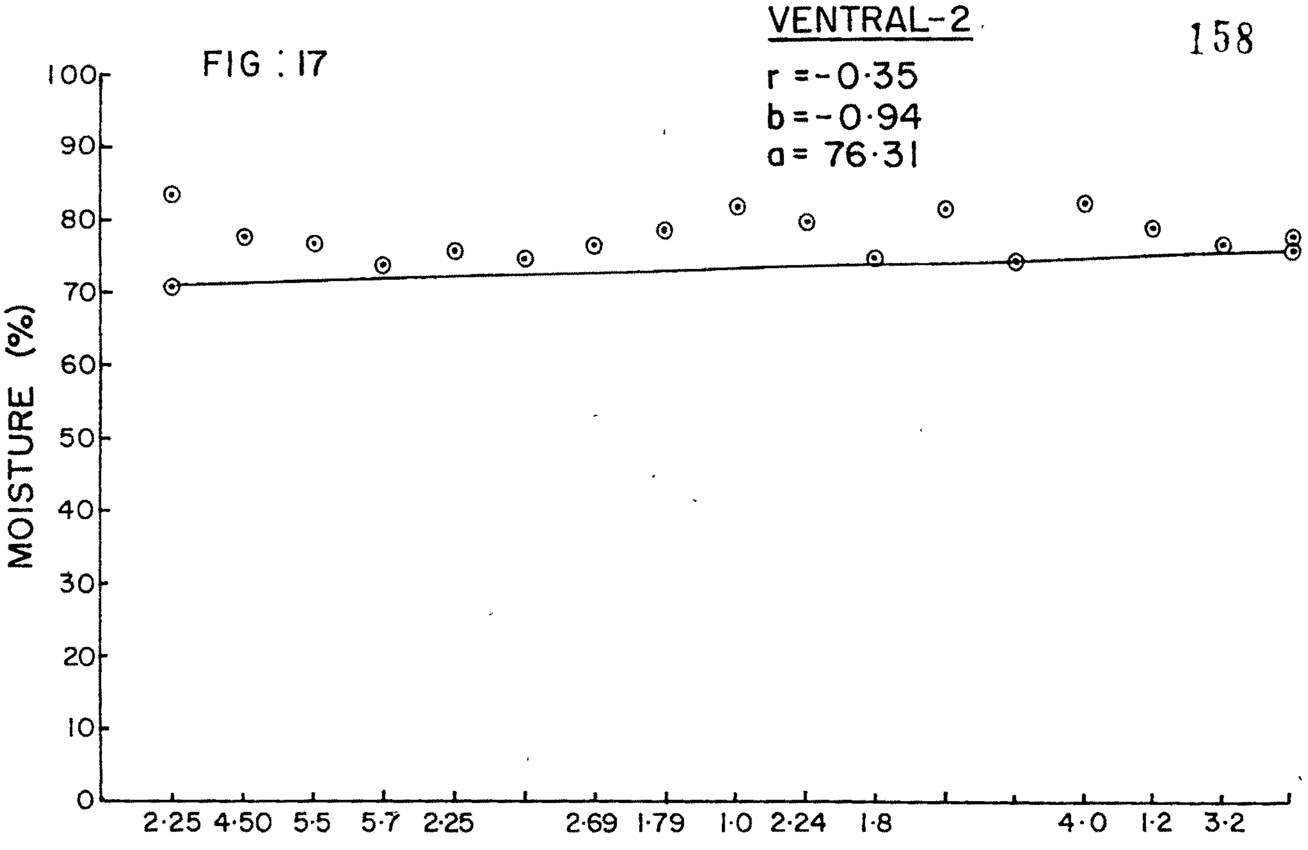

VENTRAL-2
r =-0·35
b=-0·94
a= 76·31
MOISTURE (%)
0
10
20
30
40
50
60
70
80
90
100
2·25 4·50 5·5 5·7 2·25 2·69 1·79 1·0 2·24 1·8 4·0 1·2 3·2
LIPID VALUES

FIG : 18

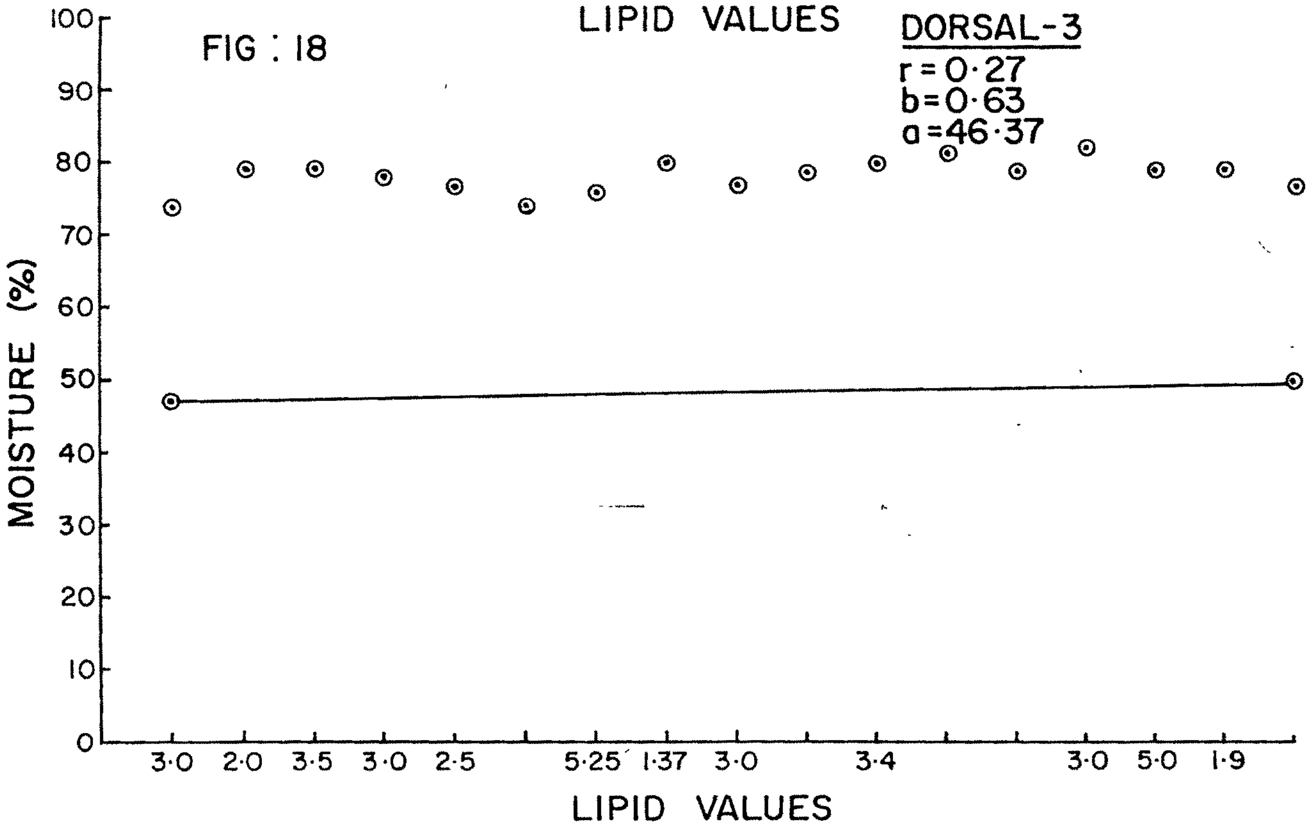
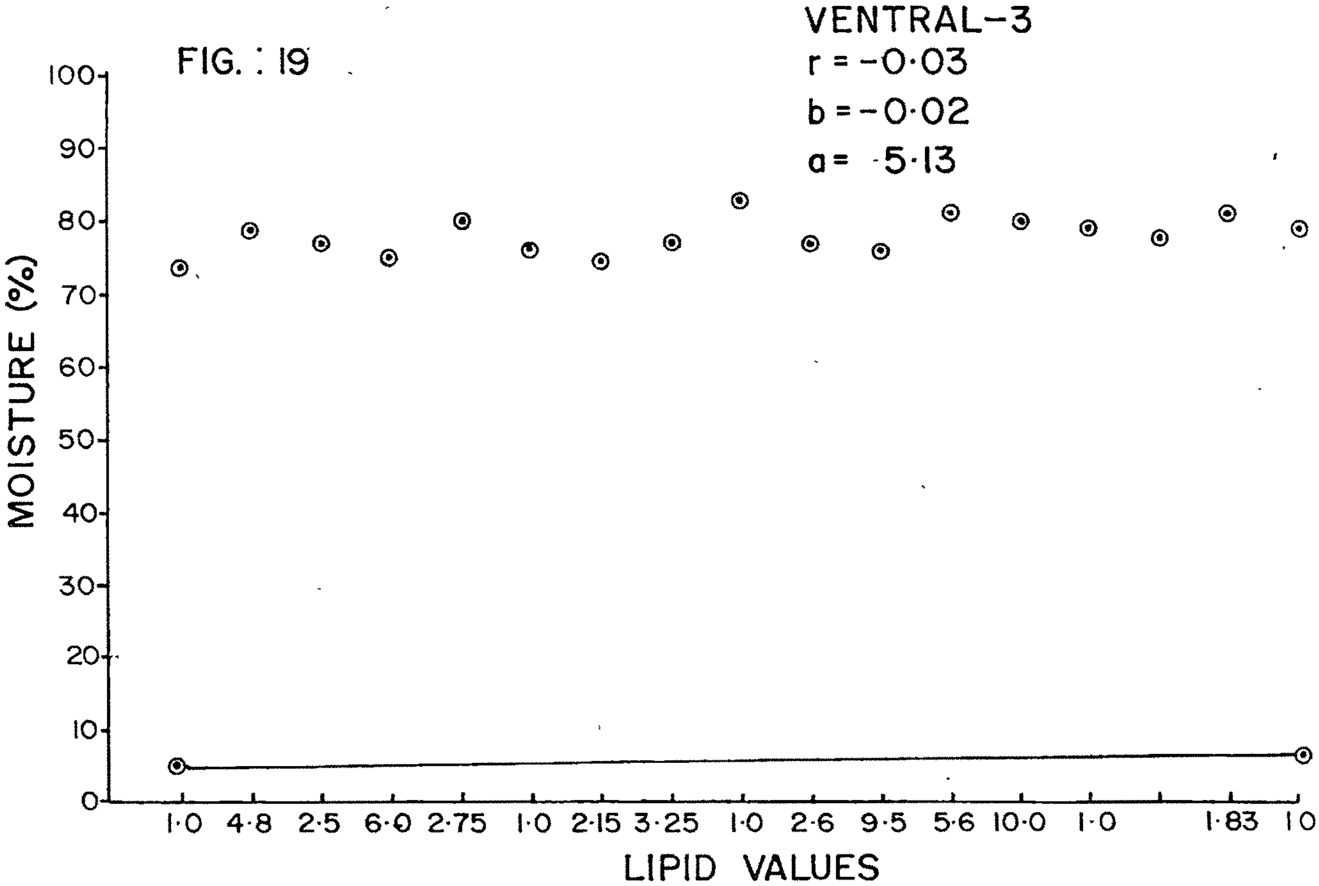

DORSAL-3
r=0·27
b=0·63
a=46·37
MOISTURE (%)
0
10
20
30
40
50
60
70
80
90
100
3·0 2·0 3·5 3·0 2·5 5·25 1·37 3·0 3·4 3·0 5·0 1·9
LIPID VALUES

FIG. : 19


VENTRAL-3
r = -0·03
b = -0·02
a = 5·13
MOISTURE (%)
100
90
80
70
60
50
40
30
20
10
0
1·0 4·8 2·5 6·0 2·75 1·0 2·15 3·25 1·0 2·6 9·5 5·6 10·0 1·0 1·83 10
LIPID VALUES

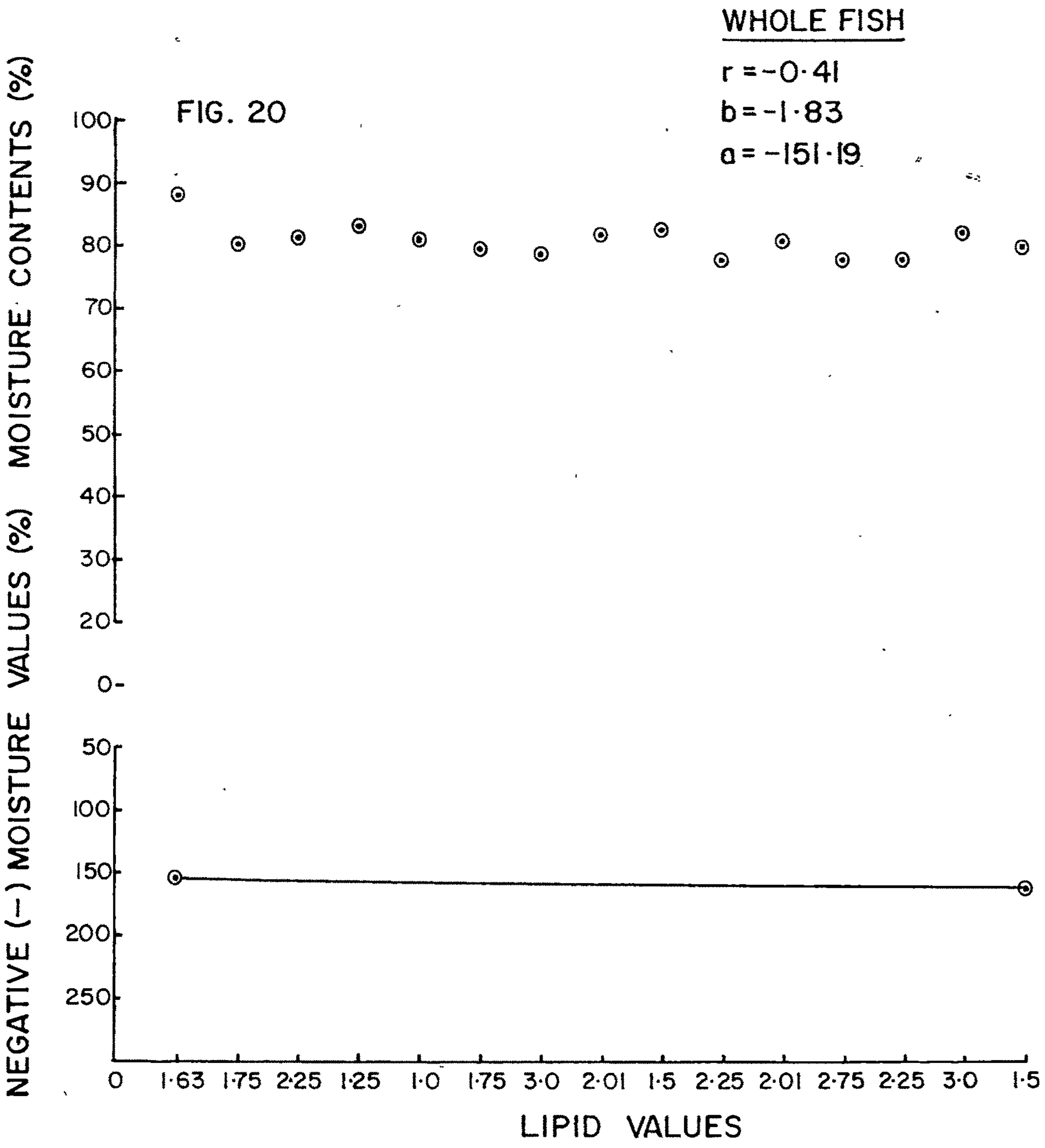


FIG. 20. RELATIONSHIP BETWEEN LIPID & MOISTURE CONTENTS OF DIFFERENT FISHES (REGRESSION LINE Y ON X)

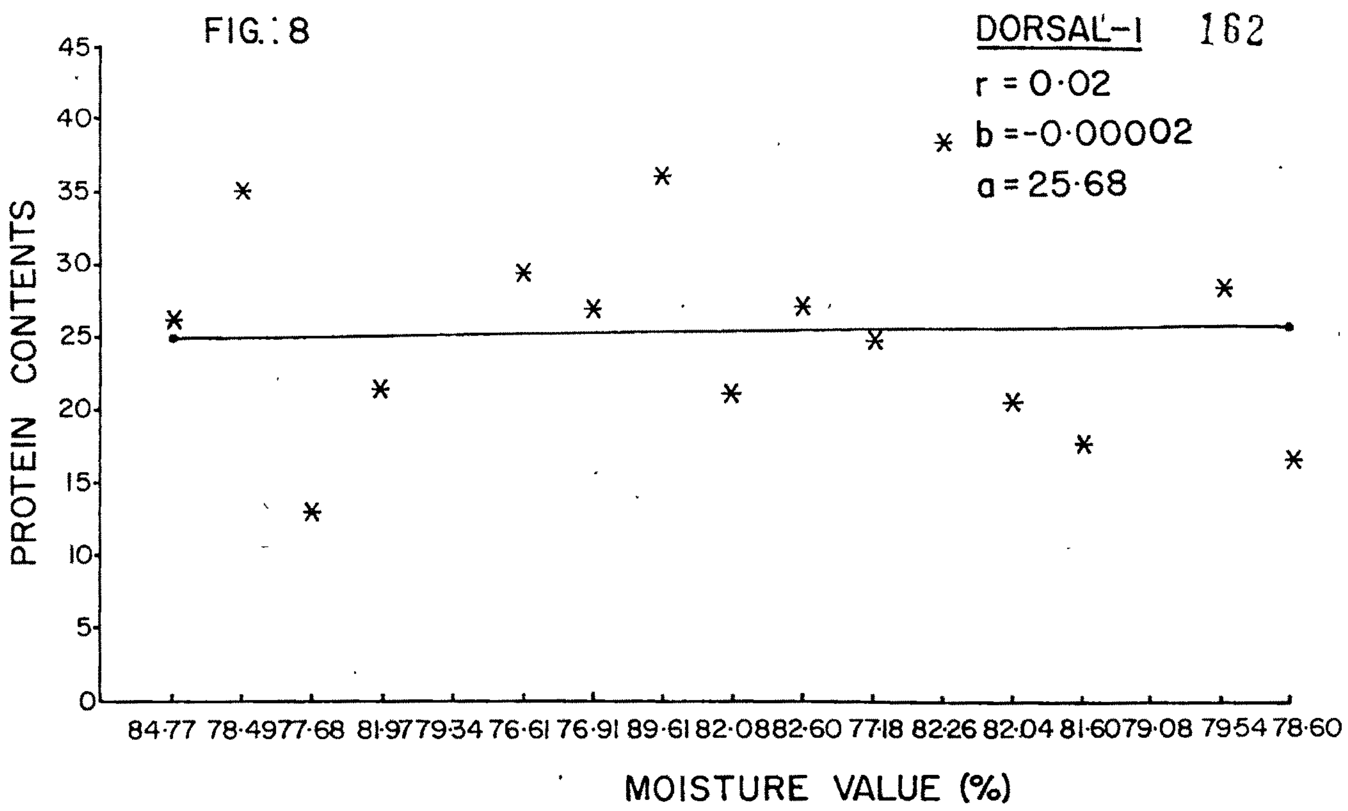
Similarly, in dorsal-2, a negative correlation was found (r = -0.12), Fig. 16; in ventral-3 (r = -0.03), Fig. 19; in ventral-2 (r = -0.94), Fig. 17. The correlation was positive in dorsal-1 (r = 0.12), Fig. 14 and in dorsal-3 (r = 0.27), Fig. 18. In whole fish samples the correlation was negative (r = -0.41), Fig. 20.

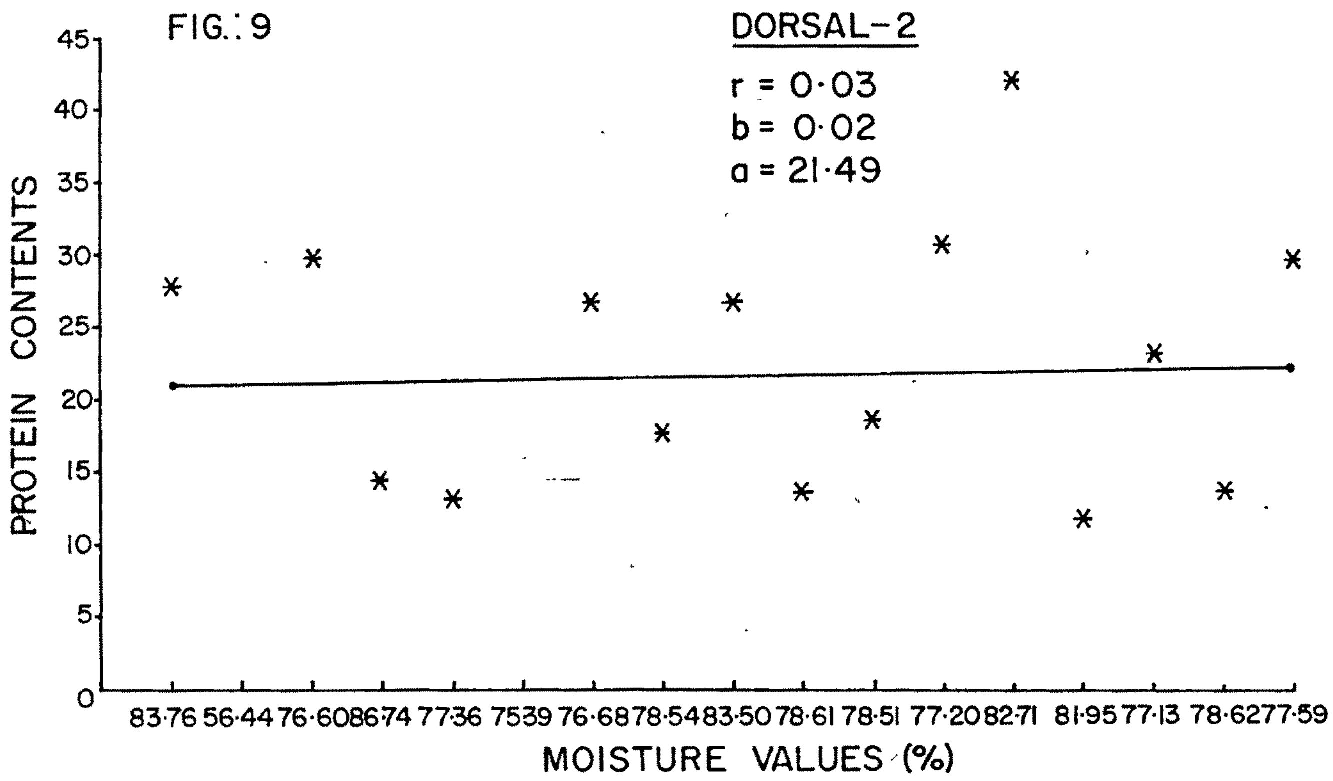
Therefore, it may be concluded, on the basis of the present findings that the correlation between moisture and lipid contents is negative. In dorsal-1 and dorsal-3 portions. The above values are negligible. The moisture and lipid content ratio is variable within the same species or genus or in any particular portions of the same species, when one value increase the other value decreases. This is the bio-chemical compositional interrelationship between moisture and lipid content within the fish body of different species.

INTERRELATIONSHIP BETWEEN PROTEIN & MOISTURE

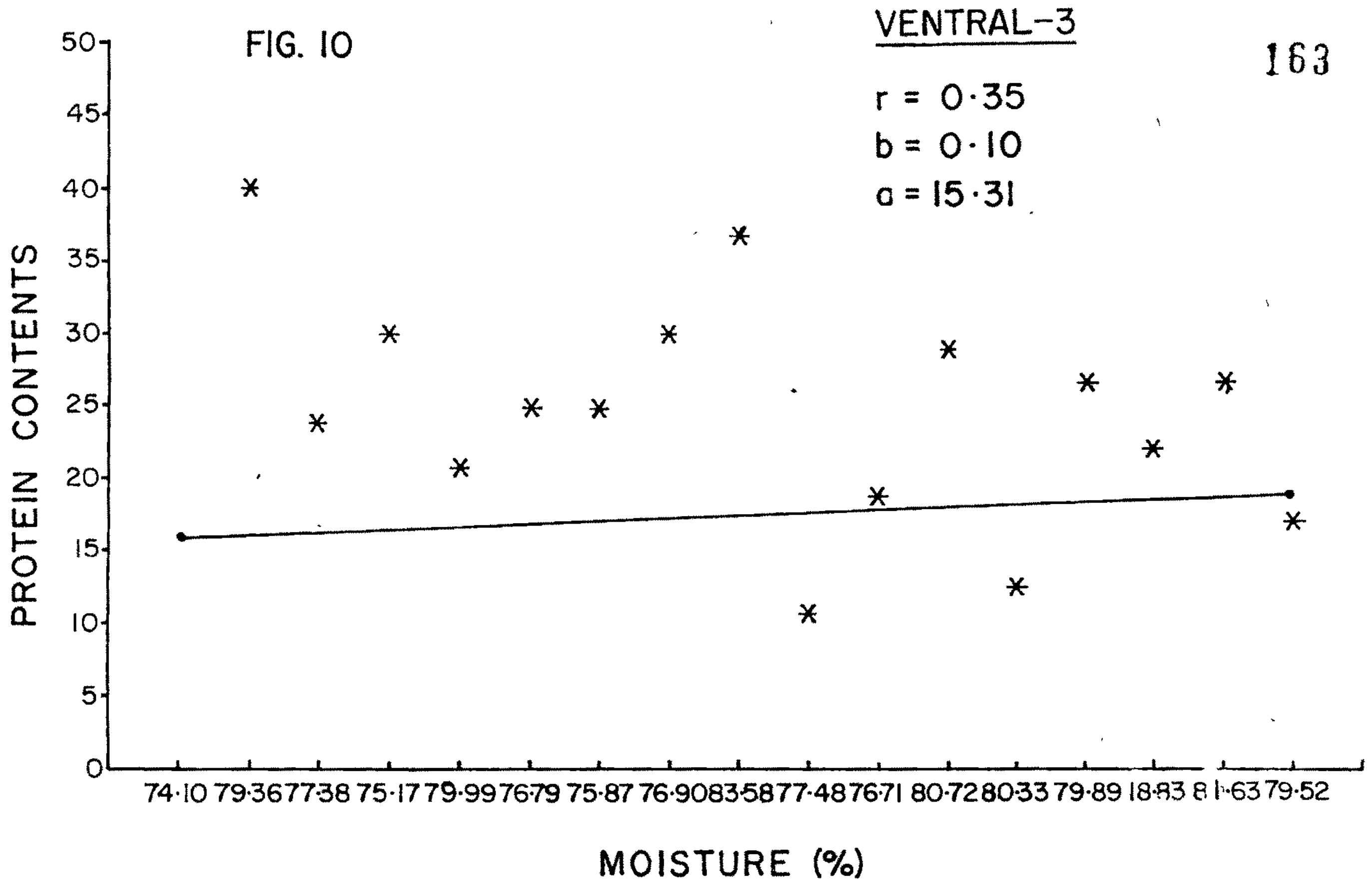
The interrelationship between protein and moisture content of different anatomical portions of marine and fresh water fish species are shown in figures as follows :

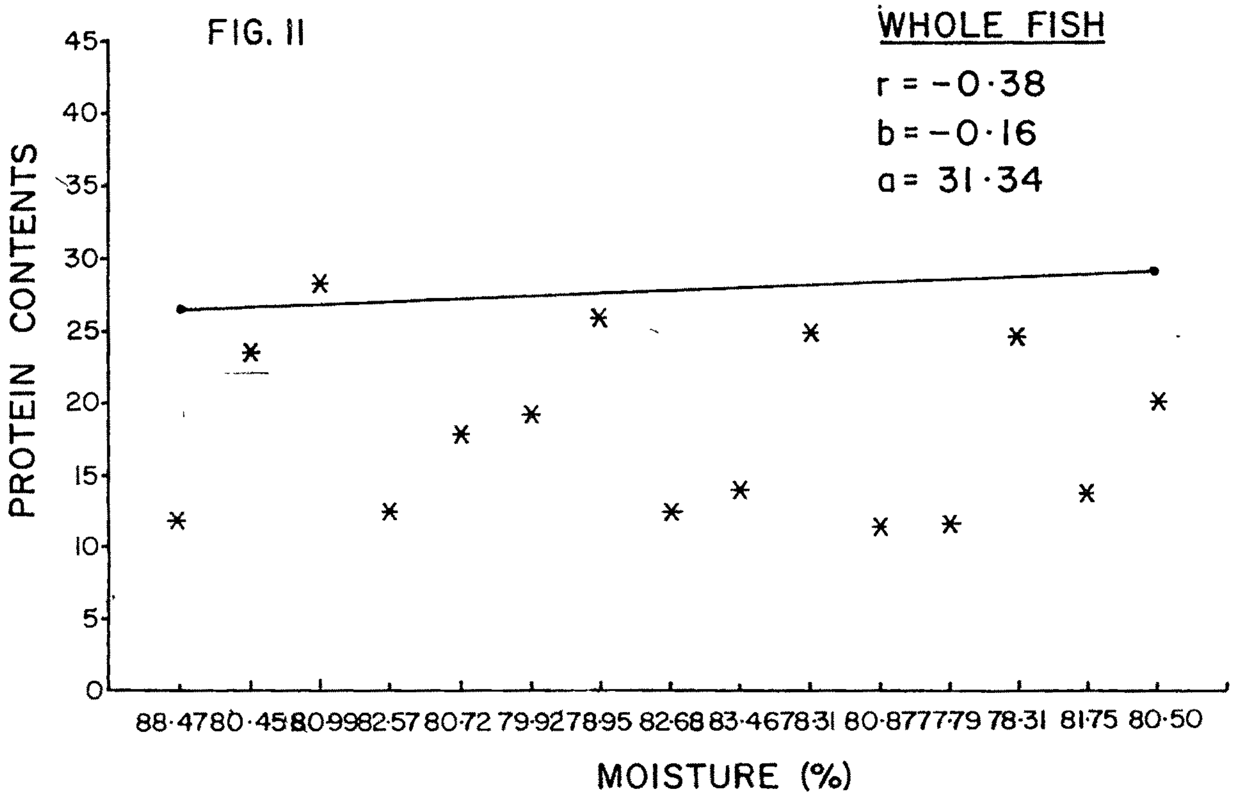
Dorsal-1	(Fig. 8)
Dorsal-2	(Fig. 9)



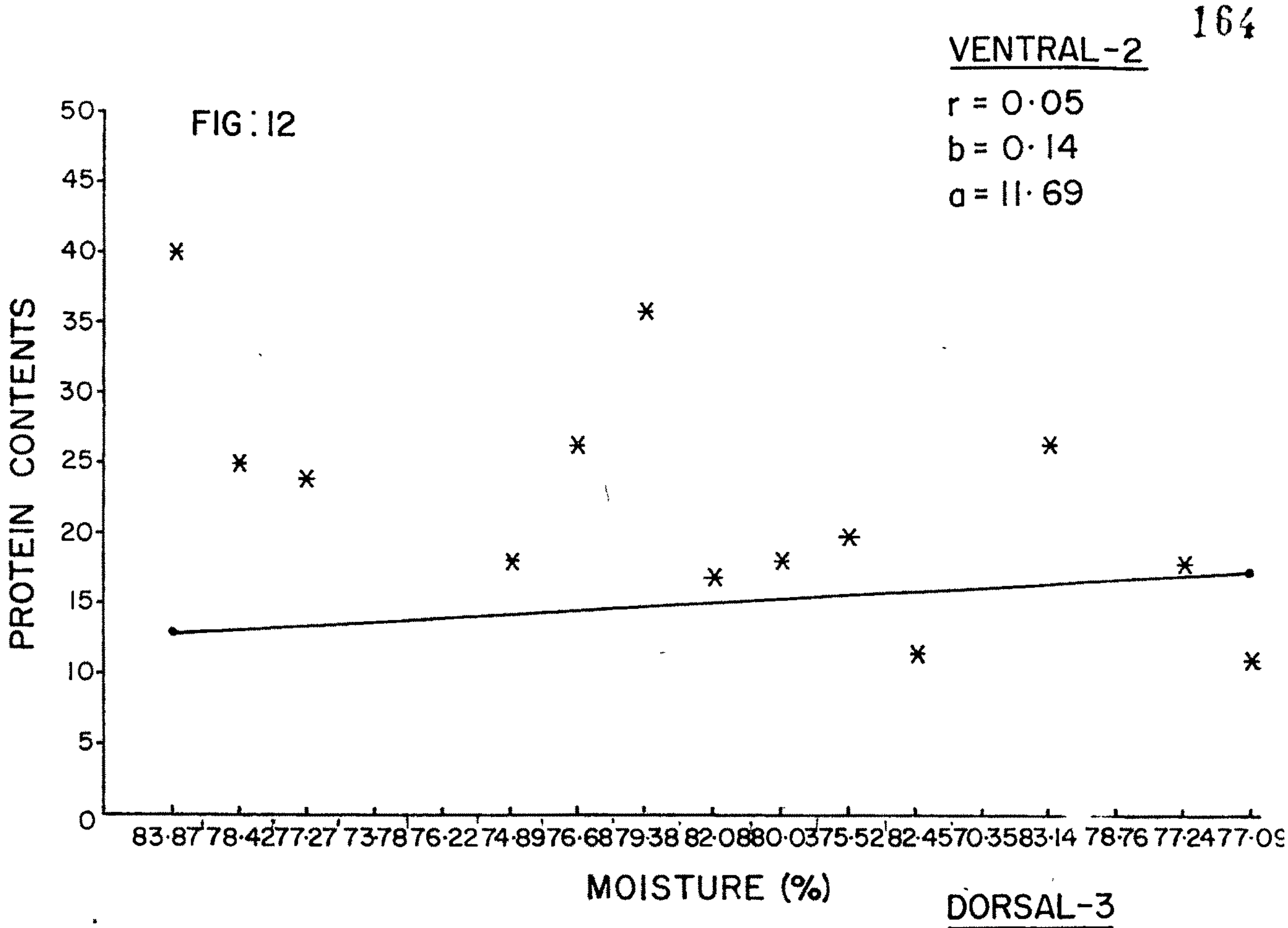


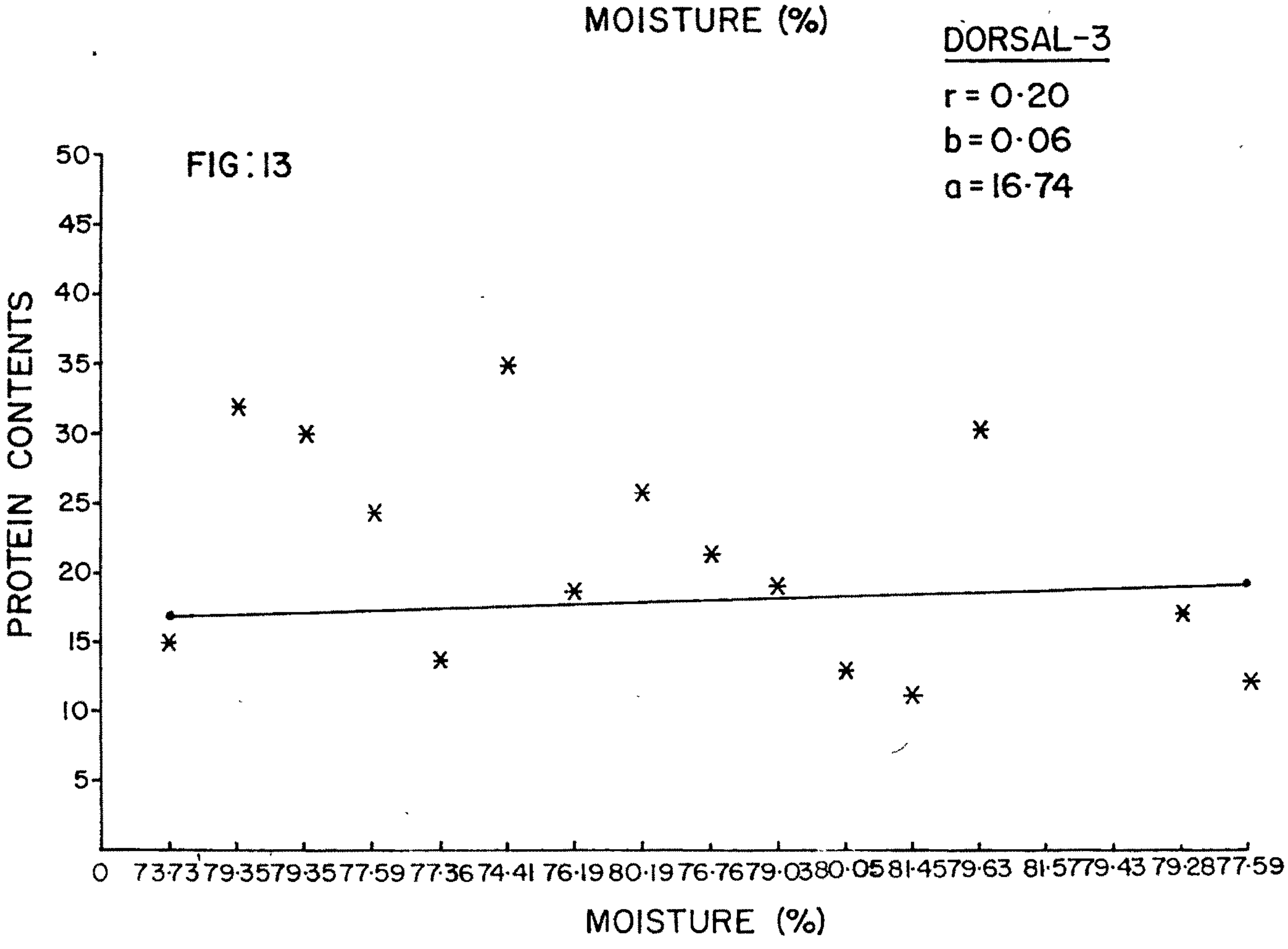
FIGS.: 8, 9, 10, 12 & 13, RELATIONSHIP BETWEEN MOISTURE & PROTEIN CONTENTS OF DIFFERENT ANATOMICAL PORTIONS OF DIFFERENT FISHES (REGRESSION LINES Y ON X)





FIGS. 10 & 11: RELATIONSHIP BETWEEN MOISTURE & PROTEIN CONTENTS OF DIFFERENT FISHES (REGRESSION LINES-Y ON X)


VENTRAL-2
r = 0·05
b = 0·14
a = 11·69
FIG: 12
PROTEIN CONTENTS
50
45
40
35
30
25
20
15
10
5
0
83·87 78·42 77·27 73·78 76·22 74·89 76·68 79·38 82·08 80·03 75·52 82·45 70·35 83·14 78·76 77·24 77·09
MOISTURE (%)


DORSAL-3
r = 0·20
b = 0·06
a = 16·74
FIG: 13
PROTEIN CONTENTS
50
45
40
35
30
25
20
15
10
5
0
73·73 79·35 79·35 77·59 77·36 74·41 76·19 80·19 76·76 79·03 80·05 81·45 79·63 81·57 79·43 79·28 77·59
MOISTURE (%)

Ventral-3	(Fig. 10)
Whole fish	(Fig. 11)
Ventral-2	(Fig. 12)
Dorsal-3	(Fig. 13)

The positive coefficient of correlations (r) were found in different anatomical portions of marine and fresh water fish species under the present investigations. The negative correlations was observed in whole body fish samples. The figures also show the diversifications of observed nutritional values (proteins with corresponding moisture values) from the linear regression lines. In ventral-1 and ventral-3 the correlation was found to be highly insignificant and hence not shown in figures.

The different regions namely; ventral 1, 2, 3 and Dorsal - 1, 2, 3 and whole body fish samples contained different nutritive values (Table-9). The pattern of distribution of nutritive values were also different in such anatomical portions.

Among different marine and freshwater species, the results of the interrelationship between proteins and lipid contents are given separately in the figures 1 to 7.

Dorsal-1	(Fig. 1)
Ventral-1	(Fig. 2)

Dorsal-2	(Fig. 3)
Ventral-2	(Fig. 4)
Dorsal-3	(Fig. 5)
Ventral-3	(Fig. 6)
Whole fish	(Fig. 7)

It was observed that there were marked differences between the proximate compositions among the different anatomical portions as well as in whole body fish samples. In different anatomical portions, the protein content were positively or negatively correlated. The diversification of the observed nutritive values from the linear regression lines are shown in each of the figures respectively.

Protein content and corresponding moisture content within the particular portions and whole body are shown in statistical correlation coefficient and linear regression lines.

Fig. 11 shows the interrelationship between protein content and corresponding moisture contents in whole body fish samples.

INTERRELATIONSHIP BETWEEN THE MAIN CONSTITUENTS OF FISH BODY (PROTEIN AND CORRESPONDING LIPID VALUES)

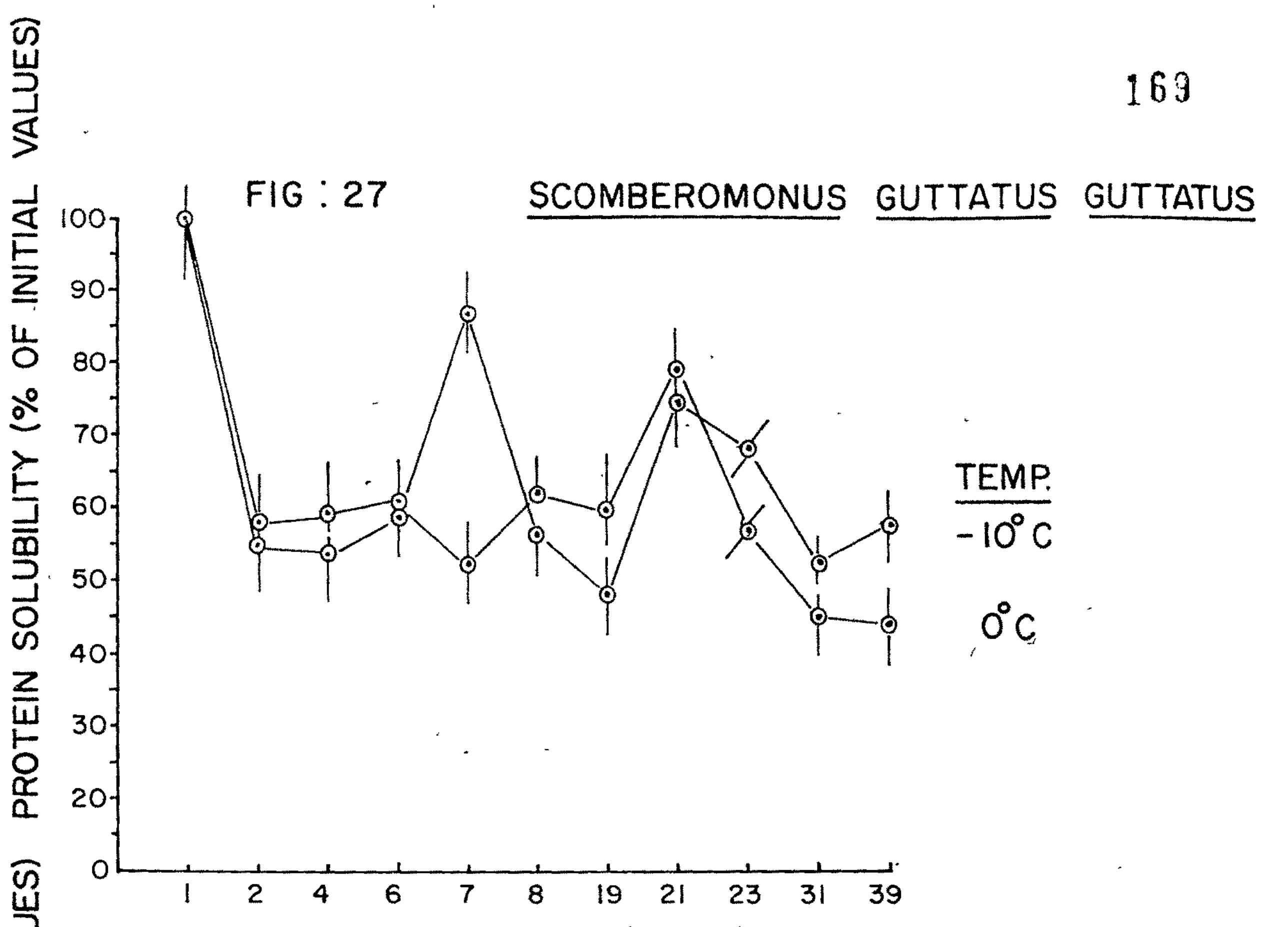
Fig. 4 (ventral-2). The coefficient of correlation

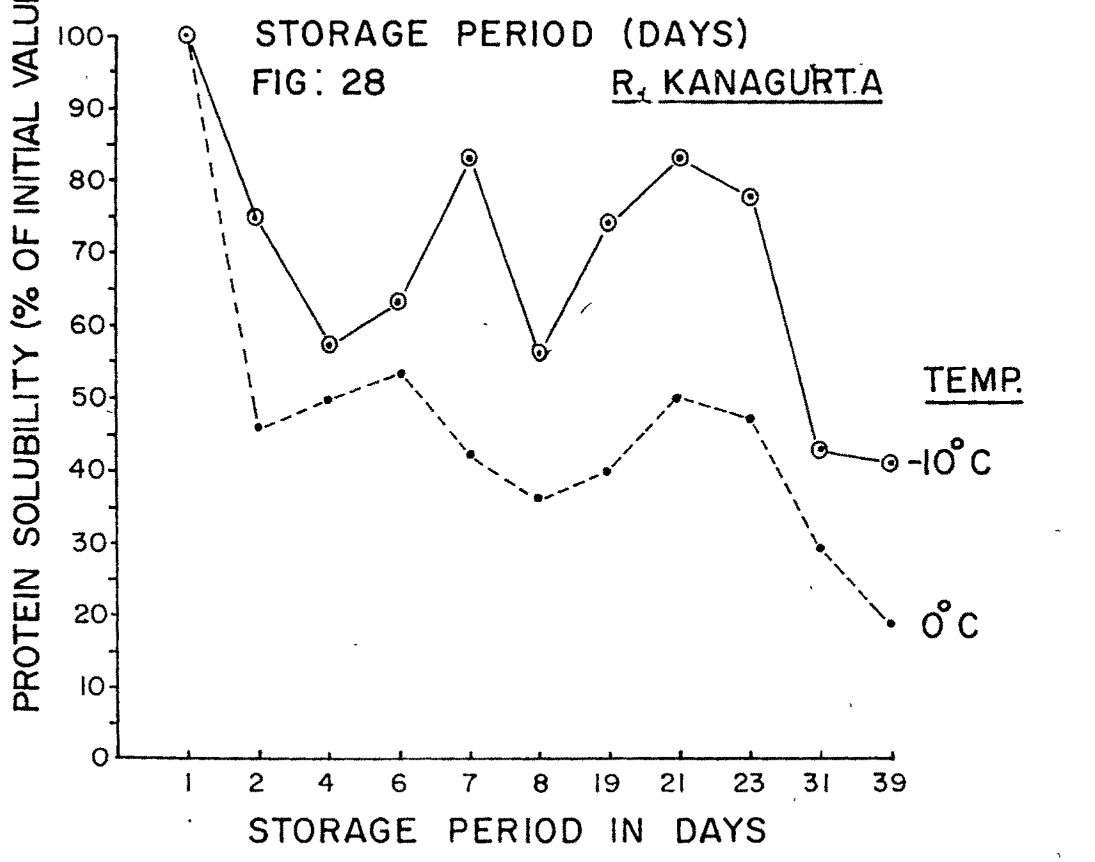
$r = -0.97$, where slope (a) = 23.11 and intercept (b) = -0.17 respectively. The linear regression equations were $Y_1 = 23.11 - 0.17X_1$ and $Y_2 = 23.11 - 0.17X_2$ ($X_1 = 40.0$ and $X_2 = 11.25$ mg.N/100 gm of fish protein, these are the highest and lowest values in ventral-2 sections.

CHANGES OF PROTEIN SOLUBILITY IN MARINE AND FRESH WATER FISHES AT LOWER TEMPERATURE OF STORAGE

The loss of protein solubility of different fish species from percentage of initial values at different storage temperatures are presented in tables and figures. The loss of protein solubility varies from species to species and within the species with length of storage period. Storage temperatures have some effect on protein solubility changes. Lower the storage temperature, lower the loss of protein solubility as compared to the higher storage temperature. In Scomberomonus guttatus guttatus species, the loss of protein solubility a was 44.80% at 0°C and 68.72% in -10°C storage period and same experimental condition, the difference of loss of protein solubility in two different storage temperature were 23.92% (from percentage of initial values). The total loss of protein solubility at 0°C and -10°C temperature were 55.2% and 31.28% (from percentage of initial values) Fig. 27; Table-1.

The loss of protein solubility in Labeo rohita species was 19.81% at 0°C and 18.23% in -10°C storage temperature at 17 days of storage. The total loss of protein solubility from percentage of initial values were 80.19% at 81.77% 0°C and -10°C storage temperatures, for the same storage period. The difference in the loss between 0°C and -10°C was 1.58% at the end of the storage period (Table-2 and Fig. 21). Similar pattern of findings were also observed in Rastrelliger kanagurta and catla catla species. The total loss of protein solubility was 80.03% in 0°C and 58.26% in -10°C storage temperature after 39 days of storage in Rastrelliger kanagurta (Table-3 and Fig. 28). In Catla catla fish the total loss of protein solubility was 86.86% and 87.24% (from percentage of initial values) at 0°C and -10°C storage temperatures after 43 days of storage (Table-4 and Fig. 25). The difference of loss of protein solubility was 0.38% between 0°C and -10°C storage temperature for the same storage period. Among all the species the protein solubility values gradually decreases. It was observed from the result that there were no marked differences in loss of protein solubility changes between marine and fresh water fishes. The storage temperature and length of storage period had great influence in protein solubility changes. With the increase of storage time the value of the protein solubility declined steadily. The loss of solubility of the denaturated protein ~~is~~ was





FIGS. : 27 & 28, PROTEIN SOLUBILITY CHANGES OF SCOMBEROMONUS GUTTATUS GUTTATUS & RASTRILAGAR KANAGURTA DURING STORAGE AT 0°C & -10°C TEMPERATURES

Table 2 : Changes of protein solubility and total volatile nitrogen of *Labeo rohita* during storage at 0°C and -10°C temperatures.

Species : Scientific name	Storage period (days)	Protein solubility (mg/gm of fish muscle) 0°C	Protein solubility (From % of initial values) 0°C	Protein solubility (mg/gm of fish muscle) -10°C	Protein solubility (From % of initial values) -10°C	TVN (mg.N/ 100 gm of fish muscle) Log values 0°C	TVN (mg.N/ 100 gm of fish muscle) Log values -10°C
Labeo rohita							
	0	3.13	100	1.79	100	1.21	0.99
	3	5.36	58.40	2.5	71.60	1.42	1.23
	5.	8.04	38.93	4.82	37.14	1.56	1.34
	7	6.25	50.08	6.25	28.64	2.30	1.44
	9	4.16	75.24	4.64	38.58	2.21	1.50
	11	4.82	64.94	2.59	69.11	2.14	1.22
	13	8.93	35.05	2.68	66.79	2.01	1.29
	15	11.61	26.96	3.50	51.14	2.28	1.20
	17	15.80	19.81	9.82	18.23	2.31	1.32
	19					2.41	2.22

Table 4 : Changes of protein solubility and trimethyle amine contents of <u>Catla catla</u> during storage at 0°C and -10°C temperatures

Species: Scientific name	Storage period (days)	Protein solubility (mg/gm of fish muscle) 0°C	Protein solubility (From % of initial values) 0°C	Protein solubility (mg/gm of fish muscle) -10°C	Protein solubility (From % of initial values) -10°C	TMA (mg.N/ 100 gm of fish muscle) Log values 0°C	TMA (mg.N/ 100 gm of fish muscle) Log values -10°C
<u>Catla catla</u>							
	1	4.60	100.0	1.61	100.0	1.31	0.26
	2	4.10	93.87	1.96	81.63	1.30	0.65
	4	5.0	92.0	4.46	36.10	1.27	0.73
	6	17.86	25.75	3.39	47.49	1.10	0.77
	7	16.07	28.62	11.64	13.83	1.43	0.99
	8	23.20	19.83	11.61	13.87	1.64	0.95
	19	32.10	14.33	9.82	16.74	1.13	0.91
	21	42.86	10.73	11.61	13.87	1.24	1.22
	23	33.03	13.93	9.11	17.67	1.20	1.14
	31	39.30	11.70	11.61	13.87	1.46	1.11
	39	39.28	11.71	12.5	12.88	1.38	1.10
	43	35.0	13.14	12.61	12.76	1.34	1.23

20

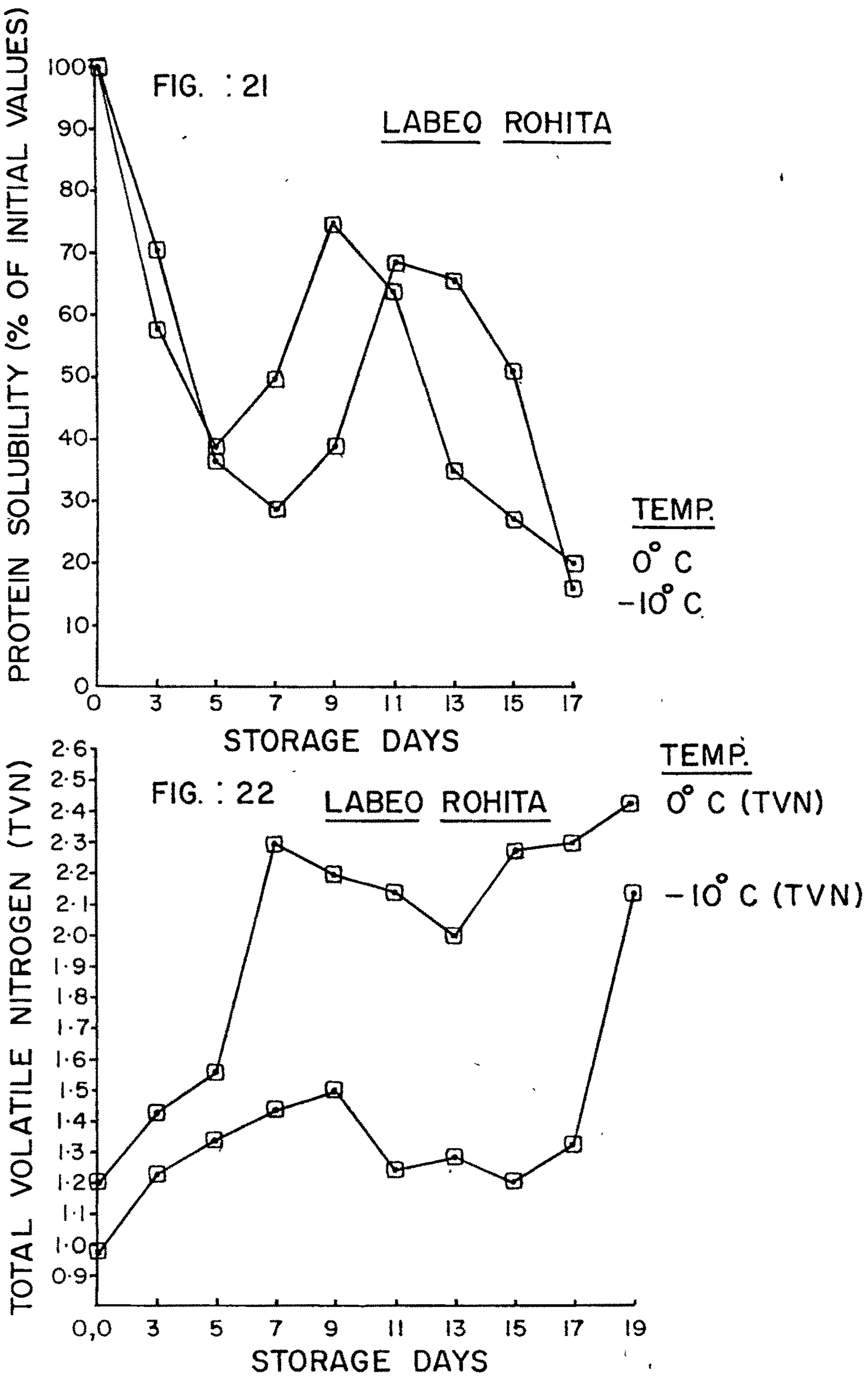
the result of reorganization of hydrophilic groups and lipophilic groups on the surface of the molecules after unfolding of the polypeptide chain. The free fatty acid attach themselves hydrophobically or hydrophilically to appropriate site on protein surface. Thus at the end it results in decrease in protein solubility (Si korski et al., 1979). The denaturation of protein measured as loss of solubility in defined salt solution decreases with lowering of storage temperatures (Finn, 1934). Dyer (1954) made extensive studyo of the denaturation of fish muscle actomyosin and found a general correlation between loss of solubility of the protein and organoleptic changes. The solubility should not be expected to reflect the textural changes beyond a certain limit. Ironside and Love (1958) reported that actomyosin denaturation may play an important part in the textural changes during freezing. Storage toughness may also result from the changes in cell membrane. Inorganic salts play a decessive role in actomyosin denaturation and that denaturation is almost arrested when the temperature fall below the cryshydratic point of NaCl. At low-temperature of storage lipid possibly offers protective action against protein denaturation (Dyer et al., 1957b). The rate of denaturation of protein was higher at higher storage temperature. Fluctuation of storage temperature shows a drastic effect. At lower temperature of storage the denaturation is

21

slower. At higher storage temperature the rate of dehydration is higher and such dehydration accelerates the protein in extractibility. The loss of protein solubility showed species variation. Hollander and Nell (1954) reported that freezing period has apparent influence on the denaturation of protein. Stiffening of the muscle reduced the solubility of actomyosin. The solubility decreases to a low value of about 30% in herring when frozen before rigor sets in and does not change during 30 days of storage (Nikkila and Linko, 1956). Protein solubility returned to normal from the low pre-rigor values. Protein is less soluble in salt solution and muscle had been able to shrink freely on defrosting (Love, 1962a). The solubility changes of different fish samples during low-temperature of storage are shown in figures. After low temperature of storage they showed a decrease in solubility. From the figures it can be seen that during chill storage, the solubility show very slight changes. However, during frozen storage a further solubility loss occurs which depends on the storage period. After frozen storage for only a week there was very little effect. The solubility loss was considerable but little change is evident. Solubility is not affected by the freezing process but is a function of storage period. Lowering of temperature by -10°C level induces a four fold increase in shelf-life (Heen, 1949). Fish muscles suffer deteriorative quality

2

changes during frozen storage. However, there is a need to find difference between fresh and frozen muscle for a meaningful discussion of fish muscle preservation. Many studies have been carried out to detect changes in the chemical and physical properties of protein for example, measurement of solubility (Dyer, 1951; Connell, 1961). They all found that these properties were affected by freezing. Protein were examined in an attempt to find out any changes in their properties as a result of freezing and to use this information to monitor changes occuring during cold storage. Salt soluble proteins were extracted from fresh and frozen muscle in order to compare their pattern of changes. The loss of protein solubility is used as one of the indicators of protein denaturation. The major part of the solubility loss occuring over the two weeks of storage happens during the first week and that the subsequent solubility loss is very slow. Washing the pieces before freezing has no great effect on the initial and final loss of solubility. The loss of solubility during the first week could be that the act of freezing was causing the effect. It could be argued that the loss of solubility was caused by the changes in pH, ionic strength or enzyme substrate concentrations brought about by the effect of freezing. However, a linear relationship between rate of solubility loss and temperature may be expected. Storage of fish for a week tend to large



FIGS. : 21 & 22, PROTEIN SOLUBILITY & TOTAL VOLATILE NITROGEN CHANGE OF LABEO ROHITA DURING STORAGE AT 0°C & -10°C TEMPERATURE.

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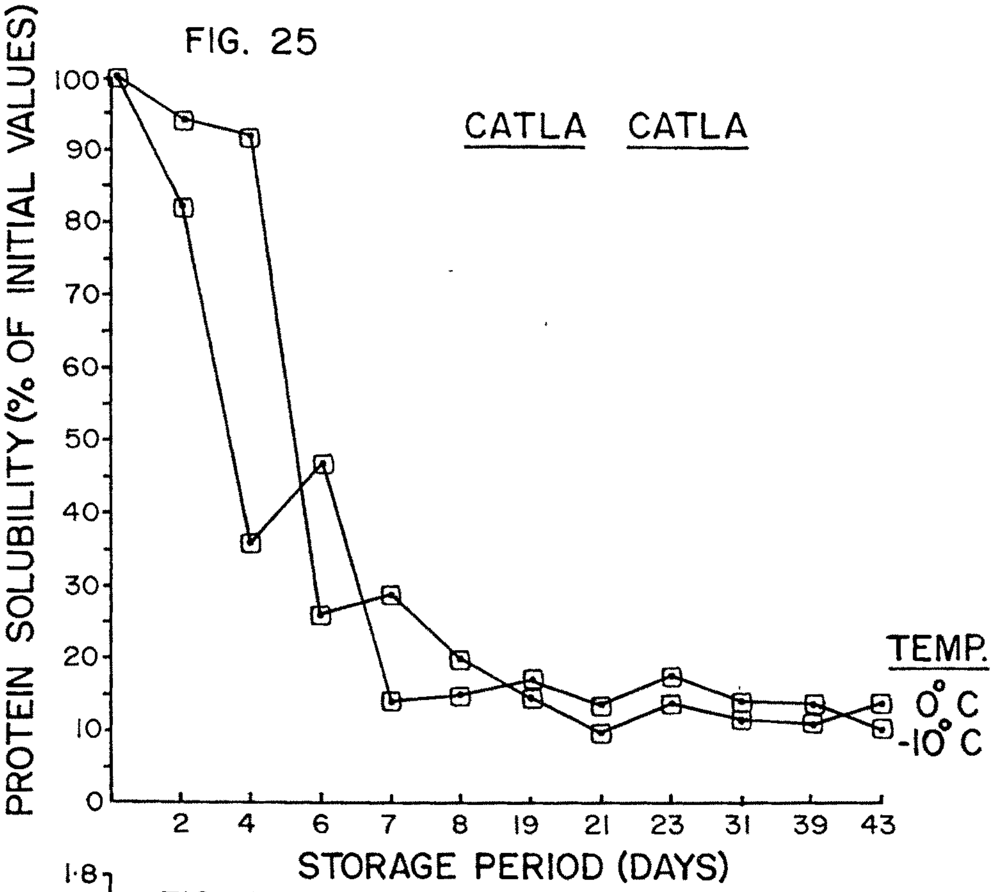
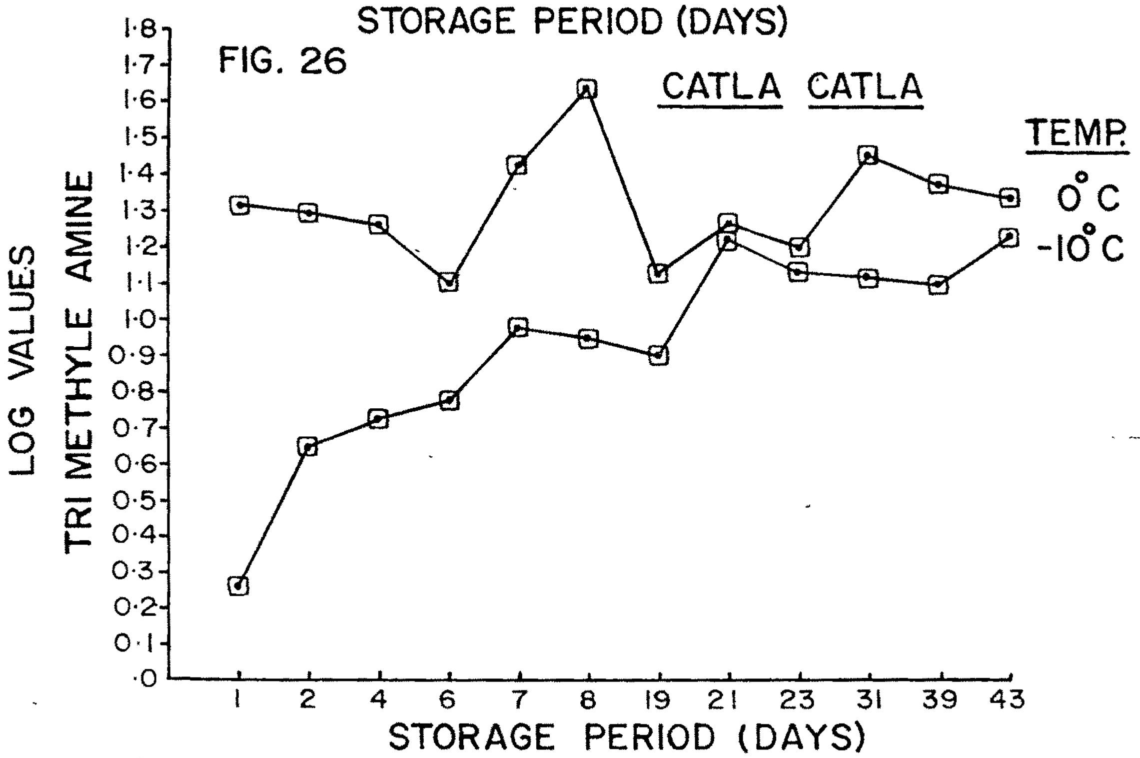
Table 3 : Changes of protein solubility and trimethyle amine contents of Rastrelliger Kanagurta

Species: Scientific name	Storage period (days)	Protein solubility (mg/gm of fish muscle) 0°C	Protein solubility (From % of initial values) 0°C	Protein solubility (mg/gm of fish muscle) -10°C	Protein solubility (From % of initial values -10°C	TMA (mg.N/ 100 gm of fish muscles) Log values 0°C	TMA (mg.N/ 100 gm of fish muscles) Log values -10°C
Rastrelliger Kanagurta	1	11.60	100	13.40	100	1.35	1.33
	2	25.00	46.40	17.90	74.86	1.32	1.45
	4	23.20	50.00	23.20	57.75	1.13	1.35
	6	21.43	54.13	21.43	62.53	1.29	1.18
	7	27.80	41.73	16.07	83.39	1.31	1.24
	8	32.14	36.09	24.10	55.60	1.18	1.19
	19	28.60	40.56	17.90	74.86	1.40	1.54
	21	23.20	50.00	16.10	83.23	1.41	1.37
	23	24.46	47.42	17.00	78.82	1.32	1.54
	31	39.29	29.52	32.14	41.69	1.19	1.19
	39	58.10	19.97	32.10	41.74	1.33	1.18





FIGS. 25 & 26, CHANGES IN PROTEIN SOLUBILITY & TRI-METHYLE AMINE OF CATLA CATLA DURING STORAGE AT 0° C & -10° C TEMPERATURES decrease in the initial solubility of the protein, different species as well as different regions of fish showed variations in loss of protein solubility. (Figs. 21, 25, 27 and 28). Protein solubility study would be helpful in determination of micronutritional quality changes in fish stored at low temperature.

STATISTICAL ANALYSES

Statistical 't' test were carried out for established the hypothesis that storage temperature had some effect on protein solubility changes in different fish species namely, scomberomorus guttatus guttatus, labeo rohita, catla catla and Rastrelliger kanagurta. From the statistical test, it was found that storage temperature had some effect on protein solubility changes and it has been found that lower the temperature, lesser were the changes of protein solubility. The pattern of solubility changes among different species (marine and fresh water) are also different. The results of statistical analyses are given in tables : 15, 15.1, 15.2 and 15.3 respectively.

Table-15 : Statistical 't' test

Species: Scomberomorus guttatus guttatus

Protein solubility x_1i(0°C)	Protein solubility x_2i(-10°C)	Difference $d_i=(x_1i-x_2i)$	n	Observed value='t'	Tabulated 't' at 1% level of significance
17.68	19.64	- 1.96			
30.40	35.70	- 5.30	n=11	1.89	
30.36	35.72	- 5.36			3.169
28.6	33.04	- 4.44			
33.9	22.32	12.59			
28.6	34.80	- 6.20			
29.5	39.50	-10.0			
22.32	25.89	- 3.57			
30.70	28.60	2.1			
39.30	37.50	1.8			
39.46	25.0	14.46			

$\bar{d} = 0.53$

Storage temperature : 0°C and -10°C

Hypothesis : Storage temperatures has some effect on protein solubility changes

Assumption : $d_1, d_2 \ldots\ldots d_n$ constitute a random sample from $n(\mu d, \sigma^2 d)$ populations.

$$\bar{d} = \frac{\sum_1^n di}{n} = 0.53$$

$$\frac{\sum_1^n (di-\bar{d})^2}{n-1 = 10} = 547.26$$

The test statistics $'t' = \frac{\bar{d}}{sd/\sqrt{n}} = 1.89$

$$S^2d = \frac{\sum_1^n (di-\bar{d})^2}{n-1} = 54.73 \qquad sd = 7.40$$

Conclusion: The observed value 't' is less than the tabulated 't' at 1% level of significance at (n-1)=10 degree of freedom, the hypothesis is accepted.

Table-15.1 : Statistical 't' test

Species: *Labeo rohita*

Protein solubility x_1i (0°C)	Protein solubility x_2i (-10°C)	Differences $d_i=(x_1i-x_2i)$	n	Observed value 't'	Tabulated 't' at 1% level of significance
3.13	1.79	1.34	n=9	3.93	
5.36	2.5	2.86			3.355
8.04	4.82	3.22			
6.25	6.25	0.00			
4.16	4.64	- 0.48			
4.82	2.59	2.23			
8.93	2.68	6.25			
11.61	3.50	8.1			
15.80	9.82	5.98			

Storage temperatures : 0°C and -10°C

Hypothesis : Storage temperatures has effect on protein solubility changes

Assumption : d_i, d_2 .... d_n constitute a random samples from $n(\mu d, \sigma^2 d)$ populations

$$\bar{d} = \frac{\sum_1^n di}{} = 3.69$$

$$\sum_1^n (di - \bar{d})^2 = 63.72$$

$n-1 = 8$

$$\text{The test statistics 't'} = \frac{\bar{d}}{sd/\sqrt{n}} = 3.93$$

$$s^2 d = \frac{\sum_1^n (di-\bar{d})^2}{n-1} = 7.97$$

sd = 2.82

Conclusion: The observed value 't' is greater than the tabulated 't' at 1% level of significance at (n-1)=8 degree of freedom, the hypothesis is accepted.

Table-15.2 : Statistical 't' test

Species: Rastrelligerkanagurta

Protein solubility x_1i (0°C)	Protein solubility x_2i (-10°C)	Differences $d_i=(x_1i-x_2i)$	n	Observed value 't'	Tabulated 't' at 1% level of significance
11.60	13.40	- 1.8			
25.0	17.90	7.1	n=11	4.22	
23.20	23.20	0			3.169
21.43	21.43	0			
27.80	16.07	11.73			
32.14	24.10	8.04			
28.60	17.90	10.7			
23.20	16.10	7.1			
24.46	17.0	7.46			
39.29	32.14	7.15			
58.10	32.10	26.0			

Storage temperatures: 0°C and -10°C

Hypothesis : Storage temperatures has effect on protein solubility changes.

Assumption : $d_1, d_2 \ldots d_n$ constitute a random samples from $n(\mu d, \sigma^2 d)$ populations.

$$\bar{d} = \frac{\sum_1^n d_i}{n} = 9.28$$

$$\sum_1^n (d_i - \bar{d})^2 = 534.67$$

$n-1 = 10$

The test statistics $'t' = \frac{\bar{d}}{sd/\sqrt{n}} = 4.22$

$$s^2 d = \frac{\sum_1^n (d_i - \bar{d})^2}{n-1} = 53.47$$

$sd = 7.31$

Conclusion: The observed value 't' is greater than the tabulated 't' at 1% level of significance at (n-1)=10 degree of freedom, the hypothesis is accepted.

Table-15.3 : Statistical 't' test

Species: *Catla catla*

Protein solubility x_1i (0°C)	Protein solubility x_2i (-10°C)	Differences $d_i=(x_1i-x_2i)$	n	Observed value 't'	Tabulated 't' at 1% level of significance
4.60	1.61	2.99			
4.10	1.96	2.14			
5.0	4.46	0.54	n=12	4.49	3.106
17.86	3.39	14.47			
16.07	11.69	4.38			
23.20	11.61	11.59			
32.10	9.82	22.28			
42.86	11.61	31.25			
33.03	9.11	23.92			
39.3	11.61	27.69			
39.28	12.5	26.78			
35.0	12.61	22.39			

Storage temperatures: 0°C and -10°C

Hypothesis: Storage temperatures has effect on protein solubility changes.

Assumption: d_1, d_2 .... d_n constitute a random sample from $n(\mu d, \sigma^2 d)$ populations.

$$\bar{d} = \frac{\sum_{1}^{n} d_i}{n} = 15.87$$

$$\sum_{1}^{n} (d_i - \bar{d})^2 = 1640.24$$

$$n - 1 = 11$$

The test statistics 't' $= \frac{\bar{d}}{sd/\sqrt{n}} = 4.49$

$$s^2 d = \frac{\sum_{1}^{n} (d_i - \bar{d})^2}{n - 1} = 149.11, \quad sd = 12.21$$

Conclusion: The observed value 't' is greater than the tabulated 't' at 1% level of significance at (n-1)=11 degree of freedom, the hypothesis is accepted.

TRIMETHYLE AMINE FOR MEASUREMENT OF FISH ACCEPTABILITY DURING STORAGE

The values of trimethyle amine gradually increase during the storage period. The highest log values of trimethyle amine 1.60 mg.N/100 gm of fish flesh stored at 0°C and 1.46 mg.N/100 gm of fish flesh stored at -10°C for 39 days of storage period were observed (Table-1 and Fig. 23). The initial values were 1.5 mg.N/100 gm of fish flesh at 0°C and 1.28 mg.N/100 gm of fish flesh at -10°C of storage. The total increase of trimethyle amine value during the same storage period was log 0.1 mg.N/100 gm of fish flesh at 0°C of storage Similarly, the total increase of trimethyle amine value at -10°C for the same storage period was log 0.18 mg.N/100 gm of fish flesh. The changes in TMA value depends on the storage temperature, length of storage period and also on the species. It is observed from the Table-1, 3, 4 and Figs. 23, 24 and 26 that higher the storage period higher the TMA values, lesser the

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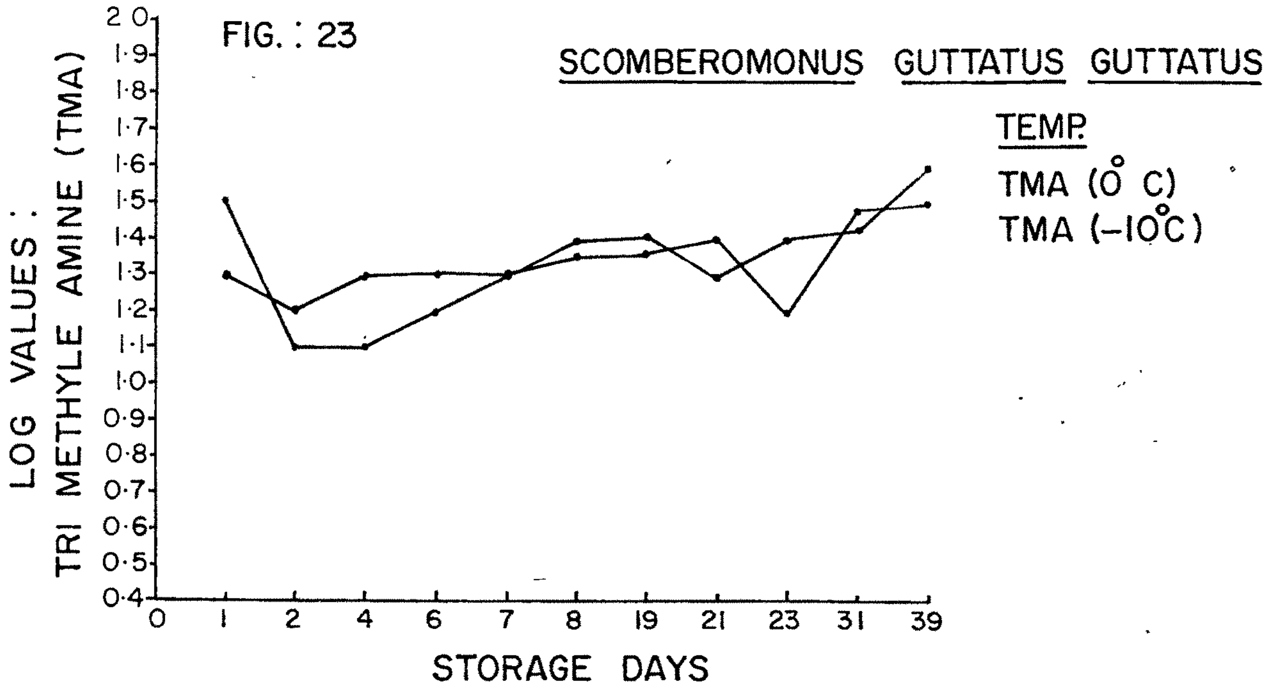
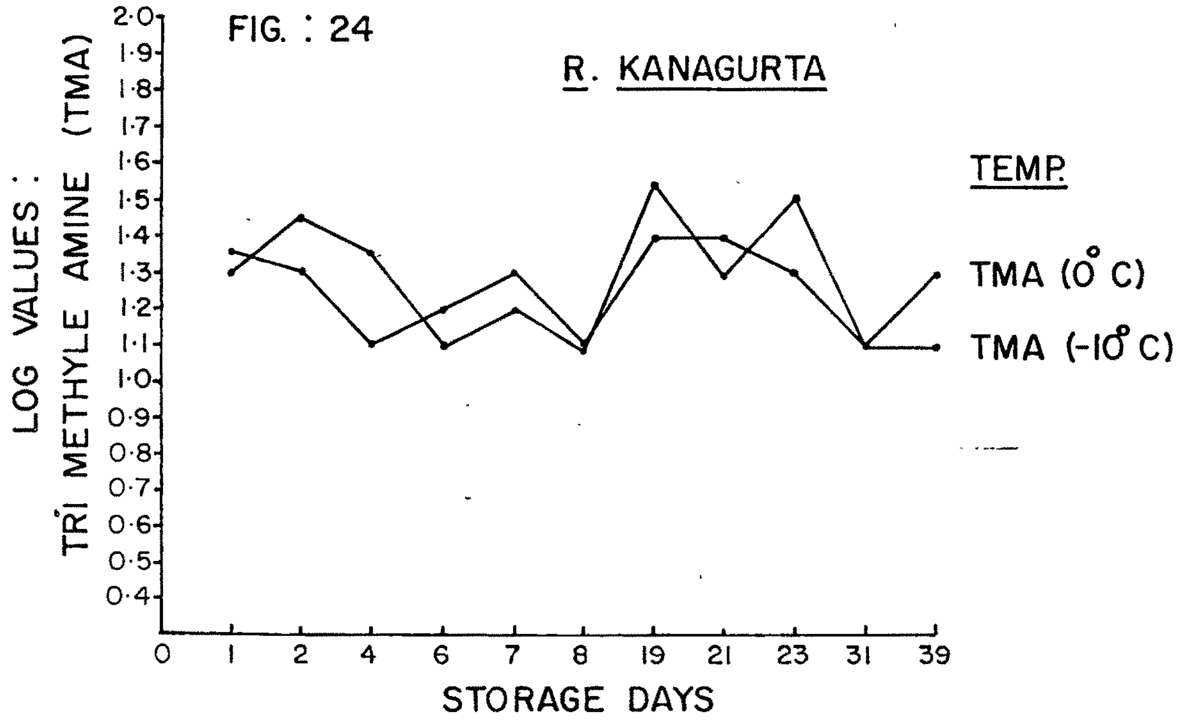
storage period lower the TMA production. Increase in TMA values did not show the same pattern. Sometime value showed fluctuations and the rate of increase was very less. In marine fish species the TMA production and pattern of increase was significant but in fresh water fish the rate of increase was very slight or negligeble. The highest and lowest TMA values were 1.35 and 1.33 mg.N/100 gm of fish flesh at 0°C and 1.33 and 1.18 mg.N/100 gm of fish flesh at -10°C found in _Rastrelliger kanagurta_ fish species at initial to 39 days of storage period. In comparison the TMA production between two different storage temperature 0°C and -10°C for the same storage days were 0.15 mg.N/100 gm of fish flesh. The -10°C temperature samples contained less amount of TMA upto the end of storage period (1.18 mg.N/100 gm of fish flesh). There were no regular pattern of increase of TMA production between the initial and final storage sample (Table-3 and Fig. 24). The results of TMA production in _Catla catla_ fish are presented in Table-4 and Fig. 26 from which it is observed that there was no regular increase of TMA production in _Catla catla_ fish during storage. The values showed fluctuations in samples both from the storage temperatures. At the end of storage period at 43 days of storage TMA value 1.34 mg.N/100 gm of fish flesh were found in 0°C storage samples and correspondingly 1.23 mg.N/100 gm of fish flesh were found in -10°C tempera-





FIGS. : 23 & 24, CHANGES OF TRI METHYLE AMINE OF SCOMBEROMONUS GUTTATUS GUTTATUS & RASTRELLIGER KANAGURTA DURING STORAGE AT 0° C & -10° C TEMPERATURES

Table-1 : Changes in protein solubility and trimethyle amine contents of *scomberomorus guttatus gattatus* during storage.

Species scientific name	Storage period (days)	Protein solubility (mg/gm of fish flesh)	Loss of protein solubility (from % of initial value)	Protein solubility (mg/gm of fish flesh)	Loss of protein solubility (from % of initial value)	Trimethyle amine (TMA) (mg.N/100 gm of fish muscle)	Trimethyle amine (TMA) (mg.N/100 gm of fish muscle)
		0°C	0°C	-10°C	-10°C	Log value (0°C)	Log value (-10°C)
Scomberomorus	1	17.68	100	19.64	100	1.5	1.28
guttatus	2	30.40	58.16	35.70	55.01	1.11	1.24
guttatus	4	30.36	58.24	35.72	54.98	1.11	1.27
	6	28.6	61.82	33.04	59.43	1.23	1.30
	7	33.9	52.15	22.32	87.99	1.28	1.31
	8	28.6	61.82	34.80	56.44	1.41	1.38
	19	29.5	59.93	39.50	49.72	1.43	1.33
	21	22.32	79.21	25.89	75.86	1.30	1.37
	23	30.70	57.60	28.60	68.64	1.40	1.24
	31	39.30	44.99	37.50	52.37	1.49	1.47
	39	39.46	44.80	25.00	68.72	1.60	1.46

tures of storage for the same storage period. Variation in TMA values were also observed within the storage temperatures both 0°C and -10°C respectively (Table-4 & Fig. 26). TMA is a reliable indicator for measurement of freshness of fish species, specially marine fishes during low temperature of storage. These are various causes for the formation of TMA in fish muscles during storage.

Trimethyle amine is higher in the muscle of marine fish but it is normally absent or present only in very small amount in fresh water fish (Shewan, 1951; Simidu, 1961; Yamada, 1969). During storage in low temperature the oxide is reduced to trimethyle amine by naturally occuring bacteria on the skin of the fish or in the viscera which invade the flesh after death. Quite a few number of publications based on the non-specific methods of analyses are available on the relationship between volatile bases and the quality of fish but comparatively little quantitative information is available on concentration of DMA in fish and fish products. There is evidence to suggest that the effect of mincing tissue is to accelerate reactions due to both bacteria and natural enzymes of the flesh and other organs (Babbit et al., 1972). There are significant difference in the rates of production of TMA from one species to another. The initial level of TMA is very little and then it relatively increases. Mackie and Thomson (1974) reported that TMA reached level 20 mg.N/100 gm of fish flesh for cod and 12 mg.N/100 gm of fish flesh for haddock kept in low temperature for 15 days. There was increase in TMA during storage of marine fish. The TMA values did not show any significant rise during storage of fresh water fish. The degradation of TMAO during low temperature storage is still mainly due to TMA. Determining spoilage by TMA has been recommended by some; others have claimed it is of little value.

As with the volatile basic nitrogen studies. The conflicting situation for TMA is partly the result of the use of different species of fish with varying composition and of the employment of different storage temperature and conditions (Borgstrom, 1965). TMA determination could be enhanced by determining its increase in fish after an incubation period. There was a variation in levels of TMA between species or the range of values were so wide and overlapping as to preclude setting up definite standards, (Good and Stern, 1955). Trimethyle amine oxide is found in considerable quantities in marine fish. After death, the alteration in the fish tissues are accompanied by a considerable increase in the di- and trimethyle amine formed as a consequences of the reduction of oxide into free bases. The free amino nitrogen varies with the season and storage conditions. The free basic amino acids which increased after death (glutamic acid, methythiotidine) and those which diminish in relative quality (Lysine, Leucine) while third group (Glycine) remains constant (Hodgkiss and Jones, 1955). Trimethyl amine value might be used as an objective measurement of quality of stored fishes. TMA log values between 0 and 2 indicate fish judged by these men to be of prime quality. TMA log values 1 and 4 indicates fish that are spoiling. When the TMA log values exceeded 3-4 the fish have reached a condition that is considered unfit for processing. At the initial storage period approximately of a week there is a slow consistent of increase in the TMA values. This is followed by an accelerated increased during next week and then almost linear increase during which TMA is formed very rapidly. This reveals that the TMA values of the fish muscle have a very significant correlation with the length of storage period (Castell and Greenough, 1958). It would appear from the results that TMA values may be used with certain limitation as an objective measure of spoilage.

The spoilage rate of fishes at a particular temperature is not uniform. The temperature effect may vary not only from one species to another but also in different spoilage test. Spoilage of fish during storage at a low temperature is a highly complex process. Very low TMA indicates very good condition of fish. The onset of spoilage during storage after a week was substantiated by an increase in TMA content to a level 4 mg.N/100 gm of fish flesh (log value) followed by a rapid rise to above 4 mg.N/100 gm of fish flesh (log value) at the end of the storage period.

The variation of TMA is due to the intrinsic biological factors associated with the fish, such as the variation in chemical constituents TMAO etc.(Love et al., 1959). Other causes are sexual cycle of the fish, food, fishing ground, together with the post-harvest conditions, particularly method of handling and temperature. The bacterial population causing spoilage is itself in a dynamic state, altering during spoilage, not only in total numbers but also in the population of the various types (Shewan et al., 1960). The slower rate of production of TMA which lead to the longer shelf life of the fish is due to retardation of bacterial and enzymatic activity at lower temperature of storage.

The effect of temperature enhance the rate of spoilage during storage, distribution and transportation. For measurement of shelf life (freshness) of fish a relative rate of spoilage chart has been prepared from 1 to 25°C temperature for definite period of storage days, on the basis of trimethyle amine content at 0°C of storage temperature. From the approximate TMA log value one can easily know the freshness of fishes. A limit of acceptable line was also drawn for the acceptable limit of the particular fish species (Table 19).

CHANGES OF TOTAL VOLATILE NITROGEN IN STORED LABEO ROHITA FISH

The log value of total volatile nitrogen at two different storage temperatures (0°C and -10°C) for 17 days of storage is presented in table-2 and Fig. 22. The total volatile nitrogen value gradually increased throughout the storage period. At 0°C storage temperature the initial TVN value was 1.21 mg.N/100 gm and final value was 2.41 mg.N/100 gm from 0 to 17 days of storage period. The rate of increase was very slow, the fish samples were acceptable upto each of storage period at 0°C temperature.

Similar pattern of log TVN values were also observed in -10°C storage samples. Here the initial TVN value was 0.99 mg.N/100 gm and final TVN value was 2.22 mg.N/100 gm from 0 to 17 days of storage.

Lower the storage temperature, lower is the TVN production. The storage temperature has a significant effect on the TVN production. Many workers have suggested that 30-40 mg.N/100 gm of fish flesh is the upper limit of acceptability during storage at low temperature. In the present findings the TVN values were always below the acceptable limit. Thus all the fish samples were in acceptable condition. Comparatively -10°C fish samples were better in quality (Fig. 22 and Table-2).

The present findings *Labeo rohita* fish agreed with the relevant literature that the muscle deterioration was delayed by lowering the temperature and the high temperature caused much more shrinkage (Burt *et al.* 1969). The results indicate that rapid extensive stiffening on chilling is not accompanied by muscle shrinkage. After post-rigor, low temperature has relatively little effect on the shelf life or still allows a sufficiently long storage period.

The TVN values showed a gradual increase during the period of storage, they remained well within the limit of acceptability. The rate of increase of TVN values at -10°C of storage was negligible. Paladino (1945) reported that volatile N - containing bases were suitable indicator of the degree of spoilage. One hundred mg. of volatile basic nitrogen per 100 gm samples of fish muscle was the critical point. When the values exceeded the fish in question were not acceptable as human food. Fish enzymes are retarded

Table-18: Statistical 't' test (Fish-salt ratio)

TVN log values x_1i(1:2) fish:salt	TVN log values x_2i(1:1) fish:salt	Differences $d_i(x_1i-x_2i)$	n=	Observed value 't'	Tabulated 't' at 1% level of significance
1.26	1.28	- 0.02			
1.09	1.65	- 0.56			
1.43	1.27	0.16	n=11	=0.32	
1.20	1.25	- 0.05			
1.40	1.36	0.04			
1.18	1.15	0.03			=3.169
1.42	1.38	0.04			
1.40	1.44	- 0.04			
1.55	1.44	0.11			
1.44	1.42	0.02			
1.43	1.51	- 0.08			
		$\bar{d}$=0.02			

Species: Hilsa ilisha

Fish-salt ratio (1:2 and 1:1), kept at room temperature ± 32°C

Hypothesis: Higher proportion of salt had a significant effect on the changes of rate of spoilage during storage at room temperature.

Assumption: $d_1, d_2 \ldots d_n$ constitute a random samples from $n(\mu d_i, \sigma^2 d_i)$ populations.

$$\bar{d} = \frac{\sum_1^n d_i}{n} = 0.02$$

$$\sum_1^n (d_i - \bar{d})^2 = 0.4222$$

$$n - 1 = 10$$

$$\text{The test statistics 't'} = \frac{\bar{d}}{sd/\sqrt{n}} = 0.32$$

$$s^2 d = \sum_1^n (d_i - \bar{d})^2 = 0.04222$$

$$sd = 0.205475$$

Conclusion: The observed value of 't' is less than the tabulated values, (3.169) at 1% level of significant at (n-1)=10 degree of freedom, the hypothesis is accepted therefore, it may be concluded from the findings that higher proportion of salt to fish, the rate of spoilage was slower during storage at ordinary room temp. without proper packaging materials.

in their activity as soon as temperature decreases to 20°C. The catalase activity of the gills also runs parallel to the degree of spoilage (Tomiyama et al., 1950). Upto an amount of 30 mg.N % volatile basic nitrogen in the flesh, the catalase activity remained almost constant.

Not only bacteria and their metabolic products are responsible for spoilage, but that the enzymes of fish muscle and intestine are also involved in the spoilage. Muscle enzymes are particularly active in the initial phases. After capture of fish the biochemical changes takes place. In storing the whole fish as is commonly done with fresh water fish (Labeo rohita) the intestinal enzymes may invade the muscle tissue, thus causing spoilage. Bacterial enzymes may exert a certain influence, but not only bacteria are responsible for the spoilage of fresh water fish. Intrinsic enzyme may participate too. The presence of certain fish muscle enzyme appears to be a pre-requisite for an optional growth of bacteria which causes spoilage.

For measurement of fish spoilage, like Labeo rohita, total volatile nitrogen is a good indicator. From the TVN results fish acceptability was easily calculated. The total volatile basic nitrogen content increased during storage of oysters and of white meat fish. Increase of the total volatile nitrogen content with fish spoilage was also found (Shewan, 1942; Riemann, 1952; Pierangeli et al., 1954). The content of ammonia or volatile bases increased appreciably during fish spoilage but if carbohydrates were present, protein decomposition with ammonia formation was depressed (Simidu and Hibiki, 1957). Spoilage of fresh water fish at temperature of 80-90°F the content of volatile base showed a rough correlation with the freshness but at 30-40°F on correlation was found between other freshness parameters.

LIMIT OF ACCEPTABILITY OF *LABEO ROHITA*

The *Labeo rohita* fish species were biochemically acceptable throughout the storage period. At -10°C storage samples were highly acceptable upto the end of storage period and 0°C storage samples were acceptable at the end of storage period of 17 days. In both the cases the TVN values were below the acceptable limit. Wierzhchowski (1956) suggested 30 to 40 mg.N/100 gm as the upper limit for fresh water fish and 60 mg.N/100 gm as the limit of marine fish. Sato (1960) found a consistent difference between bottom fish and surface fish in case of total volatile nitrogen. The present findings have some similarities with the findings of above workers. Fellers et al., (1957) recommended that the ratio of volatile basic nitrogen to the total nitrogen as a useful index of the quality of fish. It may be concluded from the results (Table-2 and Fig. 22) that <u>Labeo rohita</u> fish samples were acceptable through out the storage period of 17 days at 0°C and -10°C for storage temperatures.

CONTROL OF FISH SPOILAGE USING COMMON SALT

TOTAL VOLATILE NITROGEN

The log values of total volatile nitrogen (TVN) gradually increases upto the end of storage period. The rise of volatile nitrogen values were very slow in higher proportion of salt-fish ratio as compared to lower proportion and in control samples. The results of volatile nitrogen values are expressed in terms of log values and presented in figs. 35 and 36 respectively. In control samples (without salt treatment) the rise was from 1.17 mg.N/100 gm to 1.69 mg.N/100 gm at 0 to 8 days of storage period at room temperature ± 32°C. At the beginning of storage period, the increase of TVN was rapid which then gradually declined. The control samples were unacceptable after 2 days of storage (log TVN values 1.66 mg.N/100 gm). After 2 days of storage at room temperature (± 32°C) the fish deterioration was rapid.

FISH-SALT RATIO (1:1)

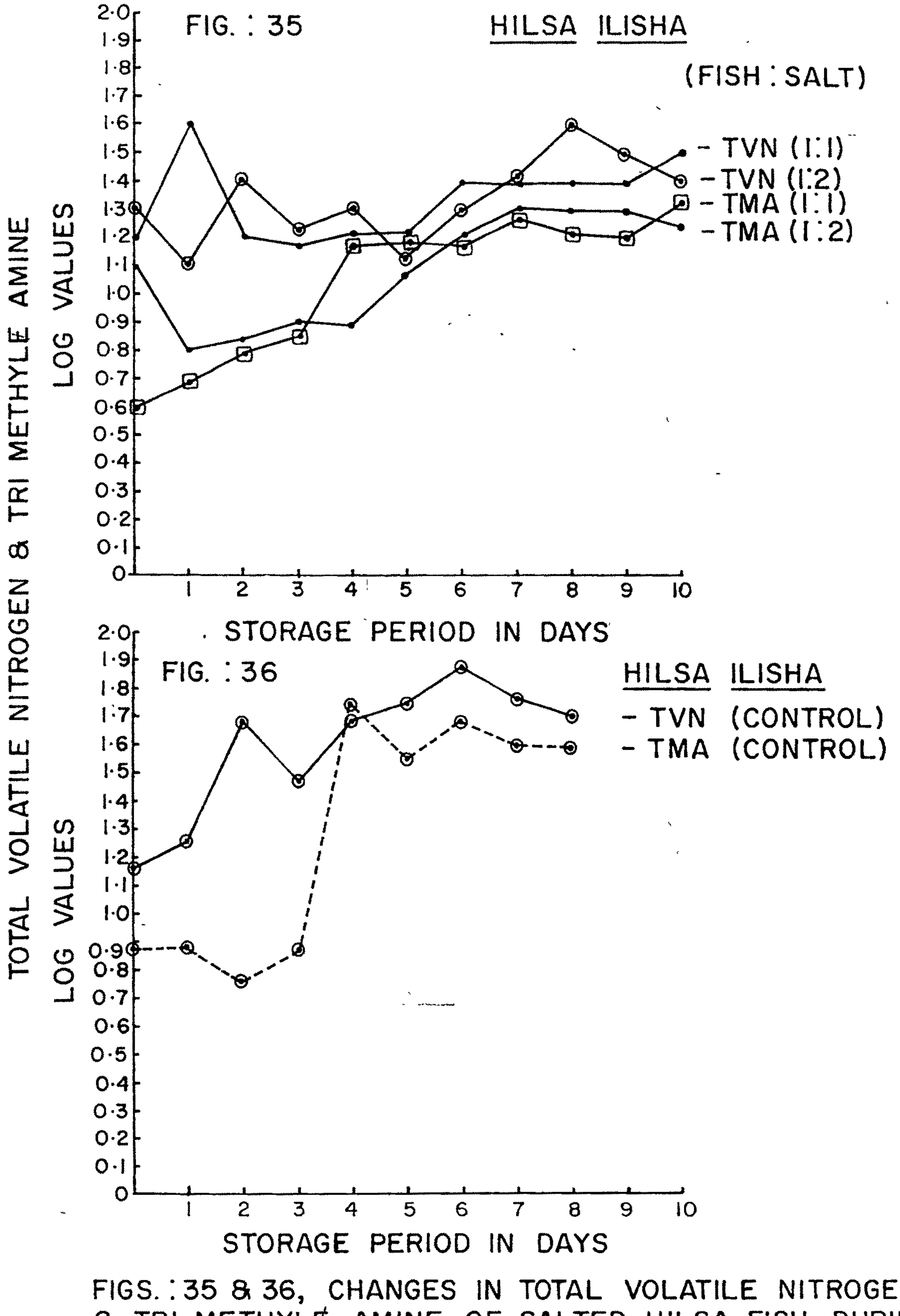
The initial TVN values was 1.28 mg.N/100 gm and final TVN value was 1.51 mg.N/100 gm of fish flesh at 0 and 10 days of storage in fish-salt ratio 1:1. The results are reported in the table and fig. After one week of storage the fish samples were moderately acceptable. At the end of storage period the log TVN values were 1.51 mg.N/100 gm of fish muscles. The fish samples were acceptable on the basis of TVN formation by using 1:1 fish-salt ratio upto the end of storage period (Figs. 35 and 36).

FISH-SALT RATIO (1:2)

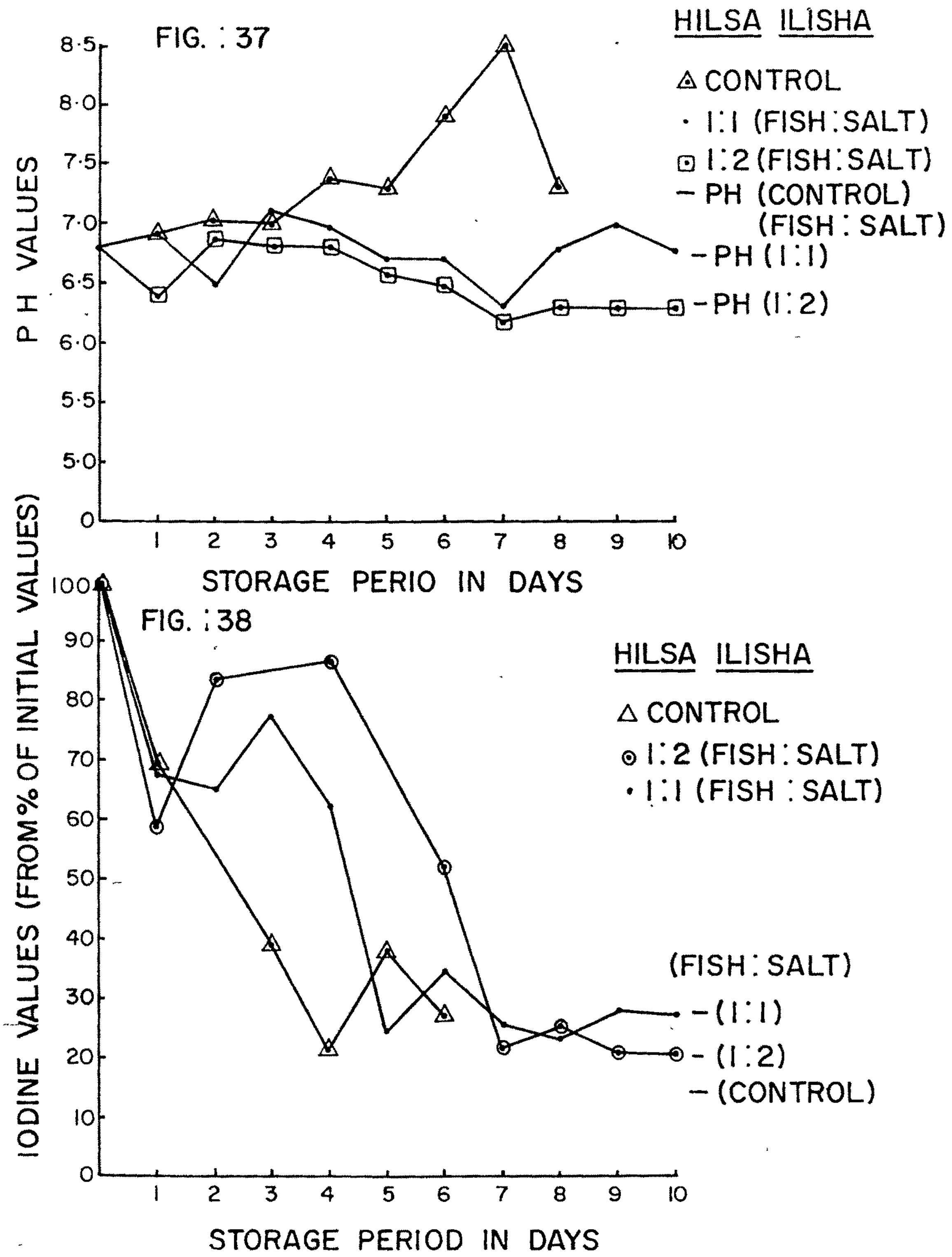
The fish samples were highly acceptable throughout the storage period at 1:2 fish-salt ratio. But slight discolouration was observed probably due to excessive salt. Fish samples became hard and slight reddish colour developed just anterior to the tail portions. Overall the TVN value increased very slowly from 1.26 mg.N/100 gm to 1.43 mg.N/100 gm of fish flesh. The rise was very negligeble as compared to 1:1 fish-salt ratio and in control samples.

COMPARATIVE TRIMETHYLE AMINE VALUES

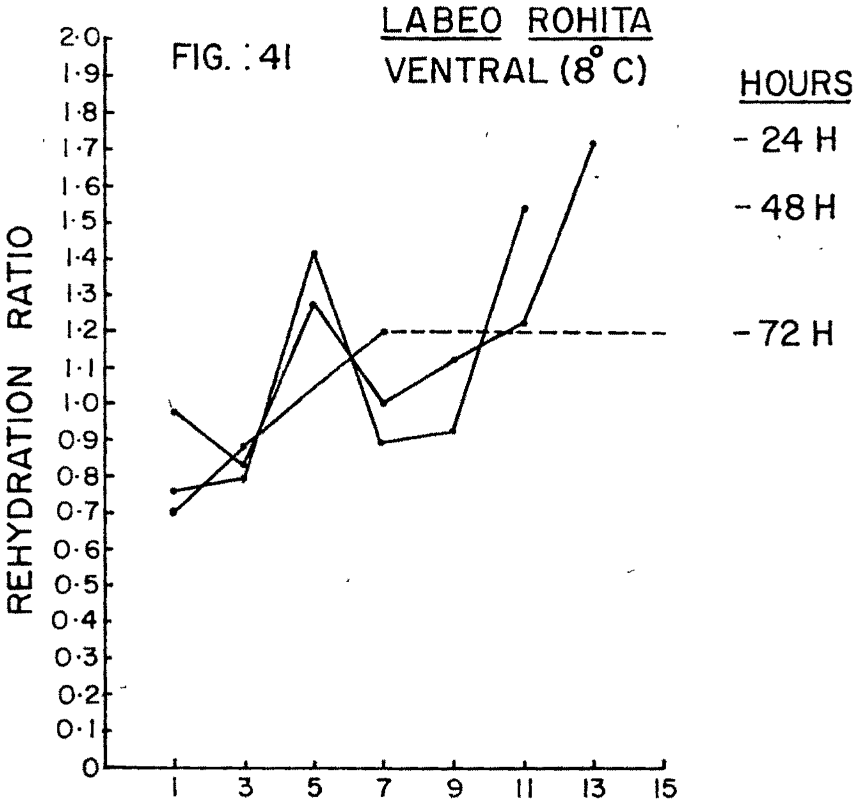
The increase of TMA values are presented in

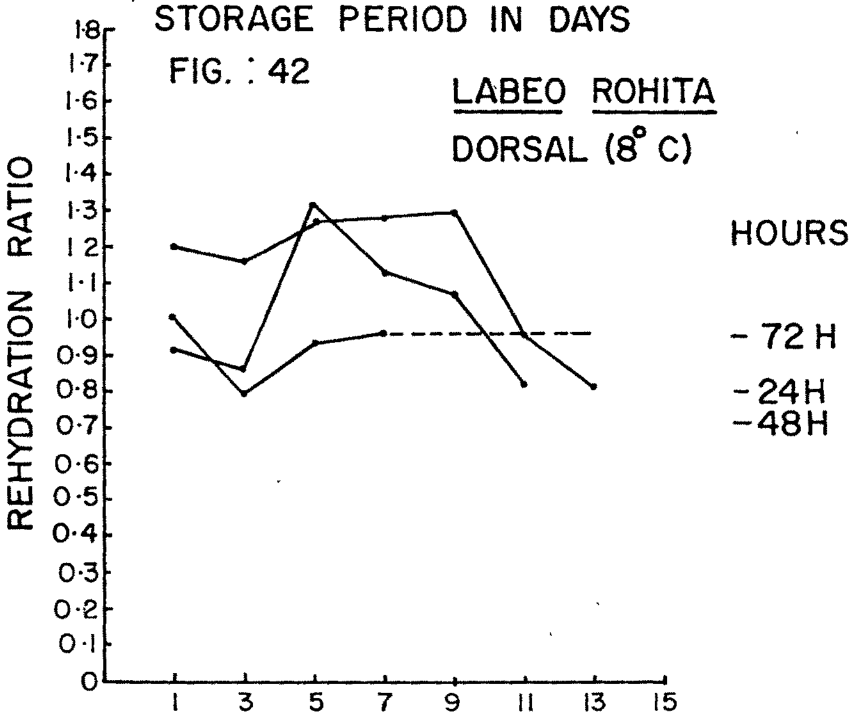


FIGS. : 35 & 36, CHANGES IN TOTAL VOLATILE NITROGEN & TRI METHYLE AMINE OF SALTED HILSA FISH, DURING STORAGE AT ROOM TEMPERATURE



FIGS : 37 & 38, CHANGES IN PH VALUES & IODINE VALUES OF SALTED HILSA FISH DURING STORAGE AT ROOM TEMPERATURES





FIGS. 41 & 42, REHYDRATION RATIO OF DORSAL & VENTRAL 8° C STORAGE LABEO ROHITA MUSCLES AT DIFFERENT SOAKING HOURS

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figs. 35 and 36. The increase of TMA value was from 0.87 to 1.60 mg.N/100 gm in control samples. Similarly, 0.69 to 1.36 mg.N/100 gm in 1:1 fish-salt ratio and 1.12 to 1.28 mg.N/100 gm of fish flesh in 1:2 fish-salt ratio respectively. The fluctuation of TMA values were observed in all the samples. The pattern of rise of TMA values in 1:1 and 1:2 fish-salt ratio were more or less similar. There was a significant difference in TMA values of hilsa fish species in control samples as compared to fish-salt ratio samples. Salt retard the TMA formation during storage by controlling the bacterial and autolytic (enzymatic) spoilage. Upto four days of storage the formation of TMA was very less, after remaining period of storage, the TMA formation was very high in all the fish samples. The TMA formation were very less in 1:2 fish-salt ratio. All the salt treated samples were acceptable at the end of 0 to 10 days of storage period (Figs. 35 and 36).

SHELF-LIFE OF THE SALTED PROCESSED PRODUCT

Different proportion of common salt is used in order to extend the keeping quality of the fish for marketing. Salt has been used as a method of long-term preservation of fish. Salt slows down the spoilage of the fish and the different proportion of salt will have an effect on the shelf-life of the fish. During preservation the shelf-life of the fish depends on many factors like storage temperature, storage time, species of the fish as well as various other condition influencing the action of the micro-organisms (Burgess et al., 1965; Shewan, 1951).

With the increase of storage period, the TVN values of the fish species increases. The overall findings of the present investigations suggests that the shelf-life of the fish could be extended by keeping the fish in salt which has got preservative action. Higher proportion of salt extended the shelf-life of the fatty fishes like hilsa.

The excessive softening of the fish texture is almost certainly due to enzymatic action. Digestive enzymes hydrolyse protein, destroying the muscle structure and allowing nutrients to be leached into the brine. Removal of the gut tissue which has particularly active digestive enzymes inhibits enzymatic spoilage. The level of enzymatic activity at a given temperature will vary from species to species, depending on the normal environment temperature in which the fish is found. The dressing procedure used seemed to have a significant effect on the extension of shelf-life of salted fish.

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VARIATION OF IODINE VALUE

The variation of iodine value in salted and unsalted hilsa fish samples are shown in fig. 38. The iodine values are calculated from percentage of initial values. The effect of salt proportion on the degradative changes of fat are measured by estimation of iodine values during storage at ordinary room temperature of fatty fishes like Hilsa ilisha. The iodine value gradually decreased during the storage period in both salted and unsalted fish samples. In control samples the total percentage (from percentage of original values) of decrease was 73.22% in 6 days of storage at room temperature.

IODINE VALUE AT 1:1 FISH-SALT RATIO

The changes of iodine value (from percentage of original value) was from 100 to 25.89% at 0 to 10 days of storage at room temperature (± 32°C). From 0 to 4 days the changes were very slow, after which the pattern of changes was very significant (from 52.39 to 25.89%).

IODINE VALUE IN 1:2 FISH-SALT RATIO

The iodine value gradually decreases from percentage of the initial value. The percentage of total decrease was 75.01% at the end of storage period. A

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regular pattern of decrease of iodine value was observed in 1:2 fish-salt ratio. From 0 to 4 days of storage the rate of decrease were very slow and after four days of storage the rate of decreases were very high. A marked difference of iodine value was observed in 0 to 4 days and 5 to 10 days of storage samples (Fig. 38). The different proportion of salt had a significant effect on the iodine value during storage. Iodine values were low in higher proportion of salt.

A statistical 't' test was carried out on the basis of the hypothesis that higher proportion of salt had a significant effect on the changes of the rate of spoilage (biochemical changes of raw fish samples) during storage at room temperature. From the statistical 't' test, it may be concluded that biochemical changes were slower in higher proportion of fish-salt ratio (1:2) as compared to 1:2 fish-salt ratio and in control samples (Table-18).

Iodine values varies according to the different region of body from which the fat was obtained (Pearson, 1962).

Even within a single muscle there may be systematic difference in fat composition and constituents. As the percentage of fatty tissue in an animal increases, the percentage of intramuscular fat also tends to increase. Moreover, on a high plane of nutrition a greater proportion of fat is synthesized from carbohydrate and such fat has consequently a lower iodine number. A high plane of nutrition increases the percentage of intramuscular fat and decreases the percentage of moisture. The moisture content of muscle may also be influenced by the nature of the diet.

The iodine value, showing regular drop during storage indicates a rapid degradation of fat. Storage temperature affects the rate of changes of iodine value during storage. In all the fish-salt ratio samples including control samples iodine value declined slowly. Fat degradation of fatty species becomes a predominent factor over the other deteriorative changes (Mus lemuddin, et al., 1984).

Iodine value of the lipid decreased from an initial value during storage period. An approximate estimate of the loss of polyunsaturated fatty acids, based on the iodine values (Ackman, 1966) showed that there was a loss of iodine value. This is of high significance from nutritional point of view. Delay in storage will adversely affect the shelf-life of the product. The significant point was the sharp fall in shelf-life for the samples which were stored in salt media for more than a week. The apparent lower level of free fatty acid production in the samples observed during this study may be due to the irreversible binding of the free fatty acids with the protein or due to oxidative degradation caused by storage. Any further delay in storage will reduce the shelf-life drastically. Varela and Wojeiech (1956) found that there was an iodine uptake by hake muscles during spoilage but the amount depended on the physical state of the fish. Iodine value varied with the species of the fish during spoilage. Lujipen (1954b) reported that in salted herring fat showed no direct relation to the storage period of temperature. The samples showed higher percentage of iodine value at the end of the storage period (26.78%) at that time fish sample were unacceptable by volatile nitrogen values. The (1:1) fish-salt ratio samples were moderately acceptable upto the end of storage period, the corresponding iodine value was 25.87% (from % of original value). Higher proportion of salted (1:2) were highly acceptable by measuring the TVN and TMA values. From figs. 37 and 38, it may be suggested that fish stored at room temperature ± 32°C the higher proportion of salt would be suitable for preservation of fatty species like hilsa but due to addition of excessive salt slight discolouration was observed and alters the physical appearence of the product. On the basis of the present findings fish-salt (1:2) would be better if fish is preserved after dressing. It reduces alteration in the biochemical quality. Such samples were acceptable in bio-chemical tests after 10 days of storage without any protective packaging materials and at ordinary room temperature.

VARIATION OF pH VALUES OF SALTED SAMPLES

The variation of pH values in salted and unsalted fish samples are shown in Fig. 37. Highest pH values were observed in control samples and lowest pH values were observed in samples at 1:1 (fish:salt) ratio. Fluctuation of pH values was observed in samples at 1:2 fish-salt ratio during the same storage period. The pH values depend on sampling procedures and spoilage pattern of the particular portion of the fish samples. The high pH has been related to the breakdown of tissues (Love et al., 1969). A relatively higher pH and percentage of protein isolubility indicated that more denaturation had taken place which resulted in a softer product. The initial fall of pH during the first week of storage is quite marked in most of the samples. The glycogen store would be depleted at the end of the storage period. An explanation for the rise in pH during salting storage and its subsequent fall is perhaps easier to find if the reaction TMAO→ FA+DMA is considered. The production of DMA will lead to a pH rises since it is a basic compound. However, there

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will be a finite amount of TMAO which can decomposed and if we consider that DMA may diffuse from the tissue, at some point the loss of DMA will exceed its production and hence the observed pH will fall. Similarly, the formation of TMA from TMAO during the extended storage period may account for the differences in pH between the samples. There are probably many other metabolities present which as a result of bacterial spoilage are affecting the pH. Washing out these basic material would lead to the initial pH being lowered. However, the subsequent pH profiles would be very similar to the unwashed samples. Love et al., (1968) attributes softness in flesh to high pH, polyphosphates and long periods in salting and temperature which breaks myofibrils at Z - band. Fish in poor condition might be expected to become less tough because of high pH.

Borgstrom (1965) reported that pH has little or no significance as a reliable index of the state of freshness of the sample. pH showed no significant correlation with the onset of spoilage in fresh fish. The chemical methods proposed to date for quality evaluation depend mostly on the presence of one or more products of degradation of the muscle constituents. pH increases in fish parallel to the increase in the content of volatile oxidizable substances. pH varies initially and it showed no real or significant

correlation with the onset of spoilage in fresh fish and salted fish. Dyer et al, (1944) suggested that pH of the surface could be used as a rapid and sample that for the degree of freshness. The usefulness of pH determination is often greatly restricted or vitiated by its variability from sample to sample and by its cyclic fluctuations during the storage period. The pH at initial stage of storage is higher around 7.0 giving rise to a condition referred to as alkaline rigor. Salt retard the spoilage and ultimately higher proportion of salted fish were found to be better in quality by observing the lower pH values (Fig. 37)
Overall pH values were lower in less spoiled as well as salted fish samples.

BIOCHEMICAL PROPERTY CHANGES IN FISH MUSCLE ON FREEZING & DRYING

VARIATIONS IN REHYDRATION RATIO AND PERCENTAGE OF DORSAL AND VENTRAL MUSCLES OF LABEO ROHITA ON SOAKING AFTER STORAGE AT 8°C AND DRYING (FIGURES: 41, 42, 43, 44 AND 45)

It was observed from the above figures and table that rehydration percentages depend on the length of soaking period and different portion of fish. Higher the length of soaking period higher is the percentage of water gained. It was also observed that the dorsal portion of fishes gained higher percentage of water as compared to the ventral portion.

The value of the rate of differences of rehydration ratio between ventral and dorsal muscles has been shown in Figs. 41, 42 & 45. It was observed that as the length of soaking tissue was increased the value of rehydration ratio also increased. Values for all the samples could not be recorded as few samples disintegrated into pieces during soaking. Main causes for the variation was that the ventral portion contained this water and dried more as compared to the dorsal portion. Overall, the rate of rehydration ratio values were more in ventral muscles as compared to the dorsal muscle.

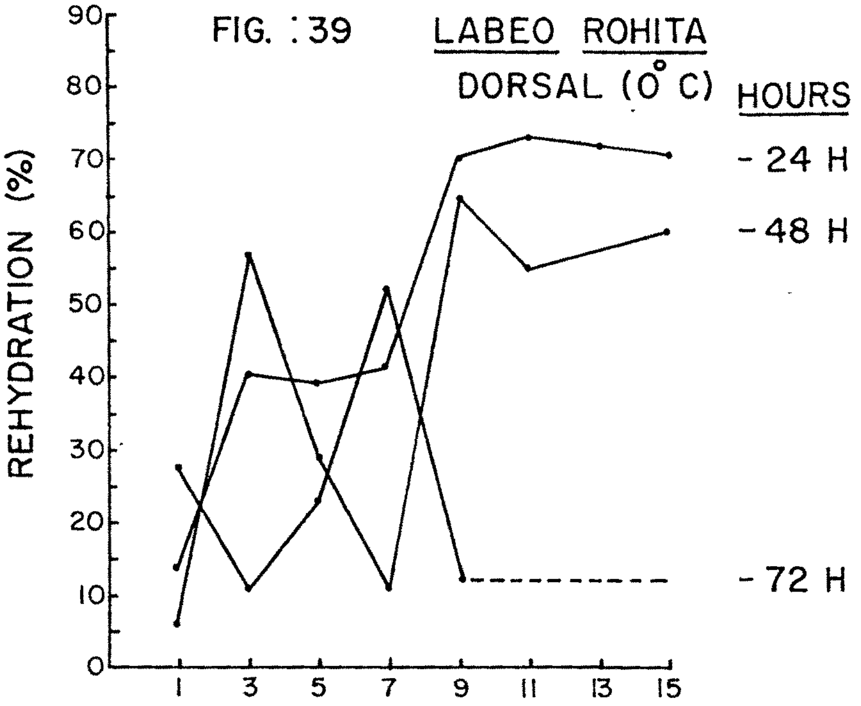
CHANGES IN REHYDRATION RATIO & PERCENTAGE OF DORSAL & VENTRAL MUSCLES OF <u>LABEO ROHITA</u> ON SOAKING AFTER STORAGE AT 8°C AND DRYING (FIGS. 39, 40 AND 46)

The rate of variation of rehydration percentage between dorsal and ventral muscles has been shown in fig, (at 24 hours of soaking period). It may be said that from the average results the rehydration ratio value decreased from the initial values. Decrease was more in dorsal muscle as compared to the ventral muscles. It was also observed that length of storage of individual samples at 0°C had effect on water intake. The variation of water intake depends on storage temperature, length of storage before rehydration, types of muscle, composition of the particular muscle, length of soaking period and original moisture content of the raw material.

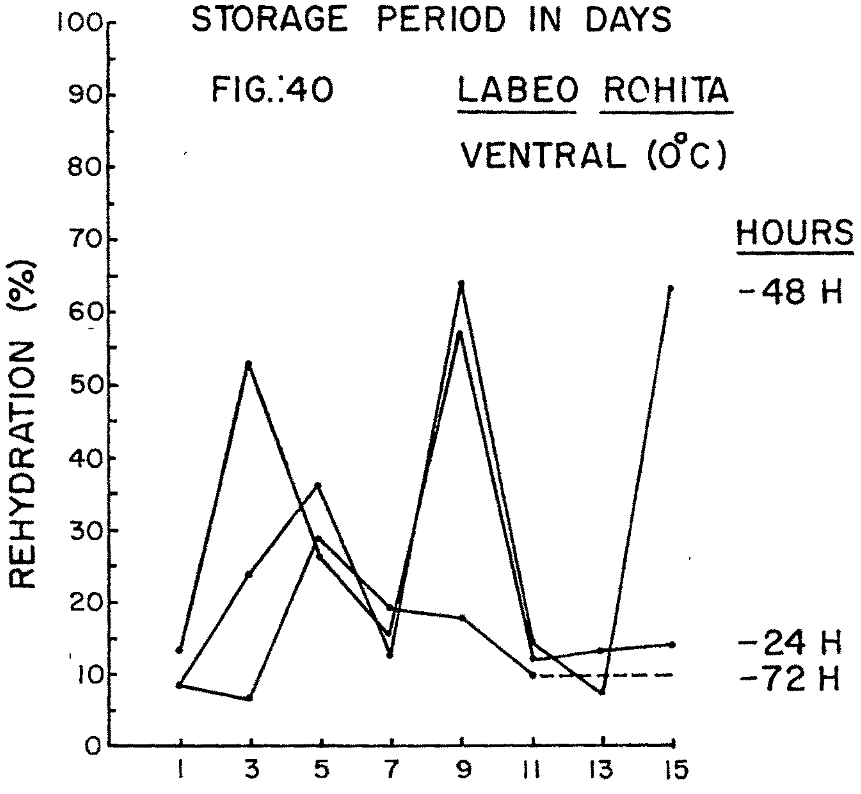
The water intake capacity of 0°C dorsal muscles were 14% to 70%; 6 to 60%; 27 to 12% at 24, 48 and 72 hours of soaking period. The above values were found in initial to final storage samples. The water intake capacity of 0°C storage ventral muscles were 13 to 14%; 8 to 63%; 8 to 10%; at 24, 48 and 72 hours in soaking. The above values were found at the initial to final storage days at 0°C temperature.

From the initial to final storage days at 8°C temperature, the water intake capacity of ventral muscle were 30 to 37%; 34 to 25%; 29 to 33% at 24, 48 and 72 hours of soaking period. The water intake capacity of dorsal muscle at the same storage period and same soaking period were 18 to 39%; 9 to 29% & 29 to 35% respectively.

On comparing the results of samples stored at 0°C and 8°C, it is seen that average water intake capacity was higher in 0°C storage samples at the same soaking period as compared to the 8°C storage

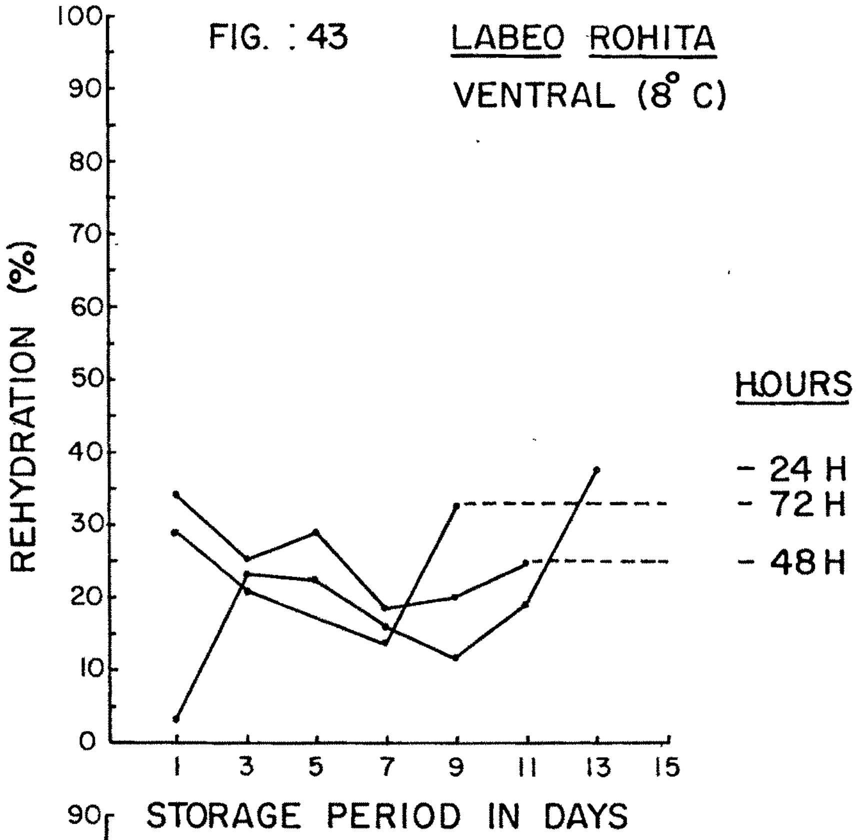


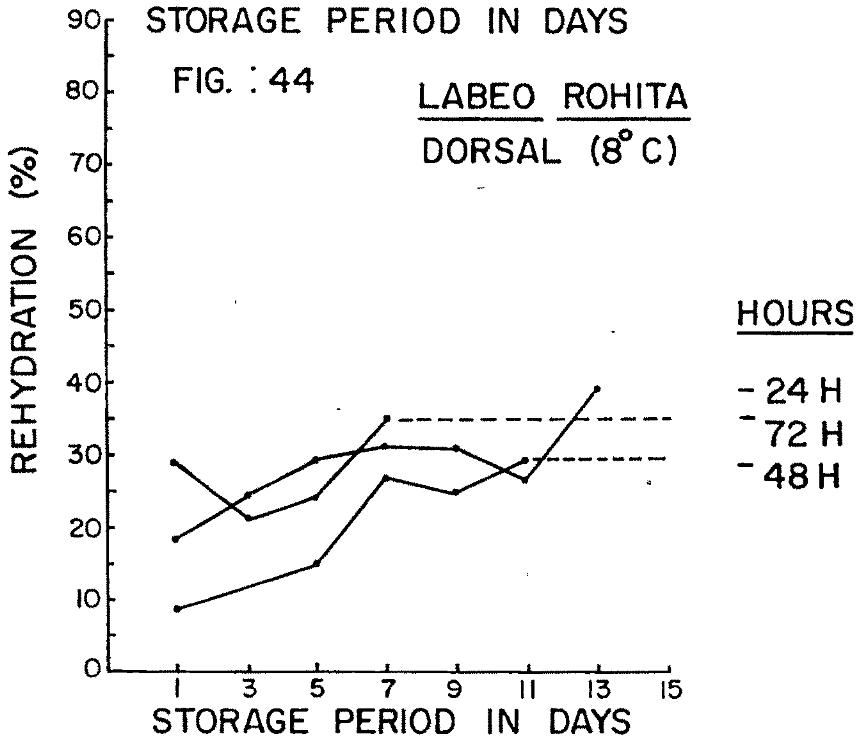
STORAGE PERIOD IN DAYS



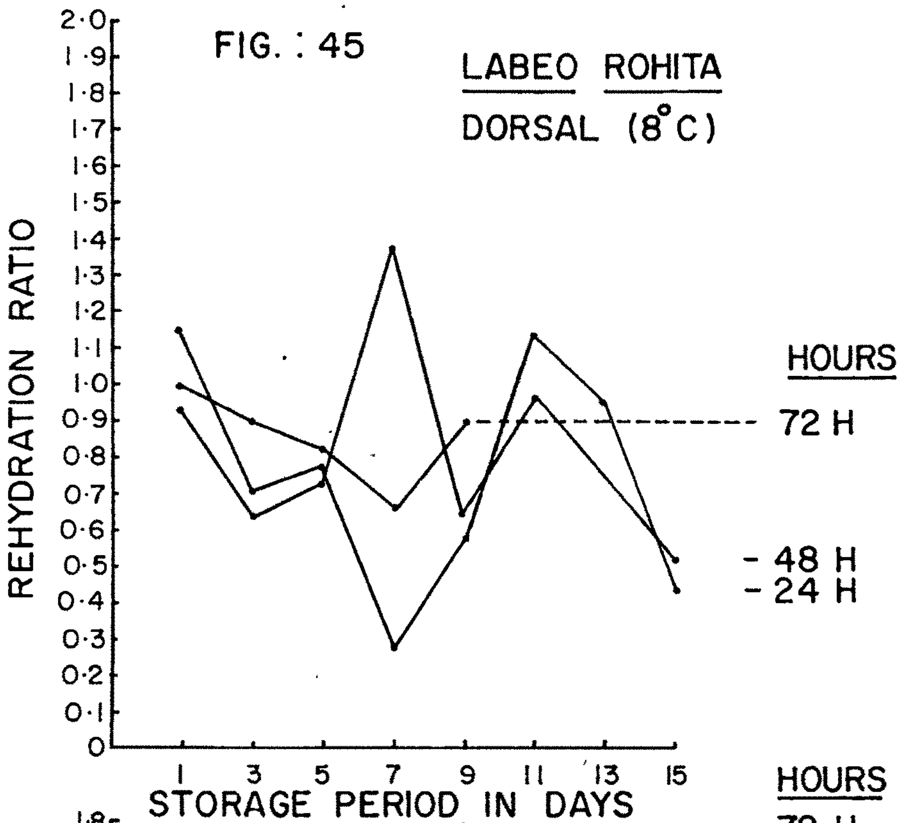
STORAGE PERIOD IN DAYS

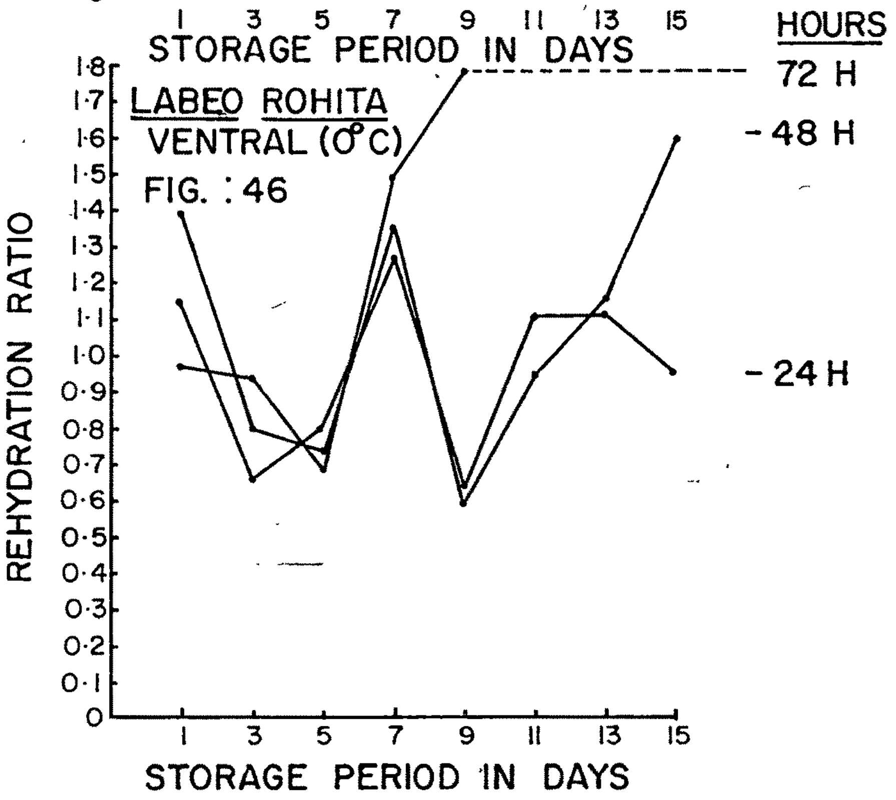
FIGS. : 39 & 40, REHYDRATION PERCENTAGE OF DORSAL & VENTRAL (0°C) LABEO ROHITA STORAGE FISH MUSCLES AT DIFFERENT SOAKING HOURS





FIGS. : 43 & 44, REHYDRATION PERCENTAGE OF VENTRAL & DORSAL 8°C STORAGE <u>LABEO</u> <u>ROHITA</u> FISH MUSCLES AT DIFFERENT SOAKING PERIOD (HOURS)





FIGS. : 45 & 46, REHYDRATION RATIO OF DORSAL (8°C) & VENTRAL (0°C) STORAGE LABEO ROHITA FISH MUSCLE AT DIFFERENT SOAKING PERIOD HOURS.

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Table-10: Variations in rehydration ratio and rehydration percentage of whole hilsa fish samples on soaking after storage and drying.

Storage days	Soaking hours	Storage Temperatures							
		30°C		8°C		0°C		-10°C	
		Rehy. ratio	Rehy %	Rehy. ratio	Rehy. %	Rehy. ratio	Rehy.%	Rehy. ratio	Rehy.%
1	24H	0.74	45.54	0.71	40.19	0.81	39.79	0.85	18.35
	48H	0.87	15.32	0.99	0.15	0.10	9.58	1.10	9.09
	72H	0.78	27.75	1.14	12.51	1.25	21.73	1.23	18.78
3	24H	0.76	31.84	0.93	17.3	0.82	21.76	1.24	45.0
	48H	0.91	10.42	0.88	30.4	1.07	6.98	1.32	52.8
	72H	0.77	30.74	0.76	31.07	1.18	35.6	1.32	51.6
5	24H	0.94	19.2	1.27	14.4	0.96	7.4	1.02	3.4
	48H	1.23	53.0	1.34	37.6	1.24	35.6	1.30	42.4
	72H	1.10	24.0	1.26	31.9	1.29	41.5	1.35	48.5
7	24H	0.71	95.4	0.67	48.81	0.88	24.5	0.80	40.0
	48H	1.04	20.70	1.10	18.4	0.99	2.6	0.80	40.0
	72H	0.90	26.0	0.95	11.5	1.13	18.5	1.19	26.6

samples. The 0°C storage samples were less deteriorated as compared to the 8°C storage samples, water intake capacity was better in better quality sample. Water intake capacity was very less in spoilage samples.

VARIATIONS IN REHYDRATION RATIO & REHYDRATION PERCENTAGE OF WHOLE HILSA FISH MUSCLE ON SOAKING AFTER STORAGE AND DRYING

Rehydration ratio and percentage are shown in Table-10. Highest water intake capacity were observed in -10°C storage samples at the end of storage days (7 days). The values were 26.6 (rehydration %) and 1.19 (rehydration ratio) at 72 hours of soaking period. The lowest rehydration percentage were 11.5 after 72 hours of soaking at 8°C storage samples at the end of 7 days of storage. The lowest rehydration ratio 0.90 was observed in 30°C storage samples at the end of 7 days storage after 72 hours of soaking period.

It is concluded that the results that lower temperature of storage sample contained highest percentage of water, because the samples were less spoiled due to effect of lower temperature. Spoiled muscles were loose. During spoilage the inter-locking pattern of muscles were brokendown. Thus the water intake capacity of the spoiled muscles was least. When increases the length of storage was increased

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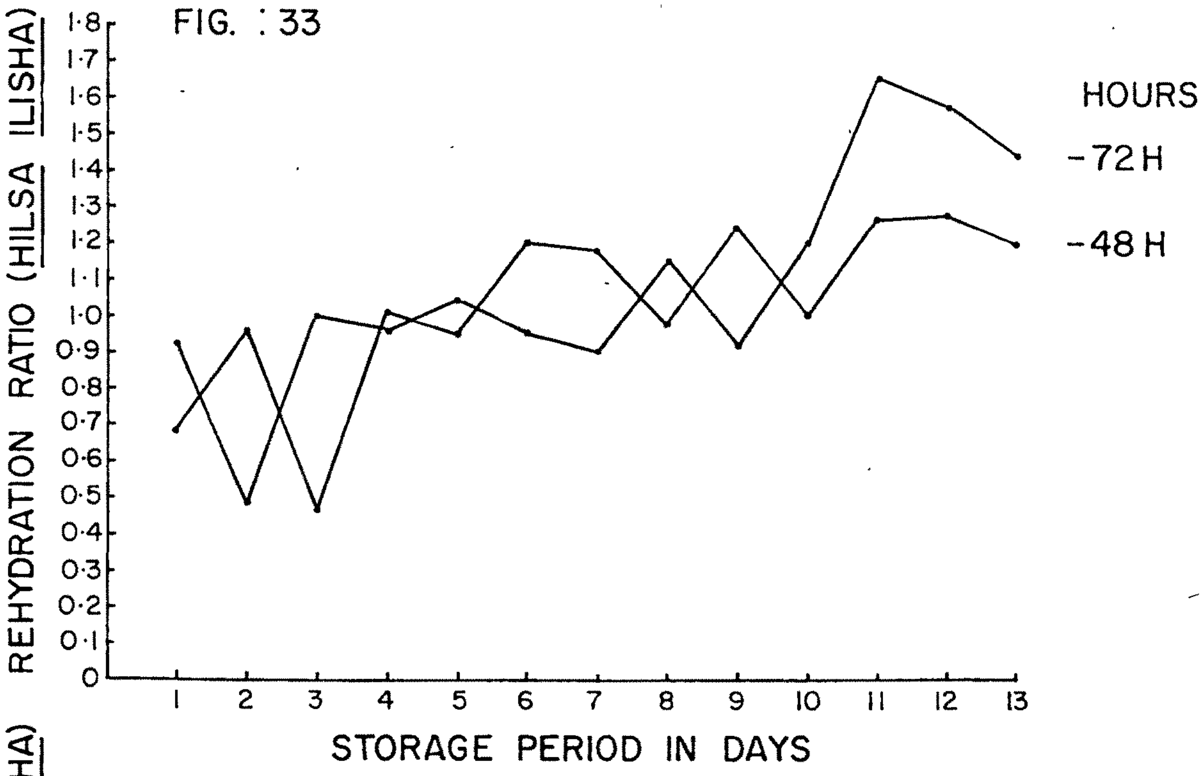
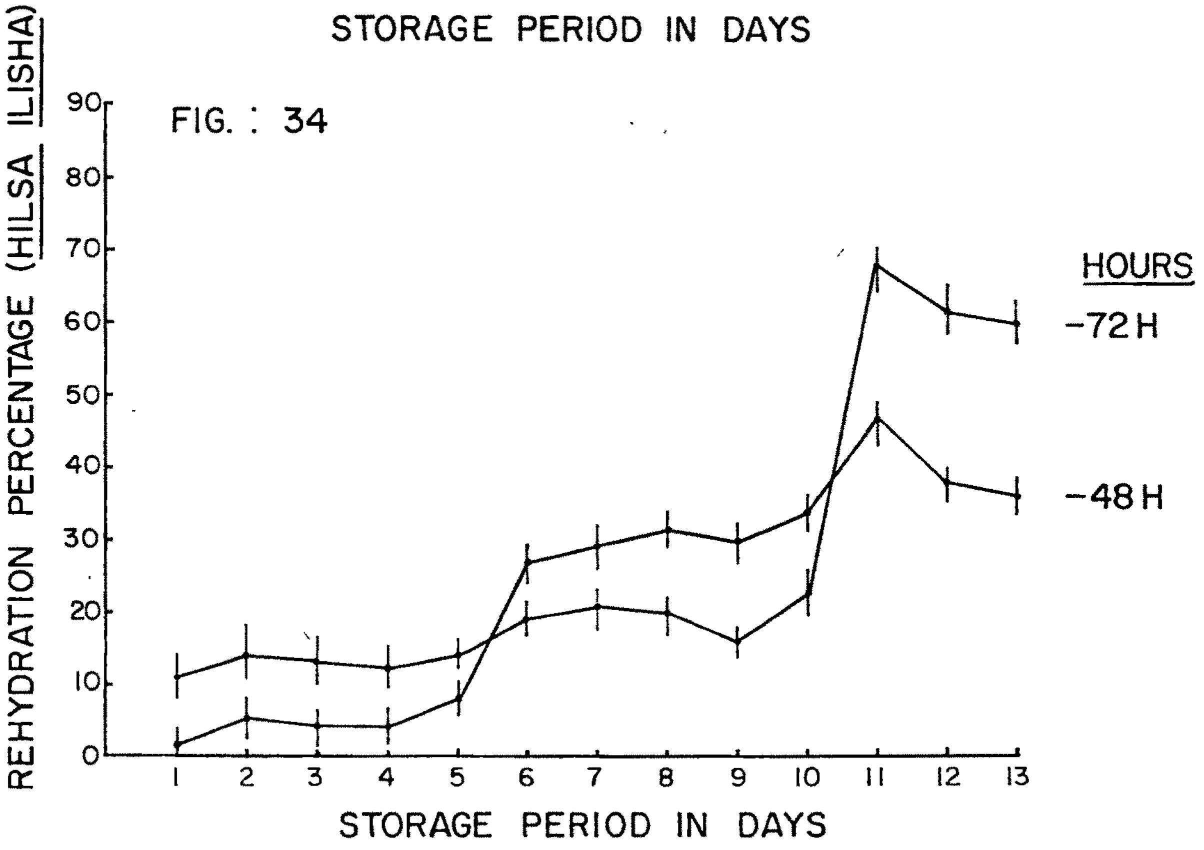
at a particular storage temperature, muscles became spoiled, as a result water intake capacity was comparatively less from the initial water intake capacity. Overall, lower temperature and length of soaking hours had some effect on water intake. At higher temperature of storage, the water intake capacity was low in most of the cases. It depends on the particular portion of the fish samples and its original quality and moisture contents. Lower temperature (-10°C) fish samples were better in water intake capacity among all the storage samples.

VARIATIONS IN REHYDRATION RATIO AND PERCENTAGE OF WHOLE HILSA FISH SAMPLES ON SOAKING IN 5% $NaHCO_3$ SOLUTION AFTER STORAGE AT 0°C & DRYING

On soaking in 5% $NaHCO_3$ for the rate of variations of rehydration at 72 and 48 after storage at 0°C for 1-23 days drying are shown in Figs. 33 and 34.

It can be concluded from the present results that rate of chemical intake depend on soaking hours and individual storage samples. Samples after longer storage contained higher percentage of chemical as compared to the shorter period of storage samples. Increase in the length of soaking hours correspondingly increases the rate of chemical intake.





FIGS. 33 & 34. REHYDRATION RATIO & PERCENTAGE OF HILSA FISH MUSCLE AT 5 % $NAHCO_3$ IN DIFFERENT SOAKING HOURS

Table-12 : Changes of rehydration ratio and percentage of 8°C and 0°C storage dorsal and ventral hilsa fish muscles at different soaking hours

Muscle/ species	Storage period (days)	Rehydration ratio(8°C)			Rehydration Ratio (0°C)			Rehydration (%)/8°C			Rehydration (%)/0°C		
		24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H
Hilsa Ilisha													
Dorsal	1	1.06	0.85	1.88	0.80	0.73	-	5.39	17.05	13.64	19.53	4.69	-
Ventral		1.27	0.97	1.10	0.93	0.73	0.89	21.41	3.37	3.36	6.60	14.07	12.89
Dorsal	3	-	-	-	0.81	0.60	0.90	-	-	-	23.24	67.02	11.14
Ventral		0.88	0.78	0.95	0.73	0.81	0.80	13.49	27.86	5.11	36.68	15.20	2.43
Dorsal	5	1.03	1.09	1.00	1.01	1.42	1.08	2.09	7.52	0.40	1.46	29:62	7.07
Ventral		1.02	1.19	1.13	0.96	0.92	1.07	2.48	15.87	11.30	3.67	8.66	8.39
Dorsal	7	1.22	1.29	-	1.22	1.29	-	18.27	22.76	-	18.27	22.76	-
Ventral		1.33	1.12	-	1.23	1.19	-	11.26	11.05	-	18.49	15.87	-
Dorsal	9	1.15	0.98	0.94	0.63	0.68	0.57	13.31	2.52	6.70	57.78	46.93	75.61
Ventral		1.08	1.0	1.08	0.76	0.79	0.49	7.17	0.45	7.17	31.22	26.93	16.54
Dorsal	11	1.17	1.01	0.87	1.14	1.06	-	14.20	1.06	14.59	12.63	5.78	6.60
Ventral		1.19	-	-	1.3	0.97	0.88	16.18	-	15	15.39	3.08	13.08
Dorsal	13	0.79	0.82	-	0.69	0.47	-	27.19	21.97	-	45.59	-	-
Ventral		0.72	0.72	-	0.50	0.38	-	38.29	38.01	-	70.50	66.08	-

Table-13 : Variations of rehydration ratio and percentage at room temperature storage dorsal and ventral hilsa fish samples, soaked at salt-treated soaking media at different hours.

Muscle/ Species	Storage period (days)	Rehydration Ratio			Rehydration (%)		
		24H	48H	72H	24H	48H	72H
Hilsa ilisha							
Dorsal (Saturated)	1	0.76	-	0.72	31.54	-	37.96
Ventral (Saturated)		0.75	-	0.70	32.99	-	43.61
Dorsal (10% salt)		0.73	-	0.70	36.75	-	42.97
Ventral (10% salt)		0.89	-	0.85	11.91	-	16.63
Dorsal (Saturated)	3	0.59	1.04	0.53	68.37	4.0	89.37
Ventral (Saturated)		0.82	1.08	0.75	22.63	8.16	-
Dorsal (10% salt)		1.06	1.06	0.93	32.96	5.79	7.71
Ventral (10% salt)		0.87	0.98	0.75	23.20	2.40	33.63
Dorsal (Saturated)	5	0.74	0.86	0.78	47.7	23.17	27.61

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COMPARATIVE ACCOUNT OF CHANGES IN REHYDRATION RATIO & PERCENTAGE IN DORSAL & VENTRAL MUSCLES OF HILSA ILISHA, AFTER STORAGE AT 8°C AND 0°C FOR MAXIMUM OF 13 DAYS AND DRYING ARE PRESENTED IN TABLE-12

There were no marked differences in rehydration and percentage in dorsal and ventral muscles at 8°C and 0°C of storage. Individual samples contained higher or lower values. From average values it was observed that dorsal muscle contained higher rehydration percentage as compared to ventral muscle. 0°C storage samples contained higher rehydration values as compared to 8°C storage samples. The length of storage at both the storage temperatures had effect on water uptake. The spoiled samples gained less water. Pieces of fish samples having more fat gained less water as compared to lean samples (dorsal portion).

VARIATIONS IN REHYDRATION RATIO AND PERCENTAGE OF DORSAL & VENTRAL MUSCLES OF HILSA FISH ON SOAKING IN SALT AFTER STORAGE AND DRYING

Dorsal and ventral muscles of Hilsa were dipped for 30 minutes in saturated 10% salt solution. After that they were stored at room temperature for 1, 3, 5 and 7 days. These were then dried in sun for 3 days and soaked in saturated 10% salt solution for 24, 48 and 72 hours. After 24 hours of soaking 10% salt treated ventral samples showed highest rehydration ratio (average 1.03). The ventral 10% salt treated muscles contained highest rehydration ratio 0.97 after 72 hours of soaking.

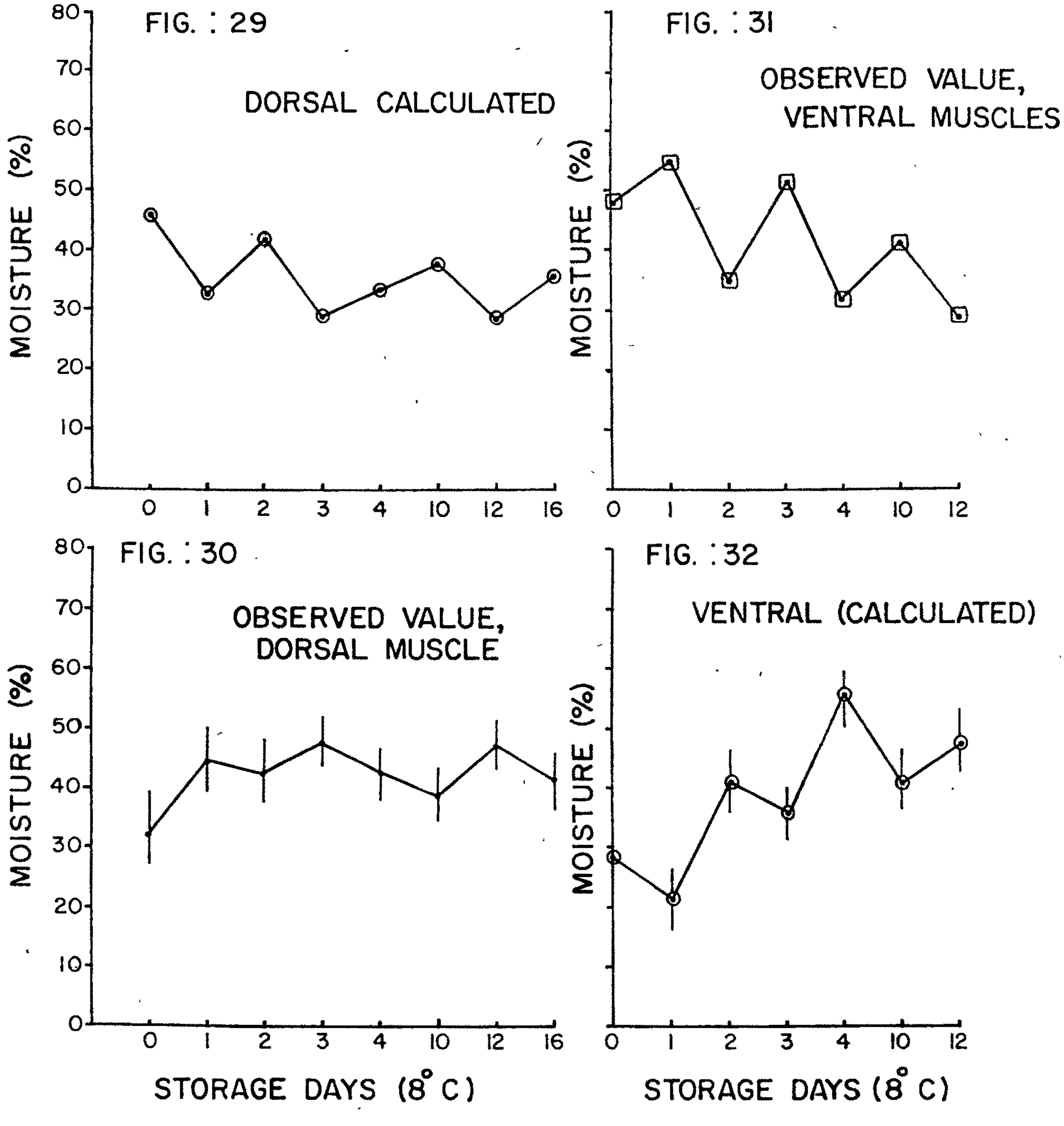
Ventral muscle samples, soaked in saturated salt solution for 24 hours contained least (0.7) average rehydration ratio (Table-13).

DISCUSSION

Dorsal muscle showed higher water content after rehydration and also the highest rehydration ratio which may be attributed to the fact that it took the shortest time for drying compared to other samples. Samples after freezing for shorter duration showed better rehydration capacity. Samples stored for longer period of soaking was not desirable as it spoiled the quality of the product. This was more evident in the case of ventral and dorsal muscle of the whole body fish sample.

The product treated with 10% salt had the greatest water binding capacity. 10% salt treated yielded a lighter coloured product than did the others. Taste panel tests indicated that the samples had an acceptable flavour, closely resembling that of traditional salted fish and that 10% salt treated samples appeared to have the best texture. The results suggested that the addition of 10% salt by weight was sufficient to yield a product with better properties. The dried products were rehydrated at different length of dipping time for the determination of the rehydration property of the storage samples. The important steps in the rehydration properties of the edible fish muscles are 1. adjustment of pH of the soaking media to improve the rehydration properties, 2. determination of rehydration capacity of the materials with varying contents of salt indicated that the salt rehydration properties to any significant extents than soaking in water.

Rehydration capacity of the product from all species was good and did not show variation with the degree of rehydration among the different samples. The relative high moisture accelerates the deterioration in physical and bio-chemical changes during storage considering the value of trade of this commodity and its steady increase it was felt necessary to workout a technique to improve its quality and shelf-life. Temperature of storage between 0°C to 8°C does not markedly affect the rehydration of certain samples. Toughness of the dehydrated fish is found to be related to swelling properties.



FIGS: 29 TO 32, CHANGES IN MOISTURE CONTENTS OF DORSAL & VENTRAL MUSCLES OF SMALL SIZE SALTED (1:2, FISH: SALT RATIO) HILSA FISH DURING STORAGE AT 8° C TEMPERATURE

Poulter and Lawrie (1977) reported that fish muscle extract pH was in range of 6.5 - 7.0 (pH) and change during spoilage (Cutting, 1953). The biochemical factors control the freshness and the spoilage pattern, which in turn are due to difference in muscle composition, bound water and pH value. The preserving effect of freezing, however is due to the extent of reduction in rate of chemical and biochemical process, when the temperature of the fish is lowered. Freezing also results in partial dehydration, which probably contributes to its preserving effect. The rate of freezing therefore, has long been considered of vital importance for obtaining the high quality product, slow rate of freezing results in greater destruction of the tissue than quick freezing.

THE RATE OF DEHYDRATION OF FREEZING STORAGE SALTED MUSCLE TISSUE

The results of the variations in moisture content of dorsal and ventral muscles of hilsa fish due to effect of low temperature (8°C) and in combination of (1:2) fish-salt ratio are shown in Figs. 29, 30, 31 and 32 respectively. The variation in moisture content depends on the rate of penetration of salt within the fish muscle. Before preservation the whole body fish samples contained 78.18% of moisture.

The dorsal portion contained higher moisture (from average value). The variation of moisture content in dorsal and ventral fish muscle depends on species, size, age and maturity of the fish. The rate of moisture removal depends on the rate of salt penetration. The rate of penetration of salt depends on the body constituents. It was observed from the Figs. 29, 30, 31 and 32 that at 12 days of storage the dorsal muscle contained 48.96% and the ventral muscle contained 29.39% of moisture. Percentage loss of moisture was higher in dorsal muscle than in ventral muscles of hilsa fish samples at 12 days storage. The rate of penetration of salt was higher in dorsal muscle resulting in removal of more moisture from dorsal muscle. Similarly, the rate of penetration of salt was lower in ventral muscles due to body composition (higher fat contents) resulting in low rate of moisture removal from ventral portions of hilsa fish samples (Figs. 29 to 32)

De Valle and Nickerson (1968) published a quick salting process of fish that entailed (i) grading fish muscle with salt (ii) mixing the salt with fish to remove water from fish and fish products and drying the salted fish to give a stable product. Adding excessive amount of salt higher than the minimum resulted in removal of water from fish tissue.

Mendelsohn (1974) proposed another process where skinless fillets were ground and mixed with saturated brine (1:1). As a result, extraction of water from the muscle took place under the influence of osmotic diffusion. As the salt penetrates deeper into the fish flesh, the rate of water diffusion is reduced. Finally, the time comes when the movement of water out from the fish ceases completely. This phenomena occurs before the end of salt penetration into the fish. The consequence is a minor increase in weight of the fish at the end of the salting period. After the salt concentration in the cellular fluid of various parts of the fish body reaches 15-20% the bound (absorption) water reverts to a free state (Levanidov, 1960). As a result of the transformation of water from the bound state to the free state, a reduction in salt concentration takes place in the cellular fluid of the fish, In fish-salt mixing method the salt on the surface of the fish prevents the brine from becoming diluted. As it dissolves in the water that comes out of the fish, an additional quality of salt brine forms. Higher proportion of salt prevents the spoilage of the inner layers of the fish flesh. In this way large-sized and fatty fishes can be slowly preserved in salt. In the initial state of salting the preserving brine is cold. Gradually, depending on the degree of thawing the salt penetrates into the fish flesh.

The water removal or loss is accompanied by a shrinkage which is not always uniform. Salting has great influence on the structural and mechanical features of the fish muscle tissues. The findings agree well with observation made by other workers. The outer layer of the fish controls the rate of salt penetration. At this stage a considerable decline occurs in the weight of the fish. No profound chemical changes have as yet taken places. The fish have the odour and taste of a raw fish. Salt has not fully penetrated into the inner layer of the fish muscle as well as the body organs in the abdominal cavity. The complex protein bodies contribute to the absorption of water. It would be more correct to assume that protein binding the sodium chloride reduces the salt concentrations in the cellular fluid. This induces the additional movement of salt molecules from the brine into the fish. The ripening of the salted fish results in the bio-chemical process that causes the changes in chemical and physico-chemical characteristics of the fish tissues. These changes are induced by enzymes, which breakdown both proteins and fats. The tissue structure of the muscles and body organs of the fish are also affected. Some of the nitrogenous substances diffuses from the fish into the salt brine. During salting, the exchange of matter in the system is accomplished chiefly by the movement of salt molecules but during the ripening period nitrogeneous substances chiefly of low molecular weight as well as fat pass from the fish into the brine.

The effect of the ratio of salt to fish and its suitability to different samples of hilsa fish have been examined. The bio-chemical changes by the effect of different proportion of fish salt ratio and their quality criteria are reported in figures Certain modifications like storing without any protective packaging materials and without dressing the fish were adopted.

Dehydration by the action of salt in whole body of small sizes hilsa fish muscles at 8°C temperature of storage is easy to attain by thoroughly removing the resulting brine. The excess of salt and soluble compounds originating from the fish would adversely influence the stability of the product.

Freshly salted fish is almost natural in flavour and remains, without additional drying, almost unchanged for several days of storage at 8°C temperature if properly protected. After desalting and proper rehydration the texture of the fish fibers is acceptably soft. Application of higher concentration of salt does not improve salting process and results in introduction of excessive amount of salt into the product. Most of the product retained an acceptable in quality for a definite period when stored at 8°C temperature. During storage at 8°C temperature the physical appearence was good, the colour slightly varied, the flavour and odour were slightly changed due to removal of water including formation of various nitrogenous substances within the fish body. The shelf-life of salted fatty fish is not considerably extended beyond that of the dehydration.

Quality of salted fish is determined by type and size of the fish, fat contents and salt penetration. Salting of fatty fishes reduced the moisture content. After salting at (1:2 fish-salt ratio) the shelf-life of the product was considered to be acceptable.

The ratio of water to fish sustances correspondingly to the water within the fish particles, obviuusly decreases with the increasing temperature denaturation of protein also increases. Under practical conditions of salting, approximately one half of the total lipid of fish is removed with the brine so that their amount is considerably reduced. Losses in protein will depend on the amount of brine. Soluble protein can be easily coagulated by heating the brine. However, the effect of factors such as p^H and salt concentration must be first investigated and the optimum condition of filteration and dehydration established.

The main disadvantages of the process is that the product is heavily salted. In essence we wanted to produce a salted fish that would retain some of the functional properties of the protein. Duerr and Dyer (1952) reported that study of the denaturation of cod muscle protein by sodium chloride shows that the myosin fraction is denatured when a critical concentration about 8 to 10% in the muscle is reached. Parallelling the rapid denaturation a sudden increase of salt uptake and of moisture loss occurs (Figs. 29 to 32) From the observation, we considered it possible to produce lightly salted fish with sufficient functional properties. An experiment was designed to determine the effects of salt (1:2) fish-salt ratio to small sizes fish species. Key factor considered in the study was water release by the action of (1:2) fish-salt ratio at 8°C temperature of storage for 16 days. The variations of moisture contents in dorsal and ventral muscle were observed and total loss from percentage of original moisture content were calculated at definite period of storage days (Figs. 29 to 32). For comparative purpose the variation in moisture content of salted dorsal and ventral muscle tissues of hilsa fish at 8°C temperature of storage were subjected to a series of laboratory test. This findings will be helpful for preservation of fatty fish by salting of small sizes hilsa fish and also helpful for long term storage and to determine the rate of penetration of salt in particular portions (dorsal and ventral muscle). On the basis of the present findings, it may be concluded and recommended that at the time of abundantly catching of hilsa fish, especially monsoon season 1:2 fish-salt ratio were given a better quality product for long term storage without deterioration.

The higher storage temperature (8°C) are sometimes used in order to accelerate the ripening. The rate of deterioration were slow in heavy salted fish (1:2 fish-salt ratio). In ventral portion the rate of spoilage was faster than the dorsal portion. The oxidative changes in the fat during salting and preservation of the fish are clearly noticeable. Fish can be brined for an extended period to give a product with a higher salt content and hence a longer storage life. High salt content would be the most important means of lowering the water activity of the product.

SHELF-LIFE MEASUREMENT OF MARINE & FRESH WATER FISHES BY RELATIVE RATE OF SPOILAGE CHART:

(TABLE No. 18: FRESH WATER FISH LABEO ROHITA AND TABLE-19 FOR MARINE FISH RASTRELLIGER KANAGURTA)

From table-18, it can be seen that storage

: Shelf-life measurement of *Labeo rohita* at 1°C to 25°C temperature of storage at different length of storage days.

Observed Log TVN values at 0°C Temp.	* The rate of decomposition of *Labeo rohita* fish at 1°C to 25°C storage temperature at different length of storage days. * The calculated values, based on log total volatibe nitrogen values at 0°C temperature of storage at different length of storage days											
	1°C	2°C	3°C	4°C	5°C	6°C	7°C	8°C	9°C	10°C	11°C	12°C	13°C
1.21	1.65	2.08	2.52	2.95	3.39	3.82	4.26	4.70	5.13	5.57	6.00	6.44	6.87
1.42	1.93	2.43	2.95	3.46	3.98	4.49	4.99	5.51	6.02	6.53	7.04	7.55	8.07
1.56	2.12	2.68	3.24	3.81	4.37	4.93	5.49	6.65	6.61	7.18	7.74	8.30	8.87
2.30	3.13	3.96	4.79	2.30	6.44	7.27	8.09	8.92	9.75	10.58	10.41	12.24	13.06
2.21	3.50	3.80	4.60	5.39	6.19	6.98	7.78	8.57	9.37	10.17	10.96	11.76	12.55
2.14	2.91	3.68	4.45	5.22	5.99	6.76	7.53	8.30	9.67	9.84	10.61	11.38	12.16
2.01	2.73	3.46	4.18	4.90	5.63	6.35	7.68	7.80	8.52	9.25	9.97	9.97	11.92
2.28	3.10	3.92	4.74	5.56	6.38	7.20	8.03	8.85	9.67	10.89	11.31	12.13	12.95
2.31	3.14	3.97	4.80	5.64	6.47	4.70	8.13	8.96	9.79	10.63	11.46	12.29	13.12
2.41	3.27	4.15	5.01	5.88	6.75	7.62	8.48	9.35	10.21	11.09	11.95	12.82	13.69

* Log (1+TVN) values at 0°C temperature (Total volatile nitrogen, mg.N/100 gm of fish muscles)
* Limit of bio-chemical acceptability, measured by Log TVN values range from 1.5 to 1.6 mg.N/100 gm of fish muscles.
* *Labeo rohita* (Fresh water species).

Contd. ....

ble-18 : (Continued)

rage riod ays)	Observed Log TVN values at 0°C Temp.	* The rate of decomposition of Labeo rohita fish at 1°C to 25°C storage temperature at different length of storage days. * The calculated values, based on log total volatice nitrogen values at 0°C temperature of storage at different length of storage days											
		14°C	15°C	16°C	17°C	18°C	19°C	20°C	21°C	22°C	23°C	24°C	25°C
tial	1.21	7.31	7.75	8.18	8.62	9.05	9.49	9.92	10.38	10.79	11.23	11.66	12.10
3	1.42	8.58	9.09	9.60	10.11	10.62	11.13	11.64	12.16	12.67	13.18	13.69	14.20
5	1.56	9.42	9.98	10.55	11.11	11.67	12.23	12.79	13.36	13.98	14.48	15.03	15.60
7	2.30	13.89	14.72	15.55	16.38	17.20	18.03	18.86	19.69	21.34	21.34	22.17	23.00
9	2.21	13.35	14.14	14.94	15.74	16.53	17.33	18.12	18.92	19.67	20.51	21.30	22.10
11	2.14	12.93	13.70	14.47	15.24	16.00	16.78	17.55	18.32	19.09	19.86	20.63	21.40
13	2.01	12.14	12.86	13.59	14.31	15.03	15.76	16.48	17.21	17.93	18.65	19.38	20.10
15	2.28	13.77	14.59	15.41	16.23	17.65	17.88	18.70	19.52	21.16	21.16	21.98	22.80
17	2.31	13.95	14.78	15.55	16.45	17.28	18.11	18.94	19.77	20.61	21.44	22.27	22.80
19	2.41	14.56	15.42	16.29	17.16	18.03	18.89	19.76	20.63	21.47	22.36	20.79	23.10

* Log (1+TVN) values at 0°C temperature (Total volatile nitrogen, mg.N/100 gm of fish muscles)
* Limit of bio-chemical acceptability, measured by Log TVN values range from 1.5 to 1.6 mg.N/100 gm of fish muscles.
* Labeo rohita (Fresh water species).

Table-18 : (Continued)

Storage period (days)	Observed Log TVN values at 0°C Temp.	* The rate of decomposition of Labeo rohita fish at 1°C to 25°C storage temperature at different length of storage days. * The calculated values, based on log total volatice nitrogen values at 0°C temperature of storage at different length of storage days											
		14°C	15°C	16°C	17°C	18°C	19°C	20°C	21°C	22°C	23°C	24°C	25°C
Initial	1.21	7.31	7.75	8.18	8.62	9.05	9.49	9.92	10.38	10.79	11.23	11.66	12.10
3	1.42	8.58	9.09	9.60	10.11	10.62	11.13	11.64	12.16	12.67	13.18	13.69	14.20
5	1.56	9.42	9.98	10.55	11.11	11.67	12.23	12.79	13.36	13.98	14.48	15.03	15.60
7	2.30	13.89	14.72	15.55	16.38	17.20	18.03	18.86	19.69	21.34	21.34	22.17	23.00
9	2.21	13.35	14.14	14.94	15.74	16.53	17.33	18.12	18.92	19.67	20.51	21.30	22.10
11	2.14	12.93	13.70	14.47	15.24	16.00	16.78	17.55	18.32	19.09	19.86	20.63	21.40
13	2.01	12.14	12.86	13.59	14.31	15.03	15.76	16.48	17.21	17.93	18.65	19.38	20.10
15	2.28	13.77	14.59	15.41	16.23	17.65	17.88	18.70	19.52	21.16	21.16	21.98	22.80
17	2.31	13.95	14.78	15.55	16.45	17.28	18.11	18.94	19.77	20.61	21.44	22.27	22.80
19	2.41	14.56	15.42	16.29	17.16	18.03	18.89	19.76	20.63	21.47	22.36	20.79	23.10

* Log (1+TVN) values at 0°C temperature (Total volatile nitrogen, mg.N/100 gm of fish muscles)
* Limit of bio-chemical acceptability, measured by Log TVN values range from 1.5 to 1.6 mg.N/100 gm of fish muscles.
* Labeo rohita (Fresh water species).

temperature has a significant effect on the changes of the rate of deterioration during storage and preservation at particular storage temperatures (1°C to 25°C). The higher the storage temperature, the rate of formation of total volatile nitrogen was also higher. Total volatile nitrogen value gradually increases from lower to higher degree of storage temperature (1°C to 25°C). The value gradually rises with increase in length of storage period.

It is clear from the present results that when storage temperature was increased TVN value also showed increase. Higher TVN values indicate the sign of higher spoilage during storage. The rate of increase depends on the storage temperature and length of period of storage. At the initial period of storage, the rise of TVN values was very slow but after certain period of storage (one week)the increase of TVN values was more rapid during the remaining period of storage. Overall, the pattern of increase of TVN value was linear.

Storage temperature and length of storage period has a significant effect on fish decomposition. In addition other associate factors are washing, handling, micro-organisms and self-degradation by enzymatic action during storage and preservation. From bio-chemical (TVN) values, fish acceptability were measured during storage at a particular storage temperature and different length of storage period.

Length of storage period has some effect on the deteriorative changes of Rastrelliger kanagurta, marine species during storage at various storage temperatures. The rate of differences of log trimethyl amine value from 1 day to 39 days of storage at 1°C to 25°C are shown in Table 19.

With higher storage temperature the rate of formation of TMA was higher. The rate of variation of TMA from lower to higher storage temperatures was very less. But in comparison with different storage temperatures, there was a marked difference in TMA value, specially at higher storage temperatures the rate of variation of TMA formation in the different length of storage period was observed. It was also found that after one week of storage at 0°C storage temperature the value of TMA gradually rises at the end of storage period.

The effect of length of storage and storage temperature both are important factors in deterioration of the fish samples.

Sometimes, physically the fish samples were acceptable in condition but biochemically the samples were unacceptable. The low temperature of storage retard the spoilage slightly but not totally. The knowledge of deterioration would be useful for identification of the consumer acceptability. It is easy to identify the bio-chemical acceptability of fishes by using the relative rate of spoilage chart. The chart would also be helpful for measurement of the storage life of fishes at different storage temperatures for different length of storage period.

LIMIT OF ACCEPTABILITY

According to Spencer and Baines (1964) the effect of temperature (Q) on the rate of spoilage (u) stored was found to be approximately linear and expressible in the form U = V(1 + C,Q), where U = spoilage rate (spoilage unit/day) at temperature Q. Q = storage temperature (1°C to 25°C temperature), U = spoilage rate at 0°C (observed standard spoilage rate), C = temperature effect (the average value of C = 0.36). Spoilage is measured by TMA. The linear temperature response 'C' is measured by relative increase in spoilage rate per degree above 0°C, and is therefore independent of the units in which spoilage is measured.

The relative rate of spoilage was calculated by the log TMA and TVN values in marine and fresh water species. In marine fishes, Shewan and Ehrenberg (1957) considers only TMA values for measurement of the freshness. In the present study values of TVN in Labeo rohita species was also carried out. According to the log formula of Shewan and Ehrenberg (1957) (10 X log (1 + TVN) and 10 X log(1 + TMA)) were used. The values increase linearly with storage time and with the storage temperature (1°C to 25°C). Temperature effect vary from species to species. Hence, variations in the results of TVN and TMA parameters were observed.

Trimethyle amine oxide is widely distributed in marine species and the significant variation of TMA values during storage were reported by many researchers.

Bio-synthesis of TMAO in fish proceeds possibly through glycine betaine, choline and TMA (Watt and Watts, 1974). More TMA is produced from TMAO by bacterial section than by fish tissue enzymes. TMA is clearly an important factor in imparting fishy odour (Jones and Billinski, 1967a). TMA levels are usually much lower than TMAO levels in fresh fish muscles. An increase of TMA content of a level 8 mg.N/100 gm followed by a rapid rise to 37 mg.N/100 gm after 16 days, indicates increased spoilage. Dyer and Dyer (1949) reported that TMA level was 4 to 5 in spoilage fillets and 15 to 30 or more in the spoiled materials. The low temperature effectively inhibited the production of TMA throughout the storage period.

The fish samples are acceptable if TVN does not exceed 16.5 to 19.7 mg.N/100 gm (Pearson and Muslemuddin, 1968). Fish can be considered as fresh if the amount of TVN is less than 20 mg.N/100 gm. Fish is stale when the value of TVN exceed of 30 mg.N/100 gm of fish muscle. The 30 mg.N/100 gm is considered the upper limit of acceptability (Pearson, 1976). Kimura and Kiamikeura (1939) recommended that basic nitrogen levels per 100 gm of fish as 10 mg or less for fresh fish, 20 to 30 mg for beginning of spoilage, and over 30 mg.N/100 gm for spoiled fish. Tanikawa and Akiba, (1955) suggested that 20 mg VBN/100 gm as the upper limit for fresh crab meat. Kawabata (1953b) reported that 30 mg VBN/100 gm as the upper limit for freshness. Wierzhochowski (1956) suggested that 30 to 40 mg.N/100 gm as the upper limit of freshness for fresh water fish and 60 mg.N/100 gm as the limit of marine fish.

Castell et al., (1958) reported that TMA value 0 to 1.0 indicates good quality fish. TMA value 1 to 5.0 indicates poorer quality fish, but still fit for human consumption. Where the TMA value exceeds 5, the fish has reached a condition that is considered unfit for processing. TMA values of the fish muscle have a very significant correlation with the storage time. Hoogland (1958) expressed TMA as log (1 + TMA value). Variation of TMA is due to TMAO originally present in the fish and differences in the bacterial flora. The present findings indicated that the samples were acceptable in quality in all and every respect of the given set of experimental conditions.

STORAGE LIFE OF FISHES AT PARTICULAR STORAGE TEMPERATURES & DEFINITE LENGTH OF STORAGE PERIOD IN DAYS

Shelf-life of fishes depends on many factors, types of the fish, storage temperatures, length of storage period and bio-chemical factors. Breakdown of the trimethyle amine oxide, production of volatile nitrogen, volatile acid etc. by the action of associated micro-organisms and external environmental bacteria, fish enzymes etc. For extension of shelf-life of fishes, it is necessary to know the actual causes of fish spoilage and how it can be prevented. The low temperature have a profound effect on the extension of shelf-life of fishes but it is necessary to know the rate of decomposition and deterioration at particular storage temperature.

It is also important to know the pattern of spoilage in marine and fresh water fishes. These finding would be useful for the extension of shelf-life of fishes at desirable storage temperature. Basic bio-chemical deterioration results would be helpful for long-term preservation of fishes. Without knowing the range of the bio-chemical acceptability of particular fishes in definite period of storage at definite degree of storage temperatures, it is impossible to measure the storage life of the fishes. From the range of bio-chemical acceptability value, it is easy to measure the scientific acceptability of particular fish species.

Various fishery researchers suggested various methods, pointing out the causes of spoilage and limit of scientific acceptability of the locally available fish species. In the present study the shelf-life was calculated using marine species <u>Rastrelliger kanagurta</u> (Table-19) and fresh water fish species <u>Labeo rohita</u> (Table-18). A limit of acceptability has been reported on the basis of this findings, on measurement of storage life of <u>R. kanagurta</u> and <u>L. rohita</u> at 1°C to 25°C storage temperatures for 39 and 19 days of storage period respectively. No information is available for the measurement of shelf-life of fishes stored at 1°C to 25°C and for different period of storage.

The effects of temperature on spoilage have

Table-19: Shelf-life measurement of R. Kanagurta at 1°C to 25°C temperatures of storage at different length of storage days.

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Storage period (days)	Observed TMA Values at 0°C temp.	* The rate of decomposition of Rastrelliger Kanagurta fish at 1°C to 25°C storage Temperatures at different length of storage days. * The calculated values, based on log TRImethyle amine values at 0°C temperature of storage at different length of storage days.												
		1°C	2°C	3°C	4°C	5°C	6°C	7°C	8°C	9°C	10°C	11°C	12°C	13°C
1	1.35	1.52	2.32	2.80	3.29	3.78	4.27	4.75	5.24	5.72	6.21	6.70	7.18	7.67
2	1.32	2.27	2.27	2.75	3.22	3.70	4.17	4.65	5.12	5.60	6.07	6.55	7.02	7.50
4	1.13	1.54	1.99	2.35	2.36	3.16	3.57	3.98	4.38	4.79	5.20	5.60	6.01	6.42
6	1.29	1.75	2.22	2.68	3.15	3.67	4.08	4.54	5.00	5.47	5.93	6.40	6.86	7.33
7	1.31	1.78	2.25	2.72	3.20	3.67	4.14	4.61	5.08	5.55	6.03	6.50	6.70	7.44
8	1.18	1.60	2.03	2.45	2.87	3.30	3.72	4.15	4.58	5.00	5.42	5.85	6.28	6.70
19	1.40	1.90	2.91	2.97	3.41	3.92	4.42	4.97	5.43	5.94	6.44	6.94	7.45	7.95
21	1.41	1.92	2.43	2.93	3.44	3.95	4.46	4.96	5.47	5.98	6.49	6.99	7.50	8.00
23	1.32	1.80	2.27	2.75	3.22	3.70	4.17	4.65	5.12	5.60	6.07	6.55	7.02	7.50
31	1.19	1.62	2.05	2.48	2.90	3.33	3.76	4.19	4.62	5.05	5.47	5.90	6.33	6.76
39	1.33	1.80	2.29	2.77	3.25	3.72	4.20	4.68	5.16	5.64	6.12	6.60	7.08	7.55

* **Log (1+TMA) values at 0°C temperature (mg.N/100 gm of fish muscles)**
* Limit of bio-chemical acceptability measured by TMA values log range from 1 to 1.3 mg.N/100 gm of fish muscles.
* Rastrelliger Kanagurta (Marine species)

Contd

Table-19 : (Continued)

Storage Period (days)	Observed TMA values at 0°C Temp.	* The rate of decomposition of <u>Rastrelliger</u> <u>Kanagurta</u> fish at 1°C to 25°C storage Temperatures at different length of storage days. * The calculated values, based on log Trimethyle amine values at 0°C temperature of storage at different length of storage days											
		14°C	15°C	16°C	17°C	18°C	19°C	20°C	21°C	22°C	23°C	24°C	25°C
1	1.35	8.15	8.64	9.13	9.61	10.10	10.58	11.07	11.56	12.04	12.53	13.01	13.50
2	1.32	7.98	8.45	8.92	9.40	9.88	10.35	10.82	11.30	11.77	12.25	12.72	13.2
4	1.13	6.83	7.23	7.64	8.05	8.45	8.86	9.27	9.67	10.08	10.49	10.89	11.3
6	1.29	7.80	8.26	8.72	9.18	9.65	10.11	10.58	11.04	11.51	11.97	12.44	12.9
7	1.31	7.91	8.38	8.86	9.33	9.80	10.27	10.74	11.21	11.69	12.16	12.63	13.1
8	1.18	7.13	7.55	7.98	8.40	8.83	9.25	9.68	10.10	10.53	10.96	11.38	11.80
19	1.40	8.45	8.96	8.46	9.97	10.47	10.98	11.48	11.98	12.49	12.99	13.50	14.00
21	1.41	8.52	9.02	9.53	10.04	10.55	11.65	11.56	12.07	12.58	13.68	13.59	14.10
23	1.32	7.97	8.45	8.92	9.40	9.87	10.35	10.82	11.30	11.77	12.25	12.72	13.20
31	1.19	7.19	7.62	8.04	8.47	8.90	9.33	9.76	10.19	10.62	11.04	11.47	11.90
39	1.33	8.03	8.51	8.99	9.47	9.95	10.43	10.91	11.39	11.86	12.34	12.82	13.30

* Log (1+TMA) values at 0°C temperature (mg.N/100 gm of fish muscles).
* Limit of bio-chemical acceptability measured by TMA values log range from 1 to 1.3 mg.N/100 gm of fish muscles.
* <u>Rastrelliger</u> <u>Kanagurta</u> (Marine species)

been recorded for a variety of fishes (Castell and MacCallum, 1950; Lujipen, 1958; Cutting et al., 1953); various expression have been used to characterize these effects, such as a linear relation between rate of spoilage and temperature (Mossel and Ingram, 1955).

Maintaining a constant temperature is very important because even at a low temperature, small amount of water remains liquid in the cell tissues, where it completely freeze only at high negative temperature. Fluctuation of temperature may increase the amount of unfrozen liquid and as the temperature decreases causes it is precipitate in the form of ice-crystal to the deteriment of the frozen food. Temperature fluctuations also causes increased evaporations. In addition, it is possible to obtain scientifically exact information concerning fat oxidation enzyme activity, texture, absorption of light and proteins breakdown. Such determinations are however, too complicated and time consuming to be used for on the spot inspection.

In recent year the scope of the method for low temperature fish has been further explored (Pearson and Muslemuddin, 1969,a): The TVN increases only slowly during the chilled storage of most fresh water fish (Nair et al., 1971). In species such as most marine fish and pike among the fresh water fish, which contained substantial amounts for TMAO, the determination of the degradation product, TMA has been used extensively as a more specific index of spoilage.

The relative rate of spoilage chart from 1°C to 25°C is shown in Tables 18 and 19. From which the deteriorative changes of the fishes could be measured in the related fish species.