

CHAPTER - 2

STUDIES ON PHYSICAL AND CHEMICAL QUALITIES OF DIFFERENT
ORGANS AND MUSCLE TISSUE OF HILSA FISH (HILSA ILISHA,
HAM-BUCH) STORED AT LOW TEMPERATURE

INTRODUCTION

Hilsa fish, the Indian shad, (Hilsa ilisha, Ham-Buch) are abundant during monsoon months of each year in the middle and lower stretches of the Ganga river (Jhingaran, 1977) and lower stretches of Narmada river (Joseph 1967). In last few years, hilsa had shown marked fluctuations in landing. Considering the abundance of this fish during monsoon period its proper utilization as an exportable commodity is of utmost importance.

Icing and storage at low temperature is a common practice for the extension of shelf life of fatty species like Hilsa. Storage at low temperature can only retard but cannot completely stop the spoilage. During storage at low temperature microbial growth occurs and pH value increases in fish resulting in undesirable physical and chemical changes, ultimately leading to spoilage (Bachhil, 1982). Fatty fishes have generally a shorter storage life than the leaner ones. Therefore, a thorough knowledge of storage qualities of hilsa fish is necessary for its effective utilization after storage.

As fish spoilage begins, various physical changes become apparent, odour deteriorates, the surface becomes

dull and the shine on it is reduced; the flesh softens; the eyes and pupils become cloudy and reddish colouration develops along the backbone (Bramsnaes, 1955). For the routine assessment of the degree of spoilage in a fish, sensory inspection of different organs is a suitable method. The odour and sight of food are common indicators of acceptability. Such sensory methods are widely employed industrially for quality control in grading the marketability of fish produced (Muller, 1977). Most of the consumers are frequently guided by such sensory inspection in deciding the choice of the food and food products.

The 9-point hedonic rating scale for overall acceptability of fishes is commonly employed in research laboratory for differentiating various fish samples (Peryam and Pilgrim, 1957). In addition to 9-point hedonic rating, sensory evaluation of organoleptic qualities was carried out in the case of different organs of this fish during storage at 0°C temperature.

Generally, fish are kept within ice for preservation, storage and transportation, but due to short supply of ice and its melting, fish and fishery products are usually transported in refrigerated vehicles. Nicol (1959) reported that foodstuffs should always be maintained below 3°C to avoid proliferation of pathogenic bacteria. Since fish bodies are poor conductors

of heat and any large mass will cool down slowly, it is desirable to know the pattern of spoilage of fish and changes in physical as well as chemical properties of hilsa fish at different low temperature. This information will help to determine conditions of storage, transport, distribution and marketing of stored fish as well as preserved products thereof.

MATERIAL AND METHODS

Twelve (average length 24 cm) iced organoleptically fresh hilsa fish, Hilsa ilisha (Ham-Buch) were procured from local market in the morning and brought to the laboratory for experiment. The fish were washed, gutted and washed again. Samples were stored at 0°C temperature for the assessment of physical and chemical quality. Every time two samples were taken from storage temperature of 0°C at intervals of 1, 8, 12, 16, 21 and 27 days respectively.

TOTAL VOLATILE NITROGEN (TVN)-& TRIMETHYLE AMINE (TMA)

PREPARATION OF EXTRACT

The extract was prepared by mixing 2 gm of the minced sample with 25 ml of 5% TCA in a mortar. After 20 minutes the samples were filtered through a filter

paper (Whatman-1) 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 TVN and TMA values. In this method boric acid solutions was used in the central compartment of the Conway dish, which was titrated against N/70 H₂SO₄ solution.

MOISTURE

Moisture content was determined by drying the samples at 100 to 105°C temperature till a constant weight was reached (AOAC, 1960).

pH

Two gms. of fish muscles were macerated in 10 ml of redistilled water. The pH was measured at room temperature with a pH meter, using a glass electrode. Each determination was performed in duplicate (Poulter, et al., 1978).

FREE FATTY ACID (FFA)

The free fatty acid content was assayed with oleic acid (1 ml 0.1 M sodium hydro-oxide = 0.0282 gm

oleic acid), as per Pearson (1976).

STATISTICAL METHOD

Co-relation coefficient and regression analyses (Mian and Miyan, 1984) was carried out. Figures 1 to 5 shows the relationship between observed parameters with storage period in day.

OVERALL ACCEPTABILITY

Nine point hedonic scale was used to judge the overall acceptability. Scores were made using the hedonic ratings scales as a measure by preference in consumer like category (Howgate, 1978).

RELATIVE RATE SPOILAGE CHART

Spencer and Baines (1964) have shown that the spoilage rate could be expressed by the formula $U = V(1+C.Q)$. Where, U = rate of spoilage at particular temperature, V = rate of spoilage at 0°C, Q = storage temperature 1 to 8°C, C = constant upto 8°C. This constant is approximately 0.25. The values of total volatile nitrogen were used in building up the relative rate spoilage chart.

ORGANOLEPTIC SCORING METHOD UTILIZED

(Point-wise characters)

According to Shewan and Jones (1957); Howgate (1978)

Eye		Dull, slightly milky white	7
Bright, clear & full	10	Watery colour/white	6
Slightly dull	9	Slightly misty/milky white	4
Moderately dull	8	Pale/white	2
Dull	7	Extremely pale/more white	0
Slightly sunken	6		
Moderately sunken	4		
Sunken	2	Fresh	
Completely sunken	0		
		Translucent	10
Body surface		Loss of translucence	9
		Slightly dull	8
Shining bright	10	Moderately dull	7
Slight loss of brightness	8	Dull	6
Loss of brightness	6	Dull red	4
Slightly dull	4	Loss of redness/slightly waxy	2
Dull & slightly reddish of tail	2	Least redish & loose	0
Completely dull	0		
		Gill	
Pupil		Bright red	10
Brightly transparent	10	Slightly dulled, pale	9
Slightly dull & misty	8	Slightly pale/white	6
		Pale/white	5

Table-1 : Changes the bio-chemical properties of hilsa fish stored at 0°C temperature.

Storage days	TVN mg.N/100 gm of fish flesh	TMA mg.N/100 gm of fish flesh	Moisture %	pH values	FFA (as % oleicacid)
1	12.43	5.88	78.48	5.9	0.76
8	16.52	6.95	76.98	6.3	1.15
12	24.31	4.21	76.45	6.5	1.26
16	30.73	7.62	74.56	6.7	1.12
21	41.57	9.11	72.69	6.6	1.36
27	54.72	8.56	72.18	6.8	1.58

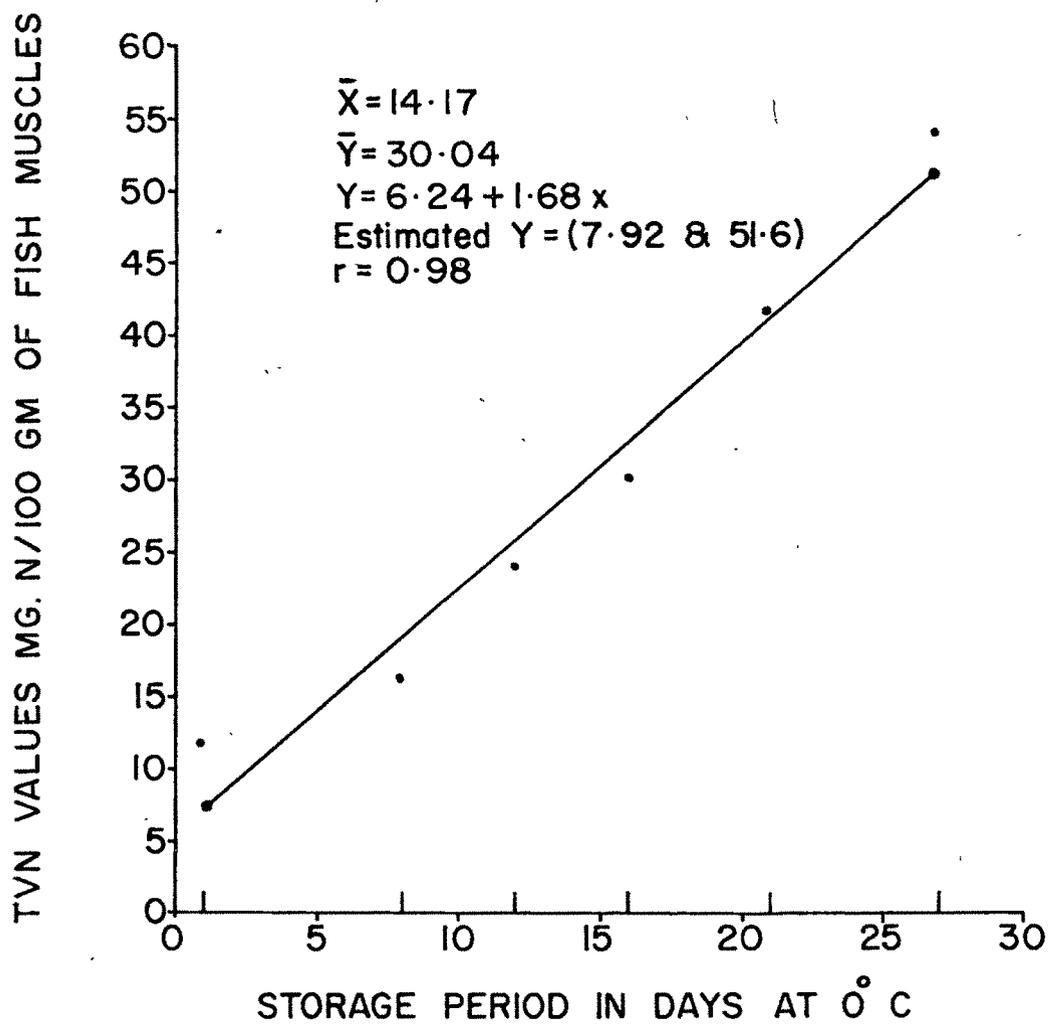


FIG. 1 TVN VALUES VERSUS STORAGE PERIOD
(REGRESSION LINE OF Y ON X)

Browish/greyish	4	Toughness	
Dark colour	2	Extremely tender	1
Blackish	0	Very tender	2
		Tender	3
Belly wall		Slightly tough	6
Normal flesh	10	Tough	7
Slight discolouration	8	Very tough	8
More discolouration	6	Extremely tough	9
Slightly digested	4		
More digested	2	Odour	
Extremely digested	0	Absent	0
		Slight	2
Texture		Moderate	4
Firm elastic	10	Strong	6
Rigor mortis	8	Very strong	8
Just post rigor stage	6	Extremely strong	10
Slightly soft	4		
Moderately soft	2	Overall acceptability	
Soft and loose	0	Like extremely	9
		Like very much	8
Dryness		Like much	7
Sloppy, watery	0	Like slightly	6
Juicy, moist	1	Neither like nor dislike	5
Slightly dry	2	Dislike slightly	4
Dry	3	Dislike very much	2
Extremely dry	4	Dislike extremely	1

Flavour		Firmness	
Absent	0	Very soft	0
Very slight	1	Softer than normal	1
Moderate	3	Firm	2
Strong	4	Slightly firmer than normal	3
Very strong	5	Slightly tough	4
		Tough	5
		Extremely tough	6

ORGANOLEPTIC METHODS (SENSORY EVALUATION)

The under mentioned score sheets were used, which are mainly based on the score sheets of Shewan and Jones (1957) and Howgate (1978). In the present work the score sheets were slightly modified to meet out requirements. In the sensory scoring technique, the fish were judged on the basis of following factors.

GENERAL APPEARENCE

Eye, pupil, gill, body surface, flesh, belly wall, texture, odour, flavour, dryness, firmness, toughness and overall acceptability. Each of the factors was given pointwise scores. Organoleptic scoring method was utilized.

RESULTS AND DISCUSSION

TOTAL VOLATILE NITROGEN (TVN)

The total volatile nitrogen value gradually increased with the passage of storage time, ranging from 12.43 to 54.72 mg.N/100 gm of fish flesh over the period of 1 to 27 days of storage at 0°C temperature (Table-I). When the TVN values exceeded 41.57 mg.N/100 gm of fish muscle by 21 days of storage the

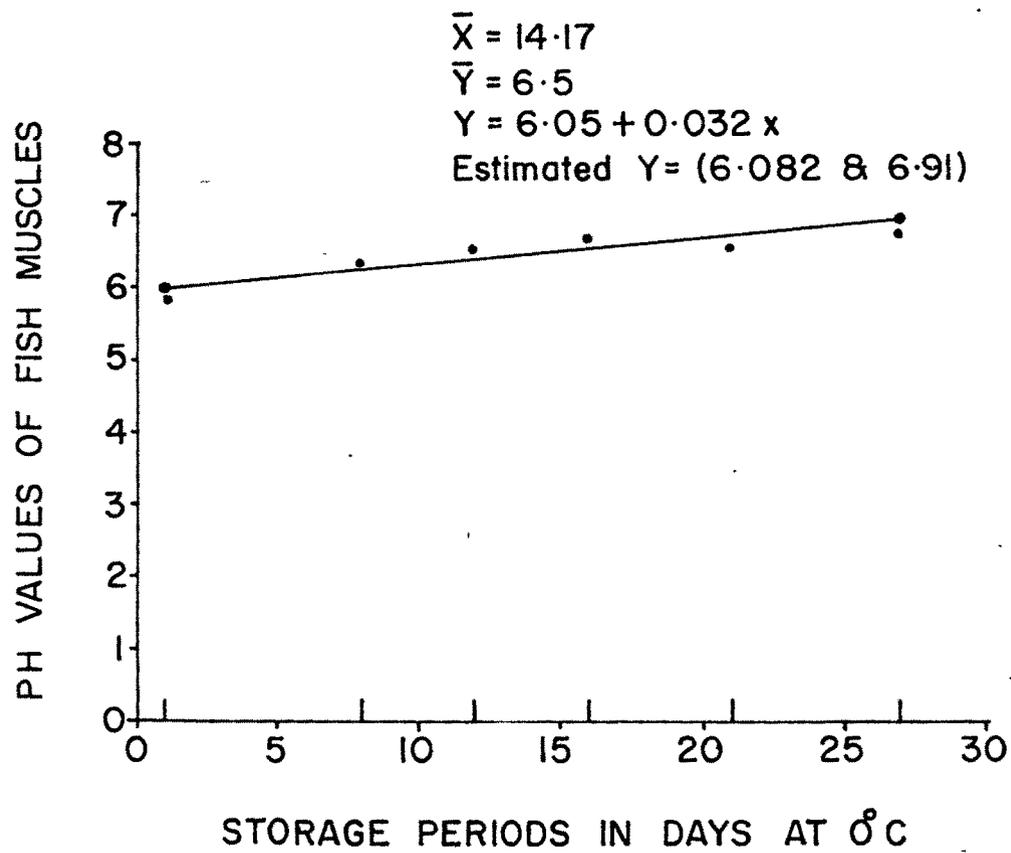


FIG. 3 PH VALUES VERSUS STORAGE PERIOD
(REGRESSION LINE Y ON X)

fish became unacceptable (Table-1). Wang *et al.*, (1967) reported that spoilage of fish stored at low temperature is due to bacterial and enzymatic action, which results in the production of various volatile compounds. The TVN value does not noticeably increase until the fish is very stale. Though the TVN value increases during the initial storage period, the same markedly increased at later stage of storage (Table-1). Ronsivalli *et al.* (1965) reported that volatile nitrogen values did not give proper indication until actual spoilage occurred. The slower rate of spoilage in the early storage periods was perhaps due to suppression of microbial growth and also due to retardation of enzymatic action at low temperature as opined by Branstedt and Aurbach (1961). At this stage only autolytic changes dominate (Tressler and Lemon, 1951).

TRIMETHYLE AMINE (TMA)

The results obtained for TMA values are represented in table-1. TMA values increased from 5.88 to 8.56 mg.N/100 gm of fish muscle during 27 days of storage. Jacquot (1961) reported that the fish tissues register considerable increase in the di- and trimethyl forms as a consequence of the reduction of oxide into free bases.

Fluctuations of TMA values were observed from 12 days to 27 days of storage, ranging from 4.21 to 9.11 mg.N/100 gm in case of fish muscle. However, the variation TMA values did not show any orderly pattern over the storage period (Table-1). According to Howgate (1978) also though trimethylæ amine content increase during spoilage due to storage, the rise is not significant. Baldwin (1957) was of the opinion that TMA values are not a proper indication of spoilage. Most of the fishery research stations maintain that fish is stable when the rate of TMA production begins to be appreciable and this corresponds well with TVN value exceeding 30 mg.N/100 gm of fish muscles (Pearson and Muslemuddin, 1971).

MOISTURE LOSS DURING STORAGE

The rate of loss of moisture observed here is presented in table-1. From the initial values of 78.48%, the moisture content was reduced to 72.18% by 27 days of storage. The magnitude of loss of moisture content due to storage at 0°C over the period of 27 days was found to be not so great. This is probably so because storage at 0°C lead to freezing of muscle water during the storage. Here, it may mentioned that dehydration of fish at low temperature of storage is dependent on the thickness of layer of muscle adhering to

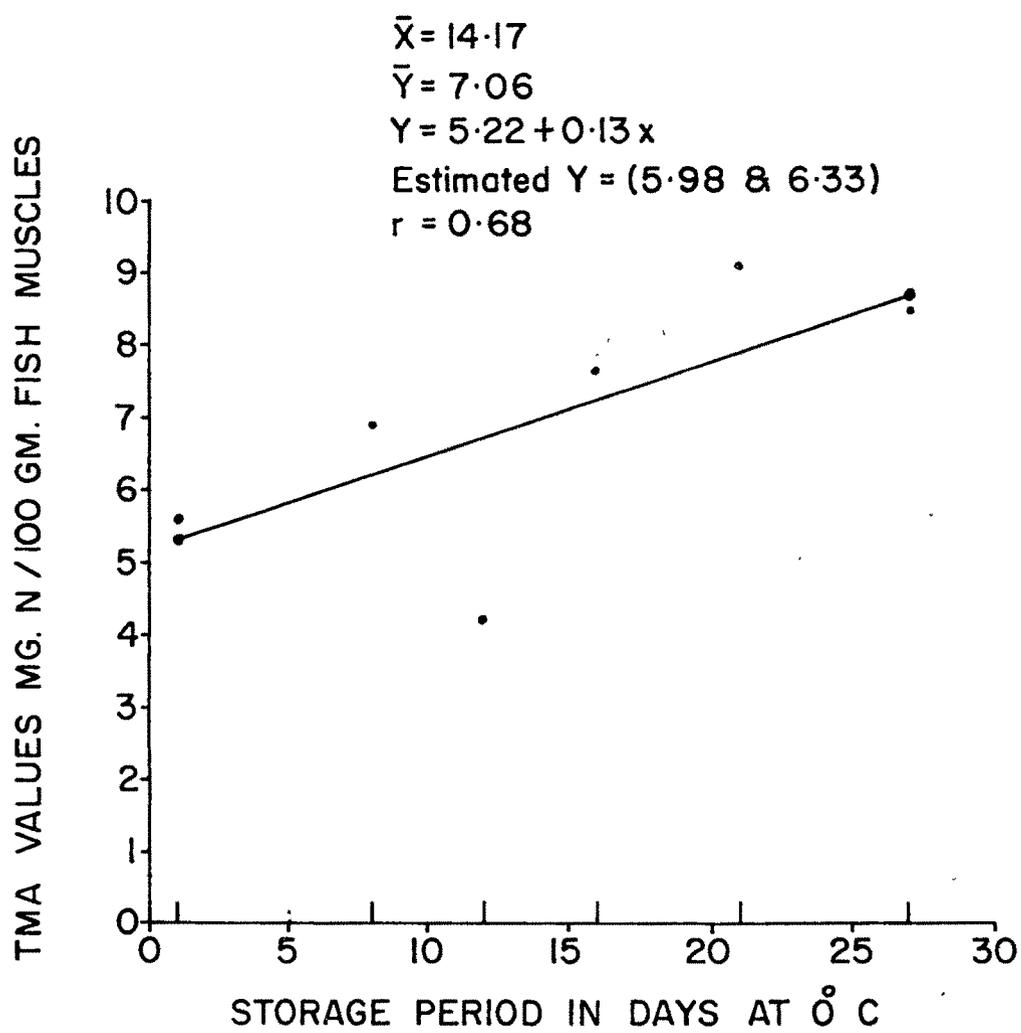


FIG. 2 TMA VALUES VERSUS STORAGE PERIOD
(REGRESSION LINE OF Y ON X)

the skin (Power et al., 1969). Thicker the layer, less is the dehydration and in case of Hilsa the muscle layer adhering the skin is found to be very thick.

pH VALUES

Table-1 shows that the pH values showed upward trend after a week of storage and reached the value of 6.8 in 27 days. According to Baldwin (1957) when the pH value exceeds 6.3 the fish is to be considered as spoiled Amlacher (1961) also reported that pH value increases with the increase in the period of storage. At the time of death the pH value is generally around 7.0 but often it may be higher or lower. Amlacher (1961) has also reported that certain fish species with a more acidic pH value 5.6 may also have good storage quality. Mestorff (1954, a & b) reported that pH value after 9 days of catch ranged between 6.8 to 7.0. Normally, the pH is 6.2 to 6.6 at the rigor stage and at this stage possible growth of micro-organisms is mainly responsible for fish spoilage (Reay and Shewan, 1949). Hjorth-Hansen (1943) suggested that pH in some species of fish kept in ice could be 5.57, about 11 days after death. Van-Deures and Hoff Joregenson (1936) proposed an upper limit of pH 7.5 for acceptability and found that spoilage ran parallel to the mounting pH of the muscle tissue. In the present investigation too,

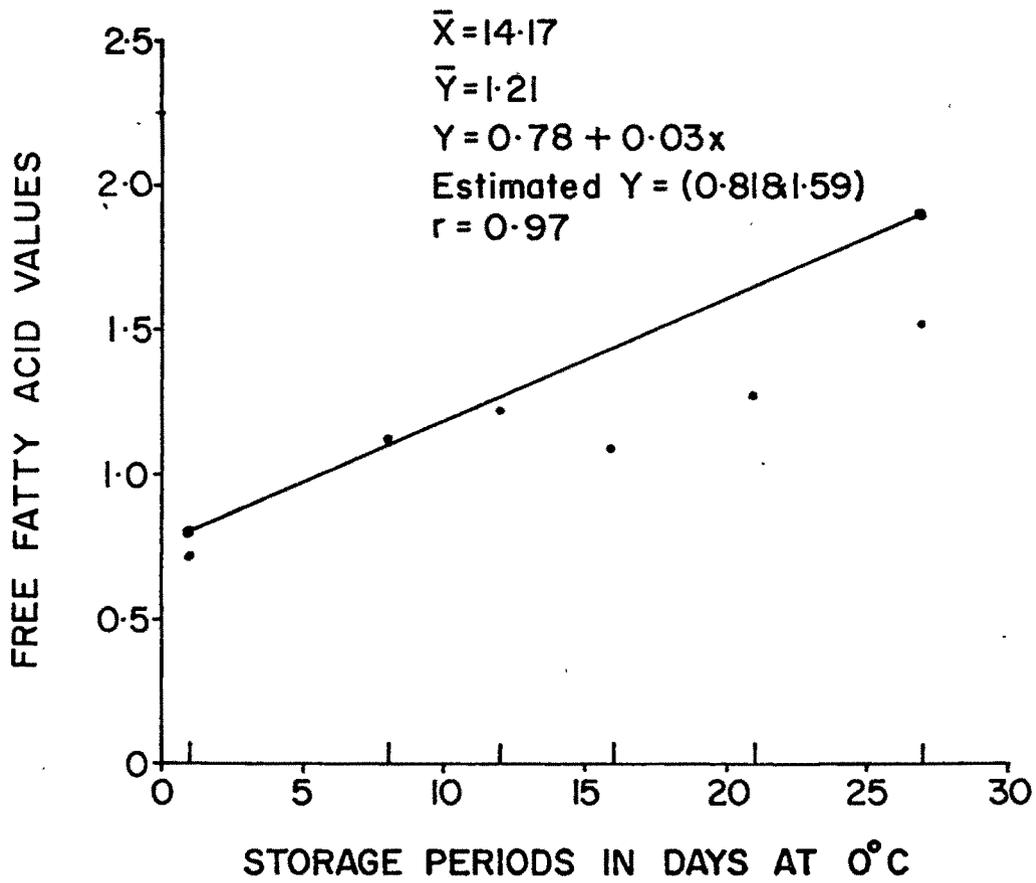


FIG. 5 FREE FATTY ACID SCORE VERSUS STORAGE PERIOD (REGRESSION LINE Y ON X)

gradual and noticeable rise in pH value was noted.

FREE FATTY ACIDS

During the course of present investigation it was observed that samples stored at 0°C temperature showed an increase in free fatty acid content of hilsa flesh over the storage period. It is obvious from table-1, that within first 7 days the rise was marked but thereafter the fluctuation was marginal. The maximum FFA level reached was 1.58% in 27 days of storage (Table-1). The FFA values continued to increase at a much slower rate during continued storage. Lessen *et al.*, (1951) found a rise in free fatty acids in fish during storage from 10.1% after 24 hours and it gradually increased in the following order 2.5, 7.8 and 8.1% after 120 hours of being kept at room temperature. Similar observations regarding gradual increase in free fatty acids have also been reported in case of different species of fish under storage (Dyer and Dingle, 1961 and Ona, 1935). Callow (1952), reported that fat hydrolysis and production of fatty acid appears to be retarded by cold storage. From the present study it is obvious that storing Hilsa ilisha at 0°C considerably retards the formation of FFA, free fatty acid though initially the rise is not suppressed.

Table-2 : Physical quality changes of hilsa fish stored at
0°C temperature

Storage period in days	Eye	Pupil	Gill	Body surface	Flesh	Belly wall	Texture	Odour	Flavour	Firmness	Dryness	Toughness	Overall acceptability
1	9	8	10	10	9	10	8	0	0	1	0	3	8
8	8	7	9	8	7	8	8	0	0	2	2	6	7
12	7	7	6	6	7	8	6	2	1	4	2	6	7
16	4	4	5	4	6	6	6	2	3	4	2	6	6
21	4	2	2	4	6	4	4	2	3	5	3	7	5
27	2	2	2	2	4	4	4	4	3	5	3	7	4

PHYSICAL QUALITY CHANGES

Results of sensory evaluation of fish stored at 0°C temperature showed significant difference which are presented in Table-2. It was found that lower storage temperature was effective in keeping the fish in sufficiently good condition upto 16 days. Later on, it gradually deteriorates and at end of 21 days it was unacceptable. A gradual decrease in physical qualities occurred and the chemical qualities deteriorated. The early drop in sensory score was due to loss of flavour rather than the development of abnormal flavour usually associated with bio-chemical changes. The texture grades of the samples decreased with the increasing time of storage. Similar pattern was found for other sensory criteria except firmness and toughness (Table-2). At the end of storage period the surface lost its brightness and became gradually sunken, the pupils became cloudy and white and the cornea became opaque. At first, the gills were seen to assume bright red colour finally turning to dark colour. The flesh gradually softened and became dull red. Belly wall exhibited slightly digested appearance. A gradual and moderate change in odour and flavour became evident, the scores for firmness and toughness increased. Nair et al., (1971) mentioned that bacterial spoilage produces more undesirable changes in the flavour, odour and overall physical acceptability of

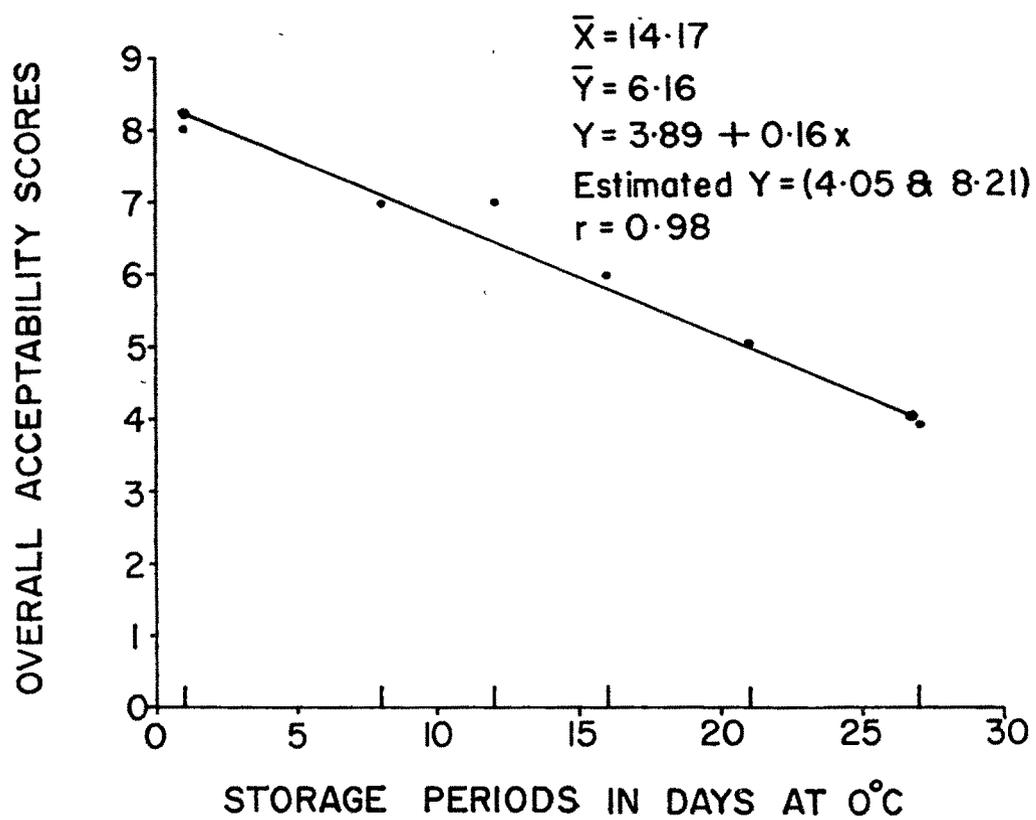


FIG. 4 OVERALL ACCEPTABILITY SCORE VERSUS STORAGE PERIOD (REGRESSION LINE Y ON X)

fishes. From the present observations, both the basis of hedonic scale scoring as well as alterations of chemical qualities, it could be seen that Hilsa ilisha fish can be stored at 0°C within limits of consumer acceptability upto a maximum period of 21 days only.

LIMITS OF ACCEPTABILITY AT DIFFERENT TEMPERATURES

It is observable from statistical analysis and the Relative Rate of Spoilage Chart extrapolated on the basis of TVN values obtained at 0°C for temperature range 1 to 8°C for hilsa flesh (Fig. 6) that when the TVN value exceeded 41.57 mg.N/100 gm the fish became unacceptable. It is also observed that the rate of deterioration of the fish was directly related to the storage temperature. Whereas in case of several other fish species many workers (Tanikawa, 1935; Shewan, 1938 and 1942; Stansby et al., 1944; Ota, 1958) suggested that 30 mg.N/100 gm fish muscles should be taken as the upper limit of acceptability. On the relative rate of spoilage chart (Fig. 6) a limitation line was drawn at the TVN value of 41.57 mg.N/100 gm of hilsa fish was within the acceptable limit in a range of 1°C to 8°C temperature. Such a limitation line on the chart with storage time in days could be used for predicting quality and shelf-life of the hilsa fish at particular storage temperatures. Burgess and Cutting (1960) suggested that such

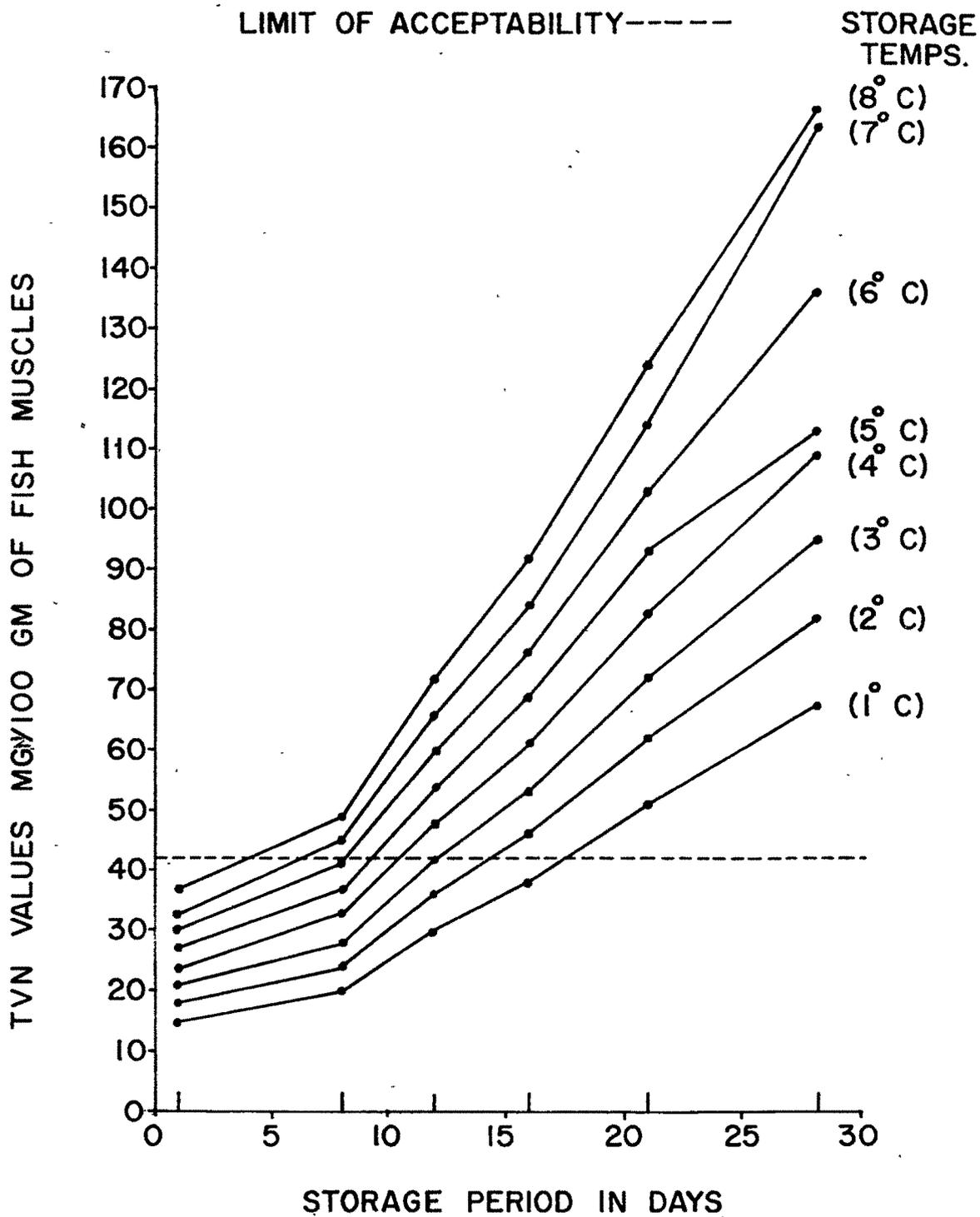


FIG. 6 RELATIVE RATE SPOILAGE CHART OF HILSA FISH (HILSA ILISHA) AT 1° C TO 8° C TEMPERATURE

acceptable limit values help in fish loading at the port, subsequent rail transport and display in the retail shops. Fig. 6 shows the limit of acceptability.

On the basis of the results obtained here it may be concluded and recommended that after 21 days of storage at 0°C the fish becomes unacceptable to consumers in the light of bio-chemical tests and sensory evaluations. Comparative low temperatures suppress the formation of volatile compound and less abnormal flavour and odour are developed. Only sensory method is not a reliable index for quality judgement in preservation at low temperature. The relative Rate of Spoilage Chart is a useful guide for quality judgement and to determine the shelf-life of hilsa fish (Hilsa ilisha) at a particular storage temperature for those concerned with fish storage, preservation, distribution, transport and marketing etc.