INFLUENCE OF COLLAGEN CONTENT ON THE TEXTURE AND STORAGE QUALITY OF FISH MINCE BASED PRODUCTS

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BY
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CERTIFICATE

This is to certify that this thesis is an authentic record of research work carried out by Smt. Femeena Hasssan, M.F.Sc. under my supervision and guidance in the School of Industrial Fisheries, Cochin University of Science and Technology in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** and that no part thereof has been submitted for any other degree.

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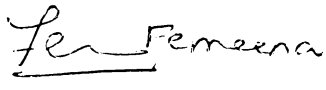
DECLARATION

I, Femeena Hassan, do hereby declare that the work presente in this thesis is the result of my own investigation and neithe the thesis nor any part thereof has been accepted, nor is bein submitted for any other degree. All the sources of informatio have been duly acknowledged.



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CONTENTS

CHAPTER 1

INTRODUCTION

The total landings from all fishery sources have been expanding to satisfy the increasing world demand for fish, shellfish and fishery products for food, for production of feeds for agriculture, aquaculture and for other industrial purposes. With increasing demand it seems logical to encourage optimum but economic utilisation of all current fishery resources for food and also to investigate additional resources.

The interest in producing minced meat arose from the need to make better use of fish available. Though many of the world's fisheries are suffering from overfishing, it is equally true that fish caught is not used as effectively as it should be. The technique of producing minced fish meat provides a solution for effective utilisation of these fishes. There is a recent trend towards the develpoment of flesh food based convenience food in the market. But the resulting product should be price competetive and meet consumers needs.

The advent of mechanical deboning of fish can be regarded as an important milestone in fish utilisation (Rao *et al*. 1990). Fish

mince is a raw material which has attracted considerable attention from food manufacturers throughout the world (Rodger *et al.*, 1980). According to Abraham *et al.* (1991), minced fish is an important ingredient in the production of a variety of seafood products in many countries for its several advantages such as better yield, easiness in incorporation with stabilisers, flexibility in product preparation and suitability in blending. Minced fish flesh is widely used as an intermediate product for fabricated foods including kamaboko, fish sausage, imitation products, fish ball, fish burger, fish stick and similar products (Dora *et al.*, 1991).

The minced fish, which is comminuted fish flesh free from the skin, bones, scales and fins of a wide range of fish species is a versatile but unstable commodity. Several studies have been done on the physico-chemical and organoleptic properties of fish mince on frozen and cold storage (Navikov, 1982; Perigreen, 1981). It is well known that once water binding and fat emulsifying capacity is lost, the mince is not at all suitable for product development. Studies have already been done on the preparation of minced meat from various fishes, its storage qualities, effect of certain additives like starch, cryoprotectants, antioxidants etc. Minced fish is found to have lesser frozen life compared to fillets and

whole fish (Joseph *et al.*, 1986). According to them, the mixing of bone marrow exudate, catheptic enzymes, enzymes from blood, lipids and inorganic constituents in the minced fish affect texture, flavour and appearance and reduce shelf life during frozen storage. The contamination of mince with skin, pigments, dark meat and blood reduce the colour. Washed mince is found to be less susceptible to deterioration during frozen storage. Washing not only removes fat and undesirable matter such as blood, pigments and odourous substances, but increases the concentration of myofibrillar protein and thereby improves gel strength and elasticity. This washed (bleached) minced meat is called surimi. With the commercial production of value added products, the demand for this proteinaceous base material has increased tremendously.

The connective tissue proteins are chiefly composed of stroma proteins and these contribute only a small fraction of the total protein content in fish meat. However, they are involved in holding together the muscle bundles (myotomes) of fish, and therefore should contribute to the overall texture of the meat. Among the connective tissue proteins, collagen is found to be the major protein and is found to influence the texture of fish meat considerably.

The texture of seafood products should not be regarded as of minor importance to the overall consumer acceptance. Any food product must have a certain degree of toughness as decided by the consumer. Further, textural aspects such as toughness or softness, elasticity and gellying properties are important considerations in the production of meat analog and other seafood products.

Objectives of the present study are

-To find out the proximate composition of 20 commercially important tropical fish species on the west coast of India.

-To determine the collagen content in these commercially important fish species and fractionation of collagen into acid soluble collagen (ASC) and hot water soluble (insoluble) collagen (ISC).

-To classify fishes according to its collagen content.

-To study the different storage characteristics in the mince based product-surimi, from different species of fishes.

-To find out a suitable collagen source to incorporate in surimi.

-To study the different storage qualities in the mince based product, surimi at different levels of collagen in different species of fishes.

-To find out optimum collagen level to get desirable texture and storage quality for mince based product.

-To develop some products from surimi with desirable level of collagen.

-To compare the products prepared from surimi of lesser collagen content fish containing desirable level of collagen with surimi prepared with high collagen content fish without collagen.

This study gains in importance as there is little information on the collagen content of different species of fishes in India. So far no attempt was made to classify fishes according to its collagen content.

Consequent to urbanisation the preference of people shifted from raw fish to ready to eat fishery products. The commercial success of mince based industry will depend greatly

upon how exactly it can absorb the existing technology as well as how innovately it can develop new products which will compete with existing products in the market. In the present study an attempt was made to achieve the above said objectives.

In tropical developing countries, fish and fishery products are important source of animal protein. Hence it is anticipated that mince based products will receive greater attention for further development, in line with the growing need for product diversification to meet the increasing demand of different ethnic groups.

CHAPTER 2

COMPOSITION OF FISH MUSCLE WITH EMPHASIS ON THE CONTENT OF COLLAGEN

2.1 *INTRODUCTION*

Fish is a protein rich food material. Proximate composition analysis is important from nutritional and biochemical point of view. The proximate composition of fish is usually determined on the skin free, bone free fillet of the fish. The proximate composition of fish shows wide variations from one species to another, within the same species and in different portions of the body of the same fish. Geographical locations of fishing ground, seasonal variation, age, size and sex of fish also affects the proximate composition. The important constituents of the fish muscle in their order of magnitude are moisture, protein, fat and minerals.

Stroma proteins refer to slightly soluble or insoluble proteins which are obtained after removing water and salt soluble proteins in the muscle. The main constituent of stroma protein is collagen. In India no systematic study has been conducted on the collagen content of fishes. In the present study collagen content of a few species of commercially important tropical fishes of

India are determined. An attempt is also done to classify these fishes based on the percentage of total collagen on total protein.

2.2 *REVIEW OF LITERATURE*

Muscle can be regarded as a two component system viz., the muscle fibers and the intra-muscular connective tissue (Rowe, 1974). The connective tissue proteins or stroma proteins refer to slightly soluble proteins which are obtained after removing water and salt soluble proteins in the muscle.

Stroma proteins have been considered to consist mainly of collagen and elastin and are the main constituents of intra-muscular connective tissue of fish (Dyer *et al.*, 1950; Hatae *et al.*, 1984; Sato *et al.*, 1988). However it has been pointed out that, connectin, a myofibrillar protein, also comes in the stroma fraction of mammalian (Maruyama *et al.*, 1976), avian (Maruyama *et al.*, 1977) and fish (Kimura *et al.*, 1981) muscle. Therefore, the muscle stroma contain not only connective tissue proteins but also some myofibrillar protein. In addition, the constituents of muscle stroma varies with the preparation procedure (Hashimoto *et al.*,1979).

The content of collagen has been estimated by different methods (Lowry *et al.*, 1941; Baker, 1954; Gustavson, 1956; Adams *et al.*, 1960; Kubota, 1967; Grand *et al.*, 1975, Culler *et al.*, 1978 and Sato *et al.*, 1988). Lowry *et al.*, in 1941 quantitatively estimated collagen by dissolving it in water by autoclaving. The nitrogen content of the gelation formed was used as a measure of collagen content. Baker (1954) described a procedure to determine collagen by estimating its hydroxyproline content. Takahashi *et al.* (1954) demonstrated that there was a direct relationship between the hydroxyproline content of collagen and its shrinkage temperature. Gustavson (1956) also has reported on the importance of hydroxyproline as an important ingredient in fish collagen. According to Eastoe (1957) though the amino acid composition of fish collagen resemble those of mammalian collagen, the contents of proline and hydroxyproline were comparatively less and the contents of serine and threonine were comparatively more in fish collagen. He also reported that fish collagen is less stable and more easily dissolved than mammalian collagen.

The collagen in fish muscle has been estimated mainly on the basis of hydroxyproline content in the hydrolysate of fish muscle by Nasedkina *et al.* (1972), Kubota *et al.* (1975) and Hatae *et al.* (1986). However, these methods isolate collagen in pure

form and determine the hydroxyproline content in collagen. The isolation of collagen in pure form from fish muscle is considerably complicated. Moreover, since there is small but measurable amount of free and peptide forms of hydroxyproline in fish muscle the non-collagenous hydroxyproline might affect the collagen content. Also the exact content of collagen could not be determined in the case of samples in which the hydroxyproline content is unknown, as hydroxyproline content varies with fish species (Kubota *et al.*, 1975; Yamaguchi *et al.* 1976). The distribution of collagen types in the muscle of fishes has not been investigated because it is difficult to isolate the native collagen in pure form due to the presence of large amount of non-collagenous proteins, mainly consisting of myofibrillar proteins. Yoshinaka *et al.* (1985) prepared crude stromata as the residual fraction of the salt extracted muscle of carp and Japanese mackeral, and it was divided into alkali soluble, acid soluble and autoclave extractable fractions.

Sato, 1988, showed that the collagen in fish muscle was quantitatively recovered only in the acid soluble and hot water soluble fractions, after extraction with water and alkali. He found that, the total collagen content in the muscles of rainbow trout, Japanese mackeral, carp and eel were 0.47, 0.5, 0.6 and

1.99 percentage by weight of the wet muscle respectively. They also determined the contents of acid soluble and insoluble collagen in the muscle and compared to the swimming movement of fish and to the texture of sliced raw meat. The total collagen was found to vary in the range from 0.34 to 2.19% of the wet tissue weight. They also noted that the solubility of muscle collagen of these fishes was generally much higher than that reported for the muscle collagen of mammals. It was also found that the musculature of fish with flexible body comprises a high proportion of collagen.

Sato *et al.*, 1989, determined the hydroxyproline content in the acid soluble collagen and showed that it varied with species in the range from 4.7 to 10%. They also reported that the factor for converting hydroxyproline content of collagen varied in the range from 10-21.3. Therefore, the same factor cannot be used for different species to estimate collagen on the basis of hydroxyproline content. The different types of collagen present in different tissues were studied by Kimura *et al.* (1988), Yoshinaka *et al.* (1988) and Sato *et al.* (1989).

Montero *et al.* (1989a) in their study, found alterations occuring in hake muscle collagen during frozen storage as a result of processing and seasonal influences.

2.3 MATERIALS AND METHODS

2.3.1 Raw material

For the study, twenty commercially important fish species of different habitats were used. The marine fishes used were Pomfret white (*Pampus argenteus*), Sardine (*Sardinella longiceps*), Mackeral (*Rastrelliger kanagurta*), Sole (*Cynoglossus semifaciatus*), Vatta (*Caranx spp.*), Ribbon fish (*Trichiurus savala*), Anchovy (*Anchoviella*), Veloori (*Kowala kowal*), Kilimeen (*Nemipterus japonicus*), Tuna (*Euthynnus affinis*), Whiting (*Sillago sihama*), Ray (*Himantura*) and Shark (*Scoliodon sorrakowah*). The brackishwater fishes include Tilapia (*Oreochromis mossambicus*), Palankanni (*Megalops cyprinoides*), Paral (*Barbus spp.*) and Mullet (*Mugil cephalus*). The freshwater used were Common carp (*Cyprinus carpio*), Rohu (*Labeo rohita*) and Catla (*Catla catla*). The marine fishes were caught off the coast of Cochin and were collected from the Cochin Fisheries Harbour. The brackishwater fishes were collected from the culture ponds of Matsyafed at Narakkal, Kerala

and the freshwater fishes from M/s Pookote Fish Farm, Trichur, Kerala. The fish caught were immediatley chilled in ice and brought to the laboratory for analysis. In the case of the marine fishes and the brackishwater fishes the analysis was started within an hour of collecting the samples. The analysis for the freshwater fishes was carried out within two hours of collecting the sample.

2.3.2 Sample preparation for chemical analysis

For the study three lots of each species were used, each lot weighing about one kilogram. The fishes were properly washed with potable water, skinned and filleted. The white muscle from the dorsal part of the trunk was used for determining the proximate composition and estimating the collagen content. Each analysis was done in triplicate.

2.3.3 Proximate composition

Moisture content was determined using 20gm of uniformly minced fish meat according to the method of AOAC (1984).

A sample of one gram of homogenised fish was used for determining the crude protein content. The nitrogen was estimated by Microkjeldahl method according to AOAC, 1984. The factor 6.25 was used for converting the total nitrogen content to crude protein content.

A sample of 10gm of fish mince was used for determining the fat content by the method of AOAC (1984).

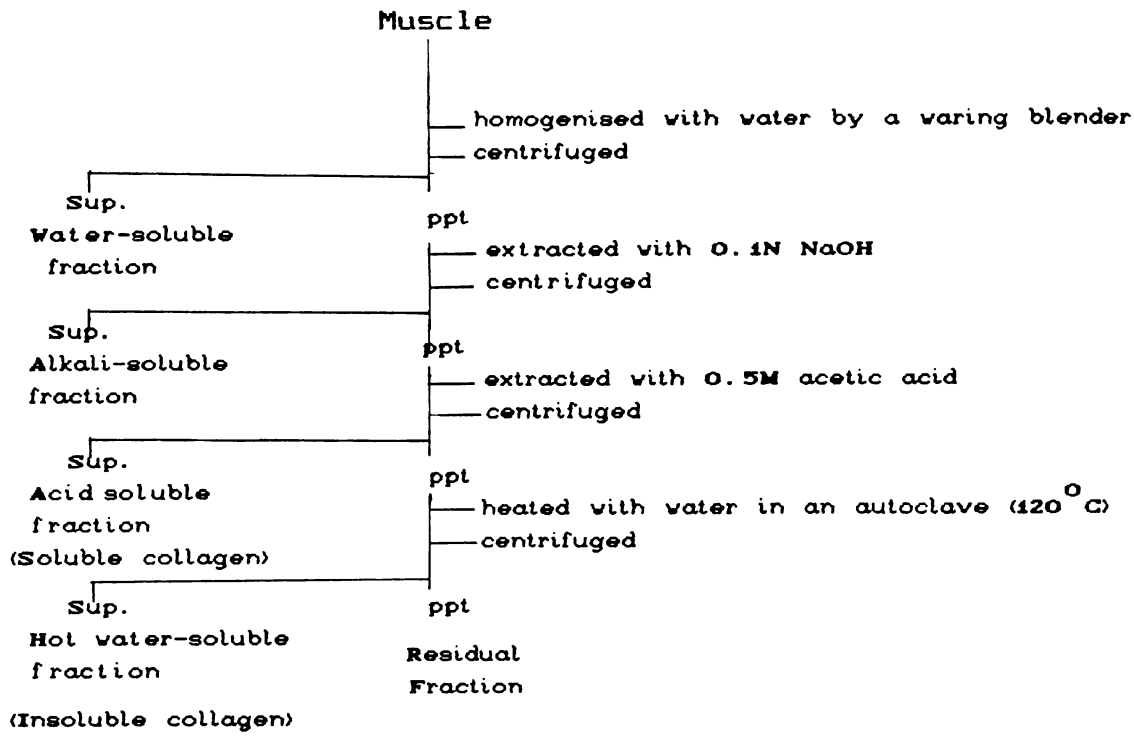
Total ash content was determined according to the method of AOAC (1984), using 5gm of fish mince.

2.3.4 Fractionation and estimation of collagen

The method of Sato (1988) was followed for extraction and estimation of collagen. About 75gm of fish fillets were uniformly minced using a meat mincer maintaining the temperature at 4^{O}C. From this minced meat 50gm was accurately weighed and blended with five volumes (V/W) of distilled water at 4^{O}C. The suspension obtained was centrifuged at 10,000 x G for 20 minutes in a refrigerated centrifuge (MB-20 Super Speed Refrigerated Centrifuge) at a temperature of 2^{O}C. The supernatent liquid which contained only soluble protein was discarded. To the

residue 20 volumes (V/W), of 0.1N sodium hydroxide was added. The suspension was stirred using a magnetic stirrer for sixteen hours, at a temperature of $8^{o}C$ ($\pm 2^{o}C$), followed by centrifugation at 10,000 x G for 20 minutes at $2^{o}C$. The alkali extraction was repeated three more times. Finally, the precipitate was washed several times with distilled water to remove traces of alkali. The alkali free residue of total collagen was suspended in 10 volumes of (V/W) 0.5M acetic acid. The suspension was kept stirred with a magnetic stirrer for three days at 8 $(\pm 2)^{o}C$, and then centrifuged at 10,000 x G for 20 minutes at $2^{o}C$. The process of acid extraction was repeated once more, with stirring for two days. The precipitate was washed free of acid by repeated washing with cold distilled water. The acetic acid extracts (supernatants) were combined to give acid soluble collagen (soluble collagen). The acid free residue was heated with five volumes (V/W) of distilled water in autoclave at $120^{o}C$ for one hour and centrifuged at 10,000 x G for 20 minutes. The supernatent consisting of hot water soluble collagen was separated. The precipitate was again extracted with hot distilled water. The supernatants were combined to give the acid soluble collagen fraction. The residue was discarded. The collagen contents of both soluble and insoluble fractions were determined by estimating total nitrogen content by the Microkjeldahl method of AOAC (1984).

Fig 2.3.4 Outline of fractionation procedure of muscle protein



The schematic representation of collagen fractionation is given in Fig. 2.3.4.

2.4 RESULTS

2.4.1 Species composition and proximate composition

The common name, scientific name, month of sampling and proximate composition of fishes studied are given in Table 2.4.1. The sampling of fish tissue for proximate composition analysis was done from the same part of fish (white muscle from the dorsal part) as the composition differs markedly from head to tail of fish and depends particularily upon the relative amounts of skin, dark muscle that are in the sample.

Of the fishes studied, the moisture content was maximum for anchovy (80.54%) and minimum for pomfret (70.86%). The protein content was maximum for whiting (22.9%) and minimum for tilapia (17.92%). The fat content was maximum for mackeral (7.5%) and minimum for shark (0.09%). The ash content was found to be high in sole (3.14%) and low in rohu (0.9%).

Table 2.4.1 PROXIMATE COMPOSITION (+/- S.D.) OF WHITE MUSCLE OF FISHES STUDIED

Common name	Scientific name	Month of sampling	Moisture gm%	S.D.	Protein gm%	S.D.	Fat gm%	S.D.	Ash gm%	S.D.
Pomfret white	Pampus argentius	MARCH	70.86	+/-2.01	18.98	+/-0.42	2.80	+/-0.002	3.00	+/-0.00002
Sardine	Sardinella longiceps	APRIL	77.36	+/-1.79	18.57	+/-0.31	3.20	+/-0.005	1.60	+/-0.00000
Mackeral	Rastrelliger kanagurta	MARCH	74.70	+/-1.82	19.53	+/-0.20	7.50	+/-0.005	1.65	+/-0.0000
Sole	Cynoglossus semifaciatus	JANUARY	72.38	+/-2.02	19.48	+/-0.22	4.70	+/-0.004	3.14	+/-0.00005
Vatta	Caranx spp.	JANUARY	75.80	+/-1.92	20.07	+/-0.28	0.50	+/-0.005	2.01	+/-0.00004
Ribbon fish	Trichurus savala	JANUARY	75.10	+/-1.86	21.98	+/-0.31	0.68	+/-0.0006	1.99	+/-0.00004
Anchovy	Anchoviella	JANUARY	80.54	+/-2.00	18.08	+/-0.28	1.02	+/-0.0005	1.58	+/-0.00002
Tilapia	Oreochromis mossambicus	MARCH	77.60	+/-1.76	17.92	+/-0.32	2.90	+/-0.005	1.10	+/-0.00000
Common carp	Cyprinus carpio	APRIL	75.20	+/-1.81	21.52	+/-0.41	3.50	+/-0.004	1.17	+/-0.00001
Rohu	Labeo rohita	APRIL	76.10	+/-0.90	18.90	+/-0.29	0.80	+/-0.0003	0.90	+/-0.0000
Palankanni	Megalops cyprinoides	JANUARY	75.90	+/-1.83	18.10	+/-0.32	1.10	+/-0.0004	1.00	+/-0.0000
Paral	Barbus spp.	MARCH	76.50	+/-2.01	18.10	+/-0.27	2.12	+/-0.003	1.40	+/-0.0000
Veloori	Kowala kowal	JANUARY	79.84	+/-2.10	18.12	+/-0.25	0.69	+/-0.0002	1.38	+/-0.0000
Kilimeen	Nemipterus japonicus	JANUARY	78.14	+/-2.36	19.32	+/-0.28	2.86	+/-0.005	1.58	+/-0.000
Mullet	Mugil cephalus	JANUARY	75.77	+/-1.76	20.22	+/-0.26	2.45	+/-0.005	1.62	+/-0.000
Catla	Catla catla	APRIL	76.00	+/-1.68	19.00	+/-0.32	1.30	+/-0.0004	0.90	+/-0.000
Tuna	Euthinus affinis	DECEMBER	74.85	+/-1.92	19.74	+/-0.28	3.98	+/-0.005	1.50	+/-0.000
Whiting	Sillago sihama	DECEMBER	75.02	+/-1.96	22.90	+/-0.41	0.30	+/-0.0005	1.73	+/-0.000
Ray	Himantura	JANUARY	75.25	+/-1.99	20.91	+/-0.42	0.50	+/-0.005	1.24	+/-0.00
Shark	Scoliodon sorrakowah	DECEMBER	72.00	+/-2.08	22.80	+/-0.42	0.09	+/-0.0001	1.50	+/-0.00

2.4.2 Collagen content in the white muscle of fishes

The contents of Acid Soluble Collagen (ASC), Insoluble Collagen (ISC) and Total Collagen (TC) of fishes studied are presented in Table 2.4.2.

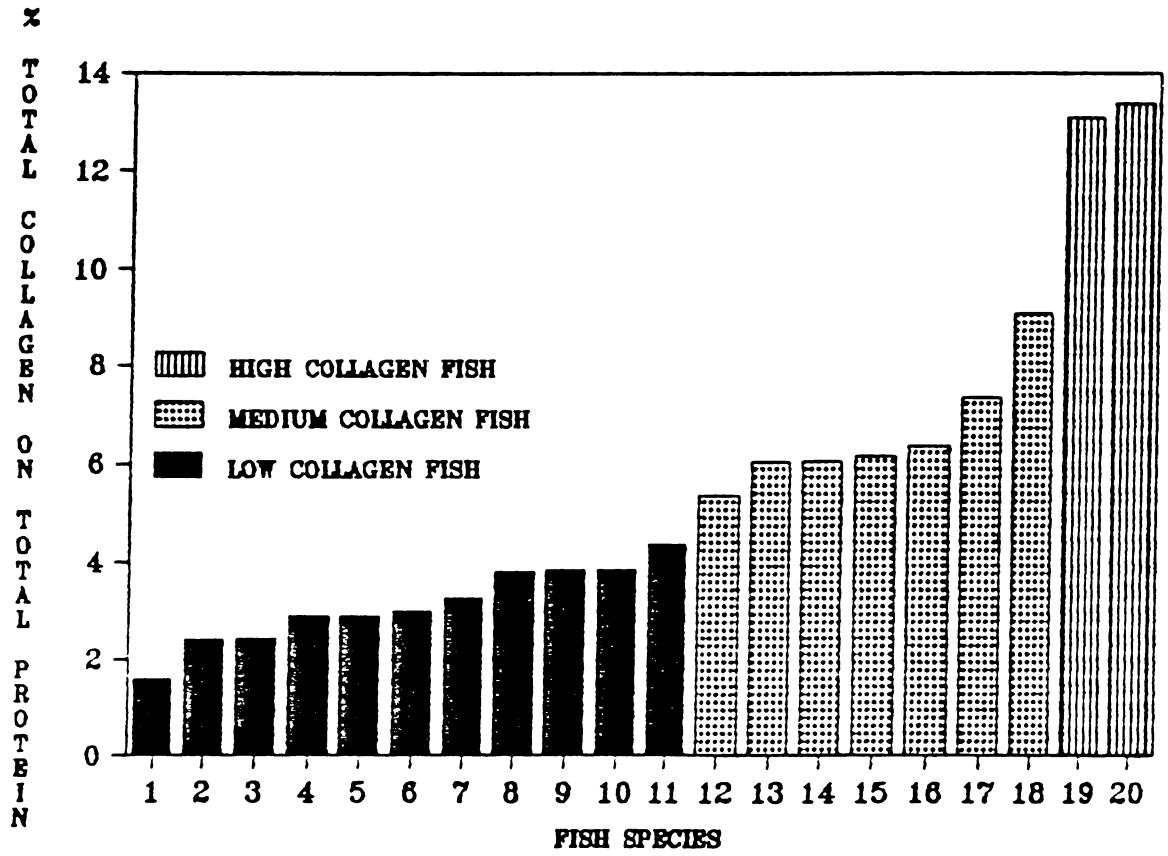
The soluble collagen content of the fishes studied vary between 0.09 to 6.2gm/100gm wet meat. The insoluble collagen content vary between 0.08 to 2.6gm/100gm wet meat. The total collagen content vary between 0.3 to 8.8gm/100gm wet meat.Percentage of total collagen on total ptotein of all the species of fishes studied is also given in Table 2.4.2.

On detailed examination of Table 2.4.2, it is found that the commercially important Indian fishes can be grouped into three according to the collagen content. So fishes having total collagen content upto 5% of total protein can be grouped as low collagen fishes, from 5-10% as medium collagen content fishes and above 10% as high collagen content fishes. The classification of fishes studied on the basis of percentage total collagen on total protein is given in Fig. 2.4.2.

Table 2.4.2 COLLAGEN CONTENT (+/- S.D.) IN THE WHITE MUSCLE OF FISHES STUDIED

Scientific Name	g/100g wet meat			% OF TC ON TOTAL PROTEIN
	ASC	ISC	TC	
P. argentius	0.19 +/-0.0003	0.11 +/-0.00003	0.30	1.58
S. longiceps	0.36 +/-0.0001	0.09 +/-0.00002	0.45	2.42
R. kanagurta	0.38 +/-0.0003	0.09 +/-0.000019	0.47	2.41
C. semifaciatus	0.40 +/-0.0002	0.18 +/-0.00004	0.58	2.98
Caranx spp.	0.50 +/-0.0002	0.08 +/-0.000009	0.58	2.89
T. savala	0.12 +/-0.0003	0.51 +/-0.00007	0.63	2.87
Anchoviella	0.09 +/-0.000005	0.60 +/-0.000077	0.69	3.81
O. mossambicus	0.47 +/-0.0002	0.22 +/-0.00005	0.69	3.85
C. carpio	0.49 +/-0.0003	0.21 +/-0.000055	0.70	3.25
L. rohita	0.28 +/-0.0003	0.46 +/-0.00007	0.74	3.86
M. cyprinoides	0.38 +/-0.0004	0.41 +/-0.00009	0.79	4.36
Barbus spp.	0.66 +/-0.0002	0.31 +/-0.00010	0.97	5.36
M. cephalus	0.66 +/-0.0002	0.62 +/-0.00009	1.22	6.03
K. kowal	0.09 +/-0.000005	1.01 +/-0.00017	1.10	6.07
N. japonicus	0.94 +/-0.0004	0.25 +/-0.00023	1.19	6.16
C. catla	0.71 +/-0.0002	0.55 +/-0.00061	1.26	6.38
E. affinis	1.06 +/-0.0008	0.39 +/-0.000096	1.45	7.34
S. sihama	1.00 +/-0.0007	1.08 +/-0.00054	1.08	9.08
Himantura	2.30 +/-0.002	0.50 +/-0.00011	2.80	13.39
S. sorrakowah	2.13 +/-0.001	0.86 +/-0.000099	2.99	13.11

Fig 2.4.2 CLASSIFICATION OF FISHES STUDIED BASED ON ITS COLLAGEN CONTENT



1. P. argentius
2. R. kanagurta
3. S. longiceps
4. T. savala
5. Caranx spp.
6. C. semifaciatus
7. C. carpio
8. Anchoviella
9. O. mossambicus
10. L. rohita
11. M. cyprinoides
12. Barbus spp.
13. M. cephalus
14. K. kowal
15. N. japonicus
16. C. catla
17. E. affinis
18. S. sihama
19. S. sorrakowa
20. Himantura

2.5 DISCUSSION

2.5.1 Proximate composition

The proximate composition of different species of fishes were worked out by several workers (Gopakumar, 1993; Govindan, 1985; Mathen, 1988). In the present study the moisture content of the fishes studied vary between 67.01% to 80.34%. The protein content vary between 17.37% to 24.3%. Fat content vary between 0.08% to 11.65%. Ash content vary between 0.9% to 3.1%. Mathen (1988), after studying several tropical fishes reported a moisture content in these fishes ranged between 70-80%, protein 14-22%, lipid 0.5-20% and minerals 0-1.5%. The proximate composition analysis was done because it is necessary that any food processor or researcher should have an idea on the proximate composition of fish.

2.5.2 Collagen content in the white muscle of fishes

Content of collagen or stroma protein in teleost muscle is reported to be in the range of 1-4% of the total protein (Dyer *et al.*, 1950; Shimizu *et al.*, 1960; Kubota *et al.*, 1975; Hashimoto *et al.*, 1979). In the present study the total collagen

content of teleost ranged between 1.58-9.08% of total protein and for elasmobranchs 13.11-13.39% of total protein. This is in agreement with the findings of Sato (1988). He also had fractionated total collagen into soluble and insoluble collagen and found that the values vary between 0.16-1.08gm per 10gm wet meat and 0.18-1.27gm per 100gm wet meat respectively. In the present study, corresponding values ranged between 0.09-2.03gm per 100gm wet meat and 0.09 to 1.08gm per 100gm wet meat respectively (Table 2.4.2). Sato (1988) found that the total collagen content vary between 0.34-2.11gm per 100gm wet meat and 1.6-12.4% of total protein.

The solubility of the muscle collagen varies significantly among the various species of fish. The difference in solubility of collagen observed in the present study may reflect the difference in the degree and properties of intra and inter-molecular cross linking of collagen. From the present study it was observed that the total collagen content in the range 0.33-2.99% wet tissue has a corresponding range of 1.58-13.39% of the total protein (Table 2.4.2). It is also noted that the variation in total collagen content among the species when expressed as percentage on total protein (1.58-13.39%) is more

distinct when compared to the total collagen content on wet weight basis.

While preparing seafood analog, texture has got an important role in giving consumer acceptance. Though there is an association of connective tissue in giving texture to meat (Sato, 1988; Hassan, 1991), no work was reported to find out the role of collagen on fish mince based products. In the following chapters this aspect of the connective tissue, collagen is studied in detail.

CHAPTER 3

FISH MINCE AND MINCE BASED PRODUCTS

3.1 INTRODUCTION

In most countries, by-catch normally receive little attention because of their low value. The quantity of by-catch caught by the shrimp trawlers have been considerably high and most of these catch were previously utilised for the production of fish meal and oil. However, these fishes are protein rich and are as nutritious as commercially important fish varieties. The important processing technique that is applied presently for the better utilisation of by-catch is surimi. Surimi and surimi products originated from Japan and are now widely popular in other Asian countries, Europe and United States. The popularity for surimi and surimi based products is on the increasing trend even in developing countries (Jayasekharan *et al.*, 1992).

Frozen surimi is now produced at sea in the factory ships equipped with freezers, cold stores and other infrastructural facilities required for surimi processing. This frozen surimi in turn is converted to a variety of products in shore based factories. Surimi technology offers great processing possibilities and provides a variety of marketing options.

3.2 REVIEW OF LITERATURE

3.2.1 Sources of raw material

In terms of consumer preference, a significant proportion of total available fish constitutes commercially important species and hence the under-utilised fish are landed as by-catch of fishing operations (James, 1986; SOFT, 1987). It has become a common practice that such fish are discarded at sea, though they are high value species in nutritional point of view (Bello, 1978). In 1979, Bello *et al.* reported that only a small portion of total resources from the sea are presently used for human consumption. According to FAO, 1981, the inherent problems in their use is the extreme homogenity of composition, bony structure, dark flesh, small size, unattractive appearance and texture, strong flavour and possible presence of toxic species.

Some under-utilised species studied by American and Canadian, workers include the sea trouts, croaker, ribbon fish, argentine (Dingle and Lall, 1979); cusk (Dingle and Lall, 1980); torbot, grey cod, thorney head (Nakayama and Yamamoto, 1977) and menhaden (Rasekh *et* al., 1976). In 1975, Bond listed some under-utilised species for mince production.

In India the preparation of frozen mince from anchovies, croaker and other species have been studied by Ghadi and Lewis (1977). Acceptable products were generated although some protein and fat degradation was noticed. In 1991, Kant estimated that India exploits only 7.7% catfish resources, 13.3% cephalopods, 25% perch and 11.5% coastal tuna resources. According to Venugopal (1995), the Indian fish industry is mainly export oriented, and process only a few selected items to meet the demand of foreign market.

The fresh water fishes also can serve as raw material for mince production. Actually these fishes are more consistent in their properties than marine resources (FAO, 1981). According to King *et al.* (1971) the delicate bone structure of most fresh water fish make filleting difficult and mincing can improve the recovery of meat.

It is seen that mince production from commercial species is confined to the developed countries (FAO, 1981). The commercial fish mainly used for mince production are gadoid fishes flat fishes (Crawford *et al.*, 1972a); rock fish (Collins *et al.*, 1980) etc. About 25% or more of fish catch worldwide is currently used for making fish meal and fish oil. These fishes are protein

rich and are as nutritious as commercially important fish varieties (Jayasekharan *et al.*, 1992). The maximum emphasis should be for utilising fish for human consumption to augment the protein deficiency gap.

3.2.2 Separation process

In 1980, Whittle *et al.*, reported that the quality of fish minces is dependant not only on the raw material but also on the nature of the separation process.

The mechanical deboning of fish was developed in Japan in the late 1940's and is now extensively used by the Japanese fish processors in the production of surimi to make kamaboko and other comminuted fish products (Miyauchi *et al.* 1973). In Europe and North America, mechanical deboning started in the early 1960's (Drew, 1976). Application of machine separation techniques (Tanikawa, 1963), that remove flesh from dressed fish in a coarse minced form with a markedly higher yield of edible fish over hand or machine filleting (Miyauchi and Steinberg, 1970; King and Carvar, 1970; Crawford et al., 1972b) has gained much interest. According to Grantham (1981), recovery of flesh by mechanical means is the first step in the development of products from under-utilised fish species.

3.2.3 Mince stabilisation

In 1981, FAO reported that mincing can accelerate the fat degradation, decrease protein functionality, affect colour and bacteriological quality.

Fish is having high levels of polyunsaturated fatty acids which are susceptible to enzyme hyrolysis and non-enzymic oxidation. Mincing accelerate these reactions through physical surface effects and through dispersion of catalytic contaminants. FAO (1981) listed out several chemical and natural antioxidants together with physical methods for limiting oxidation. However, fat stability is still the major factor limiting the use of many small pelagic and other under-utilised species in mince production.

Mince proteins are highly functional and versatile. Frozen denaturation can be minimised by a wide range of cryoprotectants, although their excessive use affect sensory properties (FAO, 1981). Mincing accelerates the deformation, aggregation, and cross linking of myofibrillar proteins (Laird *et al.* 1980; Tsuchiya *et al.* 1980). Functionality enhancers are used for their stabilisation, complex forming and gel forming effects

(Sikorski *et al.* 1968). A list of ingredients used as functionality enhancers are given in FAO Fish. Tech. paper No. 216.

The colour, flavour and functional properties of the mince depend upon the initial nature and quality of fish. Prolonged ice storage of fish and frozen storage of mince prior to processing affects protein quality, emulsification capacity, water binding capacity, cooking loss, drip loss and texture changes (Reddy *et al.* 1992). The minced meat is less stable than intact muscle. The disruption of tissue membranes and exposure of meat to air accelerates the oxidative processes during strorage. According to MacDonald *et al.* (1991), the frozen mince has an average storage life of about 6 months depending upon the fish. Longer storage may affect the protein quality with significant loss in functionality.

The major determinant of the microbiological quality of mince is the quality of raw material (Cann and Taylor, 1976). The poor storage of whole fish and unnecessary delay in mincing the fish will increase the risk of spoilage (Licciardello *et al.*, 1978 and Nickelson et al. 1980). They also reported that under good hygeinic conditions, preprocessing treatment of raw material has

very little effect on the final quality of the mince. According to Young (1978), even mincing of gut-in material leads to only small increase in counts. In 1980, Liston reported that mince prepared even under the most controlled condition are extremely susceptible to post-mincing contamination.

3.2.4 Uses of mince

Fish mince is a raw material, which has attracted considerable attention from food manufacturers through out the world (Rodger *et al.*, 1980, Abraham *et al.*, 1992). Minced fish can be used to create nutritious food from fish fillet waste (Regenstein, 1980).

Mincing offers an opportunity to excercise control over flavour, appearance and keeping quality by the incorporation of additives (Keay, 1979; Rodger *et al.*, 1980). It also helps to achieve desirable characteristics. The technique also helps to produce a variety of products such as fish cakes, fish cutlets, fish sticks, fish pastes, fish balls, fish rolls, fish spreads, fish ham, fish sausages etc.

The inherent property of minced fish is its unique

texture forming ability that make it an excellent base to manufacture a variety of seafood products (Lanier, 1981).

3.2.5 Mince based products

In Japan, fish and ham sausages are very popular. The sausage typically contains minced fish flesh with 10% pork fat,10% starch, 2.5% salt, seasoning and preservatives (Tanikawa, 1963). In United States, Carver and King (1971) developed fish frankfurters containing 76% fish plus added starch, oil and seasonings. In 1967, Venugopal *et al.* has prepared fish flakes from trash fish mince. In 1973, Patashnik developed pastes and spreads. Rudrasetty *et al.*, (1975) studied the preparation, standardisation and shelf life of fish spreads. They also showed a modified method for the preparation of canned fish paste from trash fish. Gopakumar *et al.* (1975) described the methods for the preparation of high quality fish flakes and fish soup powder from trash fish.

Baker *et al.*(1976a) described a method to prepare seafood powder. Baker *et al.*, (1976b) also gives a procedure for preparing seafood crispies. Daley *et al.* (1978), developed the seadog, a sausage type product containing minced millet, textured soyflour, tripolyphosphates and seasonings. Steinberg *et al.*, 1976

had successfully used minced fish as partial replacement for lean fish sausages. Oblleye and Spinelli (1978) managed to produce stable and palatable smoked Tilapia from minced flesh.

Uses of different methods of drying and the retention of many qualities of minced fish have been repeatedly reported by many workers (Calve and Borderias, 1979; Jensen, 1979; Niki *et al.*, 1978; Nogouchi, 1980).

Bello and Piggot (1978, 1979, 1980), carried out extensive studies on a number of species on the preparation of dried patties which were intended to be kept without refrigeration. A traditional dried product was developed by Gates and Wu in 1978 from croaker mince. Freeze dried products were prepared from the mince of Cod and Alaska Pollock (Noguchi, 1980), but both these species exhibited significant loss of protein rehydration ability. Fish protein concentrate can be prepared from fish mince by dehydartion using a solvent followed by solvent removal step (Noguchi, 1980).

Mince can be fermented by salt, acid or microbial action. For human consumption it is better to do the fermentation with lactic acid (Herborg, 1976; Herborg and Johansen, 1977). In

1977, Stunton and Yeoh, developed a fermented product suitable to use as animal feed. Here the mince was fermented with locally available carbohydrate sources and starter cultures. Silage can be prepared from the minces (Baker *et al.*, 1978; Young, 1980). Several minced based feeds and pet foods have been reported (Bremner, 1980; Suga, 1979a and b).

In 1992, Reddy *et al.*, studied the storage behaviour of frozen fish fingers from croaker and perches. Chakraborthy R., (1994) gave procedures for the preparation of wafers, soups, powders and cutlets from the minces prepared from fish by-catch. According to him these products were comparable to traditional products from expensive fish.

3.2.6 Surimi-Its importance

Minced fish is mechanically separated flesh that has not been washed and is not as stable as fish fillets on storagebecause of tissue disruption and enzyme release during mincing (Nakayame *et* al., 1977; Lee 1984; Regenstein, 1986). Water washing can improve quality and functional characteristics of minced meat (Miyanchi *et al.*, 1973; Lee *et* al., 1977). They also have the opinion that washing can remove nonprotein nitrogen compounds from the meat.

Washing of deboned flesh has been recommended since the presence of bone marrow and residues from viseral cavity increases susceptibility to rancidity development and protein instability (Dingle and Hinas, 1975; Blackwood, 1974). Sarcoplasmic proteins are deleterious from a functional point of view and it include haeme-containing compounds, which impart undesirable colour and catalyse lipid oxidation during storageenzyme systems such as trimethylamine oxidase leading to the formation of formaldehyde and subsequent protein denaturation in frozen storage and numerous other proteins which demonstrate poor functionality in terms of water binding and gelation ability (Suzuki, 1981).

Washed minced fish meat is usually more stable and acceptable than unwashed mince (Lee, 1984). Washing increases the concentration of myofybrillar protein. This will increase gel strength and elasticity (Miyauchi *et al.*, 1975; Patashnik *et al.*, 1973; Lee, 1984). Washed mince is less susceptible to deterioration during frozen storage than unwashed mince. They also have the opinion that washed minces are generally smoother and softer than unwashed mince.

Surimi is a base material made from fish that have been washed, dressed, minced, washed several times, strained, pressed, mixed with cryoprotectants, packaged and quick frozen usually in

some block form (Lee, 1986). The inherent value of surimi is its unique texture forming properties that makes it an excellent base for manufacturing a variety of seafood products (Lanier, 1981). According to him texture of seafood products should not be regarded as of minor importance to the overall consumer acceptance. Surimi is active in performing the functions of texture formation or particle cohesion and binding of fat and water in many processed muscle food systems.Babbitt, 1986 had an opinion that washing can greatly improve the colour of minced flesh. Removal of certain minerals and proteins through water washing enhances the quality characteristics of base products.

Surimi provides greater opportunities for diversification of product and has storage stability than minced fish (Yean, 1993). According to him the popularity for surimi and surimi based products is on the increasing trend in developing countries. Several convenience products such as fish sausage, kamaboko, fish burger, fish rolls, fish balls, fish pies, fish pizza, fish cakes, fish sticks and fish crackers have been developed from surimi. He also reported that, raw surimi lacks fibrous texture which is essential for the preparation of imitation products.

3.2.7 Suitable species for surimi production

The technology of surimi processing was first commercialised in 1960. By 1965 Alaska Pollock (*Thergra calcogrammis)* surimi was being produced on factory ships (Suzuki, 1981). According to him Atka mackeral (*Pleurogrammus azonus),* horse mackeral (*Trachus japonicus)* and lizard fish (*Saurida undosquamis)* were also used for production of surimi. Fish like cod, hake, whiting, Atlantic menhaden, croaker, Chilean mackeral, New Zealand hoki are found to be suitable for producing surimi (Young, 1978). Mac Donald *et al.* (1990) demonstrated the use of stabilized mince produced from New Zealand hoki (*Macruronus novaezelandiae)* as surimi source. Pacific whiting (*Merluccius productus)* has been a good source of surimi production (Chang-Lee *et al.,* 1990).

Many researchers have investigated the use of fatty fish such as herring (Hastings *et al.,* 1990; Gill *et al.,* 1992), mackeral (Shimizu, 1976; Katoh *et al.,* 1989) and sardines (Nonaka *et al.,* 1989; Roussel and Cheftel, 1990; Saeki et al., 1991) in production of surimi. Species such as Alaska pollock, croaker, jack mackeral, threadfin bream, blue whiting, sardine, lizard fish, eel, barracuda and leather jacket, have been recognised to

give good quality surimi (Lee, 1984, 1986; Yean, 1993). The technical problems associated with dark flesh fish species which influence surimi quality are high lipid content, lipid hydrolysis, large proportions of dark muscle and water soluble proteins and rapid protein deterioration (Hastings *et al.*, 1990). These problems may be overcome by removal of dark meat, washing with bicarbonate solutions, use of nitrogen-purged water for washing, adding cryoprotectants and use of an improved leaching process (Suzuki, 1981). Several techniques for separation of dark muscle from light muscle are being investigated based on physical differences between the two types of muscle.

According to Kelleher *et al.* (1992), addition of antioxidants as soon as possible after grinding helped to minimise initial damage caused by lipid oxidation. However, the initial improvement was not consistently maintained during frozen storage. In 1994, Kelleher *et al.*, could produce good quality mackeral surimi from Atlantic mackeral.

3.2.7 Cryoprotectants

Fish muscle proteins loose their gel forming ability during frozen storage. Freezing of surimi became commercially possible after the discovery of the cryoprotective role of sucrose

which prevents muscle proteins particularily actomyosin from denaturation during frozen storage (Mutsumoto, 1978). Effectiveness of cryoprotectants can be improved by increasing permeability into cell membranes (Taborsky,1979). In 1979, Back *et al.*, explained the difference in extent of proteins stabilisation by different types of sugars. Fish muscle proteins loose their gel forming ability during frozen storage. High quality surimi can only be made from fish whose myofibrillar proteins have not been denatured (Mutsumoto, 1978 and Suzuki, 1981). Although undesirable changes such as microbial growth and some chemical alterations are controlled by frozen storage, changes in functional properties can occur at low temperature (Shenouda, 1980). Cryoprotectants prevent denaturation of muscle protein during frozen storage. According to Suzuki, (1981) after the discovery of cryoprotectants, the frozen storage of surimi became commercially possible without lossing gel forming ability. He reported that, sucrose and sorbitol are the primary cryoprotectants used in surimi industry.

Low molecular weight cryoprotectants such as sucrose stabilise proteins by interacting with water throughout the system and thus prevent denaturation during freezing and cold storage (Arakawa and Timasheff, 1982). They explained, cryoprotection by sugars as its ability to increase surface tension of bound water.

Good cryoprotectants not only form an unfrozen zone, which surrounds the protein, but also prevent loss of bound water. Cryoprotectants usually are combination of sugar, sorbitol, polydextrose, polyphosphate and occasionally salt (Lee, 1984and Anonymous, 1984). In 1994 Nielsen *et al.* reported that sucrose, sorbitol blend usually represents 8% of the surimi's weight, is added to prevent denaturation during freezing.

3.2.8.Gelation

The characteristics of gel formation is the most important functional requirement in the manufacture of mince based products. When salted surimi paste of walleye pollock is incubated below 40^{O}C and subsequently heated at 90^{O}C ,a highly elastic gel is produced (Migita and Okada, 1952; Lanier *et al.*, 1981). This is termed suwari setting. A marked loss of elasticity of heat induced gels occurs when the salted paste is incubated at 50^{O}-60^{O}C. The gel degradation induced by heating of fish meat gel is called modori and impairs the textural quality of surimi based products (Okamura, 1961 and Shimisu *et al.*, 1981). The surimi paste, gellifies rapidly upon heating at 80 - 90^{O}C but slowly at 40-50^{O}C. Cooking a gel which had been slowly set at 40 - 50^{O}C, results in a stronger gel than cooking without a slow set (Okada, 1963). When a comminuted fish passes through a heating zone of 60 - 70^{O}C, a sort

of softening occurs in fish tissue. This softening is believed to be caused by an alkaline protease, since this enzyme has optimum activity at 60 - 70^{O}C (Makinodan and Ikeda, 1971; Cheng et al., 1979; Lanier et al., 1981). The weakening of the gel is overcome by using protease inhibitors (Matsumoto and Noguchi, 1992).The pre-setting at 40^{O}C may result from localised exposure of hydrophobic amino acid residues leading to inter-molecular hydrophobic interactions (Niwa *et al.*, 1982b; 1983; Wu *et al.*, 1985). Disulphide bonding does not appear to be responsible for gel formation at this temperature. Niwa *et al.*, 1982a, found no differences in gel structure resulting from addition of sulphhydryl blocking reagents to fish pastes heated at 40^{O}C for 60 minutes.

The textural profile of the heat induced gel varies with the heating schedule of salt ground meat (Katoh *et al.*, 1984). Interactions of protein-water, protein-protein and protein-lipid-water are very important for the formation of gel network structure (Regenstein, 1986). Gel formation of salted surimi paste of wall eye pollock at low temperature closely correlated with the covalent cross linking reaction of myosin heavy chain (Namakura *et al*, 1985; Saeki, *et al.*, 1992; Kamath *et al.*, 1992). In seafood industry, the use of high hydrostatic

pressure (HHP) is increasing, HHP represents a potential processing technology for high quality mince based products. HHP has been shown to decrease micro organisms, enzymatic activity and cause rheological changes in several foods (Farr, 1990; Hoover, 1993; Park *et al.*, 1994). Pressure heated gels from frozen Alaska pollock have been produced at $0^{o}C$ with treatments as slow as 2.0 K bar. According to them gelation of Pollock surimi by HHP was attributed to increase cross linkage of the myosin heavy chain. Surimi paste at atmospheric pressure also forms gels at room temperature, through the suwari or setting phenomenon (Mutsumoto and Noguchi, 1992). However, Pacific whiting and pollock suwari gels at room temperature, without heating are usually much weaker than traditional heat-set gels (Park *et al.*, 1994). Chung *et al.*, studied the effects of HHP on gel strength of Pacific whiting aand Alaska pollock surimi. HHP treated whiting, (1% beef plasma protein added) and pollock gels showed greatly increased strain values at all pressure-temperature conbinations compared to heat-set controls.

The study (Chapter 2) revealed that collagen content varies with species. In the present study an attempt was made to elucidate on the role of collagen present in the muscle on the texture and keeping quality of mince based product - surimi.

3.3 *MATERIALS AND METHODS*

Numerous biochemical reactions continue to take place in the fish muscle even after death. Biochemical and microbiological changes affect the composition of the meat and is influenced by a number of factors along the total commercial cycle of a commodity. To produce finished product of high quality, it is important to protect and monitor the integrity of the product at every stage of commercial cycle.

3.3.1 Raw material

For preparing surimi, a freshwater, brackishwater and marine fish were used. The freshwater fish used was common carp (*Cyprinus carpio*). It was collected from M/s Pookote Fish Farm, Trichur, Kerala. The brackishwater fish used was tilapia (*Oreochromis mossambicus*), and was collected from the Matsyafed Fish Farm, Narakkal, Cochin. The marine fish used were kilimeen (*Nemipterus japonicus*) and shark (*Scoliodon sorrakowah*). Both were collected from Cochin Fisheries Harbour, Cochin. Of the fishes studied, all the fishes except shark are teleosts. The fish caught

were immediately chilled in ice at 1:1 ratio and brought to the processing hall without delay.

3.3.2 Preparation of mince

In the processing hall the fish were beheaded, eviscerated, removed the inner lining of the peritoneum and washed with potable water at 10°C. If the fish is more than 20cm in length, it was filleted. This was fed to a Baader 694 Meat-Bone Separator (Nasan, Nova Scotia Corpn. NY), equipped with a 5mm diameter perforated drum. The minced meat coming out of the machine was collected in polythene covers. These covers with mince were put in vessels containing ice cubes, so that mince will always remain at a temperature less than 10°C.

3.3.3 Preparation of surimi

Washing of mince was carried out at 10°C, using potable water. This process helps to remove blood, inorganic substances, pigments, odoriferous compounds and water soluble proteins which affect the texture of surimi. The volume of water for each washing should be five times that of mince. Three washing

cycles were used. For the last washing, 0.3% sodium chloride solution was used to ease the removal of water in the further processing step. During washing, agitation was done at low speed of 300 rpm using a paddle type stirrer. The washed mince was subjected to partial dewatering by covering with cheese cloth and then squeezed in a screw press until the mince just began to come out. Then this partially dehydrated meat was mixed with 4% sucrose, 4% sorbitol and 0.3% sodium tripolyphosphate in a silent cutter for 2 minutes at 15 to 18^{0}C. The surimi thus prepared was packed in polythene bags. Each bag was filled with 350gm surimi. nine such bags were prepared in each trial. Each bag should be properly labelled. The polythene bags were frozen at -40^{0}C for 90 minutes in a contact plate freezer. After freezing one sample was taken out for analysis. This sample was designated as zero day sample. The other bags were subjected to frozen storage at -20^{0}C. From these bags samples were drawn periodically. The sampling intervals were 0, 15, 30, 45, 60, 90, 180, 270 and 360 days. Each samples drawn were subjected to physico-chemical, organoleptic and microbiological tests. Three such trials were carried out for each fish species.

3.3.4 Physico-chemical analysis

3.3.4.1 Estimation of moisture content

Moisture content was determined using 20gm of surimi by oven drying oven drying method, utilising an overnight (18hrs) drying period at 100^{0}C, (AOAC, 1984).

3.3.4.2 Determination of protein content

To determine protein content, the method of AOAC, 1984, wereKjeldahl digestion, distillation and titration procedure was used by taking a sample of 0.5gm surimi. The conversion factor 6.25 is used to convert precentage nitrogen to percentage protein.

3.3.4.3 Estimation of non-protein nitrogen

One hundred milli liter trichloroacetic acid acid extract of the surimi was prepared from 10gm surimi. From this extract 5ml was digested and the amount of protein in the digest was found out by microkjeldhal distillation

method (AOAC, 1984).

3.3.4.4 Determination of pH

Ten grams of thawed surimi was blended for 30 seconds with 90ml of distilled water and the pH of the resultant suspension was measured by a pH meter (Adair Dutt Digital pH Meter).

3.3.4.5 Estimation of trimethylamine and total volatile nitrogen

One hundred milli liter of trichloroacetic acid extract was prepared from 10gm surimi. TMA and TVN contents were determined in this extractby micro-diffusion method of conway (Conway, 1950).

One milli liter of TCA extract was taken in the outer chamber of Conways micro-diffusion unit. TVN was liberated by treating it with one milliliter saturated sodium carbonate solution. Liberated TVN was absorbed in one milli liter of N/50 H_2SO_4, taken in the inner chamber of the unit. From the amount of acid consumed TVN content was calculated.

For the determination of TMA content one milli liter of formaldehyde was added to the TCA extract taken in the outer chamber of the conways micro-diffusion unit to prevent the liberation of all the other amines (primary and secondary) and ammonia. The TMA was absorbed in N/50 H_2SO_4, and was estimated by titration.

Both TMA and TVN contents were calculated as milli gram nitrogen per 100gm surimi.

3.3.4.6 Determination of α-amino nitrogen

α-amino nitrogen was determined by the method of Pope and Stevens (1939), using 5% trichloroacetic acid extract of the sample.

α-amino nitrogen in 5ml of TCA extract was determined based on the formation of soluble copper compound through the reaction between the amino acid and the excess copper in the formation of copper phosphate. The amount of copper taken into solution was determined iodimetrically by titrating against 0.01N sodium thiosulphate using starch as indicator. α-amino nitrogen was calculated as milli gram nitrogen per 100 gram surimi.

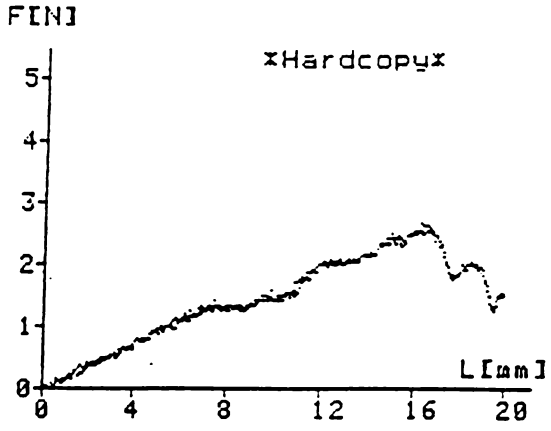
Fig.3.3.4.7 **TYPICAL GRAPH SHOWING MEASUREMENT OF GEL STRENGTH.**

INPUT PARAMETERS 7083.220

* V1	10	mm/min	* Fv	1	N	* L-E	60	mm	*
* V2	10	mm/min	* ε/F1	5	mm	* L-B	30	mm	*
* Vret	10	mm/min	* ε/F2	10	mm	* ε/F3	15	mm	*
* Vmax	500	mm/min	*			* ε/F4	20	mm	*
*			*			*			*
*			*			*			*
*			*			*			*

TEST RESULTS:

n	F-m N	L-B mm	F-1 N	F-2 N	F-3 N	F-4 N	W-Fm Nm	W Nm
1	2.68	16.35	.72	1.28	1.94	2.56	.02	.0



3.3.4.7 Determination of gel strength

Eighty gram of surimi prepared was mixed with 2% NaCl and homogenised for 3 minutes in a homogeniser with cutting blades. The resulting paste was stuffed in a cylindrical pre-fabricated mould (2cm ϕ x 6cm) and steam cooked for 20 minutes. The sample was taken out from the mould and tested in the Universal Testing Machine (Model : Zwick 1484), as per standard procedure. The procedure involved, compressing the pressed surimi to pre-set values of deformation and recording the maximum load registered along with corresponding compression value. The speed of test was fixed as 10mm per minute. A typical graph from the instrument showing compression of the sample is given in Fig. 3.3.4.7

3.3.4.8 Folding test

The sample prepared as above was cut into 3mm thick slice. This cut piece was folded into half and then into half again. Then the piece was examined for signs of structural failure (cracks). The minimum amount of folding required to produce a crack in the gel determines the score of the test. Scores were assigned as follows.

AA - No crack occurs after folding twice

A - Crack occurs after folding twice but no crack occurs after folding once

B - Cracks occur gradually after folding once

C - Cracks occur immediately after folding once

D - Breakable by finger press without folding

3.3.4.9 Determination of expressible moisture

One gram of surimi placed between two filter papers and pressed under a fixed pressure (10kg/cm^2) for 10 seconds. The weight difference compared to the weight before pressing in percentage reflects the expressible moisture.

3.3.4.10 Visual contaminants

Ten gram of thawed surimi was compressed between clear glass plates (10cm x 10cm). Objects 2mm or more in length or diameter are counted as one. Objects less than 2mm in length or diameter are counted as one half. The sum was reported as the number of visual contaminants per unit area.

3.3.5 Micro-biological studies

3.3.5.1 Preparation of media

The following media and solutions were used for the study.

i) Stock buffer solution

Dissolve 34gms of potassium dihydrogen orthophosphate (KH_2PO_4) in 1000ml of distilled water. Adjusted the pH to 7.2. Stored in a well stoppered bottle in refrigerator.

ii) Working buffer

12.5ml of the stock buffer solution was diluted to 1 liter. Adjusted the pH to 7.2. Distributed 9ml quantities in 18 x 150mm chemically cleaned test tubes and 90ml quantities in 150ml conical flasks.

iii) Dehydrated media

The following dehydrated media from DIFCO or

HIMEDIA was used. They were prepared as directed by the manufacturer.

a) Plate Count Agar (PCA)
b) Baird Parker Agar (BP Agar)
c) Tergitol - 7 Agar (T-7 Agar)
d) Bismuth Sulphate Agar (BSA)
e) Brilliant Green Agar (BGA)
f) XLD Media
g) Hektoen Enteric Agar (HEA)
h) Lactose Broth
i) Tetra Thionate Broth (TTB)
j) Selenite Cystein Broth (SCB)
k) Thiosulphate Citrate Bilesalt Sucrose Agar (TCBS Agar)
l) Alkaline Peptone Water (APW)

3.3.5.2 Enumeration of total plate count (TPC)

Asceptically transfered 10gm of surimi into a sterile mortar. Blended the sample with 90ml of sterile working buffer solution. The resulting dilution is 10^{-1}. Prepared decimal dilutions using sterile pipettes. For this, transfered 1ml of the inoculum into 9ml of sterile working buffer solution taken in a test tube. The test tubes were shakenby rotating and tilting. One milli liter of each dilutions were pipetted into sterile petri dishes in duplicates. Plate count agar

was used as plating media. Plate count agar previously molten and maintained in the molten state by keeping in a thermostatically controlled water bath at 45°C, was used for plating. Immediately after the agar was poured, the petri dishes were rotated on flat surface for proper mixing of the inoculum with the medium and the agar was allowed to solidify. Incubated the petri dishes at 37°C for 48 hours in the inverted position to prevent condensation of moisture on the surface of the agar media during incubation.

$$\text{Total Plate Count/gm} = \frac{\text{Average count x Dilution factor}}{\text{Weight of the sample}}$$

3.3.5.3 Enumeration of *E. coli*

From the solution made by mixing 10gm surimi with 90ml working buffer, 0.5ml was used for enumeration of *E. coli* using Tergitol-7 Agar. Spread plate method was adopted. After plating, the plates were incubated at 37°C for 24 hours. Yellow circular mucoid colonies were counted. *E. coli* per gram was calculated from the equation,

$$\textit{E. coli}/\text{gm} = \frac{\text{Number of colonies x 2 x Dilution factor}}{\text{Weight of the sample}}$$

3.3.5.4 Enumeration of *Staphylococcus*

From the solution made by mixing 10gm surimi with 90ml working buffer, 0.5ml was used for enumeration of *Staphylococcus*, using Baird Parker Agar. Spread plate method was adopted. After plating, the plates were incubated at 37^{o}C for 48 hours. Black colonies with narrow white margins and zones of clearance around were counted as *Staphylococcus* colonies.

$$\textit{Staphylococcus}/gm = \frac{\text{Number of colonies x 2 x Dilution factor}}{\text{Weight of the sample}}$$

3.3.5.5 Detection of *Salmonella*

Asceptically weighed 25gm of sample (surimi) and transfered into a sterile blending container. Added 225ml of sterile lactose broth and blended for 2 minutes at high speed. Asceptically transfered the mixture to a sterile wide mouthed jar of 500ml capacity and allowed the contents to stand for 60 minutes at room temperature. Ensured that the pH of mixture was 6.8 $\pm$0.2. Then loosened the cap slightly and incubated the mixture for 24 hours at 37^{o}C. Tightened the lid and shook the incubated sample mixture. Added one milli liter of the mixture to

10ml of selenite cystein broth and one milli liter of the sample to 10ml tetrathionate broth. Incubated noth the tube for 24 hours at 37^{O}C. Streaking 2 to 3mm loopful of incubated SCB and TTB was done separately on plates containing BSA, BGA, HEA and XLD Agar. The plates were incubated for 48 hours at 37^{O}C. Characteristics of typical *Salmonella* colony is as follows :

On BSA, typical *Salmonella* colonies appear as dark brown to black colour, sometimes with a metallic sheen. The surrounding medium is usually brown at first, but may turn black with increased incubation time, producing a halo effect. On BGA, the *Salmonella* colonies appear as pink colour. On HEA bluish green colonies with or without black centers, on XLD Agar, pink or yellow colonies with or without black centers.

3.3.5.6 Detection of *Vibrio cholerae*

Asceptically weighed 25gm of the composite sample of surimi and transfered into a conical flask of 250ml capacity. Added 225ml of alkaline peptone water. Prepared dried plates of TCBS Agar. Incubated alkaline peptone broth suspension overnight at 37^{O}C. Transfered a loopful of the above incubated peptone water broth suspension to the surface of TCBS plated media. Streaked to get isolated colonies. Incubated the

plates for a period of 18 to 24 hours at 37^{o}C. Inoculated 4 loopfuls of the primary enriched broth suspension into a test tube containing 10ml of alkaline peptne water and incubated at 6-8 hours at 37^{o}C for secondary enrichment. Streaked one loopful from the secondary enriched tube onto another dried TCBS plate. Incubated for 18-24 hours at 37^{o}C. Typical *Vibrio colonies* on TCBS agar plates will be larger, smooth, yellow and slightly flattened with opaque center and translucent periferies.

3.3.6 Organoleptic studies

Surimi samples were steam cooked for 20 minutes in pre-fabricated cylindrical stainless steel moulds (40mm ϕx 5mm). A sensory panel consisting of 10 judges were formed. The samples were coded and presented to the judges. The panelists were instructed to give appropriate scores to the sensory attributeds of appearence, colour, odour, texture and overall acceptability of the products in the score sheet given to them (ANNEXURE I). The textural characteristics include hardness, cohesiveness, springiness, chewiness and juiciness.

3.3.7 Statistical analysis

The data were analysed statistically (Snedecor and Cochran, 1967) to separate out significant treatments. Since all experiments were conducted in triplicates, the mean of the three values were used for the analysis. The arithmetic mean was calculated since all the experiments were conducted in triplicates. ANOVA was used to determine the variation between the days of storage and between different species on the characteristics of surimi. Wherever the treatments are found to be significant, least significant difference at 5% level were worked out to separate out significant treatments.

3.4 *RESULTS*

3.4.1 Physico-chemical characteristics

Table 3.4.1a gives the moisture content of surimi prepared from different species of fish at different storage periods. The schematic representation of this table is given in Fig. 3.4.1a. Table 3.4.1b gives the ANOVA of this data. Variation between days of storage and between species studied were significant at 0.1% level of significance. Among fishes studied, shark showed significantly lower value.

Table 3.4.1a $MOISTURE CONTENT (%) IN THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	80.12	78.16	77.78	75.00	75.52	73.90	70.00	68.62	64.20
TILAPIA	80.40	80.20	80.10	80.09	81.82	80.00	77.02	75.00	73.28
SHARK	75.12	73.83	74.62	74.48	73.53	72.92	71.68	70.98	69.96
NEMIPTERUS	82.00	80.15	79.98	79.42	78.14	76.29	74.00	72.18	71.08

$ Mean of three replicates

Table 3.4.1b ANOVA OF THE MOISTURE CONTENT OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	357.155	8.00	44.64	16.284$$$
COLLAGEN LEVEL	195.115	3.00	65.04	23.722$$$
ERROR	65.800	24.00	2.74	
TOTAL	618.070	35.00		

$$$ P < 0.001

Table 3.4.1c $TOTAL PROTEIN (gm/100gm meat) IN THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	16.38	15.48	14.28	13.12	13.06	12.68	12.32	12.05	11.93
TILAPIA	16.57	15.53	14.47	13.35	12.10	11.92	10.86	10.45	10.21
SHARK	17.01	15.06	14.24	13.86	12.74	12.65	12.50	12.21	11.80
NEMIPTERUS	15.75	14.02	13.78	12.61	11.83	10.79	10.53	10.45	9.99

$ Mean of three replicates

Table 3.4.1d ANOVA OF THE PROTEIN CONTENT OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	110.820	8.00	13.85	71.255$$$
COLLAGEN LEVEL	11.005	3.00	3.67	18.869$$$
ERROR	4.666	24.00	0.19	
TOTAL	126.491	35.00		

$$$ P < 0.001

Fig. 3.4.1a MOISTURE CONTENT OF SURIMI
FROM DIFFERENT SPECIES OF FISH

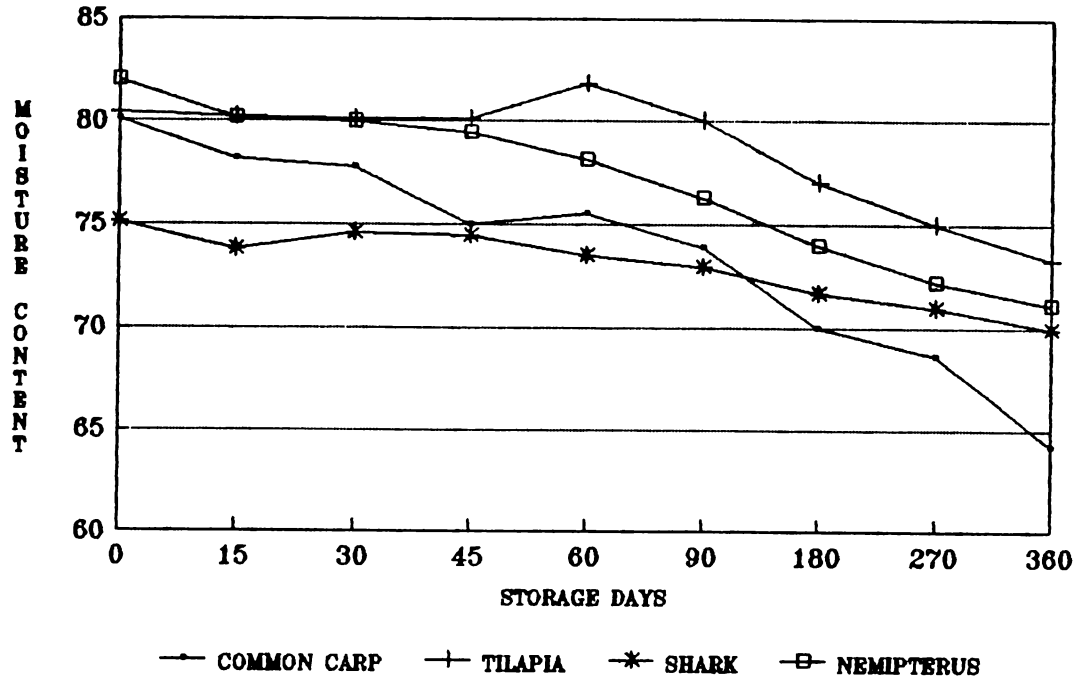


Fig. 3.4.1b TOTAL PROTEIN CONTENT OF
OF DIFFERENT SPECIES OF FISH

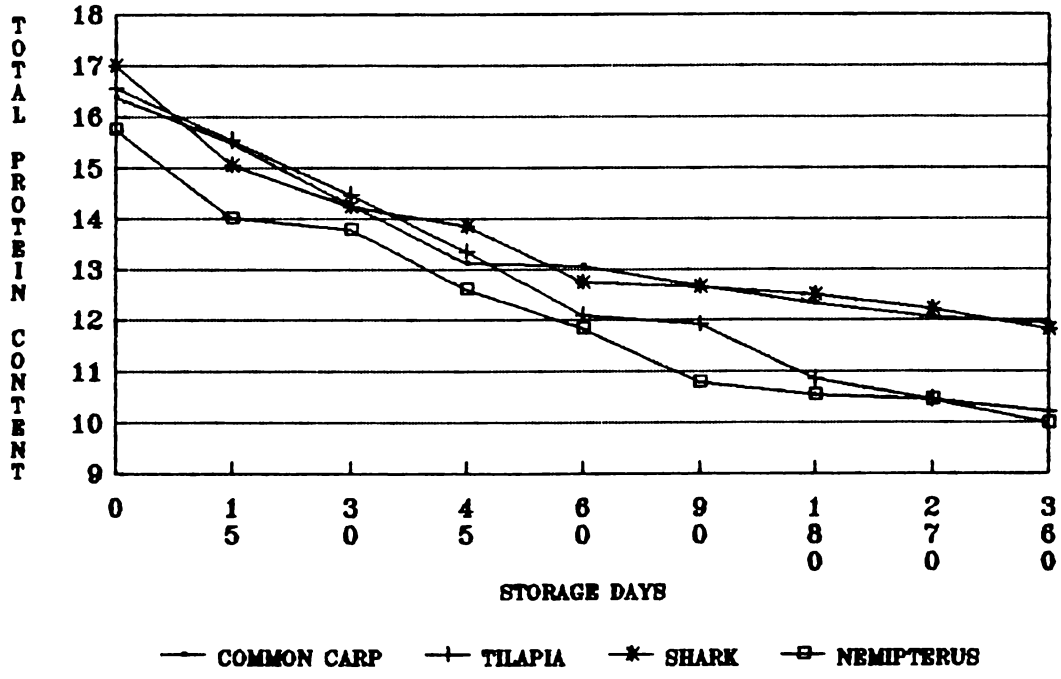


Table 3.4.1c and Fig. 3.4.1b gives the total protein content in gram percentage of surimi of different species of fishes studied. ANOVA of the total protein content of different species of fishes in the surimi is given in Table 3.4.1d. Here both variation between days of storage and between species were found to be significnant at 0.1% level. Among fishes, shark has shown highest significance.

In surimi, the non-protein nitrogen content seems to be less compared to fresh fish. Table 3.4.1e and Fig. 3.4.1c, shows NPN content in the surimi at different days of storage in different species. Highest initial NPN content was observed in shark (0.33mg %). ANOVA of the content of NPN of surimi is given in Table 3.4.1f. A 0.1% level of significance was observed between days of storage and between fish species. Shark showed highest significant value compared to other fishes studied.

The pH value of surimi prepared from different species of fishes at different storage days is given in Table 3.4.1g. Its graphical representation is in Fig. 3.4.1d. ANOVA of this data is given in Table 3.4.1h. Here variation between days of storage is significant at 5% level and variation between fishes

Table 3.4.1e * NON PROTEIN NITROGEN CONTENT IN THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0.17	0.14	0.11	0.10	0.07	0.05	0.03	0.00	0.00
TILAPIA	0.20	0.18	0.14	0.11	0.07	0.05	0.03	0.02	0.01
SHARK	0.33	0.24	0.16	0.15	0.09	0.07	0.05	0.03	0.01
NEMIPTERUS	0.22	0.17	0.14	0.09	0.09	0.08	0.07	0.05	0.05

* Mean of three replicates

Table 3.4.1f ANOVA OF THE NON-PROTEIN NITROGEN CONTENT OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	0.170	8.00	0.02	33.696***
COLLAGEN LEVEL	0.013	3.00	4.3435E-03	6.883**
ERROR	0.015	24.00	6.3102E-04	
TOTAL	0.198	35.00		

*** P < 0.001
** P < 0.01

Table 3.4.1g *pH IN THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	6.86	6.92	6.95	6.98	7.09	7.09	7.12	7.12	7.20
TILAPIA	6.81	6.93	6.92	6.93	6.95	7.03	7.13	7.20	7.22
SHARK	6.72	6.68	6.58	6.49	6.46	6.48	6.44	6.52	6.68
NEMIPTERUS	6.80	6.80	6.91	6.99	6.98	7.20	7.30	7.41	7.52

* Mean of three replicates

Table 3.4.1h ANOVA OF THE pH OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	0.49	8.00	0.06	3.240*
COLLAGEN LEVEL	1.63	3.00	0.54	28.722***
ERROR	0.45	24.00	0.02	
TOTAL	2.57	35.00		

*** P < 0.001
* P < 0.05

Fig. 3.4.1c NON PROTEIN NITROGEN CONTENT OF SURIMI OF DIFFERENT SPECIES OF FISHES

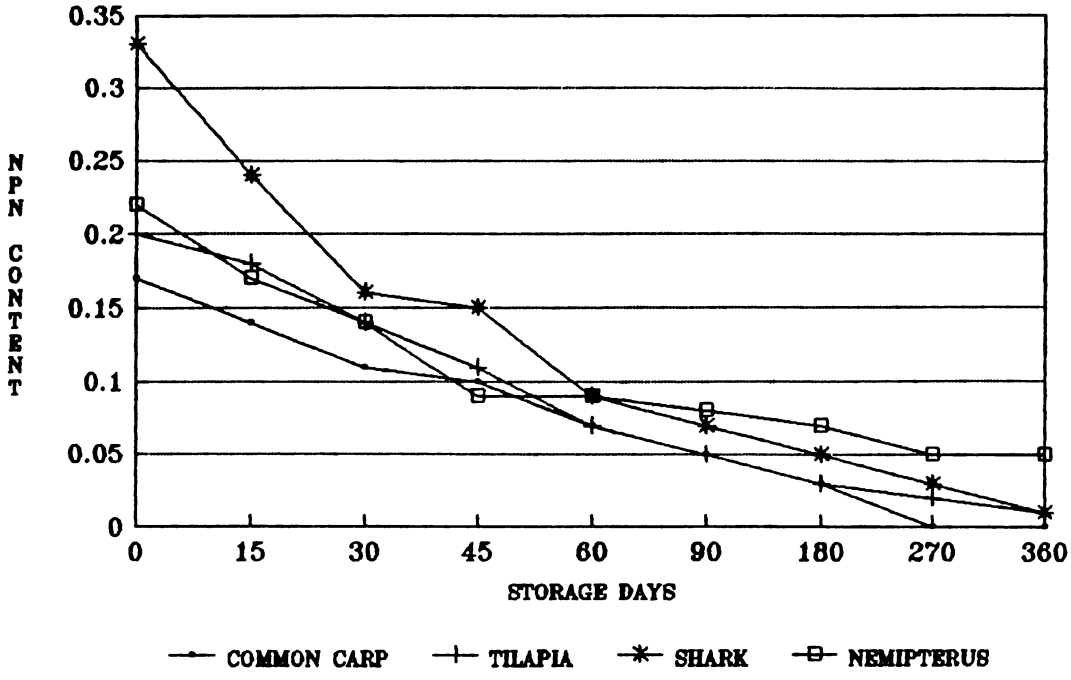
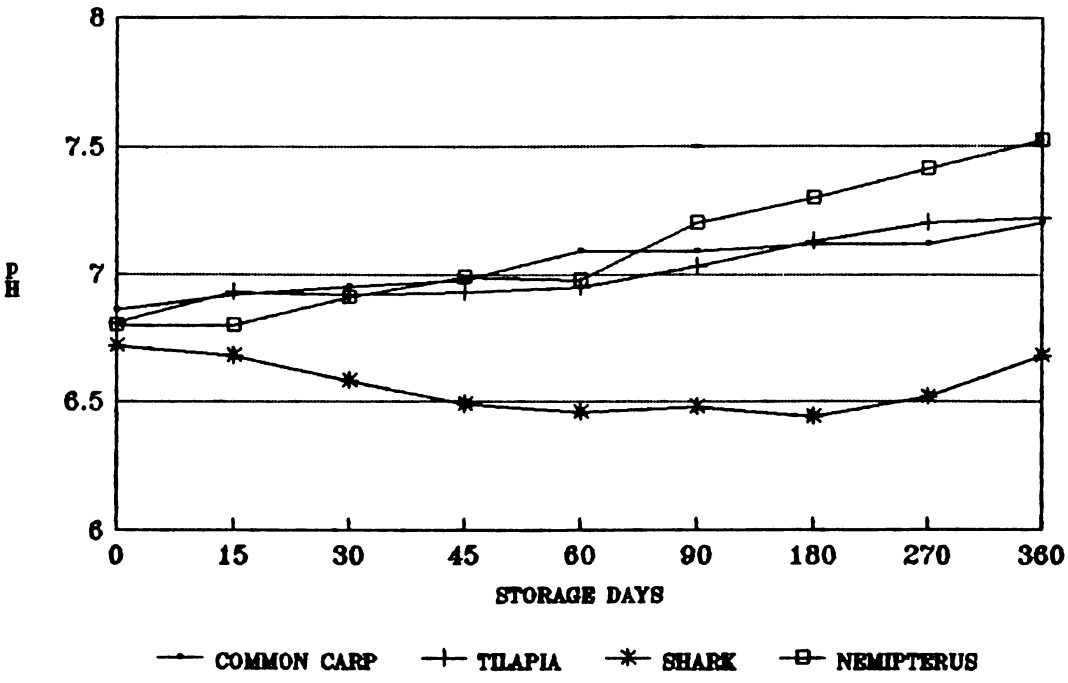


Fig. 3.4.1d pH OF SURIMI OF DIFFERENT SPECIES OF FISH



studied is significant at 0.1% level. Shark showed the highest significant value.

Table 3.4.1i, Table 3.4.1k and Table 3.4.1m, gives the content of TMA, TVN and α-amino nitrogen content of surimi of different species of fishes studied at different storage days. A schematic representation of the above three tables is presented in Fig. 3.4.1e, Fig. 3.4.1f and Fig. 3.4.1g respectively. Table 3.4.1j, Table 3.4.1l and Table 3.4.1n, represents the ANOVA of TMA, TVN and α-amino nitrogen contents respectively. The TMA value for common carp is not incuded in this table since it is a freshwater fish (Table 3.4.1i). Highest TMA content seems to be in shark (4.0 mg%). Initially the TVN of common carp was found to be 42.2mg/100g meat then it increased to 156.2mg/10g meat after 360 days of storage. The corresponding storage values for tilapia is 33.9mg/100g meat and 191mg/100g meat. In shark the initial value was 168mg/100g meat and after 360 days it was 278.8mg/100g meat. On zero day sampling the TVN content in nemipterus was 27.2mg/100g meat and it rose to 118.8 after 360 days.

The gel strength of the surimi of different species of fishes at different storage periods is given in Table

Table 3.4.1i *TMA (mg N/100gm) IN THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
TILAPIA	1.80	2.10	2.12	2.16	2.30	2.40	2.42	2.46	2.53
SHARK	4.00	4.22	4.30	4.40	4.80	5.60	6.80	7.70	9.90
NEMIPTERUS	2.10	3.18	4.00	4.42	5.68	6.12	6.82	7.00	9.95

* Mean of three replicates

Table 3.4.1j ANOVA OF THE TRIMETHYLAMINE (TMA) CONTENT OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	40.699	8.00	5.087	3.329
COLLAGEN LEVEL	204.107	3.00	68.038	44.529***
ERROR	36.673	24.00	1.528	
TOTAL	281.479	35.00		

*** P < 0.001

Table 3.4.1k *TVN (mg N/100gm) IN THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	4.50	5.20	5.90	12.80	15.60	21.00	22.20	22.60	23.20
TILAPIA	2.70	3.26	3.60	14.80	16.28	18.10	20.40	21.60	22.20
SHARK	6.20	6.80	8.80	10.20	10.80	22.80	23.60	24.80	28.80
NEMIPTERUS	8.00	8.52	10.68	12.58	14.12	15.62	15.90	17.68	22.00

* Mean of three replicates

Table 3.4.1l ANOVA OF THE TOTAL VOLATILE NITROGEN (TVN) OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	1544.00	8.00	193.00	24.192***
COLLAGEN LEVEL	46.65	3.00	15.55	1.949
ERROR	191.47	24.00	7.98	
TOTAL	1782.12	35.00		

*** P < 0.001

Fig. 3.4.1e TRIMETHYLAMINE CONTENT IN THE SURIMI OF DIFFERENT SPECIES OF FISH

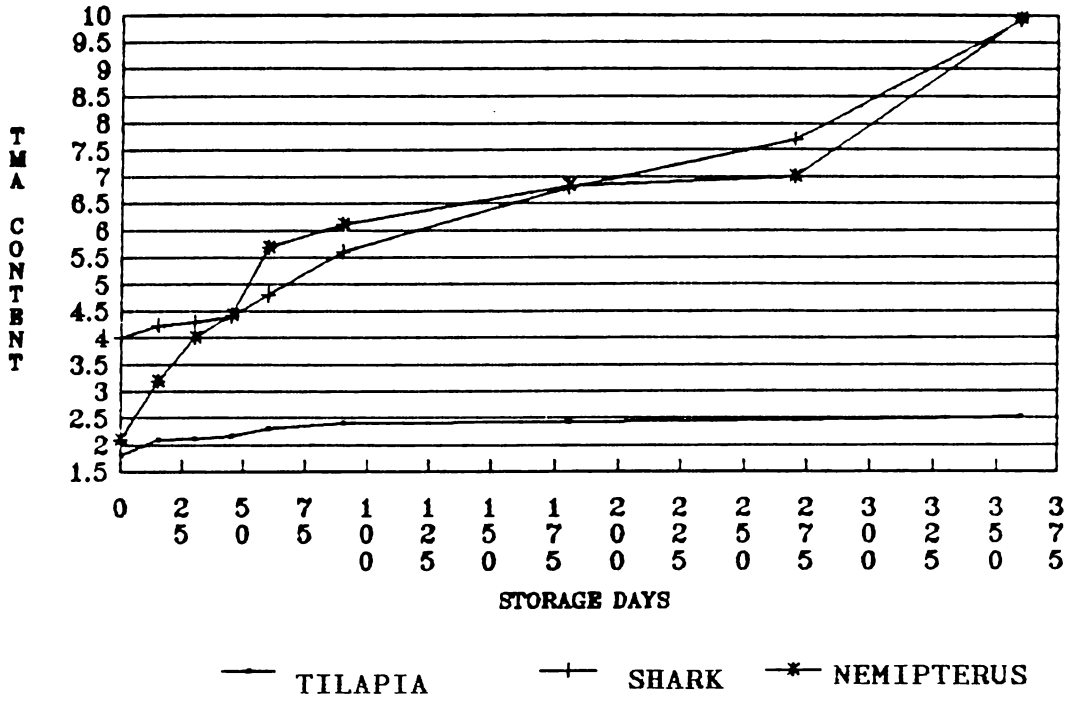


Fig. 3.4.1f TVN CONTENT OF SURIMI OF DIFFERENT SPECIES OF FISH

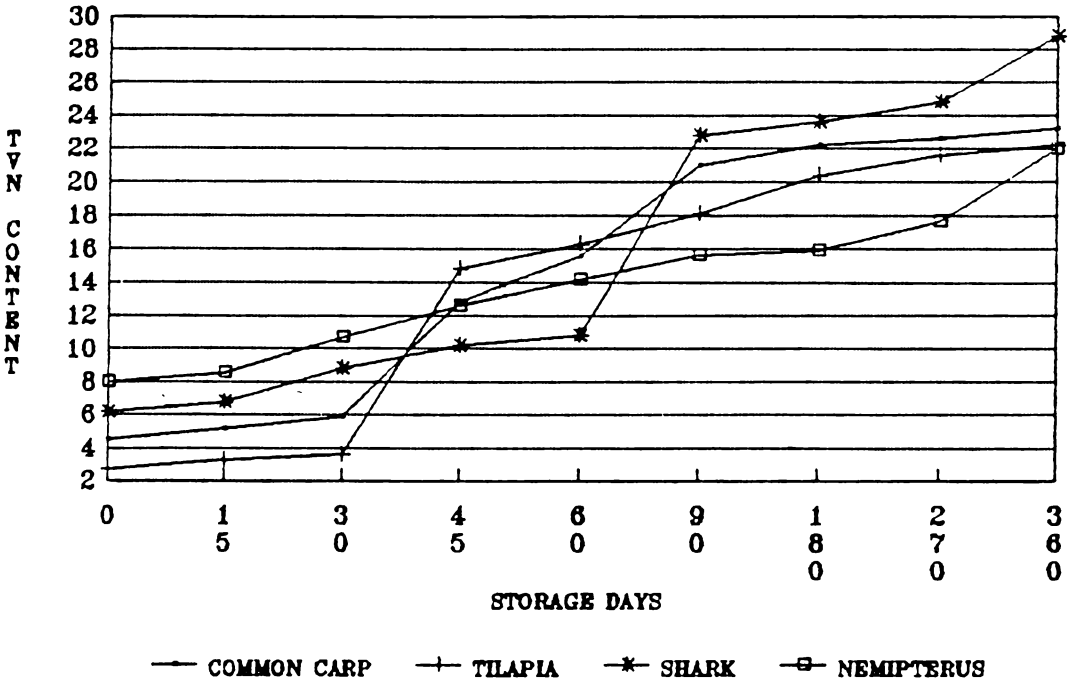


Table 3.4.1m *ALPHA AMINO NITROGEN IN THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE

NO. OF DAYS> / SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	42.20	56.00	64.40	92.40	112.00	124.40	138.40	140.60	156.20
TILAPIA	33.90	64.40	81.20	124.20	156.80	162.40	172.60	182.40	191.00
SHARK	168.00	176.40	182.00	193.20	257.60	264.20	272.00	274.40	278.80
NEMIPTERUS	27.20	32.00	45.00	49.68	56.40	64.40	92.60	110.40	118.20

* Mean of three replicates

Table 3.4.1n ANOVA OF THE ALPHA AMINO NITROGEN CONTENT OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	61545.95	8.00	7693.24	34.476***
COLLAGEN LEVEL	132290.30	3.00	44096.77	197.614***
ERROR	5355.50	24.00	223.15	
TOTAL	199191.76	35.00		

*** P < 0.001

Table 3.4.1o *GEL STRENGTH (Nm) IN THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> / SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	3.48	3.09	2.70	2.21	2.07	1.90	1.62	1.40	
TILAPIA	2.60	2.40	2.31	2.18	2.07	1.98	1.60		
SHARK	3.60	3.50	3.42	3.32	3.10	3.00	2.90	2.60	2.10
NEMIPTERUS	3.02	2.90	2.70	2.50	2.25	2.06	1.88	1.56	1.38

* Mean of three replicates

Table 3.4.1p ANOVA OF THE GEL STRENGTH OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	17.89	8.00	2.24	18.185***
COLLAGEN LEVEL	9.15	3.00	3.05	24.808***
ERROR	2.95	24.00	0.12	
TOTAL	30.00	35.00		

*** P < 0.001

Fig. 3.4.1g ALPHA AMINO NITROGEN CONTENT OF SURIMI OF DIFFERENT SPECIES OF FISH

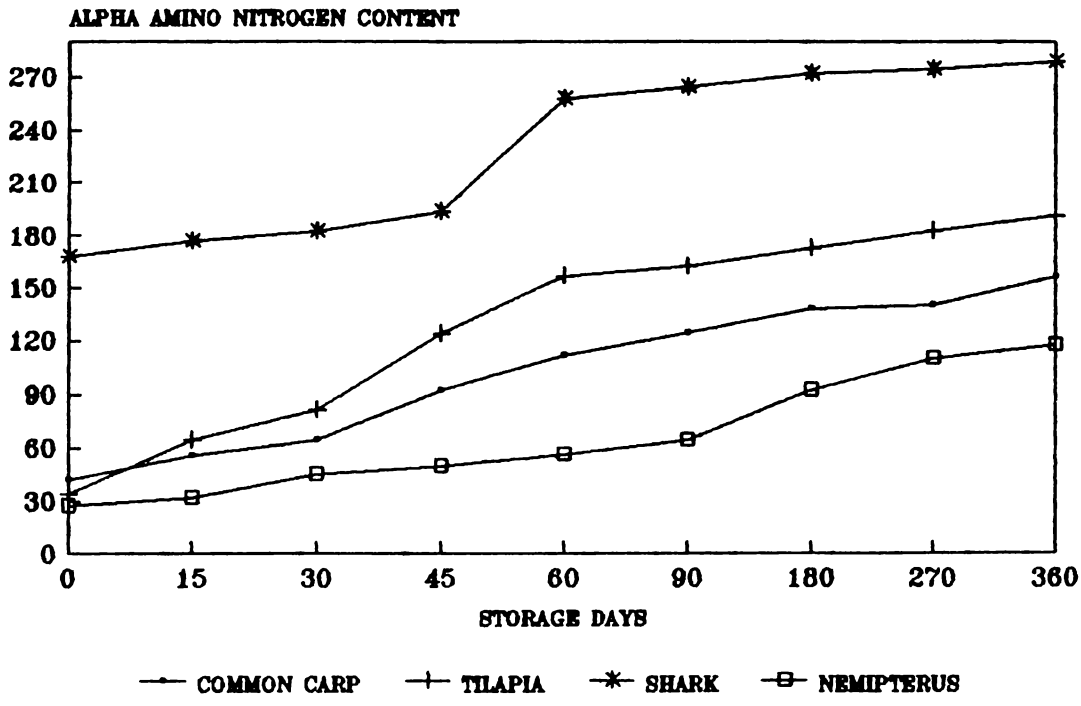
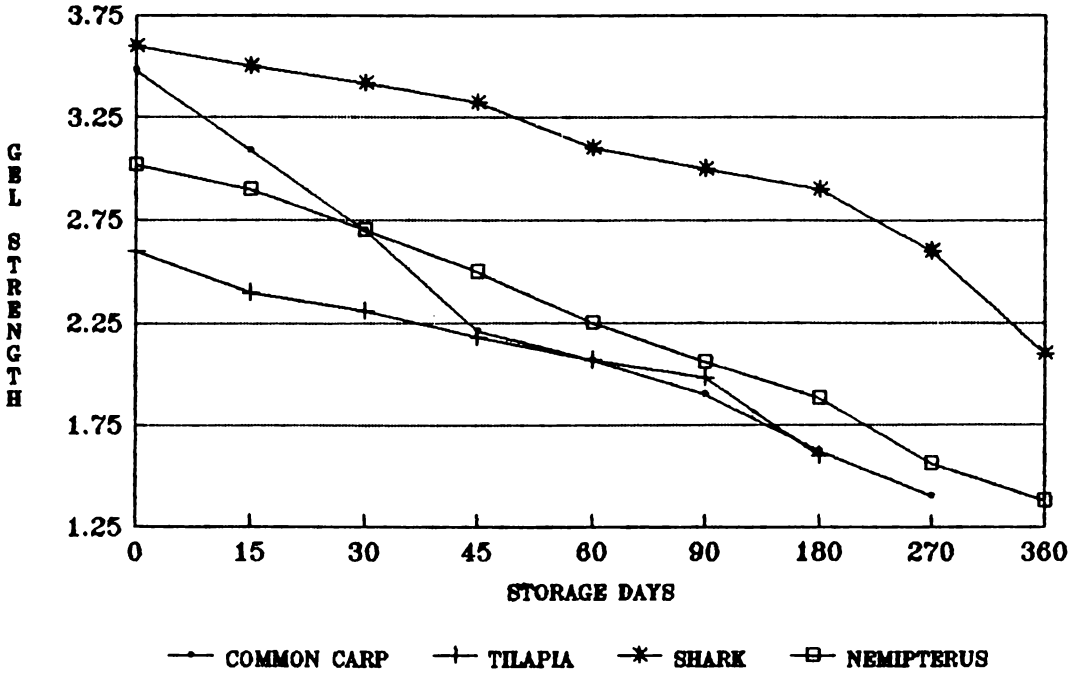


Fig. 3.4.1h GEL STRENGTH IN SURIMI OF DIFFERENT SPECIES OF FISH



3.4.1o and in Fig. 3.4.1h. Here it is noticed that the gel strength varies with species. Table 3.4.1p gives the ANOVA of this data. Here both variation between days of storage and between species of fish are significant at 0.1% level. Highest significant value was given by shark and the lowest by tilapia. From the Table 3.4.1o, it can be seen that shark surimi had highest gel strength. Common carp also had relatively high gel strength. Tilapia showed the least gel strength. By the time, the gel strength reached 1.5Nm, the surimi becomes unsuitable for product developement. Common carp and tilapia lost their gel strength by 9 months and nemipterus by 12 months. Shark retained it's gel strength to the acceptable limit even after one year.

Table 3.4.1q and Fig. 3.4.1i shows the percentage of expressible water in the surimi of different species of fishes at different storage days. Table 3.4.1r gives the ANOVA of this data. Table 3.4.1s, gives the data of the visual contaminants present in the samples prepared at different storage periods. From Table 3.4.1t, it is seen that there is no significance for this parameter for days of storage and species studied. Table 3.4.1u is the data showing folding test score, its corresponding ANOVA is given in 3.4.1v.

Table 3.4.1q ŧEXPRESSIBLE WATER (%) FOR THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	22.00	26.00	28.00	35.00	42.20	62.00	67.20	67.10	76.20
TILAPIA	36.00	42.00	48.00	49.20	57.80	62.10	66.00	68.90	69.00
SHARK	38.00	37.00	38.00	38.20	39.00	39.00	39.60	39.80	41.00
NEMIPTERUS	45.00	45.20	46.20	48.27	52.20	53.20	53.40	54.20	55.00

ŧ Mean of three replicates

Table 3.4.1r ANOVA OF THE % EXPRESSIBLE WATER OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	2856.13	8.00	357.02	4.394ŧŧ
COLLAGEN LEVEL	1304.12	3.00	434.71	5.350ŧŧ
ERROR	1949.97	24.00	81.25	
TOTAL	6110.22	35.00		

ŧŧ P < 0.01

Table 3.4.1s ŧ VISUAL CONTAMINANTS IN THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0.00	0.00	1.00	1.00	2.00	1.00	0.00	0.00	2.00
TILAPIA	4.00	2.00	1.00	3.00	1.00	3.00	2.00	3.00	1.00
SHARK	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00
NEMIPTERUS	3.00	3.00	2.00	1.00	0.00	2.00	1.00	2.00	3.00

ŧ Mean of three replicates

Table 3.4.1t ANOVA OF THE VISUAL CONTAMINANTS PRESENT IN SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	3.722	8.00	0.465	0.511ŧ
COLLAGEN LEVEL	23.667	3.00	7.889	8.672ŧŧ
ERROR	21.833	24.00	0.910	
TOTAL	49.222	35.00		

ŧŧ P < 0.01
ŧ P < 0.05

Fig. 3.4.1i EXPRESSIBLE WATER CONTENT IN THE SURIMI OF DIFFERENT SPECIES OF FISH

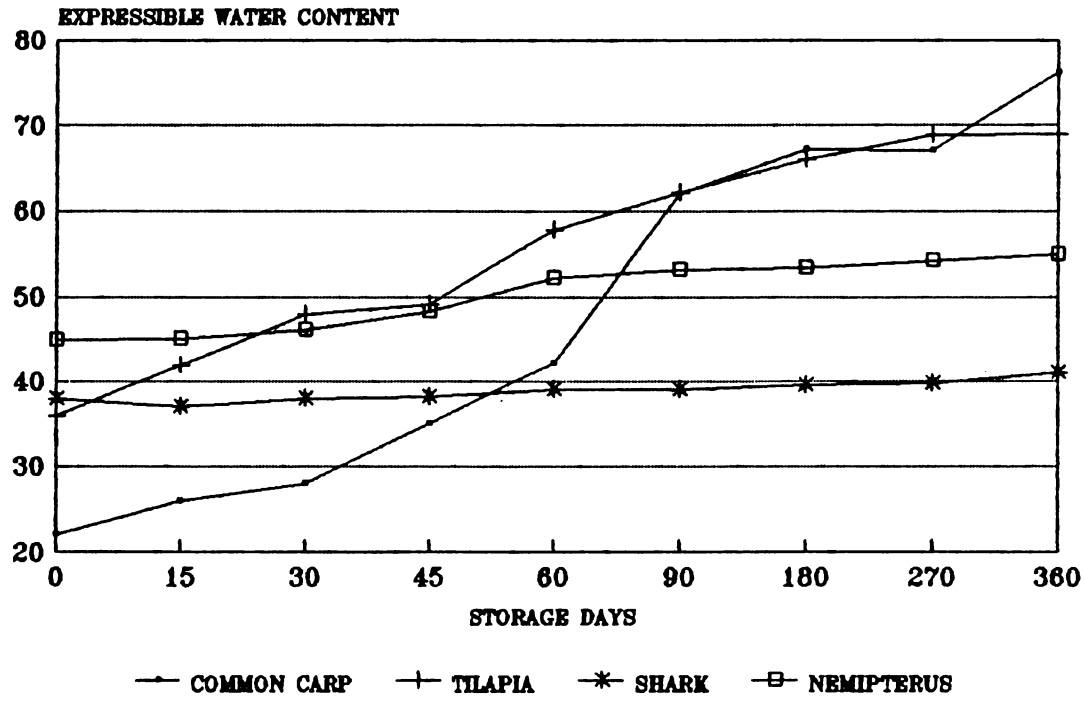


Table 3.4.1u * FOLDING TEST SCORE FOR THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> / SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	4.98	4.76	4.75	4.60	4.56	4.54	4.42	4.38	3.90
TILAPIA	3.80	3.80	3.70	3.50	3.50	3.40	3.30	3.00	3.00
SHARK	5.00	5.00	4.80	4.80	4.80	4.80	4.70	4.60	4.60
NEMIPTERUS	4.40	4.40	4.30	4.30	4.20	4.10	3.90	3.60	3.40

* Mean of three replicates

Table 3.4.1b ANOVA OF THE FOLDING TEST FOR THE SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	2.338	8.00	0.292	19.912***
COLLAGEN LEVEL	9.476	3.00	3.159	215.164***
ERROR	0.352	24.00	0.015	
TOTAL	12.166	35.00		

*** P < 0.001

Table 3.4.2a *TPC/gm IN THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> / SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	55000.00	48000.00	41000.00	3800.00	3500.00	3400.00	310.00	270.00	256.00
TILAPIA	340000.00	150000.00	130000.00	19000.00	10000.00	9100.00	8700.00	850.00	746.00
SHARK	380000.00	190000.00	92000.00	68000.00	5800.00	5000.00	390.00	363.00	276.00
NEMIPTERUS	110000.00	17600.00	13000.00	12800.00	6800.00	6000.00	590.00	420.00	216.00

* Mean of three replicates

Table 3.4.2b ANOVA OF THE TPC PER GRAM PRESENT IN SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	1.7555E+11	8.00	2.1944E+10	6.84***
COLLAGEN LEVEL	33144677398.530	3.00	1.1048E+10	3.44**
ERROR	77000888968.240	24.00	3208370373.68	
TOTAL	2.8570E+11	35.00		

*** P < 0.001
** P < 0.01

Fig. 3.4.2 TOTAL PLATE COUNT OF SURIMI
OF DIFFERENT SPECIES OF FISH

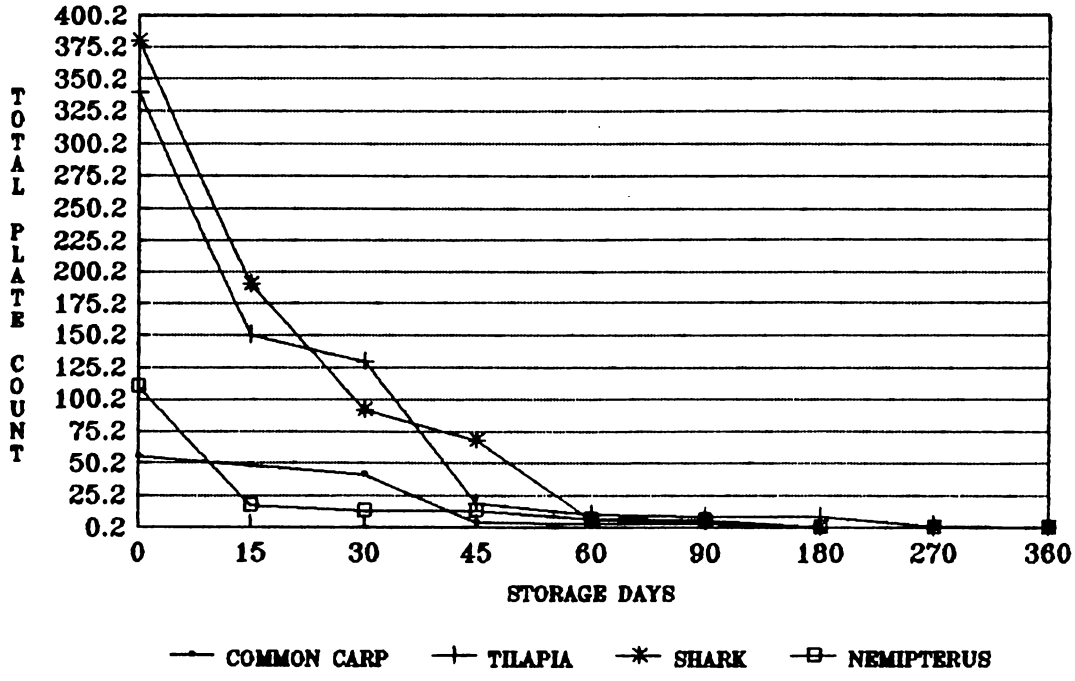


Table 3.4.2c *E. coli PER GRAM OF SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	ND	ND	ND	ND	ND	ND	ND	ND	ND
TILAPIA	4.00	1.00	ND	ND	ND	ND	ND	ND	ND
SHARK	ND	ND	ND	ND	ND	ND	ND	ND	ND
NEMIPTERUS	ND	ND	ND	ND	ND	ND	ND	ND	ND

* Mean of three replicates
ND - Not Detected

Table 3.4.2d ANOVA OF THE E. coli PER GRAM OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	5.500	8.00	0.69	1.00
COLLAGEN LEVEL	3.000	3.00	1.000	1.46
ERROR	16.500	24.00	0.69	
TOTAL	25.000	35.00		

Table 3.4.2e *STAPHYLOCOCCUS PER GRAM OF SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	5.00	2.00	ND	ND	ND	ND	ND	ND	ND
TILAPIA	6.00	3.00	1.00	ND	ND	ND	ND	ND	ND
SHARK	ND	ND	ND	ND	ND	ND	ND	ND	ND
NEMIPTERUS	4.00	ND	ND	ND	ND	ND	ND	ND	ND

* Mean of three replicates
ND - Not Detected

Table 3.4.2f ANOVA OF THE STAPHYLOCOCCUS PER GRAM OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	3.556	8.00	0.44	2.00
COLLAGEN LEVEL	0.667	3.00	0.222	1.00
ERROR	5.333	24.00	0.22	
TOTAL	9.556	35.00		

3.4.2 Microbiological characteristics

Total plate count per gram, *E. coli* per gram and *Staphylococcus* per gram of meat in the surimi of different species of fishes at different storage days is presented in Table 3.4.2a, 3.4.2c and 3.4.2e respectively. Fig. 3.4.2 is the schematic representation of Table 3.4.2a. ANOVA of total plate count per gram is given in Table 3.4.2b. There is a decrease in the number of TPC per gram in all the four species, but the variation between days of storage and between species is insignificant even at 5% level. In the case of *E. coli* per gram and *Coagulase positive staphyloccoccus* gram in the surimi, there is some occurance of these micorbes initially. But after frozen storage the number of microbes reduced to zero.

3.4.3 Sensory characteristics

Table 3.4.3a represents the mean sensory score for the attribute, appearance, for the surimi of different species of fishes at different storage days. Initially highest score for appearance is given by the sample prepared from surimi of common carp (8.6), followed by tilapia (8.4), nemipterus (8.4) and shark (8.2). Samples drawn after pre-determined intervals showed the

Table 3.4.3a *MEAN APPEARANCE SCORE FOR THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	8.60	8.59	8.10	7.10	6.20	5.00	4.90	4.70	4.00
TILAPIA	8.40	7.30	6.10	5.80	5.00	4.20	3.60	3.00	3.00
SHARK	8.20	7.80	7.40	6.80	6.00	5.60	5.30	5.00	4.00
NEMIPTERUS	8.30	7.50	7.00	6.40	5.50	5.10	5.00	4.50	4.00

* Mean of 10 X 3 replicates

Table 3.4.3b ANOVA OF THE APPEARANCE OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	88.854	8.00	11.11	111.825
COLLAGEN LEVEL	7.040	3.00	2.347	22.628***
ERROR	2.384	24.00	0.10	
TOTAL	98.278	35.00		

*** P < 0.001

Table 3.4.3c *MEAN COLOUR SCORE FOR THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	8.80	8.20	7.80	7.60	6.50	5.90	5.70	7.20	4.00
TILAPIA	8.10	7.08	6.18	5.95	5.60	5.09	4.75	4.15	3.80
SHARK	8.70	8.60	8.20	7.60	7.60	7.00	5.80	5.40	5.40
NEMIPTERUS	8.80	8.60	7.90	7.50	6.40	5.60	5.00	4.80	3.50

* Mean of 10 X 3 replicates

Table 3.4.3d ANOVA OF THE COLOUR OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	81.264	8.00	10.16	69.092
COLLAGEN LEVEL	9.058	3.00	3.019	20.536
ERROR	3.529	24.00	0.15	
TOTAL	93.850	35.00		

Fig. 3.4.3a APPEARANCE SCORE OF SURIMI
OF DIFFERENT SPECIES OF FISHES

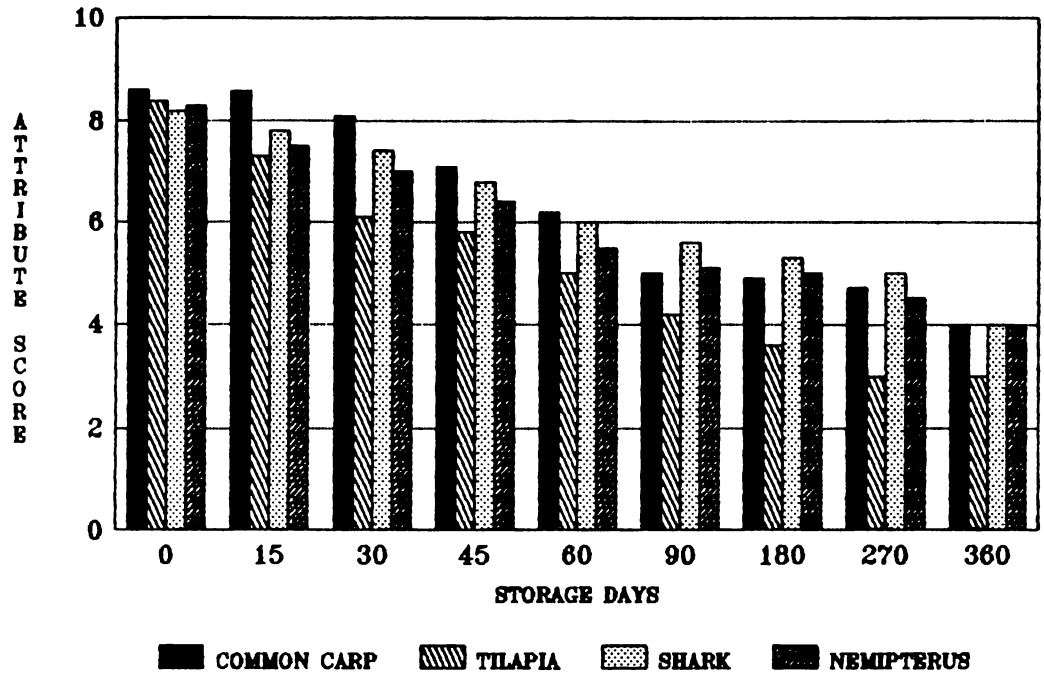
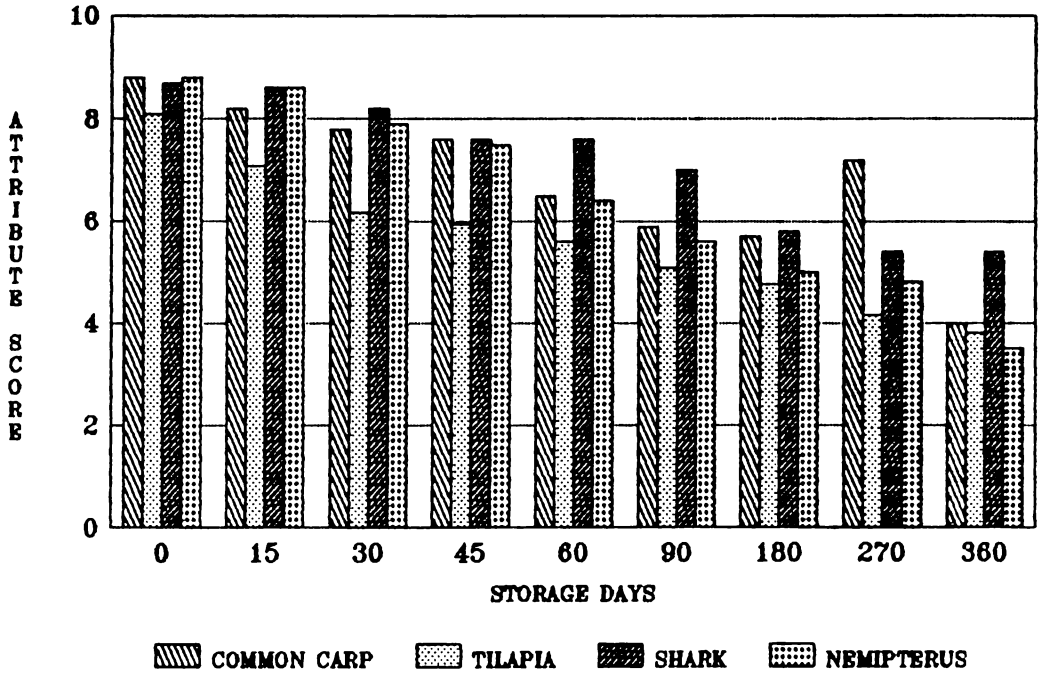


Fig. 3.4.3b COLOUR SCORE OF SURIMI
OF DIFFERENT SPECIES OF FISHES



decline in the scores. Table 3.4.3a gives the ANOVA for the appearance attribute. Here between days of storage there is no significance, but between species there is significance at 0.1% level.

From Table 3.3.4c and Fig. 3.4.3b, a declining trend can be seen in the colour attribute in the surimi of all species. the highest score was given by common carp and nemipterus (8.8), followed by shark (8.7) and least initial score was given (8) by tilapia. Even after 360 days, the colour attribute for shark surimi was found to be acceptable. Table 3.4.3d, gives the ANOVA of the colour of surimi prepared from different species without adding collagen. Here variation between days of storage and variation between species are significant at 0.1% level. Highest significance was shown by shark, followed by common carp, nemipterus and tilapia.

Mean attribute score for odour of surimi is given in Table 3.4.3e and Fig. 3.4.3c. Here also the score decreases with increase in storage time. From the ANOVA (Table 3.4.3f), it is clear that between days of storage there is no significance at 0.1% level. Between species the highest significance was given by common carp and the least by shark.

Table 3.4.3e *MEAN ODOUR SCORE FOR THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	8.21	8.00	7.60	6.20	5.01	4.11	3.58	2.99	2.70
TILAPIA	8.40	7.50	7.01	6.06	5.10	4.00	3.50	2.90	2.00
SHARK	8.40	7.00	6.10	5.60	4.00	3.20	2.30	2.01	2.00
NEMIPTERUS	8.60	7.80	6.22	5.90	4.80	4.40	3.80	2.90	1.80

* Mean of 10 X 3 replicates

Table 3.4.3f ANOVA OF THE ODOUR OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	159.311	8.00	19.91	177.38
COLLAGEN LEVEL	3.804	3.00	1.268	11.296***
ERROR	2.694	24.00	0.11	
TOTAL	165.809	35.00		

*** P < 0.001

Table 3.4.3g *MEAN TEXTURE SCORE FOR THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE DAYS

NO. OF DAYS> SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	7.60	7.00	6.60	6.20	6.00	5.70	5.00	4.00	3.20
TILAPIA	7.60	7.20	6.30	6.01	5.50	4.00	3.50	2.00	1.80
SHARK	8.30	8.00	7.90	7.80	8.00	6.70	5.20	4.00	3.20
NEMIPTERUS	7.60	7.20	6.00	6.20	5.40	5.00	4.30	2.40	1.00

* Mean of 10 X 3 replicates

Table 3.4.3h ANOVA OF THE TEXTURE OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	113.571	8.00	14.20	66.574***
COLLAGEN LEVEL	14.196	3.00	4.732	22.191***
ERROR	5.118	24.00	0.21	
TOTAL	132.885	35.00		

*** P < 0.001

Fig. 3.4.3c ODOUR SCORE OF SURIMI
OF DIFFERENT SPECIES OF FISH

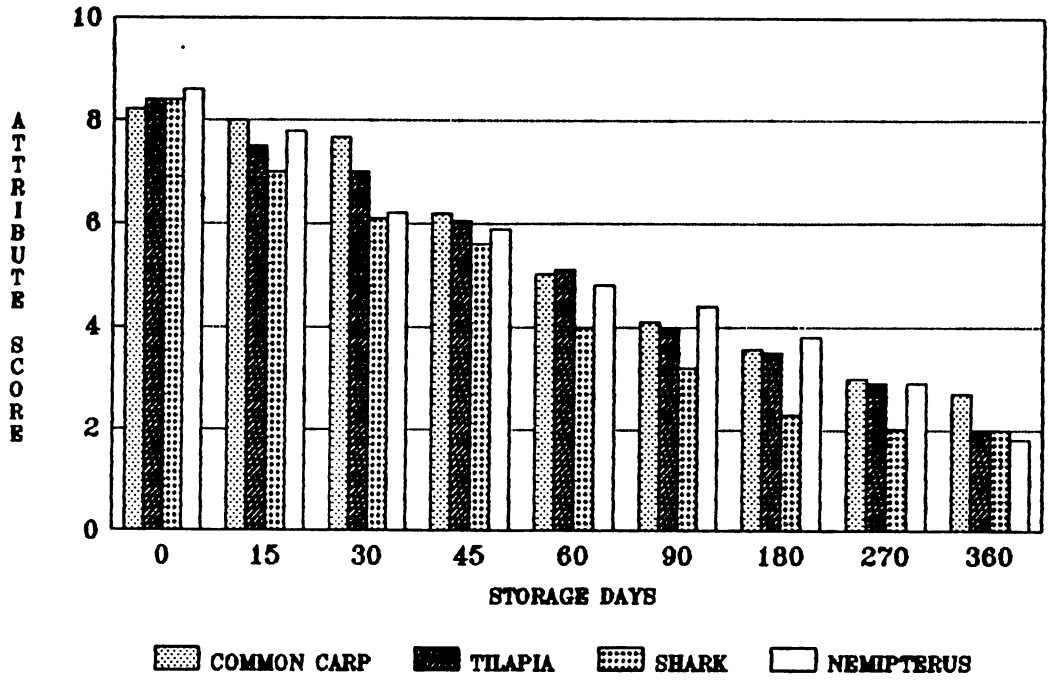


Fig. 3.4.3d TEXTURE SCORE OF SURIMI
OF DIFFERENT SPECIES OF FISH

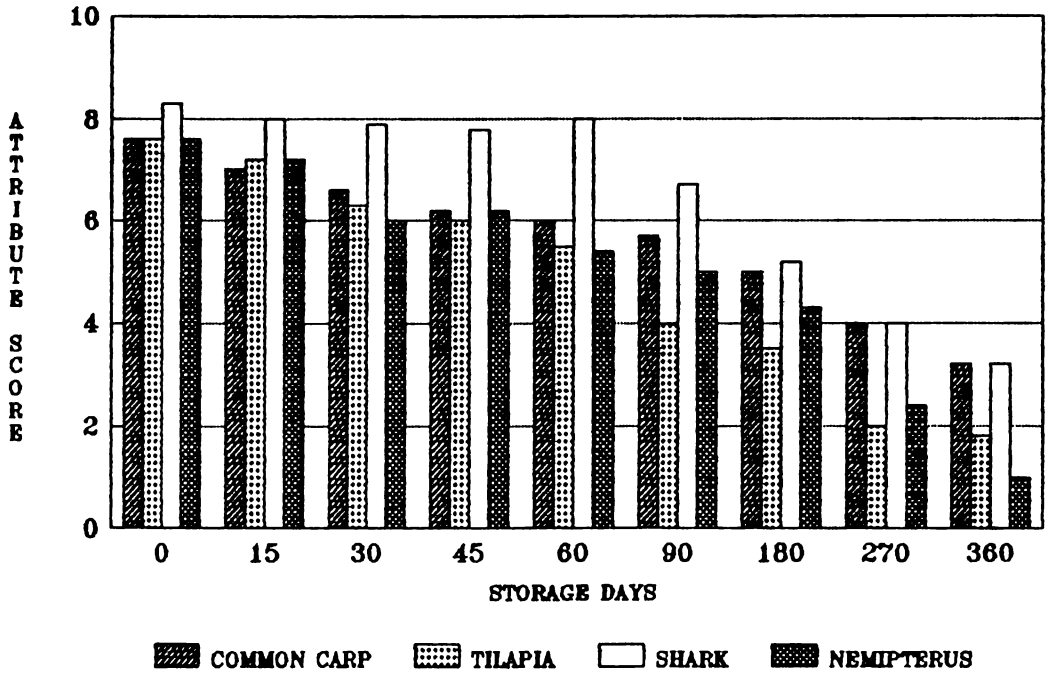


Table 3.4.3i *MEAN OVERALL ACCEPTABILITY SCORE FOR THE SURIMI OF DIFFERENT SPECIES OF FISHES STUDIED AT DIFFERENT STORAGE

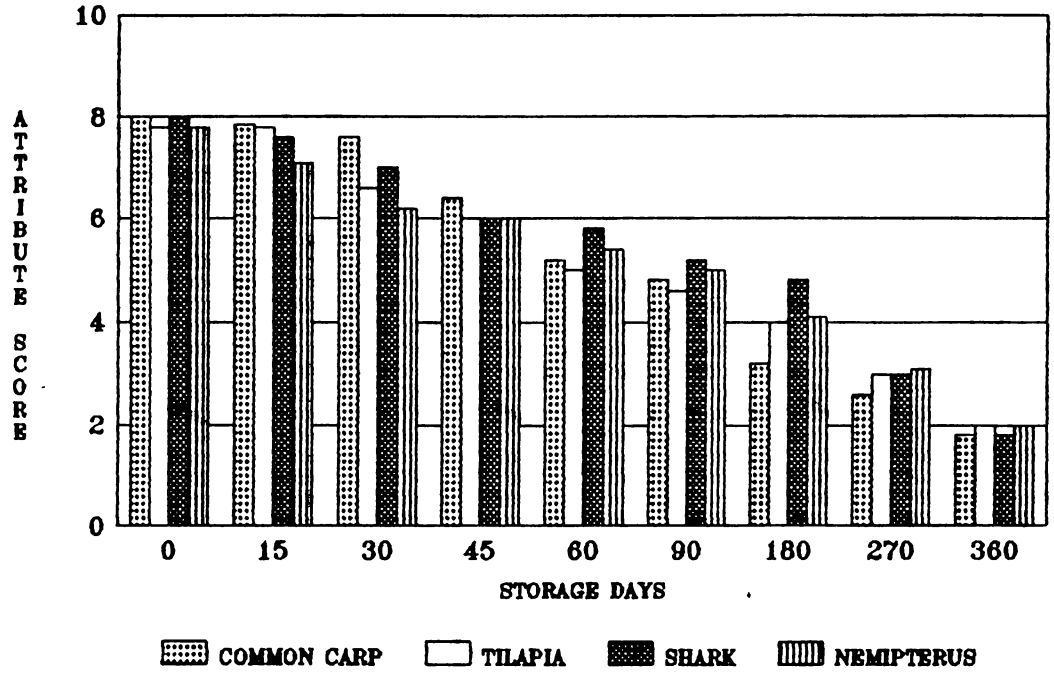
NO. OF DAYS> / SPECIES	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	8.00	7.85	7.60	6.40	5.20	4.00	3.20	2.60	1.80
TILAPIA	7.80	7.80	6.60	6.00	5.00	4.60	4.00	3.00	2.00
SHARK	8.00	7.60	7.00	6.00	5.80	5.20	4.80	3.00	1.80
NEMIPTERUS	7.80	7.10	6.20	6.00	5.40	5.00	4.10	3.10	2.00

* Mean of 10 X 3 replicates

Table 3.4.3j ANOVA OF THE OVERALL ACCEPTABILITY OF SURIMI PREPARED FROM DIFFERENT SPECIES OF FISHES

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	136.084	8.00	17.01	129.171
COLLAGEN LEVEL	0.446	3.00	0.149	1.13
ERROR	3.161	24.00	0.13	
TOTAL	139.691	35.00		

Fig. 3.4.3e OVERALL ACCEPTABILITY SCORE
OF SURIMI OF DIFFERENT SPECIES OF FISH



The texture attribute scores are given in Table 3.4.3g and Fig. 3.4.3d. Here the highest initial score was for shark (8.3). ANOVA of this attribute is given in Table 3.4.3h. Here both for days of storage and between species there is significance at 0.1% level.

Table 3.4.3i, gives the overall acceptability score for the surimi of different species of fishes. Fig. 3.4.3e gives it's schematic representation. Here also there is an inverse relation between storage days and sensory score for overall acceptability. ANOVA of this attribute is given in Table 3.4.3j. Here both for days of storage and between species there is no significance even at 5% level.

3.5 *DISCUSSION*

3.5.1 Physico-chemical characteristics

Initially nemipterus showed a nigh moisture content compared to surimi of other species (80%). Shark showed the least moisture content (75.12%). Similar results were reported by several workers. Poulter *et al.*, (1983) obtained the moisture content of surimi as 80.63%, 78.77%, 77.96% and 78.3% from Gulf croaker, Bronze stripped grand, Orange mouth corivna and Cabai

cuchos, respectively. Babbit (1986), reported 90.3% and 88.01% for rock fish and pollock respectively. Hastings *et al.*, (1990) reported moisture of 84.06% and 84.6% for the surimi of whiting prepared with different particle size. Spencer *et al.*, (1992), obtained moisture content of 81.07% for white hake, 78.5% for herring, 76.98% for Atlantic mackeral and 75.8% for dogfish. Chang Lee *et al.*, (1990), obtained 77.26% in the Alaska pollock Surimi. Yean (1993), on studying surimi, the surimi of *Nemipterus tolu*, reported a value of 81.5% $\pm$2.2. In 1995, Saeki, reported 73.8% moisture in salmon surimi.

The physical and rheological properties of surimi is affected by the moisture present in it. When water is immobilized within the three dimensional protein matrix, a gel is formed. So a certain amount of moisture is necessary for adequate solubilization of protein and formation of a gel network responsible for a elastic cohesive gel. From Table 3.4.1a and Fig. 3.4.1, a declining trend is noticed in the moisture content of surimi as storage days advances. This may be due to the decrease in water holding capacity of proteins due to frozen storage. From Table 4.4.1o, a decrease in gel strength is seen as days of storage increases. This is in agreement with the results obtained by Lee *et al.*, (1976) who reported after studying the unwashed

mince of Spanish mackeral, that when the moisture level is decreased the gel resilience and cohesiveness is deccreased and as a result they noted an increase in the toughness.

From the Table 3.4.1c and Fig. 3.4.1b it can be noticed that the protein content decreases with increase in number of storage days. This may be because of protein denaturation due to frozen storage. Here highest protein content was given by shark (17.01%) and lowest by nemipterus (15.75%). Similar values were reported by several workers. Chang Lee *et al.*, (1990) reported 13.24% for the surimi of Alaska pollock. In 1986, Babbit on studying rockfish, croaker and Alaska pollock obtained protein content as 9.5%, 17% and 10.75% respectively. Spencer *et al.*, (1992) on studying white hake, herring and Alaska pollock, reported protein content as 10.23%, 10.7% and 12% respectively. Poulter *et al.*, (1981) has reported, protein nitrogen content in surimi as 2.61%. 2.73% and 2.87% for Gulf croaker, Bronze stripped grand, Orange mouth corivna and Cabai cuchos, respectively. In 1993, Yean *et al.*, reported the protein content in the surimi of *N. tolu,* as 13.5% $\pm$0.5. The non-protein nitrogen content is showing decreasing trend with days of storage. It is absent or negligible towards the 360 days of storage. The bacteria invading the fish meat utilise the non-protein nitrogen compounds and

reduce it to simpler compounds. This may be the reason for the lowering of value with storage days. The non-protein nitrogen compounds are water soluble fractions. The composition of NPN fractions, extracted from fish meat tissues varies with species. Initially, common carp gave a pH value of 6.86, tilapia, 6.81, shark, 6.72 and nemipterus, 6.8. Poulter *et al.*, (1981) has reported similar values. They obtained pH value as 6.5, 6.4, 6.5 and 6.8 for the surimi of Gulf croaker, Bronze stripped grand, Orange mouth corivna and Cabai cuchos, respectively. After studying the pH value of surimi of white hake, herring and Atlantic mackeral, Spencer *et al.*, (1992), reported the value as 6.6, 7.2 and 6.9 respectively. In salmon surimi, Saeki *et al.*, (1992), reported a value of 6.9. From the Table 3.4.4a and Fig. 3.4.4, it is observed that as period of storage increases the pH value also found to increase, except in shark. In shark there is a gradual decrease in pH first and then there is an increase in pH. This increase in pH value with storage days can be explained on the basis of accumulation of basic nitrogenous compounds with storage days.

Lee (1992), reported that rheological property of surimi is affected by muscle pH. The strength of surimi gel is dependant on the pH value of comminuted fish meat. The optimum pH

varies with species, formulation of surimi, ingrdients added etc. The optimum pH was found to be in the range of 6 to 7. Minimum gel strength was reported to be at pH 5 and beyond pH 7, there is a decrease in gel strength.

Trimethylamine oxide is a natural component in muscles and many organs of marine fish but is completely lacking or is present in very small amounts in freshwater fish (Dyer, 1952). Micro-organisms invade the flesh and reduce TMAO to TMA as spoilage advances. The TMA content is often used as an indicator for decomposition in fish. According to Babbit *et al.*, (1972) TMA has been linked to fishy odour and flavour. The TMA content seems to be less in surimi compared to fish fillets since it is water soluble. Govindan (1985) reported TMA value of fresh shark as 245mg%. Occurrence of significant quantities of TMA content of marine fish and lesser contents or absence in freshwater fish indicates its role in osmoregulation. Reddy *et al.* (1992), could obtain a correlation between TMA nitrogen and total volatile basic nitrogen with organoleptic scores.

Gel forming ability of frozen surimi is the most important functional requirement imposing good quality surimi based products. In processed meat products gelation of muscle

protein contributes desirable texture and stabilisation of fat and water. From the Table 3.4.1o and Fig.3.4.1h, it is noticed that gel strength varies with species. This may be due to the consequence of intrinsic difference in myosin, initial pH, protein extractability and functional properties of muscle proteins in different species. According to Samejima *et al.*, (1992) formation of gel with desirable texture depends not only on ionic strength, pH, heating temperature, rate of heating and post-mortem history of the muscle but also on animal species and muscle type. According to Iwata *et al.*, (1961) gel forming ability of surimi made from fresh fish in good condition does not change significantly upto one year when held at a constant temperature below $-20^{O}C$. In 1971, Iwata *et al.*, reported that when the surimi is stored at $-10^{O}C$, the gel forming ability decreases and it becomes useless after 3 months.

In the present study, a decrease in the value of gel strength was observed with frozen storage. This may be due to the protein denaturation. Once the protein is denatured, the water holding capacity of protein is lost. For a surimi to perform its physical and rheological properties, a certain amount of water is needed. As the storage days increase there is a decrease in total protein (Table 3.4.1c) and moisture content (Table 3.4.1a).

This may in turn cause a decrease in gel strength with increase in days of storage. Table 3.4.1u gives the folding test score and 3.4.1v gives the ANOVA.

3.5.2 Microbiological characteristics

The microbial load of a frozen product depends upon various factors such as the nature of raw material, its pre and post-process treatment, sanitary conditions of the processing factories, the rate and nature of freezing (Chen *et al.*, 1990). The temperature and period of storage,the original numbers and stages of growth of microrganisms present,thawing process and protection offered also affects the microbial load. In the present study, initially, common carp had a bacterial load of 5.5×10^4 per gram of meat and tilapia had 3.4×10^5 per gram, shark had 3.8×10^5 per gram and nemipterus had 1.1×10^5 per gram of meat.

Raccah *et al.*, 1978, reported that aerobic plate count (APC) of mechanically deboned cod, pollock and whiting ranged from 4.7×10^5 per gram to 7×10^5 per gram. Blackwood (1973), found that there is a bacterial load of 10^6 per gram of meat for the minced fish samples. Licciardello *et al.*, (1978) reported, aerobic plate count at 21^0C in Alaska pollock as 10-30,00,000/gm whereas in cod mince the value ranged from 30,000

- 30,00,000/gm. Nickelson *et al.*, (1980) after studying six fishes reported APC of fish mince ranged between 1.2 x 10^5/gm to 2.6 x 10^8/gm. In frozen stored mince the value ranged from 7.9 x 10^3/gm to 7.9 x 10^6/gm. Himelbloom *et al.*, (1991), on investigating microbial conditions of Alaska pollock during processing of surimi reported APC for analogue grade surimi as 5.5 x 10^4/gm and for non-analogue grade surimi 3.0 x 10^5/gm.

The flesh, of fish caught from the sea is sterile. After death bacterial attack from surface, gut and gill increase the microbial load of fish muscle. So the environment has got a role in contributing to the bacterial load of muscle. During processing of surimi due to beheading, gutting and washing, there is reduction in bacterial load, but at the same time the increased handling will increase the number of bacteria in the surimi. The ingredients like cryoprotectants also will increase the bacterial load.

Both *E. coli* and *Staphyloccoccus* are sensitive to low temperature, so they are destroyed by frozen storage. Initial incidence of these bacteria in the surimi and further dissappearance on frozen storage can be thus explained. Licciardello *et al.*, (1978) reported in Alaska pollock surimi, the

coliforms (MPN) per gram as less than three and coagulase positive *Staphyloccoccus* per gram as less than 3. In the present study there was no incidence of *Salmonella* and *Vibrio* in the surimi prepared from any fish species studied. This does not mean that these bacteria will not occur in the surimi. While preparing surimi utmost care should be taken to prevent the incidence of pathogenic bacteria, since surimi is meant for human consumption. Harvesting, processing, transportation and marketing must operate as an unified system by taking extra care in each step to prepare surimi products of high quality.

3.5.3 Sensory characteristics

Sensory evaluation is the oldest and still the most widespread means of evaluating the acceptability and edibility of fish. According to him the line dividing fish that are still fresh from those with some early signs of spoilage is not well defined and is most often subject to difference in personal opinion. According to Park *et al.* (1994) gel colour is an important characteristic of surimi. Spencer *et al.* (1992) reported colour of surimi in descriptive terms. He explained the colour of mackeral surimi as dark or more greyish, corresponding colorimeter reading is L-value between 63.5-66.9. Gopakumar *et al.*, (1992) also described the colour of surimi in descriptive terms.

Thecolour of surimi is influenced by the protein content of the raw material. The difference in colour of surimi of different species can be due to the difference in protein content. The difference in colour noted in the present study can also be explained in this manner.

Surimi is expected to have no fishy odour. But during storage some biochemical reactions occur in the muscle and it contributes some off odour to the meat which is not desirable. It is also possible that surimi found unacceptable by organoleptic examination may show one or more chemical indices that will indicate lower value for its acceptance.

Meullenet *et al.*, (1994) reported that sensory evaluation is the traditional method for determining the acceptable texture. Texture is an important attribute contributing to the acceptability of the consumer. Protein gelation has got a role in contributing to the final texture of the product. In the present study the texture of shark surimi is showing high value, which may be due to its high content of connective tissue protein. Hassan (1991), reported that in high collagen fishes the texture of raw and cooked meat is influenced by the collagen content.

The strength of surimi is also dependant on the amount of water and pH (Lee, 1992). The present study is in agreement with this statement. According to Rodger *et al.*, 1980, the texture is an important sensory attribute on which protein denaturation has a profound influence. In the present study also it is noted due to frozen storage there is protein denaturation which in turn leads to loss of texture.

From the results obtained it is seen that there is a variation in acceptability with species. So by standardising methodology for production of surimi in commercial line, separate procedures should be adopted for each speciesto get better consumer acceptability. By repeated washing during manufacturing, most of the odour imparting compounds, pigments, water soluble proteins and other undesirable materials are removed and a translucent, bland material is obtained, which is surimi. To improve the organoleptic qualities, some methods can be adopted by incorporating some ingedients or by altering the existing technology, but at the same time attention has to be given to ratain the physico-chemical and microbiological parameters within the acceptable limit.

CHAPTER 4

INFLUENCE OF COLLAGEN ON TEXTURE AND KEEPING QUALITY OF SURIMI

4.1 *INTRODUCTION*

Freezing and frozen storage are the important methods of preservation of fishery products. Although undesirable changes such as microbial growth and other biochemical alterations are controlled by frozen storage, changes in functional properties occur. Since surimi can be used as an ingredient in a variety of food formulations, physico-chemical, miro-biological and oraganoleptic data will be useful in establishing standards for the products.

The connective tissue proteins are chiefly composed of stroma proteins and these contributes only a small fraction of the total protein content in fish meat. However they are involved in holding together the muscle bundles (myotomes) of fish, and therefore contributes to the overall texture of meat. Connective tissue (myocommata) proteins contribute to the toughness of fish meat. Among connective tissue proteins collagen is found to be the major protein and is seen to influence the texture of fish meat and also properties such as gelling, emulsification, elasticity etc.

In this study an attempt is made to find the influence of collagen, on texture and keeping quality of surimi prepared from different species of fishes.

4.2 *REVIEW OF LITERATURE*

4.2.1 Collagen

4.2.1.1 General concept

Soluble collagen from the swim bladder of teleosts was perhaps the first collagen to be characterised (Boedtker and Doty, 1955). Another similar material studied was a soluble form of collagen from shark swim bladder (Leach and Barrett, 1967). Fish collagen acquired some economic importance in the past as glue but has been replaced by synthetic products. Kubota and Kimura (1967), found a special collagen in shark fin, elastoidin, with a special characteristic cysteine content.

At the temperature of 40^{o}C, the collagen molecules were ruptured and converted into elements of lower molecular weight (Balian and Bowes, 1977). The partially solubilized samples were sensitive to heat, probably because their collagen molecules were unfolded by prior solubilisation. According to Aberle and Mills, 1983, the basic collagen structure

known as tropocollagen, consista of three polypeptide chains, each twisted in a left handed helix, coiled around each other to form a right handed triple super helix. As temperature is increased, the collagen's regular structure breaks and the chains separate and fold into random structures without any residual native structure. In 1984, Bailey, reported that collagen is polymerised through the formation of covalent cross links. Reducible cross links are involved in head to tail longitudinal cross linking. This confers considerable tensile strength to the collagen fibers. An additional transverse, non-reducible, inter-fibrillar cross links prevent myofibril slippage during mechanical stress. Any change in one cross linking state of collagen will cause change in texture.

4.2.1.2 Collagen content and texture of fish

Initial work led to the conclusion that the quantity and strength of connective tissue determined the toughness of meat (Mackintosh *et al.*, 1963). It has been demonstrated that the content of collagen of stroma protein in the muscle of teleost is usually within a range from 1 to 4 percent of the total protein content (Dyer *et al.*, 1950; Shimizu *et al.*, 1960; Nasedkina *et al.*, 1972; Kubota *et al.*, 1975, Hatae *et al.*, 1986). The same authors have also demonstrated that the collagen

content in the white muscle of fish significantly varied with species and that the texture of raw meat is affected by the content of collagen in the muscle. Culler *et al.*, 1978, found that tenderness does not vary significantly with total collagen content but it varies significantly with soluble collagen. The textural properties of cooked fish meat depends primarily on the state of myofibrillar proteins, rather than the connective tissue, as pointed out by Dunajesky, 1979. However, he also indicated that the texture of cooked meat is affected by gelatin, derived from the muscle collagen. Hatae *et al.*, (1984 and 1986) also studied the contribution of connective tissues to the textural differences of various fish species in raw and cooked condition. It was observed that the firmness of raw meat increases with increase in collagen content.

Sato, 1988, observed that the total collagen content in the white muscle of fishes significantly vary with species, and the texture of raw meat is affected by the content of collagen in the muscle.

Sikorski *et al.*, (1984) reviewed the possibilities of collagen as functional material. Montero and Borderias (1989), reported on collagen for manufacture of casings,

as an emusifying agent, forming agent amnd gellying agent. They also reported that collagen from skin was more soluble than muscle collagen. The collagen could be used for binding water in blocks of fish mince during frozen storage (Montero *et al.*, 1989b, Montero *et al.*, 1995). They reported that heat denaturation of collagen commenced before 30^{o}C and was substantial at 40^{o}C. The freeze dried samples showed less heat solubilization than the frozen samples. The freeze dried samples are generally less soluble (Montero, 1989) and require higher temperature to augment collagen solubilization. If the collagen is intended for incorporation in a reconstituted product, it is important that it should denature at the cooking temperature. Considering that cooking temperature for some foods may be as low as 60^{o}C, frozen collagen will be more suitable than freeze dried collagen.

According to Beltram *et al.*, (1991) for consumers, toughness is probably the most critical quality parameter of meat. Meat toughness is a complex property depending mainly on the two protein structures, connective tissue and myofibrils, which give the muscle its mechanical properties. Each of the structural components of the connective tissue make a distinct contribution to the overall toughness of meat.

Meullenet *et al.*, (1994) evaluated textural differences of chicken frankfurters made with 0, 2, 4, 6, 8% added collagen fibers and 10, 15, 20, 25, 30% water using a torsion test and sensory texture profile analysis. Here the addition of collagen fibers resulted in harder, springier and less juicy frankfurters. Added water resulted in softer, less springy and juicier frankfurters. The protein gelation process determines the final texture and an understanding of the factors affecting gelation is fundamental to the formulation of analogs. Some factors influencing gelation are protein source, processing conditions and non-meat ingredients (Montejano *et al.*, 1984; Saliba *et al.*, 1987; Amato *et al.*, 1989).

4.2.2 Keeping quality studies

The review of this aspect is presented under three heads

4.2.2.1 Physico-chemical studies

Shimizu *et al.*, (1954) reported that myosin solubility is responsible for gel strength of fish sausage. The development of toughness in frozen cod muscle was correlated with the amount of extractable actomyosin (Dyer *et al.*, 1956). It has

been reported that myofibrillar proteins are responsible for the desired textural properties in muscle protein-based gel and emulsion type comminuted products (Fakazawa *et al.*, 1961; Swift, 1965; Samejima *et al.*, 1969;Nakayama and Sato, 1971; Trai *et al.*, 1972). According to Tarr, 1966, the pH in fish muscle correlated with water binding capacity and meat toughness. It has been demonstrated that there is considerable varibility in the relationship between myofibrillar protein solubility of raw fish tissue and texture of finished products (Umemoto, 1971; Webb *et al.*, 1976).

The temperature increase, as in cooking, has opposite effect on the proteins of connective tissue and myofibrils. Collagen degrades most of it, being converted to gelatin, at the cooking temperature, and this transition increases the tenderness of meat. The coagulation and degradation of myofibrillar protein, reduces the toughness. Consequently different muscles react differently when coooked and for the same species, the ultimate textural properties depends upon the temperature time conditions during cooking (Szczeniak *et al.*, 1965 and Howe *et al.*, 1994). They also demonstrated that the extent of muscle protein degradation during thermal processing was closely related to the texture of processed fish gels, and suggested that

some proteolytic factors in minced fish tissue were probably active during thermal processing of fish gels. They also have shown that by controlling these proteolytic factors, product texture can be controlled. Cheng *et al.*, (1979b) indicated that low correlation between protein solubility of raw tissue and gel texture of cooked fish gels, probably resulted from variation in protein degradation during thermal processing. They also showed the relationship between texture and water holding capacity of cooked fish gels and indicated that the muscle proteins like connective tissue and actomyosin play a key role in influencing these properties.

Burgarella (1985a,b) suggested that additives function by filling the interstitial spaces of myofibrillar protein network of gels, do not seem to interact with myosin and have a tendency to weaken the gel. Their results also indicated that egg white additive produced a weaker gel than whey protein concentrate, and that a gel of surimi alone had more desirable textural attributes.

The emusifying ability of muscle proteins is one of the important functional property for manufacturing processed meat products. In general, it is known that the

functionality of myofibrillar proteins determines the characteristics of finished processed meat. Good correlation between compression and penetration values were seen by Lee *et al.*, (1989) in surimi gels prepared with and without added ingredients. In 1992, Gopakumar *et al.*, on studying the properties of surimi from *Sphyraena, Nemipterus japonicus, O. mossambicus and M. dobsoni,* found that prawn surimi is the hardest because of its high concentration of fibrils brought about by the easy extraction of the soluble nitrogen fraction. They also observed that the gels of the fishes studied have more or less the same textural quality.

During frozen storage of fish muscle alteration in fish myofibrillar proteins have been largely accepted as the principal cause of loss of protein functional properties (Shenouda, 1980). Myofibrillar protein insolubilisation was a main factor affecting functional properties (Mutsumoto, 1980; Borderias *et al.*, 1985). It is not known which chemical indicators are applicable to the detection of decomposition of these products. Total volatile acids (TVA), and total volatile bases (TVB) are well known chemical indicators of decomposition for seafood products. Cadavarine, putrescine and histamine have also been suggested as chemical indicators of decomposition (Mietz and Karmas, 1978; Struszkiewicz and Bond, 1981; Farn and Sims,

1987; Taylor and Sumner, 1987). Wekell *et al.*, 1987, suggested that TVA and TVB may be used as chemical indicators for surimi based fabricated seafood products.

4.2.2.2 Microbiological studies

According to Shewan (1951), freezing causes and initial drop in the number of bacteria of the order of 60-90%. The heavier the initial load the greater the number of survivors. Tressler and Evers, (1957) discussed the salient features of microbiology of freezing of fish. According to him, freezing does not sterilize the fish, but is causing considerable reduction in bacterial population.

In 1974, Blackwood found that 40% of minced fish samples he examined had a bacterial count of more than 10^6/gm. Blackwood (1974) and Babbit *et al.* (1974) also dealt with bacteriology of minced fish flesh. According to Bond (1975) bacteriological limit for minced fish in some countries is a maximum standard plate count level of 10^5/gm. The major determinant of microbiological quality of minced fish is raw material itself (Cann and Taylor, 1976).

Raccah *et al.*, (1978) noted that mechanical deboning of several species increased the microbiological count ten fold. Licciardello *et al.*, (1978) compared minced washed Alaska pollock produced in Japan with minced fish from North Atlantic. They found that surimi produced in Japan contained lower number of aerobic heterotrophs, coliforms, faecal streptococcus and coagulase positive staphylococcus than found in blocks of minced pollock, cod and other fish produced in Canada, Green Land and Europe. Licciardello and Hill (1978) examined 208 frozen minced blocks from cod, pollock, haddock, hake, lingcod and ocean catfish from Japan, Canada, Denmark, Green Land, Iceland, Norway and Poland. Aerobic plate counts (APC) at 21^{o}C ranged from 10 to 30,00,000/gm. Alaska pollock blocks had the lower counts (10-100,000/gm), where as counts of cod frame mince were high (30,000-30,00,000/gm). Raccach and Baker, 1978, reported on the microbial properties of mechanically deboned flesh from white sucker, cod, pollock and whiting. APC of finished mechanically deboned products ranged from 4,70,000 - 700,000/gm, when plates incubated at 25^{o}C. Similarily aerobic heterotropic counts increased by one log cycle during mechanical deboning of fish flesh from washed cod frames (Raccach and Baker, 1978).

Himelbloom *et al.*, (1991a) investigated microbial conditions of Alaska Pollock, during processing of surimi. Aerobic plate count was 2.0 x 10^3 per gram after mincing, 2.3 x 10^3 per gram after washing or screening, 4.2 x 10^4 per gram after refining and 1.6 x 10^4 per gram after dewatering. Aerobic plate count analog grade surimi was 5.5 x 10^4 per gram and highest total coliform most probable number was found to be more than 1100 per gram.

Bacterial profile of fresh fish, fish mince and spoiled fish mince from *Jhonnius dussumieri*, at refrigerated storage was studied by Abraham *et al.*, (1992). In 1992, Anguilera *et al.*, on studying the stability of washed and unwashed mince from spanish sardines treated by mild heating, pH 5.7 to 6.0, 0.05 to 0.2% potassium sorbate and 2.0 to 6.0% sodium chloride, found that the partly cooked product was microbiologically stable at 15^{o}C for at least 15 days.

4.2.2.3 Organoleptic studies

A sensory property is one that is perceived by one of the senses with which the food consumer evaluates the product (Kramer, 1973). According to Kramer, (1952) sensory

evaluation panels can be a precise tool if properly designed, if judges are carefully selected, if proper physical facilities are available and if statistical analysis are used. Kramer, (1973) reported that a consumer panel is subjected to more extraneous influences than a trained laboratory panel which has been taught to perform in an analytical manner. However, in the latter cases, the extraneous influences cannot be totally eliminated.

Szczesniak *et al.*, (1963) believes that sensory methods of measuring food quality are time consuming and expensive. They lack precision because of the variability from person to person, time to time and likes and dislikes of each person. He further stressed that, despite these serious obstacles, sensory measurement of texture is a very important aspect that cannot be ignored. He also state that, sensory evaluation is the best method of evaluating texture of new types of fabricated foods in the early stages of development, and for providing a basis on which instrumental methods might be later developed for use as a quality measure and production control tool.

According to Rodger *et al.*, (1980) the sensory attribute 'texture of meat', is an important attribute on which protein denaturation has a profound effect. Though a variety of

mechanical methods were developed and used for measuring the texture of meat, there has been some agreement that tenderness and juiciness are adequately described sensory qualities.

Hollingworth *et al.*, (1990) stated that for reasons of simplicity and rapidity the generally accepted and established method for the determination of decomposition in seafood is sensory analysis. On studying the deteriorative change in the pink perch during frozen storage Reddy *et al.*, (1992) observed a decrease in organoleptic scores of the mince, which was significant throughout the storage period. Park *et al.*, (1994) reported that for measuring product colour the world surimi industry uses three instruments: Hunter, Minolta and Nippon Denshoka colorimeters. All are tri-stimulus filter calorimeters based on the same technological principles.

No work has so far been done on the influence of collagen content on the texture and storage qualities of fish mince based porducts. Here an attempt is made to study this aspect in detail. For this three species of fish from different environments and of comparatively low collagen content are selected and different levels of collagen is incorporated to the surimi prepared.

4.3 *MATERIALS AND METHODS*

4.3.1 Preparation of collagen from the air bladder

Collagen was prepared and purified from the air bladder of common carp as described by Gallop and Seifter (1963). Approximately 500gm of freshly collected swim bladder from common carp was blended in a waring blender for 1-2 minutes at $5^{O}C$, with pre-chilled 0.5M sodium acetate solution. The volume of the homogenate was then brought to 2 liters with the same reagent. The mixture was stirred mechanically for 15-18 hours at $5^{O}C$ and then centrifuged at 2000 RPM at 5^{O} for 1 hour. The supernatent was discarded. The pasty residue obtained was transfered to a clean muslin cloth and squeezed until most of the liquid was removed. The material was then extracted once more with 2 liters of pre-chilled 0.5M sodium acetate at $5^{O}C$ for 15-18 hours. The mixture was centrifuged as before and the residue was squeezed free of gross liquid. The process of extraction with sodium acetate solution was performed two more times, yielding finally a residue from which non-collagenous soluble proteins and polysaccharides have been removed.

The residue was then suspended in 2 liters of cold distilled water and centrifuged at 2000 rpm at $5^{O}C$ for one hour. The supernatent was discarded and the precipitate was again suspended in 2 liters of water and centrifuged. A third washing

with water was also carried out in an identical manner. The precipitate was then transfered to a muslin cloth and squeezed free of gross liquid.

The crude collagen obtained in this manner can be directly incorporated in the surimi or it can be dried in oven at 30-35^{o}C and stored in a refrigerator. As and when needed this dried collagen has to be soaked overnight in water at 4^{o}C and ground to get a homogenate with a resultant moisture content comparable to that of surimi. The amino acid composition of collagen from the air bladder of carp is given in Table 4.3.1.

4.3.2 Sample preparation

Surimi was prepared from common carp (*Cyprinus carpio)*, tilapia (*Oreochromis mossambicus)* and shark (*Scoliodon sorrakowah)* and nemipterus (*Nemipterus japonicus)* as described in 3.3.3 Inorder to prepare surimi with different levels of collagen, collagen prepared as above was incorporated to the surimi prepared from different species. The levels of collagen fixed for the study was 0%, 5%, 10% and 15% of the total protein content of the fish (w/w). No extra collagen was added to shark surimi being a high collagen content fish. After collagen was added surimi was put in a homogeniser and mixed for three minutes. For each treatment

Table 4.3.1 THE AMINO ACID COMPOSITION FROM THE AIR BLADDER OF CARP
(Residues of amino acids/1000 total residues)

AMINO ACIDS	QUANTITY
Glycine	325
Alanine	126
Valine	18
Isoleucine	10
Leucine	21
Proline	116
Hydrxyproline	81
Phenylalanine	14
Tyrosine	2
Serine	37
Threonine	29
Methionine	13
Cystine	< 1
Hydroxyline	7.4
Lycine	2.6
Hystidine	3.8
Arginine	53
Aspartic acid	47
Glutamic acid	71

Source : J. Biol. chem. 235(1960):995-997

sampling was done in triplicate. The samples thus prepared was sub-divided into smaller lots, for easy withdrawal for analysis at different periods of frozen storage.

The rest of the procedures including sampling intervals and analysis were same as described in Chapter 3. The physico-chemical parameters studied include moisture, protein, NPN, pH, TMA, TVN, α amino nitrogen, gel strength, expressible water and folding test. Sensory evaluation and microbiological analysis were also carried out in the samples at different sampling periods. The details of the procedures are given in 3.3.4, 3.3.5 and 3.3.6.

4.3.4 Statistical analysis

The data were analysed statistically to separate out significant treatments since all experiments were conducted in triplicates, the mean of the three values were used for the analysis. ANOVA was used to determine significance of variation between different storage days of surimi at different levels of collagen and also to determine significance of variations between different levels of collagen on the characteristics of surimi. Wherever the treatments were found to be significant LSD at 5% level were worked out to separate out significant treatments.

4.4 *RESULTS*

The physico-chemical, microbiological and sensory characteristics of surimi from different species of fishes at varying levels of collagen was studied for a period of one year frozen storage.During the study a general decline in quality was observed over the months of storage. In samples with incorporated collagen, there is a significant change in physico-chemical and organoleptic characteristics. Actually the trend of change in parameters is same. There is a progressive increase or decrease in the parameters when collagen is added. This can be clearly seen from the corresponding figures.

4.4.1 Physico-chemical analysis

4.4.1.1 Moisture

Moisture content of surimi of different species of fishes at different levels of collagen and at different storage period is given in Table 4.4.1.1a. In the case of moisture content in the surimi of common carp, an increase from 80.12% to 81.2% was noted initially, as collagen content increased from 0 to 15%. After 360 days, the moisture content reduced from 80.12% to

Table 4.4.1.1a *MOISTURE CONTENT (%) IN THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS>	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
	COLLAGEN									
COMMON CARP	0%	80.12	78.16	77.78	75.00	75.52	73.90	70.00	68.62	64.20
	5%	80.58	79.92	81.39	73.68	70.27	73.98	72.00	69.98	66.92
	10%	80.80	79.98	81.48	75.00	76.73	76.12	76.01	72.18	70.88
	15%	81.20	81.20	83.33	77.27	76.67	75.00	76.18	75.02	74.66
TILAPIA	0%	80.40	80.20	80.10	80.09	81.82	80.00	77.02	75.00	73.28
	5%	81.00	80.09	80.07	79.98	82.76	77.78	77.68	76.19	75.98
	10%	81.02	81.20	80.08	80.02	81.08	76.74	78.90	77.82	76.18
	15%	81.10	82.05	80.98	80.88	81.20	80.00	78.98	78.68	77.19
SHARK		75.12	73.83	74.62	74.48	73.53	72.92	71.68	70.98	69.96
NEMIPTERUS	0%	82.00	80.15	79.98	79.42	78.14	76.29	74.00	72.18	71.08
	5%	81.18	81.06	80.02	79.89	79.15	77.39	75.63	73.42	72.23
	10%	80.08	80.02	80.02	79.62	78.03	78.01	77.00	74.27	73.19
	15%	80.88	77.26	80.08	80.02	79.19	78.67	77.05	75.80	73.98

* mean of three readings

Table 4.4.1.2a TOTAL PROTEIN (gm/100gm meat) IN THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	16.38	15.48	14.28	13.12	13.06	12.68	12.32	12.05	11.93
	5%	16.42	15.52	14.36	13.28	13.14	12.76	12.53	12.13	12.02
	10%	16.91	15.64	14.44	13.35	13.28	12.93	12.61	12.35	12.12
	15%	16.99	16.68	14.56	13.47	13.30	13.01	12.80	12.47	12.35
TILAPIA	0%	16.57	15.53	14.47	13.35	12.10	11.92	10.86	10.45	10.21
	5%	16.58	15.57	14.51	13.42	12.80	12.03	10.92	10.83	10.42
	10%	16.61	15.65	14.60	13.50	12.95	12.34	11.26	10.99	10.64
	15%	16.65	15.72	14.64	13.53	13.08	12.54	11.35	11.02	10.85
SHARK		17.01	15.06	14.24	13.86	12.74	12.65	12.50	12.21	11.80
NEMIPTERUS	0%	15.75	14.02	13.78	12.61	11.83	10.79	10.53	10.45	9.99
	5%	15.82	14.14	13.82	12.73	11.92	10.88	10.63	10.48	10.22
	10%	15.96	14.16	13.94	12.78	11.95	10.89	10.67	10.52	10.35
	15%	16.01	14.20	13.98	12.82	11.99	11.07	10.98	10.67	10.47

Table 4.4.1.1b ANOVA OF THE MOISTURE CONTENT OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	547.88	8.00	68.49	22.615***
COLLAGEN LEVEL	101.24	3.00	33.75	11.143***
ERROR	72.68	24.00	3.03	
TOTAL	721.80	35.00		

*** P < 0.001

Table 4.4.1.1c ANOVA OF THE MOISTURE CONTENT OF SURIMI PREPARED FROM TILAPIA

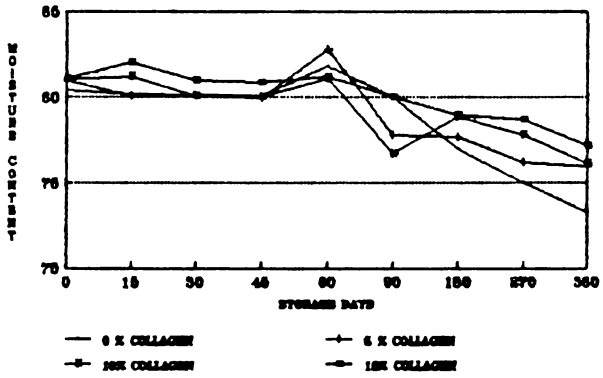
SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	128.16	8.00	16.02	17.177***
COLLAGEN LEVEL	8.75	3.00	2.92	3.129*
ERROR	22.38	24.00	0.93	
TOTAL	159.29	35.00		

*** P < 0.001
* P < 0.05

Table 4.4.1.1d ANOVA OF THE MOISTURE CONTENT OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	274.10	8.00	34.26	30.885***
COLLAGEN LEVEL	5.67	3.00	1.89	1.705
ERROR	26.63	24.00	1.11	
TOTAL	306.40	35.00		

*** P < 0.001



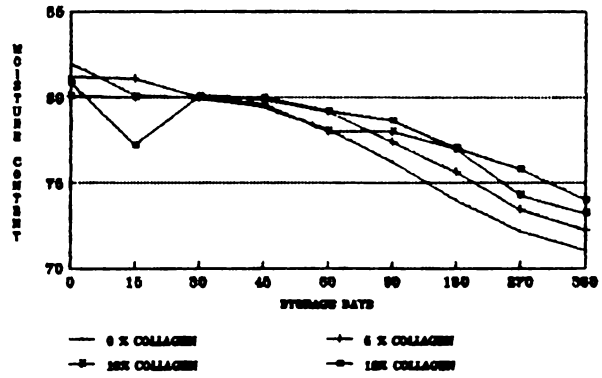
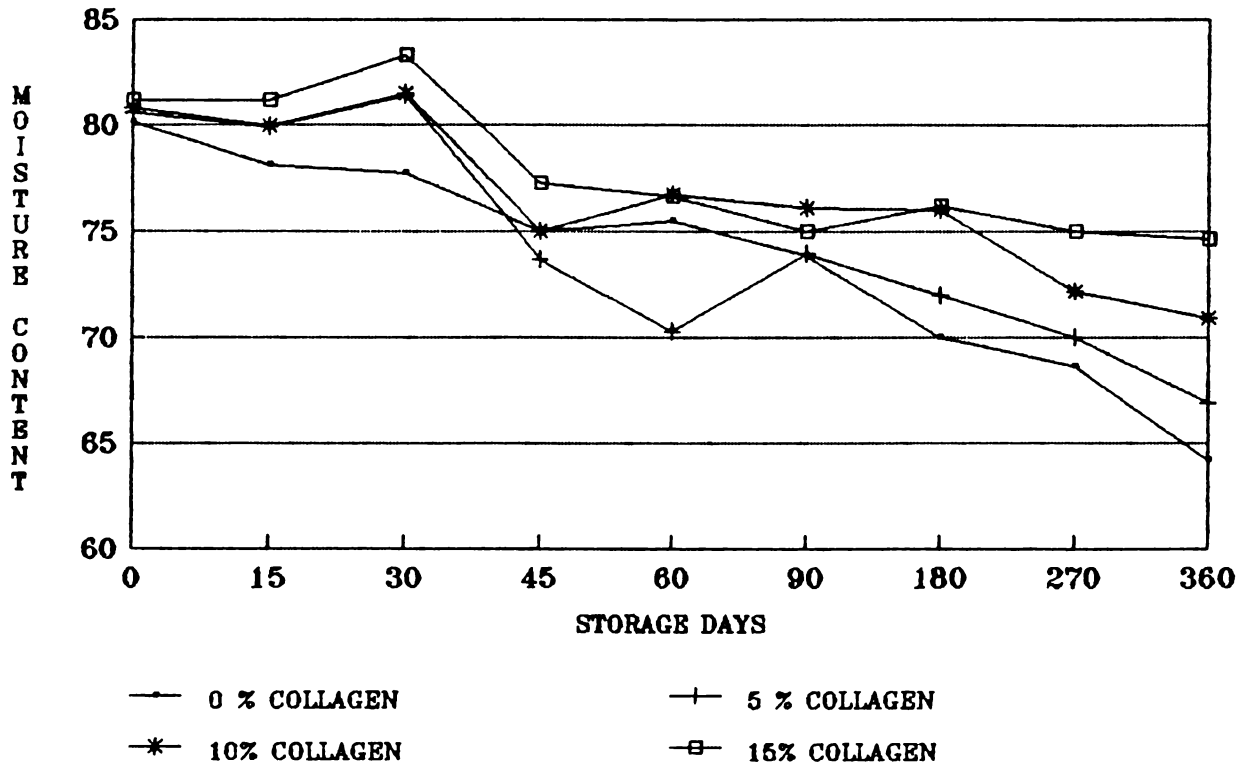


Fig. 4.4.1.1a MOISTURE CONTENT OF SURIMI
OF COMMON CARP



64.2% in the case of surimi of common carp without collagen. But this much decrease in moisture content is not seen in samples with increased levels of collagen. Even after 360 days, there is a moisture content of 74.66% in the surimi of common carp with 15% collagen. The same is the case with tilapia and nemipterus. So, with the increase in collagen level, the rate of decrease of moisture content in surimi on storage is found to minimum. Fig.4.4.1.1a, Fig. 4.4.1.1b and Fig. 4.4.1.1c represents the contents of moisture in the surimi of common carp, tilapia and nemipterus respectively at different storage periods. Table 4.4.1.1b, Table 4.4.1.1c and Table 4.4.1.1d gives ANNOVA of moisture content of surimi prepared from common carp, tilapia and nemipterus respectively at different storage periods at different levels of collagen. In these tables the variation between storage days is found to be significant at 0.1% level of significance in all the three species. Among concentrations there is a 0.1% level of significance for common carp, 5% for tilapia and there is no significance even at 5% level for nemipterus.

4.4.1.2. Total protein

Table 4.4.1.2a gives the total protein content of surimi of different species of fishes at different levels of

Table 4.4.1.2b ANOVA OF THE PROTEIN CONTENT OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	79.80	8.00	9.98	1688.885***
COLLAGEN LEVEL	0.74	3.00	0.25	41.573***
ERROR	0.14	24.00	5.9065E-03	
TOTAL	80.68	35.00		

*** P < 0.001

Table 4.4.1.2c ANOVA OF THE PROTEIN CONTENT OF SURIMI PREPARED FROM TILAPIA

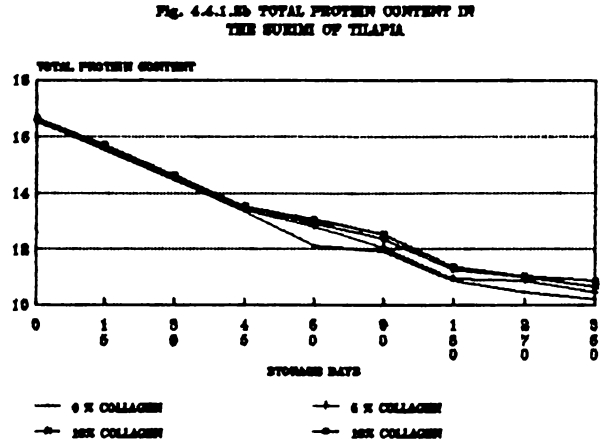
SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	150.28	8.00	18.79	897.109***
COLLAGEN LEVEL	0.99	3.00	0.33	15.744***
ERROR	0.50	24.00	0.02	
TOTAL	151.77	35.00		

*** P < 0.001

Table 4.4.1.2d ANOVA OF THE PROTEIN CONTENT OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	123.27	8.00	15.41	4217.91***
COLLAGEN LEVEL	0.35	3.00	0.12	31.901***
ERROR	0.09	24.00	3.6532E-03	
TOTAL	123.71	35.00		

*** P < 0.001



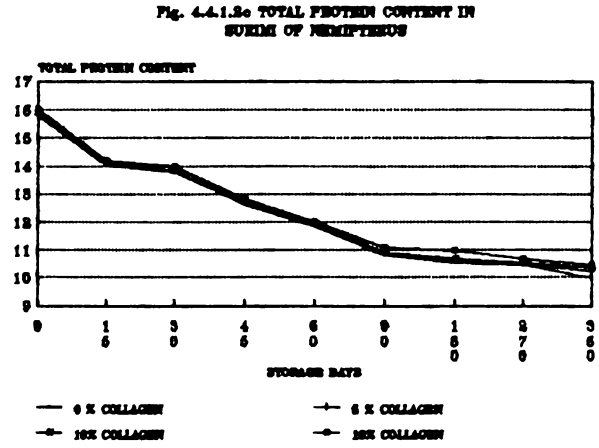
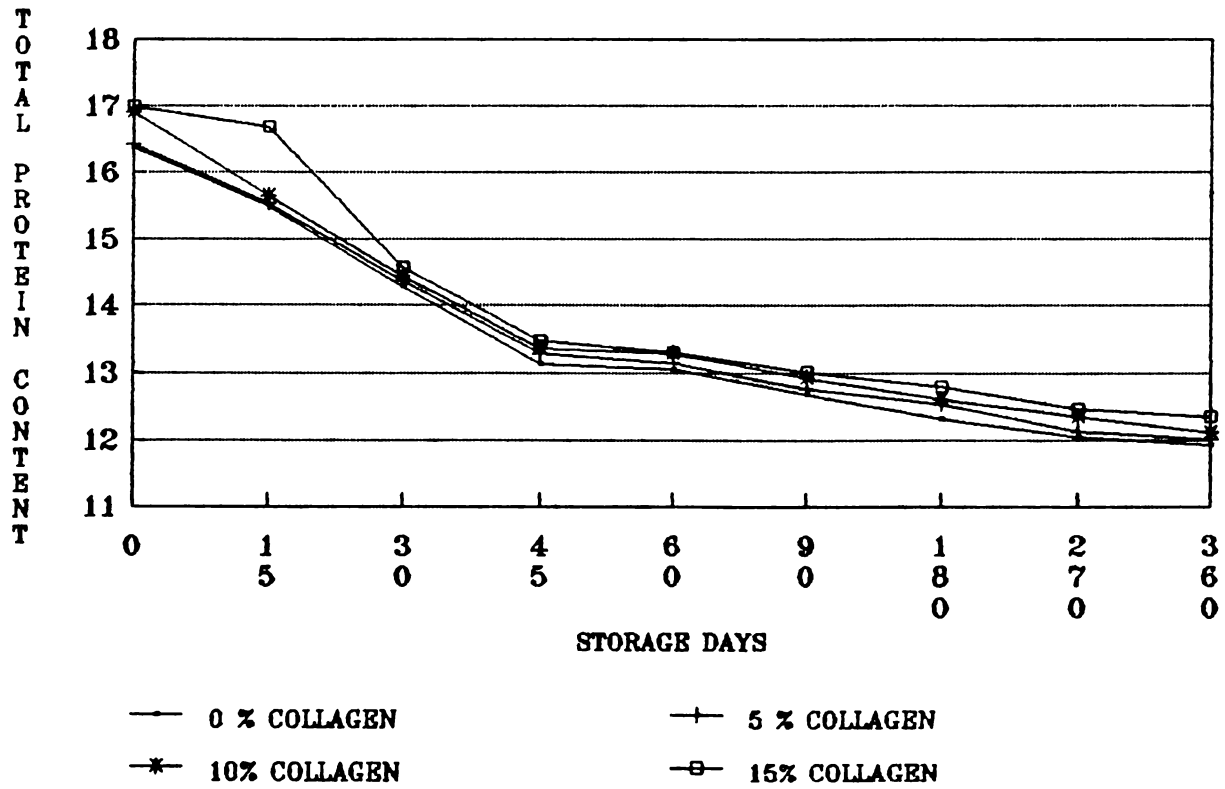


Fig. 4.4.1.2a TOTAL PROTEIN CONTENT IN THE SURIMI OF COMMON CARP



collagen. Graphical representation of Table 4.4.1.2a is given in Fig. 4.4.1.2a, Fig. 4.4.1.2b and Fig 4.4.1.2c. With increase in storage period there is a decrease in total protein. The decrease is from 16.38% to 11.93%, 16.42% to 12.02%, 16.91% to 12.12% and 16.99% to 12.35% in the surimi of common carp with 0%, 5%, 10% and 15% of collagen respectively. In tilapia surimi the decrease is from 16.57 to 10.21%, 16.58 to 10.42%, 16.61 to 10.64% and 16.65 to 10.85% for 0%, 5%, 10% and 15% collagen respectively. In nemipterus the decrease was from 15.75 to 9.99%, 15.82 to 10.22%, 15.96 to 10.35% and 16.01 to 10.47% in the surimi with 0%, 5%, 10% and 15% collagen respectively.

ANOVA of total protein content of surimi of common carp, tilapia and nemipterus is given in Table 4.4.1.2b, Table 4.4.1.2c and Table 4.4.1.2d respectively. In these tables the variation between the days of storage is insignificant even at 5% level and the variations between concentrations are significant at 0.1% level.

4.4.1.3 Non-protein nitrogen

Non-protein nitrogen (NPN) content of different species of fishes at different levels of collagen at

Table 4.4.1.3a NON-PROTEIN NITROGEN PRESENT IN SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

NO. OF DAYS		0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	0.17	0.14	0.11	0.10	0.07	0.05	0.03	0.00	0.00
	5%	0.18	0.13	0.10	0.10	0.06	0.05	0.02	0.01	0.00
	10%	0.17	0.12	0.10	0.09	0.06	0.04	0.01	0.01	0.00
	15%	0.16	0.13	0.08	0.07	0.04	0.03	0.00	0.00	0.00
TILAPIA	0%	0.20	0.18	0.14	0.11	0.07	0.05	0.03	0.02	0.01
	5%	0.20	0.17	0.13	0.09	0.07	0.04	0.02	0.01	0.00
	10%	0.19	0.16	0.12	0.09	0.06	0.03	0.01	0.00	0.00
	15%	0.18	0.14	0.11	0.07	0.05	0.03	0.00	0.00	0.00
SHARK		0.33	0.24	0.16	0.15	0.09	0.07	0.05	0.03	0.01
NEMIPTERUS	0%	0.22	0.17	0.14	0.09	0.09	0.08	0.07	0.05	0.05
	5%	0.19	0.17	0.13	0.08	0.06	0.06	0.05	0.03	0.03
	10%	0.21	0.19	0.13	0.08	0.07	0.05	0.04	0.04	0.03
	15%	0.20	0.18	0.12	0.07	0.06	0.05	0.05	0.02	0.02

Table 4.4.1.4a pH IN THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

NO. OF DAYS		0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	6.86	6.92	6.95	6.98	7.09	7.09	7.12	7.12	7.20
	5%	6.83	6.80	6.81	6.90	7.01	7.02	7.04	7.13	7.15
	10%	6.74	6.74	6.82	6.86	6.96	6.98	7.02	7.12	7.18
	15%	6.75	6.78	6.81	6.87	6.96	7.02	7.04	7.10	7.12
TILAPIA	0%	6.81	6.93	6.92	6.93	6.95	7.03	7.13	7.20	7.22
	5%	6.75	6.90	6.89	6.91	6.87	6.90	6.98	7.22	7.22
	10%	6.77	6.91	6.90	6.91	6.91	6.92	6.98	7.12	7.28
	15%	6.69	6.92	6.91	6.92	6.92	6.93	6.99	7.18	7.24
SHARK		6.72	6.68	6.58	6.49	6.46	6.48	6.44	6.52	6.68
NEMIPTERUS	0%	6.80	6.80	6.91	6.99	6.98	7.20	7.30	7.41	7.52
	5%	6.71	6.74	6.91	6.91	6.91	7.18	7.21	7.23	7.32
	10%	6.71	6.81	6.82	6.91	7.01	7.19	7.20	7.23	7.27
	15%	6.67	6.72	6.80	6.82	6.91	7.15	7.18	7.21	7.23

Table 4.4.1.3b ANOVA OF THE NON-PROTEIN NITROGEN CONTENT OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	0.11	8.00	0.01	295.404***
COLLAGEN LEVEL	1.6972E-03	3.00	5.6574E-04	12.039***
ERROR	1.1278E-03	24.00	4.6991E-05	
TOTAL	0.11	35.00		

*** P < 0.001

Table 4.4.1.3c ANOVA OF THE NON-PROTEIN NITROGEN CONTENT OF SURIMI PREPARED FROM TILAPIA

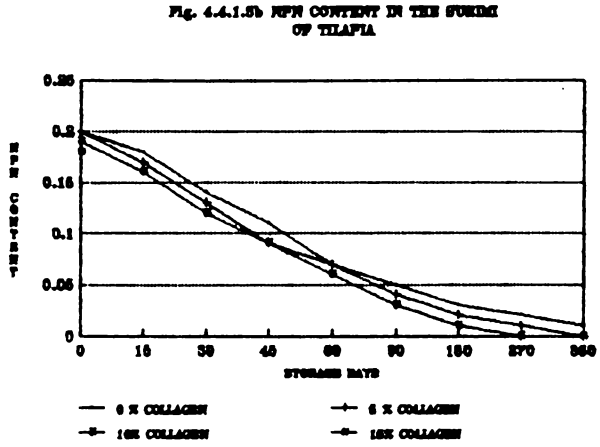
SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	0.16	8.00	0.02	734.39***
COLLAGEN LEVEL	3.2111E-03	3.00	1.0704E-03	40.209***
ERROR	6.3889E-04	24.00	2.6620E-05	
TOTAL	0.16	35.00		

*** P < 0.001

Table 4.4.1.3d ANOVA OF THE NON-PROTEIN NITROGEN CONTENT OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	0.13	8.00	0.02	251.081***
COLLAGEN LEVEL	2.3194E-03	3.00	7.7315E04	12.325***
ERROR	1.5056E-03	24.00	6.2731E05	
TOTAL	0.13	35.00		

*** P < 0.001



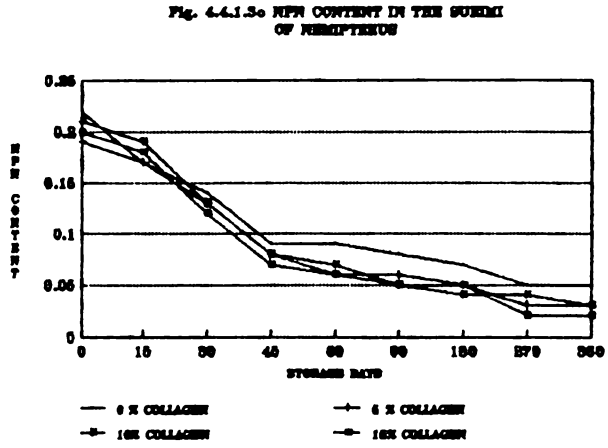
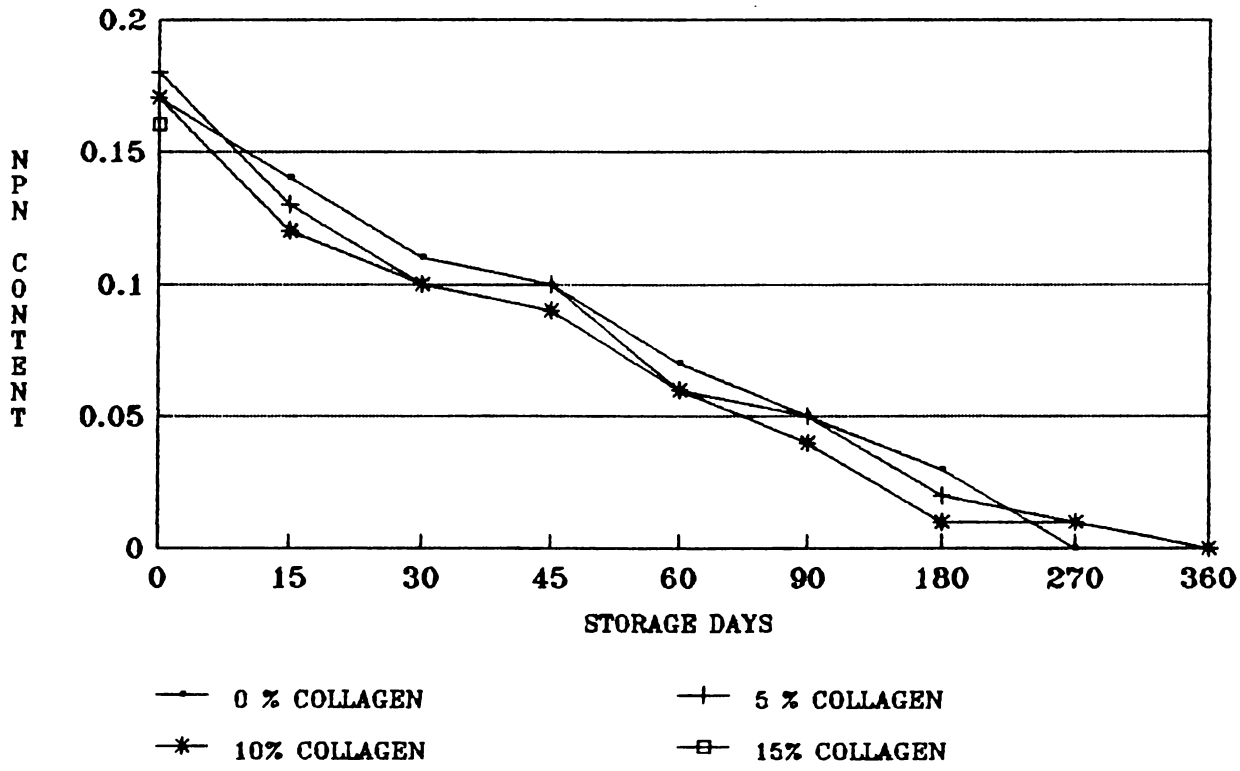


Fig. 4.4.1.3a NPN CONTENT OF THE SURIMI
OF COMMON CARP



different storage period is given in Table 4.4.1.3a. Schematically the data is represented in Fig. 4.4.1.3a, Fig. 4.4.1.3b and Fig. 4.4.1.3c. As the days of storage progressed there is a decrease in NPN content. In some cases the value even reached to zero within 360 days of storage. Table 4.4.1.3b, Table 4.4.1.3c and Table 4.4.1.3d, gives the ANNOVA of NPN contents of surimi prepared from common carp, tilapia and nemipterus respectively at different levels of storage at different levels of concentration.

4.4.1.4 pH

The pH value ranged from 6.75 to 6.86 in the surimi of common carp as collagen level increased form 0 to 15%. The corresponding increase in tilapia was 6.69 to 6.81 and for nemipterus the value was 6.67 to 6.8. The pH value of surimi of different species of fishes at different levels of collagen at different storage periods is given in Table 4.4.1.4a. It's graphical representation is given in Fig. 4.4.1.4a, Fig. 4.4.1.4b and Fig. 4.4.1.4c. With increase in collagen a decrease in pH value is seen. It is also observed that there is an increase in pH with an increase in storage days. From the ANOVA tables (Table 4.4.1.4b, Table 4.4.1.4c and Table 4.4.1.4d), for pH of surimi of different species of fishes, the variation between days of storage is found to be insignificant at 5% level for common carp and

Table 4.4.1.4b ANOVA OF THE pH OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	0.58	8.00	0.07	90.999***
COLLAGEN LEVEL	0.06	3.00	0.02	25.342***
ERROR	0.02	24.00	7.9954E-04	
TOTAL	0.66	35.00		

*** P < 0.001

Table 4.4.1.4c ANOVA OF THE pH OF SURIMI PREPARED FROM TILAPIA

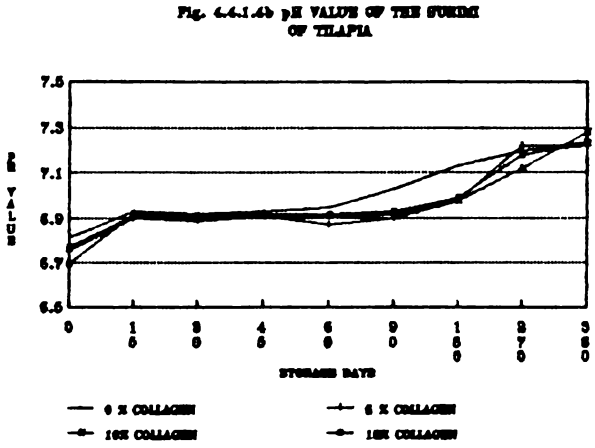
SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	0.72	8.00	0.09	71.825***
COLLAGEN LEVEL	0.02	3.00	5.4667E-03	4.381***
ERROR	0.03	24.00	1.2479E-03	
TOTAL	0.76	35.00		

*** P < 0.001

Table 4.4.1.4d ANOVA OF THE pH OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	1.65	8.00	0.21	98.957***
COLLAGEN LEVEL	0.09	3.00	0.03	13.598***
ERROR	0.05	24.00	2.0866E-03	
TOTAL	1.79	35.00		

*** P < 0.001



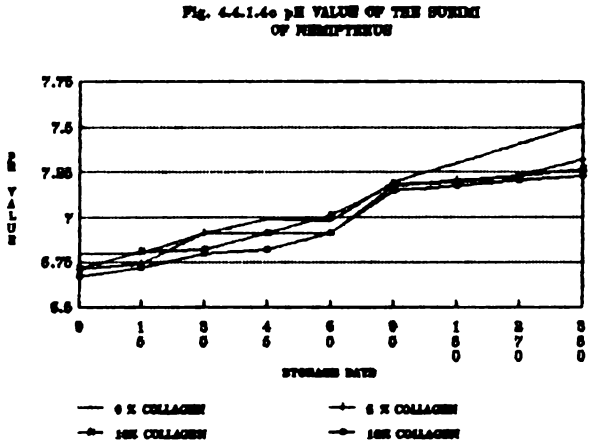
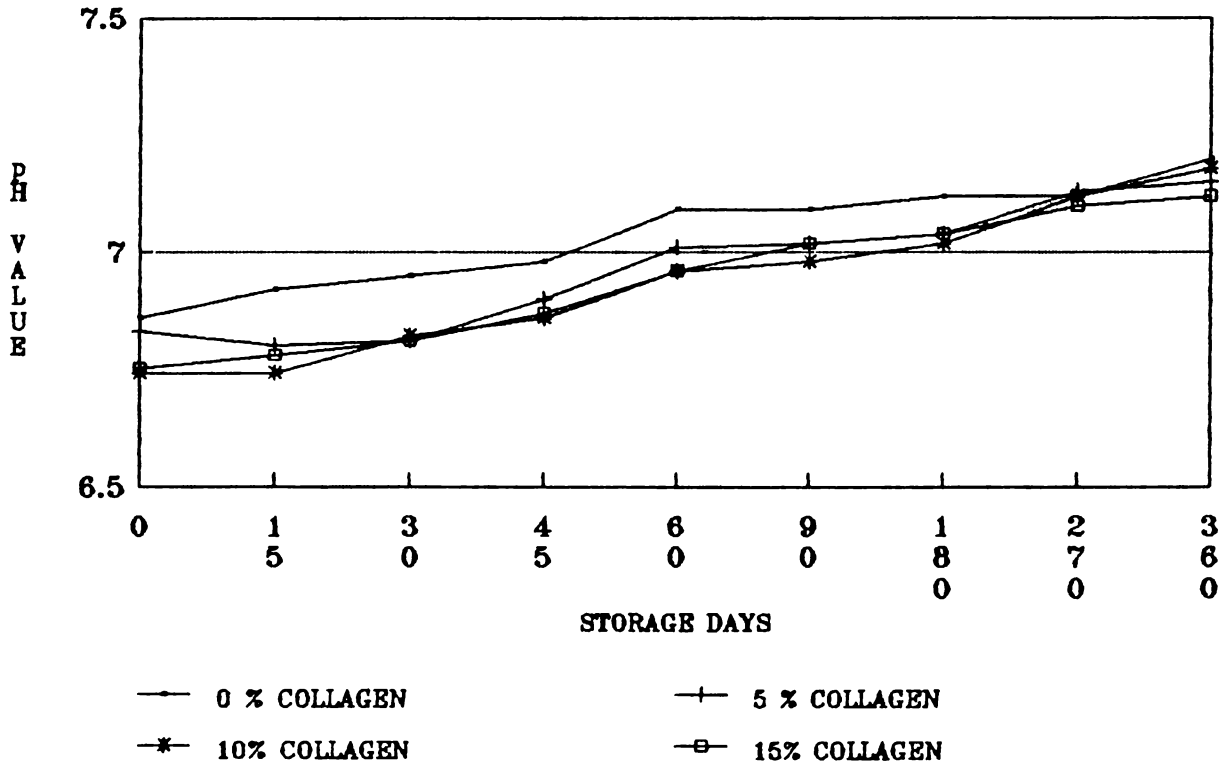


Fig. 4.4.1.4a pH VALUE OF THE SURIMI
OF COMMON CARP



nemipterus. Between levels of collagen the variation is significant at 0.1% level in all the three cases.

4.4.1.5 Trimethlyamine (TMA)

Initially, TMA content of the surimi of tilapia was 1.8mg percentage to 1.78mg percentage, in surimi with 0% and 15% collagen respectively. Its value ranged from 1.8 to 2.53mg percentage in surimi of tilapia with 0% collagen as the days of storage reached to 360 days. By the end of one year the TMA value rose from 1.7 to 2.26 mg percentage, 1.82 to 2.41mg percentage, 1.78 to 2.26mg percentage in the surimi of tilapia with 5%, 10% and 15% collagen levels. In nemipterus the value rose from 2.1 to 9.95mg percent, 2.13 to 9.99mg percent, 2.21 to 11.12mg percent, 2.32 to 11.18mg percent in surimi with 0%, 5%, 10% and 15% level of collagen respectively. The shark being an elasmobranch showed initially the highest value (4mg %), which increased to 9.9mg % after 360 days. Common carp, being a freshwater fish, contains no TMA. Table 4.4.1.5a shows TMA content in the surimi with different levels of collagen in different species of fishes at different storage days. The diagrammatic representation of the data in the above Table is given in Fig.4.4.1.5a, 4.4.1.5b and 4.4.1.5c and ANOVA of this data in Table 4.4.1.5b, Table 4.4.1.5c and Table

Table 4.4.1.5a TMA (mg N/100gm) IN THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
TILAPIA	0%	1.80	2.10	2.12	2.16	2.30	2.40	2.42	2.46	2.53
	5%	1.70	2.00	2.00	2.12	2.08	2.00	2.20	2.22	2.26
	10%	1.82	1.98	2.10	2.10	2.16	2.14	2.28	2.26	2.41
	15%	1.78	1.86	2.06	2.04	2.04	2.08	2.06	2.22	2.26
SHARK		4.00	4.22	4.30	4.40	4.80	5.60	6.80	7.70	9.90
NEMIPTERUS	0%	2.10	3.18	4.00	4.42	5.68	6.12	6.82	7.00	9.95
	5%	2.13	3.22	4.02	4.46	5.72	6.15	6.82	7.09	9.99
	10%	2.21	3.25	4.13	4.52	5.83	6.15	6.83	7.21	11.12
	15%	2.32	3.42	4.15	4.61	5.84	6.16	6.84	7.19	11.18

Table 4.4.1.6a TVN (mg N/100gm) IN THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	4.50	5.20	5.90	12.80	15.60	21.00	22.20	22.60	23.20
	5%	3.90	6.00	10.00	14.80	22.40	28.00	28.00	29.80	32.10
	10%	4.90	6.80	11.20	16.00	22.40	28.00	32.00	29.60	30.20
	15%	5.20	7.80	8.90	16.90	21.80	28.20	29.40	30.00	31.80
TILAPIA	0%	2.70	3.26	3.60	14.80	16.28	18.10	20.40	21.60	22.20
	5%	2.62	3.24	3.72	14.90	15.81	18.00	20.60	21.80	22.40
	10%	2.58	3.00	3.48	14.12	15.68	18.20	20.80	22.20	22.80
	15%	2.70	3.24	3.39	14.18	15.79	17.80	21.00	22.00	22.90
SHARK		6.20	6.80	8.80	10.20	10.80	22.80	23.60	24.80	28.80
NEMIPTERUS	0%	8.00	8.52	10.68	12.58	14.12	15.62	15.90	17.68	22.00
	5%	8.10	8.62	10.68	12.68	14.24	15.68	16.10	17.89	23.38
	10%	8.12	8.68	10.72	12.87	14.42	15.83	16.54	17.98	24.00
	15%	8.13	8.92	11.00	13.13	14.68	15.85	16.73	18.25	24.12

Table 4.4.1.5b ANOVA OF THE TRIMETHYLAMINE (TMA) CONTENT OF SURIMI PREPARED FROM TILAPIA

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	0.979	8.00	0.122	30.087***
COLLAGEN LEVEL	0.244	3.00	0.081	19.981***
ERROR	0.098	24.00	4.0694E-03	
TOTAL	1.321	35.00		

*** P < 0.001

Table 4.4.1.4c ANOVA OF THE TRIMETHYLAMINE (TMA) CONTENT OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	197.38	8.00	24.67	558.814***
COLLAGEN LEVEL	0.48	3.00	0.16	3.657*
ERROR	1.06	24.00	0.04	
TOTAL	198.92	35.00		

*** P < 0.001
* P < 0.05

Fig. 4.4.1.5a TMA CONTENT IN THE SURIMI OF TILAPIA

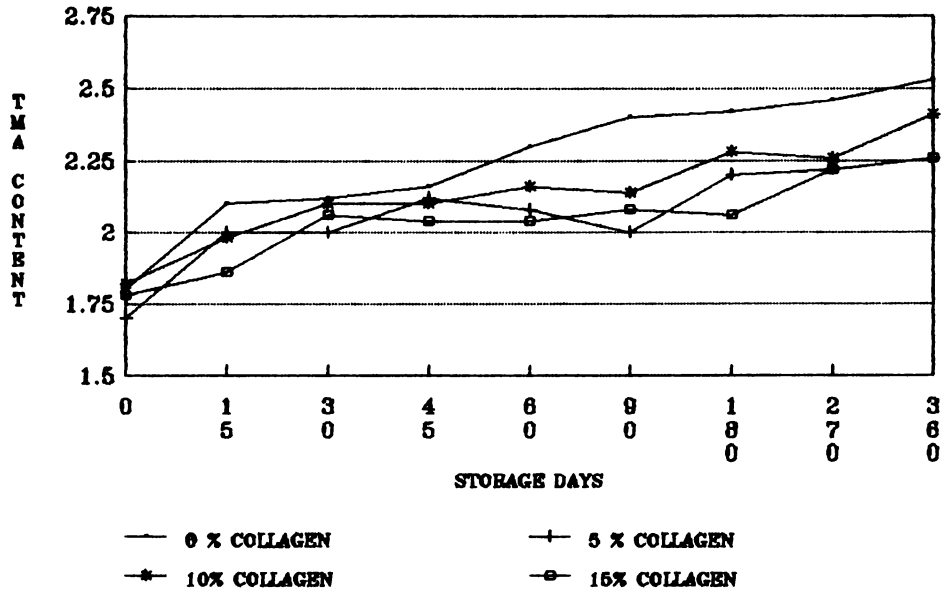


Fig. 4.4.1.5b TMA CONTENT IN THE SURIMI OF NEMIPTERUS

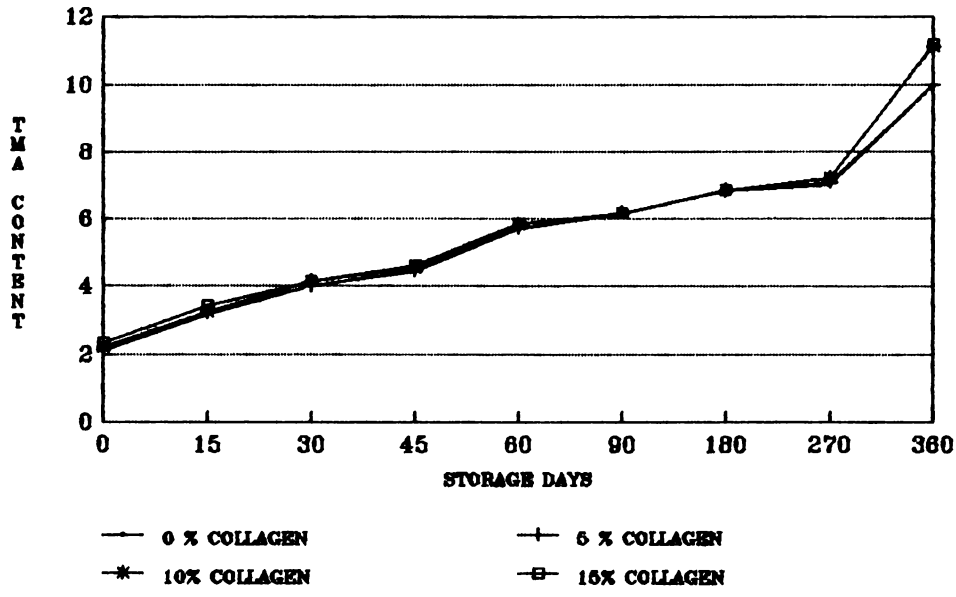


Table 4.4.1.6b ANOVA OF THE TOTAL VOLATILE NITROGEN (TVN) OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	3200.50	8.00	400.06	145.097***
COLLAGEN LEVEL	176.39	3.00	58.80	21.325***
ERROR	66.17	24.00	2.76	
TOTAL	3443.06	35.00		

*** P < 0.001

Table 4.4.1.6c ANOVA OF THE TOTAL VOLATILE NITROGEN (TVN) OF SURIMI PREPARED FROM TILAPIA

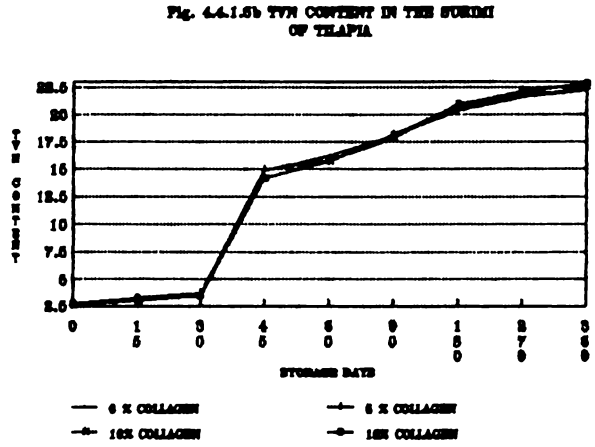
SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	2219.37	8.00	277.42	4094.293***
COLLAGEN LEVEL	3.0556E-03	3.00	1.0185E-03	0.02
ERROR	1.63	24.00	0.07	
TOTAL	2221.02	35.00		

*** P < 0.001

Table 4.4.1.6d ANOVA OF THE TOTAL VOLATILE NITROGEN (TVN) OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	744.54	8.00	93.07	1119.693***
COLLAGEN LEVEL	2.02	3.00	0.67	8.090***
ERROR	2.00	24.00	0.08	
TOTAL	748.55	35.00		

*** P < 0.001



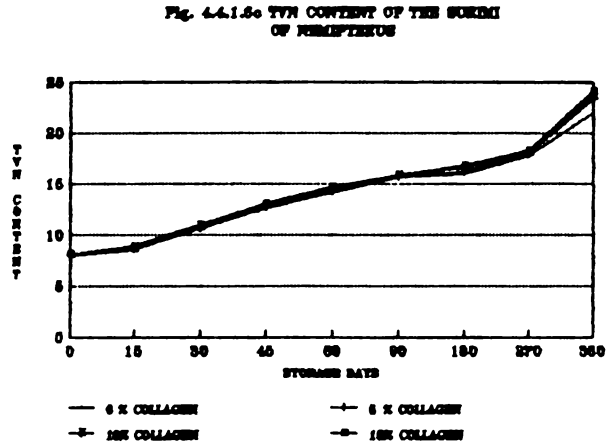
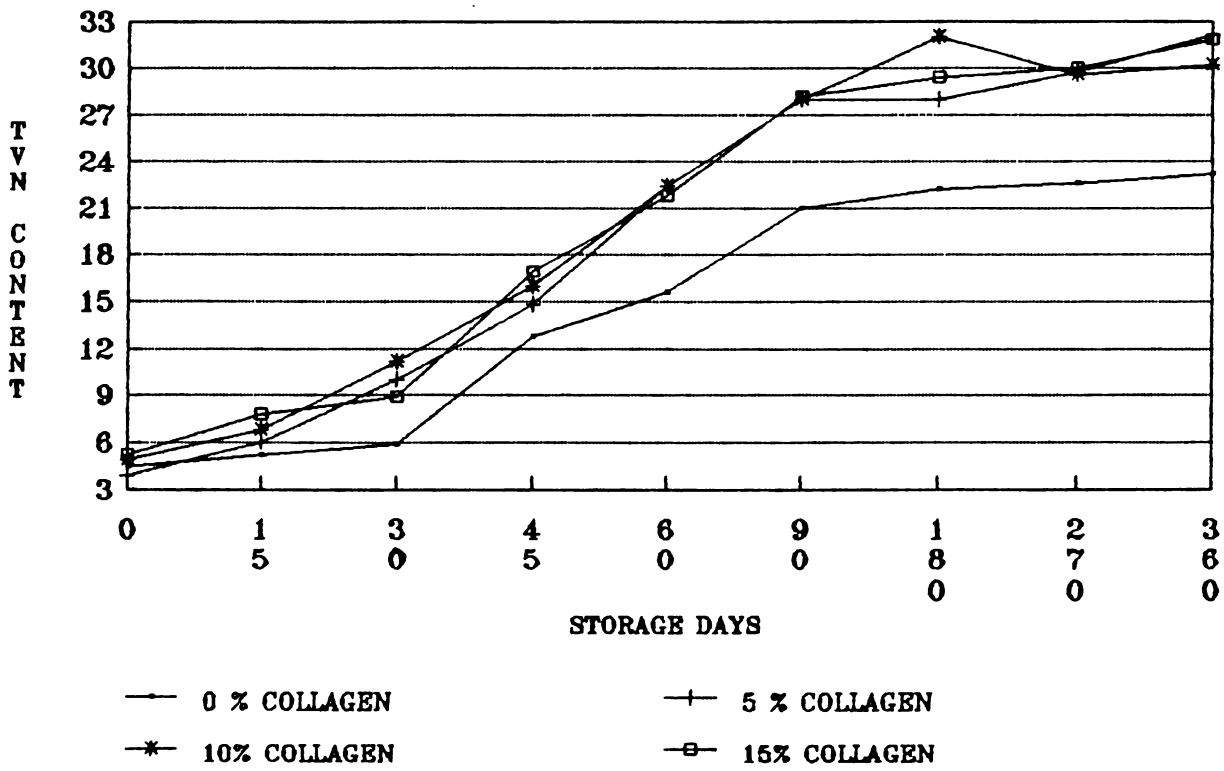


Fig. 4.4.1.6a TVN CONTENT IN THE SURIMI
OF COMMON CARP



4.4.1.5d respectively.

4.4.1.6 Total volatile nitrogen (TVN)

Table 4.4.1.6a shows the TVN content in the surimi with different levels of collagen in different species of fishes at different storage days. With increase in days of storage and collagen level an increase in the TVN content is seen. The schematic representation of the data is given in Fig. 4.4.1.6a, Fig. 4.4.1.6b and 4.4.1.6c. Among the fishes studied, for 0 day of storage, tilapia represented the lowest value (2.7mg %), followed by common carp (4.5mg %), then shark (6.2mg %) and the highest value for nemipterus (8mg %). Table 4.4.1.6b, Table 4.4.1.6c and Table 4.4.1.6d, represents ANOVA of the TVN content in the surimi of common carp, tilapia and nemipterus respectively.

4.4.1.7 Alpha-amino nitrogen

The content of alpha-amino nitrogen in the surimi of fishes with different levels of collagen at different days of storage is given in Table 4.4.1.7a. Fig. 4.4.1.7a, Fig. 4.4.1.7b and Fig. 4.4.1.7c shows the graphical representation of the data in Table 4.4.1.7a. With the increase in storage period

Table 4.4.1.7a ALPHA AMINO NITROGEN IN THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	42.20	56.00	64.40	92.40	112.00	124.40	138.40	140.60	156.20
	5%	36.40	58.80	70.00	112.00	140.00	156.60	174.20	186.80	192.40
	10%	56.00	67.20	72.80	98.00	126.40	182.40	186.80	188.80	194.40
	15%	58.80	75.60	81.20	126.00	198.20	202.10	204.20	207.80	209.20
TILAPIA	0%	33.90	64.40	81.20	124.20	156.80	162.40	172.60	182.40	191.00
	5%	42.10	70.00	92.40	140.00	145.60	156.40	161.00	168.40	171.00
	10%	53.20	92.40	123.20	168.00	176.40	188.00	192.20	199.40	205.40
	15%	52.70	98.00	162.40	196.00	218.40	224.20	234.60	244.80	250.20
SHARK		168.00	176.40	182.00	193.20	257.60	264.20	272.00	274.40	278.80
NEMIPTERUS	0%	27.20	32.00	45.00	49.68	56.40	64.40	92.60	110.40	118.20
	5%	32.50	35.20	48.28	51.44	54.20	65.20	98.20	112.20	114.20
	10%	35.20	36.40	48.68	53.56	58.80	65.60	102.00	112.80	115.40
	15%	33.30	38.20	50.06	58.02	59.20	66.00	106.80	114.00	117.80

Table 4.4.1.8a GEL STRENGTH (N) IN THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	3.48	3.09	2.70	2.21	2.07	1.90	1.62	1.40	
	5%	3.56	3.31	3.00	2.30	2.18	2.00	1.70	1.50	
	10%	3.74	3.42	3.20	3.01	2.82	2.67	2.42	2.18	1.99
	15%	3.80	3.50	3.20	2.98	2.88	2.51	2.38	2.08	1.80
TILAPIA	0%	2.60	2.40	2.31	2.18	2.07	1.98	1.60		
	5%	2.62	2.42	2.32	2.20	2.18	2.12	1.90		
	10%	3.10	2.90	2.70	2.62	2.47	2.31	2.20	2.08	1.87
	15%	3.11	2.90	2.71	2.58	2.48	2.32	2.21	2.10	1.90
SHARK		3.60	3.50	3.42	3.32	3.10	3.00	2.90	2.60	2.10
NEMIPTERUS	0%	3.02	2.90	2.70	2.50	2.25	2.06	1.88	1.56	1.38
	5%	3.10	3.00	2.91	2.72	2.50	2.30	2.20	1.94	1.64
	10%	3.50	3.42	3.28	3.12	3.00	2.80	2.69	2.76	2.42
	15%	3.58	3.42	2.26	3.10	2.98	2.78	2.68	2.60	2.30

Table 4.4.1.7b ANOVA OF THE ALPHA AMINO NITROGEN CONTENT OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	99786.40	8.00	12473.30	69.791***
COLLAGEN LEVEL	11193.87	3.00	3731.29	20.878***
ERROR	4289.35	24.00	178.72	
TOTAL	115269.62	35.00		

*** P < 0.001

Table 4.4.1.7c ANOVA OF THE ALPHA AMINO NITROGEN CONTENT OF SURIMI PREPARED FROM TILAPIA

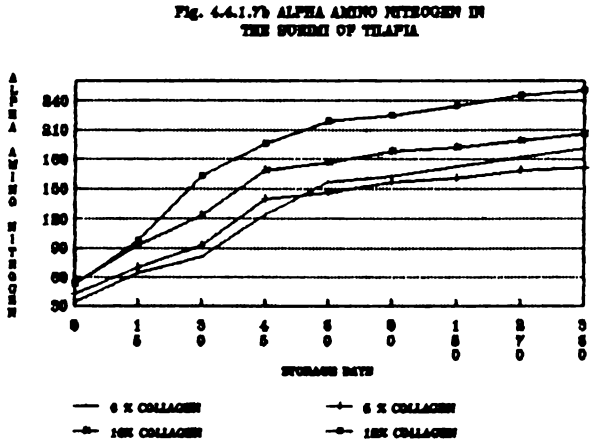
SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	102217.96	8.00	12777.25	97.653***
COLLAGEN LEVEL	20.68	3.00	6893.49	52.685***
ERROR	3140.23	24.00	130.84	
TOTAL	126038.66	35.00		

*** P < 0.001

Table 4.4.1.7d ANOVA OF THE ALPHA AMINO NITROGEN CONTENT OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	33727.10	8.00	4215.89	871.243***
COLLAGEN LEVEL	138.71	3.00	46.24	9.555***
ERROR	116.13	24.00	4.84	
TOTAL	33981.94	35.00		

*** P < 0.001



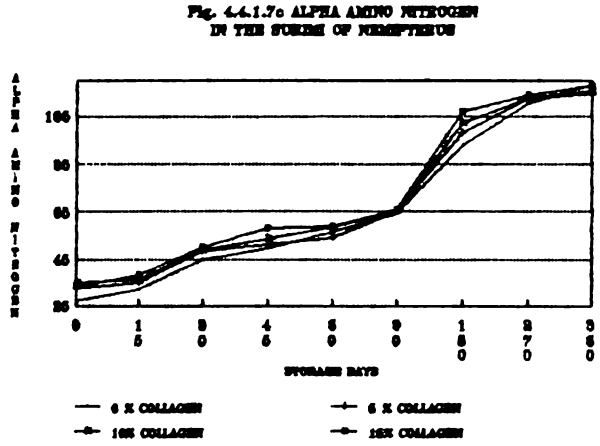
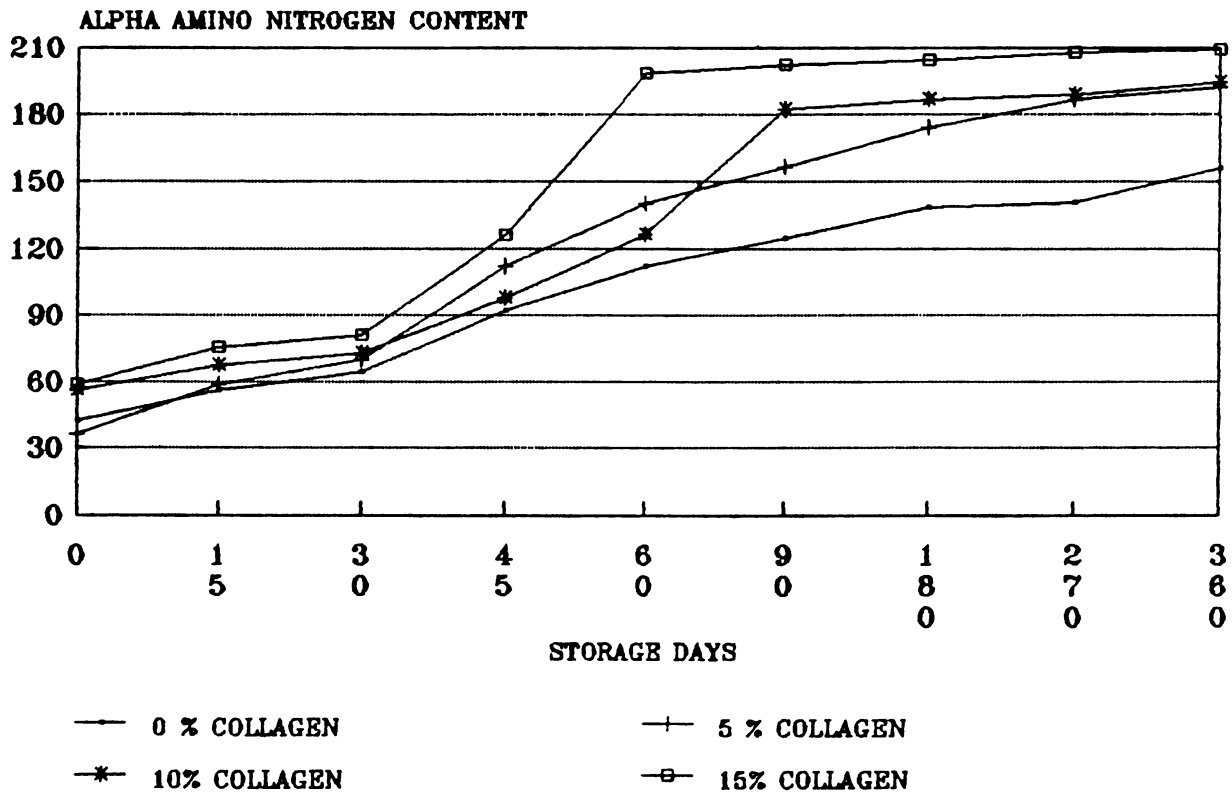


Fig. 4.4.1.7a ALPHA AMINO NITROGEN IN THE SURIMI OF COMMON CARP



and collagen level, there is an increase in alpha amino nitrogen content. Table 4.4.1.7b, Table 4.4.1.7c and Table 4.4.1.7d gives the ANOVA of alpha amino nitrogen content in the surimi of common carp, tilapia and nemipterus respectively. In these tables, variation between collagen levels are significant at 0.1% level.

4.4.1.8 Gel strength(GS)

Table 4.4.1.8a gives the gel strength of surimi, prepared from different species of fishes at different levels of collagen at different storage days. Common carp showed an increase in gel strength from 3.48Newton-meters(Nm) to 3.8Nm, as collagen level rose to 15%, from 0%. On the initial day of storage, from 3.48Nm, the GS decreased to 0 after 360 days, in the case of surimi of common carp without any collagen. the GS decreased from 3.56Nm to 1.5Nm by the end of 270 days of storage, in the case of surimi of common carp with 5% level of collagen. The GS decreased from 3.74Nm to 1.99Nm and from 3.8Nm to 1.8Nm after 360 days of storage in the case of common carp surimi with 10% and 15% level of collagen. Gel strength of tilapis surimi was from 2.6Nm to 0.0Nm, 2.62Nm to 0.0Nm, 3.1Nm to 1.87Nm and 3.11Nm to 1.9Nm for the collagen levels of 0%, 5%, 10% and 15% repectively upto 360 days of storage. In the case of nemipterus

Table 4.4.1.8b ANOVA OF THE GEL STRENGTH OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	21.86	8.00	2.73	32.414***
COLLAGEN LEVEL	4.45	3.00	1.48	17.603***
ERROR	2.02	24.00	0.08	
TOTAL	28.34	35.00		

*** P < 0.001

Table 4.4.1.8c ANOVA OF THE GEL STRENGTH OF SURIMI PREPARED FROM TILAPIA

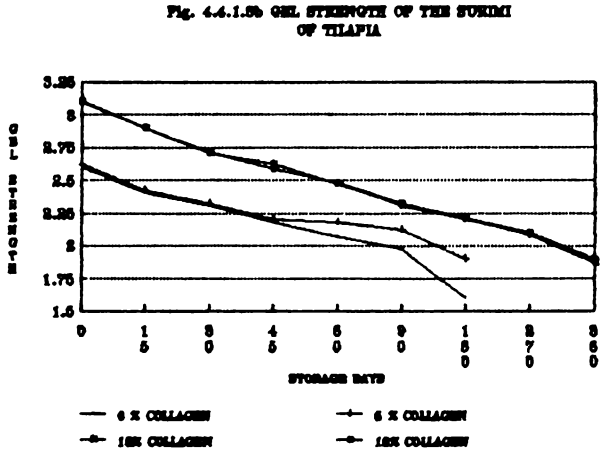
SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	14.61	8.00	1.83	10.996***
COLLAGEN LEVEL	5.21	3.00	1.74	10.449***
ERROR	3.99	24.00	0.17	
TOTAL	23.80	35.00		

*** P < 0.001

Table 4.4.1.8d ANOVA OF THE GEL STRENGTH OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	6.33	8.00	0.79	17.374***
COLLAGEN LEVEL	3.18	3.00	1.06	23.258***
ERROR	1.09	24.00	0.05	
TOTAL	10.60	35.00		

*** P < 0.001



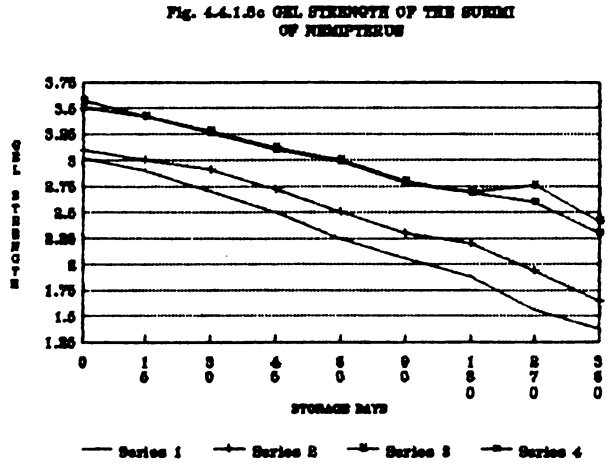
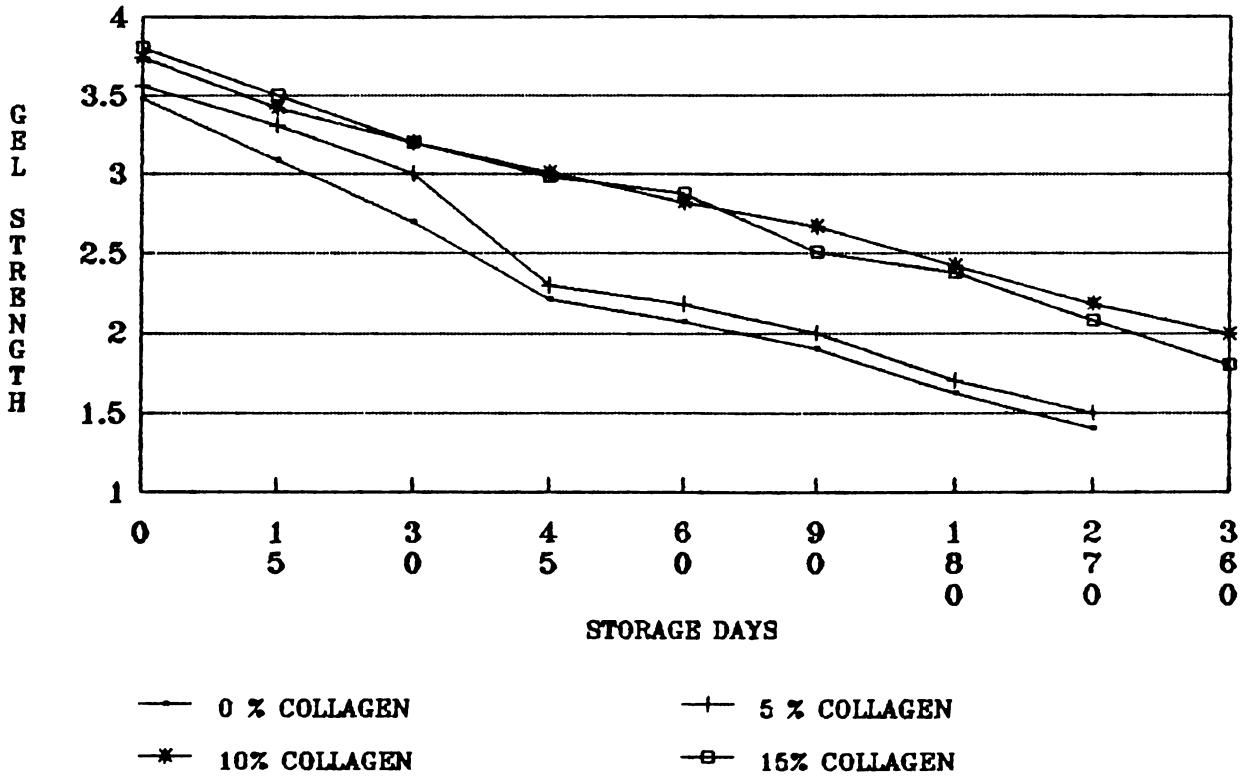


Fig. 4.4.1.8a GEL STRENGTH OF THE SURIMI OF COMMON CARP



the GS ranged from, 3.02Nm to 1.38Nm, 3.1Nm to 1.64Nm, 3.5Nm to 2.42Nm and 3.58Nm to 2.3Nm, for surimi containig collagen levels of 0%, 5%, 10% and 15% respectively, when studied upto 360 days of frozen storage. Fig.4.4.1.8a, Fig.4.4.1.8b and Fig.4.4.1.8c shows the graphical representation of the data. Table 4.4.1.8b, Table 4.4.1.8c and Table 4.4.1.8d gives ANOVA of the GS of surimi of different species of fishes studied at different levels of collagen. In these Tables, variation between days of storage and levels of collagen showed 0.1% level of significance. The highest significant variation was given by 10% level of collagen.

4.4.1.9 Expressible water (EW)

Percentage of expressible water present in the surimi of different species of fishes studied at different levels of collagen is given in Table 4.4.1.9a. Fig. 4.4.1.9a, Fig. 4.4.1.9b and Fig. 4.4.1.9c gives the schematic representation of data. With increase in collagen level there is a decrease in expressible water. There is an increasing trend in EW with increasing days of storage. Table 4.4.1.8b, Table 4.4.1.8c and Table 4.4.1.8d gives ANOVA of the EW of surimi of different species of fishes at different levels of collagen. The variation between days of storage and levels of collagen showed 0.1% level

Table 4.4.1.9a EXPRESSIBLE WATER (%) FOR THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVEL OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	22.00	26.00	28.00	35.00	42.20	62.00	67.20	67.10	76.20
	5%	20.00	25.00	26.00	28.20	31.80	51.20	66.80	68.20	69.80
	10%	16.80	17.80	19.00	20.20	26.80	34.60	46.20	58.80	63.40
	15%	15.80	16.90	18.00	20.00	24.20	30.60	38.20	47.20	62.90
TILAPIA	0%	36.00	42.00	48.00	49.20	57.80	62.10	66.00	68.90	69.00
	5%	28.00	38.00	42.00	44.60	58.60	59.90	62.00	64.30	66.30
	10%	29.00	35.00	36.00	37.20	39.00	42.00	44.50	48.10	50.20
	15%	29.00	32.00	33.00	35.00	36.20	38.00	40.20	42.10	46.20
SHARK		38.00	37.00	38.00	38.20	39.00	39.00	39.60	39.80	41.00
NEMIPTERUS	0%	45.00	45.20	46.20	48.27	52.20	53.20	53.40	54.20	55.00
	5%	48.00	48.00	48.20	49.10	50.20	51.20	52.00	52.30	55.00
	10%	43.60	44.00	45.40	46.00	46.60	47.40	48.80	50.80	51.00
	15%	39.20	39.10	39.40	39.80	39.90	42.50	43.10	43.20	43.80

Table 4.4.1.10a UNDESIRABLE PARTICLES PRESENT IN SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	0.00	0.00	1.00	1.00	2.00	1.00	0.00	0.00	2.00
	5%	1.00	1.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
	10%	1.00	2.00	1.00	0.00	0.00	2.00	1.00	2.00	1.00
	15%	0.00	0.00	0.00	0.00	1.00	1.00	2.00	0.00	1.00
TILAPIA	0%	4.00	2.00	1.00	3.00	1.00	3.00	2.00	3.00	1.00
	5%	3.00	3.00	3.00	4.00	3.00	4.00	4.00	4.00	5.00
	10%	3.00	4.00	4.00	5.00	2.00	5.00	1.00	5.00	4.00
	15%	2.00	3.00	2.00	2.00	0.00	4.00	2.00	2.00	3.00
SHARK		0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00
NEMIPTERUS	0%	3.00	3.00	2.00	1.00	0.00	2.00	1.00	2.00	3.00
	5%	4.00	2.00	1.00	4.00	3.00	2.00	2.00	3.00	4.00
	10%	2.00	3.00	2.00	2.00	2.00	3.00	1.00	2.00	1.00
	15%	3.00	1.00	3.00	3.00	1.00	3.00	4.00	3.00	0.00

Table 4.4.1.9b ANOVA OF THE % EXPRESSIBLE WATER OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	11174.16	8.00	1396.77	63.148***
COLLAGEN LEVEL	1670.49	3.00	566.83	25.174***
ERROR	530.86	24.00	22.11	
TOTAL	13375.50	35.00		

*** P < 0.001

Table 4.4.1.9c ANOVA OF THE % EXPRESSIBLE WATER OF SURIMI PREPARED FROM TILAPIA

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	2798.77	8.00	349.85	18.135***
COLLAGEN LEVEL	2141.92	3.00	713.97	37.011***
ERROR	462.98	24.00	19.29	
TOTAL	5403.67	35.00		

*** P < 0.001

Table 4.4.1.9d ANOVA OF THE % EXPRESSIBLE WATER OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	246.17	8.00	30.77	18.878***
COLLAGEN LEVEL	478.88	3.00	159.63	96.893***
ERROR	39.54	24.00	1.65	
TOTAL	764.59	35.00		

*** P < 0.001

Table 4.4.1.10b ANOVA OF THE VISUAL CONTAMINANTS PRESENT IN SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	3.000	8.00	0.38	.648
COLLAGEN LEVEL	1.861	3.00	0.620	1.07
ERROR	13.889	24.00	0.58	
TOTAL	18.750	35.00		

Table 4.4.1.10c ANOVA OF THE VISUAL CONTAMINANTS PRESENT IN SURIMI PREPARED FROM TILAPIA

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	18.39	8.00	2.30	2.662*
COLLAGEN LEVEL	18.78	3.00	6.26	7.249**
ERROR	20.72	24.00	0.86	
TOTAL	57.89	35.00		

** P < 0.01
* P < 0.05

Table 4.4.1.10d ANOVA OF THE VISUAL CONTAMINANTS PRESENT IN SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	6.000	8.00	0.75	0.59
COLLAGEN LEVEL	4.306	3.00	1.44	1.13
ERROR	30.444	24.00	1.27	
TOTAL	40.750	35.00		

of significance in these tables. The lowest significant variation was given by 10% level of collagen.

4.4.1.10 Undesirable particles

Table 4.4.1.10a gives number of undesirable particles in 1gm of surimi prepared from different species of fishes at different levels of collagen. Table 4.4.1.10b, Table 4.4.1.10c and Table 4.4.1.10d gives ANOVA of the number of undesirable particles in the surimi of different species of fishes at different levels of collagen. The variation between days of storage and levels of collagen showed no significance even at 5% level in these tables.

4.4.2 Microbiological studies

Table 4.4.2a, 4.4.2b and 4.4.2c give the TPC per gram, number of *E. coli* per gram and the number of *Staphylococcus* per gram repectively, in the surimi of different species of fishes at different levels of collagen. In all these cases, a decreasing trend is observed with increase in the number of storage days. Fig. 4.4.2, shows the graphical representation of total plate count per gram. From the Table 4.4.2a and Fig. 4.4.2 it is seen

Table 4.4.2a TPC/gm IN THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	55000.00	48000.00	41000.00	3800.00	3500.00	3400.00	310.00	270.00	256.00
	5%	59000.00	50000.00	48000.00	41000.00	3700.00	320.00	3300.00	390.00	310.00
	10%	600000.00	500000.00	59000.00	57000.00	41000.00	3700.00	3100.00	210.00	276.00
	15%	590000.00	520000.00	500000.00	58000.00	47000.00	39000.00	3900.00	300.00	362.00
TILAPIA	0%	340000.00	150000.00	130000.00	19000.00	10000.00	9100.00	8700.00	850.00	746.00
	5%	370000.00	202000.00	152000.00	32000.00	19000.00	10000.00	8900.00	898.00	776.00
	10%	370000.00	202000.00	152000.00	32000.00	19000.00	10100.00	9100.00	890.00	812.00
	15%	380000.00	211000.00	168000.00	40000.00	222000.00	12000.00	9400.00	910.00	880.00
SHARK		380000.00	190000.00	92000.00	68000.00	5800.00	5000.00	390.00	363.00	276.00
NEMIPTERUS	0%	110000.00	17600.00	13000.00	12800.00	6800.00	6000.00	590.00	420.00	216.00
	5%	120000.00	18000.00	15000.00	10100.00	8000.00	6000.00	920.00	690.00	307.00
	10%	145000.00	21000.00	16000.00	9900.00	7200.00	5900.00	1000.00	720.00	407.00
	15%	160000.00	24000.00	17500.00	9730.00	6900.00	5200.00	1100.00	890.00	501.00

Table 4.4.2b E. coli PER GRAM FOR THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	ND	ND	ND	ND	ND	ND	ND	ND	ND
	5%	ND	ND	ND	ND	ND	ND	ND	ND	ND
	10%	ND	ND	ND	ND	ND	ND	ND	ND	ND
	15%	ND	ND	ND	ND	ND	ND	ND	ND	ND
TILAPIA	0%	4.00	1.00	ND	ND	ND	ND	ND	ND	ND
	5%	2.00	ND	ND	ND	ND	ND	ND	ND	ND
	10%	2.00	1.00	ND	ND	ND	ND	ND	ND	ND
	15%	4.00	1.00	ND	ND	ND	ND	ND	ND	ND
SHARK		ND	ND	ND	ND	ND	ND	ND	ND	ND
NEMIPTERUS	0%	ND	ND	ND	ND	ND	ND	ND	ND	ND
	5%	2.00	ND	ND	ND	ND	ND	ND	ND	ND
	10%	2.00	ND	ND	ND	ND	ND	ND	ND	ND
	15%	4.00	ND	ND	ND	ND	ND	ND	ND	ND

Table 4.4.2d ANOVA OF THE TPC PER GRAM PRESENT IN SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	5.3311E+11	8.00	6.6639E+10	3.56*
COLLAGEN LEVEL	2.1002E+11	3.00	7.0008E+10	3.74*
ERROR	4.4922E+11	24.00	1.8718E+10	
TOTAL	1.1924E+12	35.00		

* P < 0.05

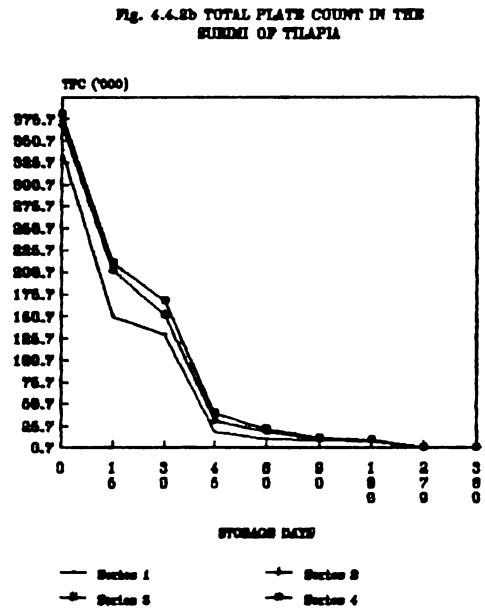
Table 4.4.2e ANOVA OF THE TPC PER GRAM PRESENT IN SURIMI PREPARED FROM TILAPIA

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	5.0793E+11	8.00	6.3491E+10	641.19
COLLAGEN LEVEL	18889747448.33	3.00	629915816.11	6.361**
ERROR	2376492066.66	24.00	99020502.78	
TOTAL	5.1219E+11	35.00		

** P < 0.01

Table 4.4.2f ANOVA OF THE TPC PER GRAM PRESENT IN SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	58052306988.00	8.00	7256538373.50	126.261
COLLAGEN LEVEL	234743023.00	3.00	78247674.62	1.361
ERROR	1379337640.89	24.00	57472401.70	
TOTAL	59666387652.75	35.00		



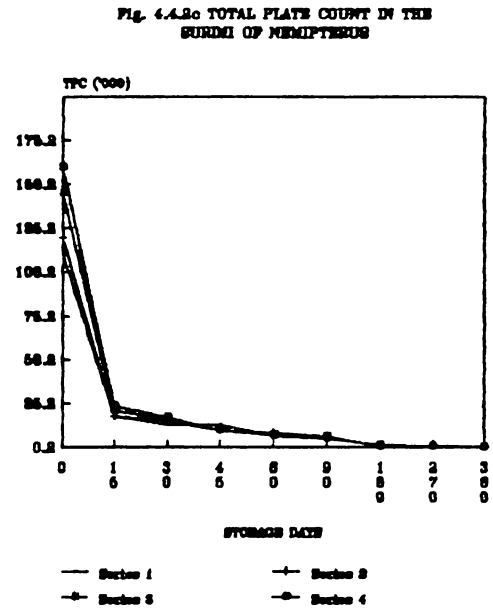


Fig. 4.4.2a TOTAL PLATE COUNT IN THE SURIMI OF COMMON CARP

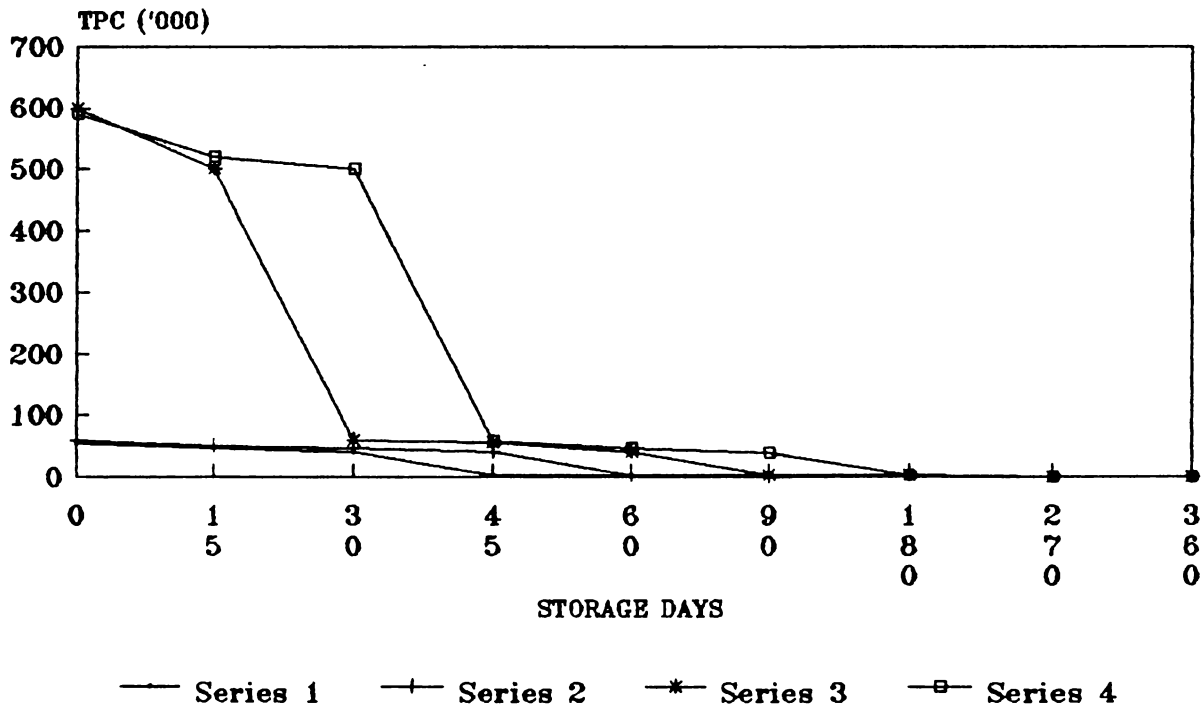


Fig. 4.4.2 TOTAL PLATE COUNT OF DIFFERENT SPECIES OF FISHES

Table 4.4.2c Staphyloccoccus PER GRAM FOR THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	5.00	2.00	ND	ND	ND	ND	ND	ND	ND
	5%	5.00	ND	ND	ND	ND	ND	ND	ND	ND
	10%	4.00	2.00	ND	ND	ND	ND	ND	ND	ND
	15%	4.00	ND	ND	ND	ND	ND	ND	ND	ND
TILAPIA	0%	6.00	3.00	1.00	ND	ND	ND	ND	ND	ND
	5%	4.00	ND	ND	ND	ND	ND	ND	ND	ND
	10%	4.00	ND	ND	ND	ND	ND	ND	ND	ND
	15%	2.00	ND	ND	ND	ND	ND	ND	ND	ND
SHARK		ND	ND	ND	ND	ND	ND	ND	ND	ND
NEMIPTERUS	0%	4.00	ND	ND	ND	ND	ND	ND	ND	ND
	5%	2.00	ND	ND	ND	ND	ND	ND	ND	ND
	10%	1.00	ND	ND	ND	ND	ND	ND	ND	ND
	15%	2.00	ND	ND	ND	ND	ND	ND	ND	ND

that with an increase in collagen there is an increase in TPC per gram in all species of fishes. Table 4.4.2d, Table 4.4.2e and 4.4.2f gives ANOVA of TPC per gram, number of *E. coli* per gram and the number of *Staphylococcus* per gram repectively, in the surimi of different species of fishes at different levels of collagen.

4.4.3 Organoleptic studies

4.4.3.1 Appearance

Table 4.4.3.1a gives attribute scores for sensory quality, appearance for the surimi of different species of fishes at different levels of collagen at various days of storage.

A decreasing trend was observed with increase in storage days.

Table 4.4.3.1b, Table 4.4.3.1c and Table 4.4.3.1d gives ANOVA of appearance of surimi prepared from common carp, tialpia and nemipterus respectively. In these tables the variation between days of storage is not significant at 5% level. The variation between different levels of collagen is found to be significant at 1% level for common carp, and at 0.1% level for

Table 4.4.3.1a MEAN APPEARANCE SCORE FOR THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	8.60	8.59	8.10	7.10	6.20	5.00	4.90	4.70	4.00
	5%	8.61	8.60	8.00	7.02	6.00	5.20	5.10	4.50	4.50
	10%	8.59	8.58	8.00	7.08	7.00	5.50	5.50	4.90	4.80
	15%	8.58	8.58	8.01	7.04	7.10	5.70	5.50	4.90	4.60
TILAPIA	0%	8.40	7.30	6.10	5.80	5.00	4.20	3.60	3.00	3.00
	5%	8.38	7.40	7.20	6.20	6.00	4.80	4.00	3.20	3.00
	10%	8.41	7.80	7.60	7.00	6.20	5.50	5.20	4.60	4.40
	15%	8.42	7.78	7.40	6.80	6.10	4.80	4.30	3.70	3.50
SHARK		8.20	7.80	7.40	6.80	6.00	5.60	5.30	5.00	4.00
NEMIPTERUS	0%	8.30	7.50	7.00	6.40	5.50	5.10	5.00	4.50	4.00
	5%	8.30	7.52	7.40	6.50	6.10	5.60	5.50	4.80	4.28
	10%	8.40	7.58	7.46	7.00	6.30	5.40	5.61	5.10	4.46
	15%	8.40	7.60	7.42	6.90	6.20	5.40	5.60	4.80	4.00

Table 4.4.3.2a MEAN COLOUR SCORE FOR THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	8.80	8.20	7.80	7.60	6.50	5.90	5.70	7.20	4.00
	5%	8.80	8.18	7.81	7.60	6.49	5.91	5.70	4.50	4.00
	10%	8.80	8.21	7.81	7.62	6.51	5.90	5.75	4.30	4.10
	15%	8.80	8.22	7.82	7.62	6.51	5.90	5.75	4.00	4.00
TILAPIA	0%	8.10	7.08	6.18	5.95	5.60	5.09	4.75	4.15	3.80
	5%	8.00	7.06	6.18	5.96	5.60	5.09	4.70	4.14	3.80
	10%	8.00	7.08	6.20	5.95	5.60	5.08	4.75	4.15	3.70
	15%	7.90	7.07	6.20	5.94	5.61	5.09	4.75	4.15	3.80
SHARK		8.70	8.60	8.20	7.60	7.60	7.00	5.80	5.40	5.40
NEMIPTERUS	0%	8.80	8.60	7.90	7.50	6.40	5.60	5.00	4.80	3.50
	5%	8.70	8.61	7.90	7.50	6.40	5.60	4.90	4.91	3.50
	10%	8.70	8.62	7.89	7.50	6.38	5.61	4.90	4.68	3.51
	15%	8.70	8.61	7.90	7.50	6.40	5.61	4.90	3.68	3.50

Table 4.4.3.1b ANOVA OF THE APPEARANCE OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	85.776	8.00	10.72	216.695***
COLLAGEN LEVEL	0.769	3.00	0.256	5.183**
ERROR	1.188	24.00	0.05	
TOTAL	87.733	35.00		

** P < 0.01
*** P < 0.001

Table 4.4.3.1c ANOVA OF THE APPEARANCE OF SURIMI PREPARED FROM TILAPIA

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	114.24	8.00	14.28	264.68***
COLLAGEN LEVEL	3.27	3.00	1.09	20.219***
ERROR	1.30	24.00	0.05	
TOTAL	118.80	35.00		

*** P < 0.001

Table 4.4.3.1d ANOVA OF THE APPEARANCE OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	58.782	8.00	7.35	185.732***
COLLAGEN LEVEL	1.172	3.00	0.39	9.878**
ERROR	0.949	24.00	0.04	
TOTAL	60.904	35.00		

** P < 0.01
*** P < 0.001

Table 4.4.3.2b ANOVA OF THE COLOUR OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	93.082	8.00	11.64	2164.796***
COLLAGEN LEVEL	0.013	3.00	4.2769E-03	0.80
ERROR	0.129	24.00	5.3748E-03	
TOTAL	93.224	35.00		

*** P < 0.001

Table 4.4.3.2c ANOVA OF THE COLOUR OF SURIMI PREPARED FROM TILAPIA

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	59.37	8.00	7.42	6443.047***
COLLAGEN LEVEL	2.8306E-03	3.00	9.4352E-04	0.82
ERROR	0.03	24.00	1.1519E-03	
TOTAL	59.40	35.00		

*** P < 0.001

Table 4.4.3.2d ANOVA OF THE COLOUR OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	114.149	8.00	14.27	233.075***
COLLAGEN LEVEL	0.228	3.00	0.08	1.242
ERROR	1.469	24.00	0.06	
TOTAL	115.847	35.00		

*** P < 0.001

nemipterus and tilapia. Among collagen concentrations, the 10% level gave highest significance for common carp and 5% and 10% for tilapia and and 5% of nemipterus.

4.4.3.2 Colour

Mean scores for the sensory attribute colour for the surimi prepared from different species of fishes at different levels of collagen at various days of storage is given in Table 4.4.3.2a.

Table 4.4.3.2b, Table 4.4.3.2c and Table 4.4.3.2d gives ANOVA of colour of surimi prepared from common carp, tialpia and nemipterus respectively. In these tables the variation between days of storage and variation between collagen concentration were insignificant even at 5% level.

4.4.3.3 Odour

Table 4.4.3.3a gives the mean scores for the sensory attribute, odour for the surimi prepared from different species of fishes at different levels of collagen at various days of storage.

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Table 4.4.3.3a MEAN ODOUR SCORE FOR THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	8.21	8.00	7.68	6.20	5.01	4.11	3.58	2.99	2.70
	5%	8.20	8.00	7.70	6.14	5.01	4.12	3.56	2.98	2.68
	10%	8.18	8.18	7.61	6.16	5.03	4.13	3.57	2.98	2.70
	15%	8.10	8.10	7.62	6.14	5.02	4.11	3.56	2.90	2.70
TILAPIA	0%	8.40	7.50	7.01	6.06	5.10	4.00	3.50	2.90	2.00
	5%	8.30	7.60	7.10	6.04	5.11	4.10	3.68	2.80	2.01
	10%	8.31	7.60	7.00	6.04	5.11	4.01	3.50	2.80	2.01
	15%	8.32	7.62	7.02	6.02	5.10	4.00	3.50	2.78	2.02
SHARK		8.40	7.00	6.10	5.60	4.00	3.20	2.30	2.01	2.00
NEMIPTERUS	0%	8.60	7.80	6.22	5.90	4.80	4.40	3.80	2.90	1.80
	5%	8.60	7.80	6.28	5.80	4.82	4.30	3.70	2.60	1.60
	10%	8.50	7.70	6.21	5.71	4.80	4.00	3.20	2.50	1.00
	15%	8.40	7.70	6.22	5.71	4.70	3.90	3.00	2.50	1.00

Table 4.4.3.4a MEAN TEXTURE SCORE FOR THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	7.60	7.00	6.60	6.20	6.00	5.70	5.00	4.00	3.20
	5%	7.70	7.08	6.62	6.23	6.08	5.74	5.24	5.12	4.40
	10%	8.20	7.20	6.73	6.41	6.16	6.02	6.00	5.90	5.50
	15%	8.10	7.30	6.74	6.40	6.15	6.10	6.01	5.92	5.57
TILAPIA	0%	7.60	7.20	6.30	6.01	5.50	4.00	3.50	2.00	1.80
	5%	7.62	7.22	6.38	6.07	5.60	4.90	4.00	3.90	3.70
	10%	7.67	7.35	7.08	7.01	6.20	5.90	5.80	5.70	4.60
	15%	7.64	7.38	7.12	7.02	6.26	5.90	5.70	5.50	4.70
SHARK		8.30	8.00	7.90	7.80	8.00	6.70	5.20	4.00	3.20
NEMIPTERUS	0%	7.60	7.20	6.00	6.20	5.40	5.00	4.30	2.40	1.00
	5%	7.60	7.40	7.00	6.80	6.00	5.64	5.24	4.80	4.00
	10%	7.90	7.80	7.30	7.20	6.96	6.88	6.79	6.60	5.80
	15%	7.90	7.70	7.30	7.01	6.90	6.81	6.70	6.27	5.50

Table 4.4.3.3b ANOVA OF THE ODOUR OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	154.499	8.00	19.44	11554.891***
COLLAGEN LEVEL	5.4528E-03	3.00	1.8176E-03	1.08
ERROR	0.040	24.00	1.6822E-03	
TOTAL	155.545	35.00		

*** P < 0.001

Table 4.4.3.3c ANOVA OF THE ODOUR OF SURIMI PREPARED FROM TILAPIA

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	163.50	8.00	20.44	693.558***
COLLAGEN LEVEL	0.16	3.00	0.05	1.78
ERROR	0.71	24.00	0.03	
TOTAL	164.37	35.00		

*** P < 0.001

Table 4.4.3.3d ANOVA OF THE ODOUR OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	172.813	8.00	21.60	359.714***
COLLAGEN LEVEL	1.168	3.00	0.39	6.482**
ERROR	1.441	24.00	0.06	
TOTAL	175.422	35.00		

** P < 0.01
*** P < 0.001

Table 4.4.3.4b ANOVA OF THE TEXTURE OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	31.199	8.00	3.90	29.139***
COLLAGEN LEVEL	3.772	3.00	1.257	8.106**
ERROR	3.723	24.00	0.16	
TOTAL	38.695	35.00		

** P < 0.01
*** P < 0.001

Table 4.4.3.4c ANOVA OF THE TEXTURE OF SURIMI PREPARED FROM TILAPIA

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	60.71	8.00	7.59	20.821***
COLLAGEN LEVEL	14.19	3.00	4.73	12.977***
ERROR	8.75	24.00	0.36	
TOTAL	83.64	35.00		

*** P < 0.001

Table 4.4.3.4d ANOVA OF THE TEXTURE OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	44.511	8.00	5.56	10.539***
COLLAGEN LEVEL	23.364	3.00	7.79	14.752***
ERROR	12.670	24.00	0.53	
TOTAL	80.546	35.00		

*** P < 0.001

storage advanced a decrease in quality attribute for odour is noticed. Table 4.4.3.3b, Table 4.4.3.3c and Table 4.4.3.3d gives ANOVA of odour of surimi prepared from common carp, tilapia and nemipterus respectively. In these tables the variation between days of storage is not significant at 5% level but the variation between collagen concentration were insignificant even at 5% level in the case of common carp and tilapia but the variation is significant at 1% level in the case of nemipterus.

4.4.3.4 Texture

Table 4.4.3.4a gives the means scores for the sensory attribute, texture for the surimi prepared from different species of fishes at different levels of collagen at various days of storage.

Table 4.4.3.4b, Table 4.4.3.4c and Table 4.4.3.4d gives ANOVA of texture of surimi prepared from common carp, tilapia and nemipterus respectively. In these tables the variation between days of storage and variation between collagen levels are significant at 0.1% level. In common carp the highest significance was obtained at 10% levels of collagen; for tilapia at 5% and 10% levels and for nemipterus at 10% level.

In contributing to texture of surimi 10% collagen is found to have better effect. So to get desirable texture for the surimi, 10% collagen is to be incorporated.

4.4.3.5 Overall acceptability

Table 4.4.3.5a gives the mean score for the sensory attribute for the overall accepteability of surimi of different species of fishes with different levels of collagen at different storage periods. Fig 4.4.3.5a, Fig 4.4.3.5b and Fig. 4.4.3.5c gives the graphical representation of the data. Tables 4.4.3.5b, 4.4.3.5c and 4.4.3.5d shows ANOVA of the overall aceptability score for surimi with different levels of collagen in common carp, tilapia and nemipterus respectively. The variation between concentrations of collagen were significant at 0.1% level for all the fishes studied. Highest significance was given by 10% level. So 10% collagen has better effect in contributing to overall acceptability.

Table 4.4.3.5a MEAN OVERALL ACCEPTABILITY SCORE FOR THE SURIMI OF DIFFERENT SPECIES OF FISH STUDIED WITH DIFFERENT LEVELS OF COLLAGEN AT DIFFERENT STORAGE DAYS

	NO. OF DAYS	0.00	15.00	30.00	45.00	60.00	90.00	180.00	270.00	360.00
COMMON CARP	0%	8.00	7.85	7.60	6.40	5.20	4.80	3.20	2.60	1.80
	5%	8.20	7.60	7.20	6.80	6.00	5.41	5.00	4.20	3.70
	10%	8.08	7.90	7.80	7.50	7.00	6.64	6.21	5.82	5.81
	15%	8.04	7.82	7.66	7.40	6.50	5.60	5.20	5.00	4.80
TILAPIA	0%	7.80	7.80	6.60	6.00	5.00	4.60	4.00	3.00	2.00
	5%	7.90	7.10	6.70	6.20	5.02	4.68	4.10	3.20	2.40
	10%	8.00	7.16	6.76	6.21	5.76	5.72	5.30	4.40	4.42
	15%	7.90	7.02	6.19	5.92	5.50	5.01	4.31	4.20	4.00
SHARK		8.00	7.60	7.00	6.00	5.80	5.20	4.80	3.00	1.80
NEMIPTERUS	0%	7.80	7.10	6.20	6.00	5.40	5.00	4.10	3.10	2.00
	5%	7.60	7.00	6.60	6.08	5.60	5.00	4.40	4.00	2.60
	10%	7.70	7.10	6.80	6.10	6.00	5.60	4.80	4.10	3.40
	15%	7.60	7.10	6.82	6.11	6.06	5.00	4.20	4.00	3.00

Table 4.4.3.5b ANOVA OF THE OVERALL ACCEPTABILITY OF SURIMI PREPARED FROM COMMON CARP

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	74.153	8.00	9.27	22.451***
COLLAGEN LEVEL	13.815	3.00	4.605	11.154***
ERROR	9.909	24.00	0.41	
TOTAL	97.877	35.00		

*** P < 0.001

Table 4.4.3.5c ANOVA OF THE OVERALL ACCEPTABILITY OF SURIMI PREPARED FROM TILAPIA

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	75.75	8.00	9.47	51.778***
COLLAGEN LEVEL	5.79	3.00	1.93	10.555***
ERROR	4.39	24.00	0.18	
TOTAL	85.93	35.00		

*** P < 0.001

Table 4.4.3.5d ANOVA OF THE OVERALL ACCEPTABILITY OF SURIMI PREPARED FROM NEMIPTERUS

SOURCE	SUM OF SQUARES	DF	MSS	F-RATIO
STORAGE DAYS	83.102	8.00	10.39	172.636***
COLLAGEN LEVEL	1.449	3.00	0.48	8.029**
ERROR	1.444	24.00	0.06	
TOTAL	85.995	35.00		

Fig. 4.4.3.5b OVERALL ACCEPTABILITY SCORE FOR SURIMI OF TILAPIA

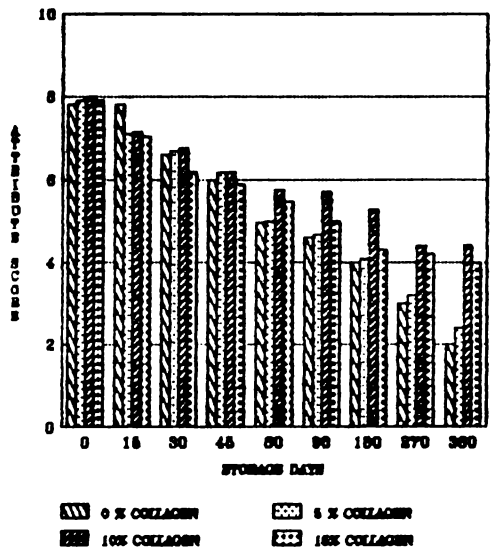


Fig. 4.4.3.5c OVERALL ACCEPTABILITY SCORE FOR SURIMI OF NEMIPTERUS

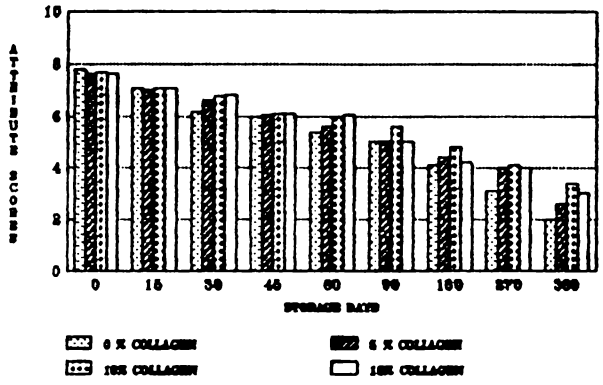
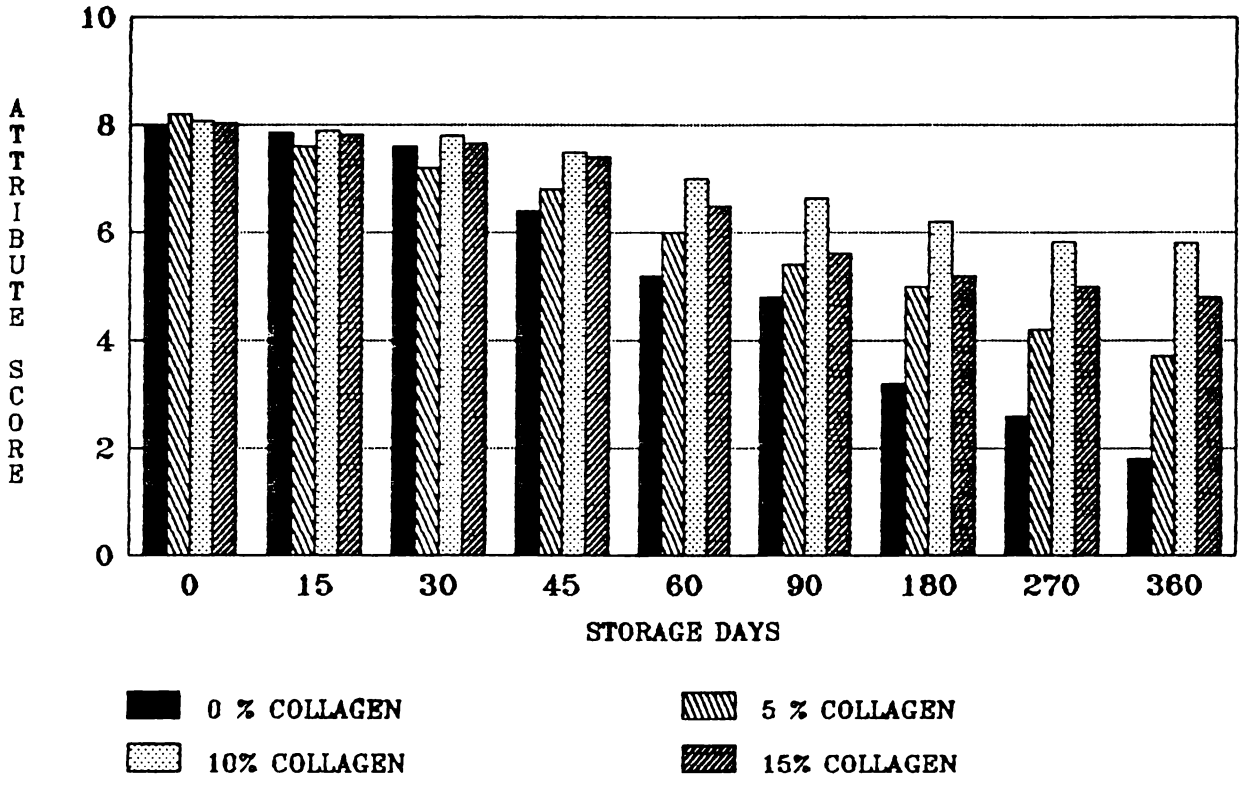


Fig. 4.4.3.5a OVERALL ACCEPTABILITY SCORE FOR SURIMI OF COMMON CARP



4.5 DISCUSSION

4.5.1 Physico-chemical analysis

Lee *et al.*, 1976, after studying the unwashed mince from spanish mackeral reported a decrease in moisture, gel resilience and cohesiveness on frozen storage. A certain amount of moisture is necessary for surimi to perform physical and rheological properties. With the increase in collagen level an increased moisture content is seen in all species of fishes and with increase in storage time a decrease in moisture content is seen at all levels of collagen (Table 4.4.1.1a and Fig. 4.4.1.1a, Fig. 4.4.1.1b and 4.4.1.1c).

In fish muscle 4-5% of total water of the muscle is tightly bound to the muscle proteins. This water is not affected by changes in the protein structure. The remaining water as free water, is retained within the protein molecules and this free water is affected by changes in protein structure. During frozen storage there is denaturation of protein and hence the water holding capacity of protein is decreased, as a result there is decrease in moisture content in the surimi after frozen storage.

The increased content of moisture initially in the surimi is due to the washing of mince. The added collagen facilitates the greater water absorption. The absorbed water enters the interstitial spaces of the protein matrix, leading to the development of sponginess of the gel and elasticity of the product. In other words, added collagen provides more open structure which helps to absorb water and hence moisture content increases with increase in collagen level.

Lee (1992) had an opinion that at a given moisture level gel strength is determined by the degree of water binding within a matrix. The tighter the water is bound, the stronger is the gel. This can be explained by the inverse relationship between gel strength and percentage of expressible water. Low gel strength coupled with large amounts of expressible water means the gel is structurally loose and does not hold water within the matrix. A similar finding was obtained by Gopakumar *et al.* 1992.

There is an increase in total protein with the increased addition of collagen level in all the three species studied (Table 4.4.1.2a). From Table 4.4.1.8a, it is seen that with an increase in collagen level there is an increase in gel strength. This confirms the results of Hamann (1988), who reported

that shear stress was primarily sensitive to protein concentration. Kim *et al.*, (1987) and Montejaro *et al.*, (1984) also had the opinion that shear stress is influenced by protein concentration. Here the shear stress is decreased with increase in collagen content, but shear strain or gel strength is increased. According to Hamann, (1988) shear strain at failure (gel strength) was sensitive to protein functionality. The shear strain is influenced by the type of proteins in the protein matrix. Collagen did not prticipate in the formation of gel matrix. It gets denatured at $65^{o}C$, and acts as filler of the protein matrix. Fish collagen is noted for their easy gelatinisation. On heating collagen is degraded to water soluble gelatin. Gelation of muscle protein contributes to desirable texture and stabilisation of fat and water. The NPN level has been used to determine the freshness of fish (Shenouda *et al.*, 1979). Table 4.4.1.3a, shows that as storage period is increased there is a decrease in NPN content. Initially also the content of NPN is very less. Even the shark surimi showed a value of 0.33% only. The low content of NPN in the surimi is probably due to the repeated washing involved in the manufacture of surimi. This is in agreement with the findings of Bugueras, 1983, who reported that washing removes higher proportion of NPN content. The loss in NPN during frozen storage

can be attributed to bacterial utilisation and/or leaching of these compounds.

The pH of surimi is found to increase with the increase in storage period and also with the collagen level, in all the species studied. The increase in pH may be due to the presence of amino acids in the collagen added. On frozen storage there is an increase in total volatile nitrogen (Table 4.4.1.6a), trimethylamine (Table 4.4.1.5a) and alpha-amino nitrogen (Table 4.4.1.7a). These increase in values will naturally increase the pH value of surimi.

pH is found to have a role in contributing to the texture of meat and it also has got a role in the keeping quality of meat at a given level of collagen. The contribution of connective tissue on meat tenderness was studied by many workers (Goll *et al.*, 1964a, b; Herring *et al.*, 1967a). Another factor infuencing meat tenderness is the water holding capacity (WHC), of the meat proteins (Hamm, 1960; Deatherage, 1963; Bouton *et al.*, 1972). Changes in WHC have been shown to be closely related to the pH of the muscle or muscle homogenate (Hamm, 1959).

In the present study, at a given collagen level, when pH increases, WHC decreases and hence gel strength decreases. This in turn will decrease the texture of surimi. When collagen level increases, though the pH increases, the effect of collagen in increasing the gel strength was more prominant than the change in pH.

TMA, TVN and Alpha amino nitrogen contents are some spoilage indicating parameters. With the increase in collagen level all these parameters increases. This may be because collagen contains some amino acids. Stansby (1957), reported TVN as the quality index of fish. He fixed the limit for spoilage as 25mg %. In the present study, common carp with added collagen reached the level of spoilage in 90 days, but tilapia and nemipterus did not cross the borderline even after 360 days. Hellig *et al.*, 1958, reported the maximum value of alpha amino nitrogen for decomposition as 261mg per 100gm. In the present study though there is an increase in alpha amino nitrogen content with storage period and collagen level, only the value given by shark surimi gave values higher than this limit.

Gel strength was found to increase with increase in collagen content but it showed a decreasing trend as the frozen

storage days increased (Table 4.4.1.8a). MacDonald *et al.*, 1990, after studying the gel strength of washed and unwashed mince before and after frozen storage found that the strength of gel decreased with storage. Sodium chloride and collagen with surimi absorb water and impede its free movement through the gel leading to greater cohesiveness. Both shear stress and strain at failure are good indicators of sensory properties such as springiness, hardness, cohesiveness for gels (Montejano *et al.*, 1985; Lanier *et al.*, 1985). According to Pruthiarenum *et al.*, (1985) the gel strength is significantly affected by the setting condition, leaching, grinding, heating etc. In the present study all experiments were conducted under similar conditions. So possibility of variation gel strength due to setting condition is negligible.

The gel strength of surimi is affected by the moisture content present in it. When water is immobilized within a three dimensional protein matrix a gel is formed. The water holding capacity of collagen allows more water to be held in the surimi and hence the increase in collagen level helps to increase the gel strength. Water soluble protein was reported to retard gel network formation by interfering with actomyosin cross linking (Okada, 1964; Shimizu *et al.*, 1974; Mashimoto *et al.*, 1985).

The increase in gel strength with collagen level can be explained as follows. When gelatinising, collagen absorbs water and become pliable and elastic, which improves the texture of surimi. The higher resistance offered by gelatinised collagen to mechanical force than that of muscle protein, helps to increase gel strength of surimi.

Percentage of expressible water was inversely correlated with compressive force and penetration force (Yoon *et al.*, 1991). The present study gave values supporting this statement. In Table 4.4.1.9a, a decrease in percentage of expressible water was noted with increase in collagen level. But gel strength was found to increase with increase in collagen level. In the present study the gel strength is measured in terms of the firmness and compressibility of the sample. This is found to be a good indicator of water binding ability of protein.

Of the fishes studied, nemipterus gave higher expressible water initially (39.2% to 45%). Common carp gave a low initial value (15.8% to 22%) irrespective of the collagen level. the percentage of expressible moisture was found to increase as storage period increased. This shows that with increase in storage

period, there is a decrease in water binding ability and decrease in gel strength.

Undesirable particles are visual contaminants defined as black membranes, small bones etc.. The number of undesirable particles is no way related to the storage quality of the surimi, though it contributes to the degree of good manufacturing practice. Collagen level is also found to have no significance in contributing to the number of undesirable particles.

4.5.2 Microbiological evaluation

Total plate count (TPC) of surimi in this study were lower than fresh or frozen mechanically deboned fish. Initial TPC per gram of surimi ranged from 5.5 x 10^4 to 6.0 x 10^5 per gram. In 1978, Raccach *et al.*, reported counts of mechanically deboned cod, pollock and whiting, ranged from 4.7 X 10^5 per gram to 7 x 10^5per gram. Blackwood (1979), reported bacterial counts of products drom a plant operating under good practices at 25^0C, as 6.7 x 10^4per gram. According to the International Commission on Microbiological Specifcation for Foods (ICMSF), 1974, the freshly minced croaker, sand trout, tilapia and black drum will be acceptable at counts not higher than 10^7 per gram, mullets and

sheep heads at 10^6 per gram. At 25^0C aerobic plate count for fish mince ranged from 1.2 x 10^5 per gram (sheep heads) to 2.6 x 10^8 per gram (tilapia).

Himelbloom *et al.*, (1991) had the opinion that during manufacture of surimi, the micro organisms per gram is actually increasing. Their view was that micro organisms got entrapped in fish flesh during mincing and were not dislodged by washing and screening. Nickelson *et al.*, 1980, reported aerobic plate count in minced fin fish between 10^6 per gram and 10^8 per gram.

After passing through the mincing machine, an increase in count is expected. This is not only because of contact contamination from equipment but also because of increased handling and increased growth of bacterial cells due to the release of rich nutritive cellular material during mincing. In the present study the count was less, may be because of washing and freezing. Freezing will cause sub-lethal injury of cells and death of psychrophobic bacteria. The addition of collagen will increase the microbial load due to handling during preparation of collagen from air bladder.

In the present study, presence of *E. coli* per gram is very less. After 15 days of frozen storage, there was no

incidence of *E. coli* in any case. It was well established that coliform bacteria are sensitive to freezing (Ordal *et al.*, 1976). Licciardiello and Hill, (1978) reported coliform bacteria ranging from 3 to 43 per gram in frozen minced fish samples. According to Nickelson *et al.*, (1980) coliform counts of fresh minced fish ranged from 11.2 to 1100 per gram. They also detected faecal coliform in the frozen mince in the range of 1.2 to 254.3 per gram. In the present study, no significant variation was observed between days of storage and between collagen levels. So it can be concluded that days of storage and collagen levels is not having any influence on the number of *E. coli*. Since the presence of *E. coli*, is an indication of faecal contamination, the absence will indicate that the raw material was not contaminated by feacal matter.

The presence of *Staphylococcus* will emphasise the need for hygeinic handling. In the present study, only a few *Staphylococcus* colonies were seen, that too only in the initial day of storage. The number of colonies were within the tolerance limit.

During the study there was no incidence of *Salmonella* and *Vibrio*.

4.5.3 Sensory evaluation studies

The consumers appreciation of food quality is very important in determining the importance of sensory properties. The precievable sensory attributes, appearance, colour, odour, texture, overall acceptability etc. are the deciding factors in food acceptance. One of the aims of this work is to find out the influence of collagen on the texture and keeping quality of mince based products. There is significant variation in all attributes studied between different days of storage in all species of fishes at different levels of collagen (Table 4.4.2.1a, 4.4.2.2a, 4.4.2.3a, 4.4.2.4a and 4.4.2.5a). But between levels of collagen, the significant variation at 0.1% level was noted only in the case of texture and overall acceptability. In these two cases, the highest significant variation was given by 10% level of collagen. The other attributss did not show significant variation even at 5% level, between collagen levels. So these attributes- appearance, colour and odour were not affected significantly by the addition of collagen. But there is variation in these due to frozen storage.

In the case of texture and overall acceptability, 10% level of collagen has some desirable effect. As

far as consumers appreciation and enjoyment of food is concerned it is better to incorporate surimi with 10% collagen on the total protein content. Texture is an important factor that decides palatibility.

On studying frozen storage changes, Reddy et al., (1992) could also observe a significant ($P < 0.05$) decrease in organoleptic scores in pink perch. This in turn will increase the water holding capacity of the product. According to Okazaki (1994), washing removes odouriferous substances from the fish mince and impart higher gel forming ability to surimi. The washing process also removes pigments and gives a translucent appearance to surimi. But on frozen storage a change in colour due to decomposition. An increase in firmness, on frozen storage was also noted (Nakayama *et al.*, 1977; Patashnik *et al.*, 1973; Rodger *et al.*, 1980). The toughness may be due to the progressive denaturation of muscle protein. In the present study also there is decrease in desirable texture of surimi as evaluated from the sensory tests and measurement of gel strength. The decrease in sensory scores is more for surimi without collagen. In 1994, Borderias *et al.*, reported an improved sensory porperty in minced cod when collagenous material was added during frozen storage.

It can be concluded that all treatments with added collagen changed the textural properties. The treatments with 15% level of collagen, showed undesirable toughness. Treatments with 5% level of collagen showed an increase in texture, but more acceptable level is 10%. When the level exceeded 15%, the surimi gave strong objectionable taste to the final product. So the additon of collagen should not exceed 10% of total protein content of the tissue.

CHAPTER 5

PRODUCT DEVELOPMENT

5.1. *INTRODUCTION*

Consequent to urbanisation, the preference of people shifted from raw fish to ready to eat fishery products. Consumer acceptance of these products are based largely on their appearance, sensory qualities and economic value. Surimi, a refined form of minced fish meat, has great potential as a functional protein ingredient which can be substituted for a variety of traditional animal and vegetable proteins. According to Spencer *et al.*, (1992) surimi products are being consumed in increasing quantities every year.

In Japan surimi has been used extensively to develop a variety of fabricated foods. In Japan starch is often added to surimi to improve textural properties. The effect of starch on the strength of cooked fish gels has been investigated by several Japanese workers (Okada and Yamazaki, 1959 and Wu *et* al., 1985). In USA, surimi technology led to the development of commercially acceptable shellfish analogs, which were not successful when soyprotein was used. Surimi when mixed with salt and heated, it is transformed into elastic gel. In India no attempt has been identified to introduce gel type seafood products based on surimi.

The functional quality of surimi varies with fish species and it is necessary to improve the quality of low grade surimi using additives or modified processing schedules. In this study an attempt has been made to develop some products with surimi as one of the ingredients.

5.2 *REVIEW OF LITERATURE*

Nature of surimi products is affected by the functional properties of surimi, ingredients used and temperature time relationship during heat setting (Okada, 1963; Lee, 1984; Wu *et al.*, 1985; Kim *et al.*, 1987). By the incorporation of gel forming polymers into products and mechanical texturisation processes, a variety of seafood analogue products have been developed (Okada, 1963; Akahame *et al.*, 1984; Kim and Lee, 1987; Chung and Lee, 1990). For successful formulation in developing new products, the understanding of the functional effects of ingredients on texture of final product is important. The textural properties of the product reflect the characteristics of a network structure whcih forms through protein gelation where, protein-protein and protein-solvent interactions are balanced.

Fabricated foods are made by structuring, shaping or blending various ingredients into finished food products. The

ingredients used must be readily available, economical, safe and must serve a useful function. Surimi is a protein source and has unique gelation characteristics, which can form fabricated foods with visco-elastic properties (Lanier, 1986). Close correlation between myofibrillar protein solubility and gel structure has been reported for products from fish tissue (Cheng *et al.*, 1979). According to Shimizu *et al.* (1954), maximisation of myosin solubility is the key factor in the gel strength of fish sausage.

According to Lee (1984), unlike soyprotein, surimi produces an elastic and chewy texture which can be made to resemble that of shellfish because of it's high concentration of myofibrillar protein. Surimi based products passed through three stages of gel formation, during conventional processing (Montejano *et al.*, 1983, 1984; Wu *et al.*, 1985). The first stage occurs as the surimi is initially heated. the gel increases in strength upto $13^{O}C$, followed by a passive temperature range from, $13^{O}C$ to $30^{O}C$. A second "heat set" occurs between $30^{O}C$ to $40^{O}C$ and a final cook occurs between $50^{O}C$ to $90^{O}C$, during which the maximum gel strength is realised.

In 1983, Codex Alimentarius Commission, proposed hydrocolloids such as, caraageenan, pectin and alginates as additives for minced fish. Lee (1984) reported that surimi based

products are prepared by extending surimi paste into various shapes. In 1985, Ismond *et al.*, stated that incorporation of fibers into an appropriately flavoured and gelled surimi matrix would yield a prototype resembling seafood. Hydrocolloid gums such as, Xanthan, alginate and methyl cellulose etc. have extensive loss of rigidity and alasticity in muscle protein, although they are known to have an excellent water retention ability (Lanier, 1986; Forgeding and Ramsey, 1987).

According to Chung *et al.*, 1991, a variety of formulated seafood analog products have been developed through the modification of functional and textural properties. Ismond *et al.*, 1994, described a method to texturise analogs. Meullenet *et al.*, 1994, studied the textural properties of chicken frankfurters with added collagen fibers. According to them the addition of collagen fibers resulted in harder, springier and less juicy frankfurters.

In Chapter 4, it was found that 10% collagen when added to surimi will enhance consumer acceptability and will provide good texture to the product. In the present Chapter, seven speciality products with 10% collagen of the total protein percentage were prepared with common carp surimi and with shark surimi without collagen. A comparative study of sensory

characteristics of products prepared with these two types of surimi is done in this Chapter.

5.3 *MATERIALS AND METHODS*

From the results obtained in Chapter 4, it was found that surimi incorporated with 10% collagen of the total protein has some better functional properties as far as product development is concerned. On the basis of this result, seven products were prepared from the base materials a) Surimi prepared from common carp incorporated with 10% collagen b) Surimi prepared from shark without collagen.

5.3.1 Materials

5.3.1.1 Base material

a) Surimi prepared from common carp incorporated with collagen at 10% level of the total protein.

b) Shark surimi.

5.3.1.2 Ingredients

The ingredients are given along with recipe (5.3.2) of each product.

5.3.1.3 Mould

To get an attractive appearance moulds were prepared. These moulds were cast in brass for hygienic handling of the product.

5.3.2 Methods

The method of preparation along with the recipe of each product is given below. The total weight of the ingredients added for each of the products prepared weighed 10 kilogram.

The products prepared out of common carp surimi incorporated with collagen at 10% of the total protein percentage and shark surimi without collagen are given below.

5.3.2.1 Modified mince cake

The product must be cooked before consumption. It can be fried, steam cooked or grilled. The ingredients required are

Ingredients	Weight in kg
Fish surimi	4.85
Cheese	3.00

Rusk	1.00
Water	1.00
Corainder	0.02
Pepper	0.01
Salt	0.12
Total weight	10.00

Surimi was mixed with other ingredients in the chopper for 4 minutes and 30 seconds. The mixture was filled in the mould. The perishable product retrieved from the mould can be kept chilled or frozen. It will keep in good condition for 4 days at $4^{o}C$; when deep frozen it will keep for more than six months at $-20^{o}C$.

5.3.2.2 Moulded surimi sausage

This is a ready to eat cooked product or can be fried if desired. The ingredients incorporated include

Ingredients	Weight in kg
Fish surimi	7.00
Cheese	1.30
Water	0.65
Rusk	0.50
Pepper	0.22
Salt	0.20

Potato starch	0.10
Garlic	0.03
Total weight	10.00

Surimi is mixed with other ingredients in the chopper for five minutes. The mixture is allowed to set in the mould. It was then steam cooked for 2 hours and then cooled for 30 minutes. The product should be kept chilled or frozen; it will keep in good condition for a week at $4^{O}C$ and for more than six months at $-20^{O}C$.

5.3.2.3 Fish mince crisps

The product has to be fried for 10 seconds in vegetable oil at $200^{O}C$ before serving. The ingredients required are

Ingredients	Weight in kg
Fish surimi	4.40
Starch	4.30
Water	1.00
Salt	0.30
Total weight	10.00

Surimi is mixed with other ingredients for 15 minutes in a dough mixer, until the mixture is firmly bound and comes away cleanly from the side of the bowl. The mixture was spread to a thickness of 2mm in a stainless steel container and pressure cooked at 15 lbs for 30 minutes. The cooked mass was cut into different shapes as desired and then dried in a current of warm air at 35 to 40^{o}C, until they are hard and brittle. It was then packed in air tight containers. This will keep in good condition for six months at room temperature.

5.3.2.4 Fish mince chips

The chips were ready for consumption after frying in oil at 200^{o}C, till they are light brown in colour. The ingredients added include

Ingredients	Weight in kg
Fish surimi	3.00
Water	3.50
Dried potato powder	2.50
Starch	0.60
Salt	0.10
Total weight	10.00

The surimi with the ingredients are mixed in a food mixer until the mixture is smooth and pliable. The mixture is spread into thin sheets of 2mm thickness, then cut into suitable shape. The chips were fried by immersing in vegetable oil at 200^{o}C for 2 seconds, freed of excess fat and then quick frozen at -40^{o}C and kept in cold storage at -20^{o}C. In this condition the product will remain in good condition for more than nine months.

5.3.2.5 Savoury mince fingers

The thawed product can be mixed in bread crumbs or batter and then fried in vegetable oil at 200^{o}C or grilled. The ingredients used include

Ingredients	Weight in kg
Fish surimi	5.80
Tomato sauce	1.80
Milk	1.70
Rusk	0.60
Salt	0.06
Pepper	0.04
Total weight	10.00

Surimi with the ingredients were mixed in a food mixer into a pulp form. The pulp was then shaped in a mould and the moulded product was frozen at $-40^{o}C$ and cold stored at $-20^{o}C$. In this condition the product will remain in good condition for more than six months.

5.3.2.6 Fish mince paste

This product can be used as a savoury spread on bread or biscuits. The following ingredients were added

Ingredients	Weight in kg
Fish surimi	7.70
Cheese	1.00
Starch	0.50
Tomatc sauce	0.40
Salt	0.40
Vinegar	0.08
Cinnamon	0.016
Pepper	0.008
Ginger	0.008
Cloves	0.004
Total weight	10.00

Surimi and cheese were pulped in a food mixer and then mixed with the remaining ingredients in the mixer itself, so that the whole mass was converted into a smooth paste. The paste was taken in glass jars and then sealed. The sealed jars with the mix was heat processed at 15 lbs pressure for 30 minutes. This product will remain in good condition for two years.

5.3.2.7 Shrimp analog

The product should be fried in vegetable oil at 200^{o}C or steam cooked before serving. It can also be battered and fried in vegetable oil or grilled.

Ingredients	Weight in kg
Fish surimi	6.80
Water	1.30
Starch	1.10
Egg white	0.49
Vegetable oil	0.25
Shrimp flavoring	0.02
Total weight	10.00

The ingredients were mixed in food processor until a pasty consistency was obtained. The paste was moulded. The

product will keep in good condition for a week at $4^{o}C$ and for more than six months at $-20^{o}C$.

5.4 *RESULTS*

The enjoyment value of food is very difficult to assess because this must take into account all those properties of food such as, appearance (visual appeal), odour, texture, taste etc. The overall acceptability of the food depends upon all these parameters.

The organoleptic evaluation of all products were done by a panel of ten judges. The samples were coded and presented to the judges, who were instructed to evaluate the product by giving scores for different attributes like appearance, colour, odour, taste, texture and overall acceptability. The quality of samples were evaluated for appearance and colour by visual feel, odour by olfaction, taste by tongue and texture by biting. Sensory feel ratings were recorded in a score sheet and these ratings were based on previously assigned numerical scores.

5.4.1.Moulded mince cake

Table 5.4.1. gives the sensory evaluation scores

Table 5.4.1 Mean sensory evaluation score for modified mince cake

ATTRIBUTES	* ATTRIBUTE SCORES	
	COMMON CARP SURIMI WITH 10% COLLAGEN	SHARK SURIMI
APPEARANCE	4.8	4.8
COLOUR	4.7	4.8
ODOUR	4.7	4.5
TASTE	4.8	4.3
TEXTURE	4.6	4.2
OVERALL ACCEPTABILITY	4.5	4.0

* MEAN OF 10 X 3 READINGS

Table 5.4.2 Mean sensory evaluation score for moulded surimi sausage

ATTRIBUTES	* ATTRIBUTE SCORES	
	COMMON CARP SURIMI WITH 10% COLLAGEN	SHARK SURIMI
APPEARANCE	4.8	4.8
COLOUR	4.7	4.7
ODOUR	4.8	4.5
TASTE	4.5	4.4
TEXTURE	4.7	4.6
OVERALL ACCEPTABILITY	4.6	4.1

* MEAN OF 10 X 3 READINGS

for different attributes of moulded mince cake prepared with surimi of common carp incorporated with collagen and also with surimi of shark without collagen. Here the appearance score is same for both the products. Colour score is better for products prepared with shark surimi. Odour, taste and texture and also overall acceptability scores were better for common carp surimi. Plate 5.4.1a shows the steamed moulded mince cake and Plate 5.4.1b shows the fried product.

5.4.2 Moulded surimi sausage

Mean sensory evaluation score for the product is given in Table 5.4.2. Here the scores for appearance and colour are same for both the products. But the score for taste, odour, texture and overall acceptability was found to be higher for common carp surimi. Shark is having higher content of non-protein nitrogenous matter and urea. So all attributes connected with olfaction and tongue shows a decreasing score. Plate 5.4.2a and Plate 5.4.2b shows the steamed and fried products respectively.

5.4.3 Fish mince crisps

Result of the mean sensory evaluation tests for the product is given in Table 5.4.3. Here the score for appearance,

Table 5.4.3 Mean sensory evaluation score for fish mince crisps

ATTRIBUTES	* ATTRIBUTE SCORES	
	COMMON CARP SURIMI WITH 10% COLLAGEN	SHARK SURIMI
APPEARANCE	4.9	4.9
COLOUR	4.8	4.8
ODOUR	4.7	4.7
TASTE	4.7	4.7
TEXTURE	4.7	4.7
OVERALL ACCEPTABILITY	4.7	4.7

* MEAN OF 10 X 3 READINGS

Table 5.4.4 Mean sensory evaluation score for fish mince chips

ATTRIBUTES	* ATTRIBUTE SCORES	
	COMMON CARP SURIMI WITH 10% COLLAGEN	SHARK SURIMI
APPEARANCE	4.8	4.8
COLOUR	4.8	4.8
ODOUR	4.7	4.5
TASTE	4.8	4.5
TEXTURE	4.8	4.6
OVERALL ACCEPTABILITY	4.8	4.8

* MEAN OF 10 X 3 READINGS

colour, texture and overall acceptability is same for the two species of fishes used to make product. Plate 5.4.3a and Plate 5.4.3b, shows the appearance of fish mince crisps before frying and the fried product respectively.

5.4.4. Fish mince chips

Table 5.4.4. gives the mean values of organoleptic test of the product prepared from both species. Here the score for appearance, colour and overall acceptability are same for both species. Fish mince chips before frying is shown in Plate 5.4.4a and the product after frying is shown in Plate 5.4.4b.

5.4.5 Savoury mince finger

The sensory evaluation score for appearance attribute for both species of fish was 4.7. In the case of other attributes some noted difference in organoleptic score is seen. This can be seen in Table 5.4.5. Plate 5.4.5a shows the steamed product and Plate 5.4.5b the fried product.

5.4.6. Fish mince paste

The average organoleptic score for the two species of fishes for the product is given in the Table 5.4.6. The product is

Table 5.4.5 Mean sensory evaluation score for savoury mince fingers

ATTRIBUTES	* ATTRIBUTE SCORES	
	COMMON CARP SURIMI WITH 10% COLLAGEN	SHARK SURIMI
APPEARANCE	4.7	4.7
COLOUR	4.6	4.6
ODOUR	4.7	4.2
TASTE	4.6	4.2
TEXTURE	4.5	4.3
OVERALL ACCEPTABILITY	4.6	4.0

* MEAN OF 10 X 3 READINGS

Table 5.4.6 Mean sensory evaluation score for fish mince paste

ATTRIBUTES	* ATTRIBUTE SCORES	
	COMMON CARP SURIMI WITH 10% COLLAGEN	SHARK SURIMI
APPEARANCE	4.8	4.6
COLOUR	4.7	4.5
ODOUR	4.6	4.5
TASTE	4.6	4.5
TEXTURE	4.6	4.5
OVERALL ACCEPTABILITY	4.6	4.5

* MEAN OF 10 X 3 READINGS

Table 5.4.7 Mean sensory evaluation score for shrimp analog

ATTRIBUTES	* ATTRIBUTE SCORES	
	COMMON CARP SURIMI WITH 10% COLLAGEN	SHARK SURIMI
APPEARANCE	4.7	4.5
COLOUR	4.6	4.6
ODOUR	4.6	4.2
TASTE	4.6	4.4
TEXTURE	4.5	4.4
OVERALL ACCEPTABILITY	4.6	4.5

* MEAN OF 10 X 3 READINGS

shown in Plate 5.4.6. The overall acceptability scores for this product prepared from common carp was 4.6 and from shark was 4.5. The other attributes also are not showing much difference. All attributes show better value for common carp.

5.4.7 Shrimp analog

Table 5.4.7 gives the attribute score for the shrimp analogue prepared from common carp and shark. Here except for the attribute score for colour, all other attributes give better value for the product prepared from common carp. The steamed shrimp analog prepared is shown in Plate 5.4.7a and Plate 5.4.7b shows the fried analog.

5.5 *DISCUSSION*

Surimi is a good protein source with unique gelation characteristics and visco-elastic properties. In the present study, surimi was used as base material. Being the source of protein, when incorporated as an ingredient for making product, the nutritive value of the product shoots up. The seven products prepared were found to have good consumer acceptability regardless of species difference. Since products are meant for human consumption, sensory evaluation of the product is very important.

The sensory evaluation is the oldest and still the most widepsread means for evaluating the acceptability and edibility of fish and fishery products.

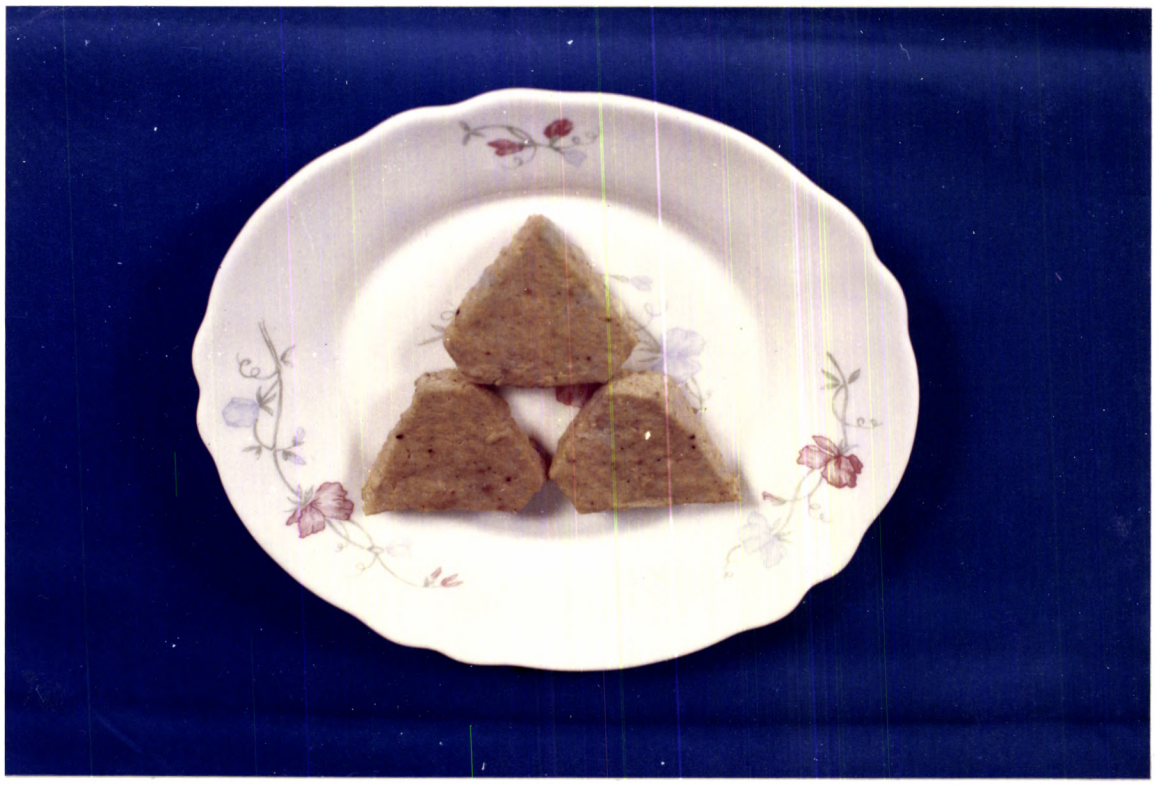
The fabricated products were prepared by blending and shaping or structuring various ingredients into finished products. Here the ingredients used are readily available, economical and safe. A variety of seafood analogs were prepared by Jayasekharan *et al.*, (1992), by incorporating non-fish protein to comminuted meat. Ismond *et al.* (1994), successfully texturised surimi by incorporating fibers.

The consumer acceptability of the products evaluated by sensory evaluation was excellent. One of the reasons for this may be, the acceptable odour and taste of the product. Due to repeated washing while preparing surimi, most of the odouriferous compounds were removed and the resultant surimi was almost free of fish odour. The good texture of these product may be due to the heat denaturation of collagen and its consequent convertion to gelatin. The ability to undergo thermal transition and dispersion of the ingredients are responsible for the difference in texture modifying effect.

From the results of sensory evaluation it was also noticed that shark surimi, eventhough had good consumer acceptability its overall acceptability is less compared to the products prepared from surimi of common carp with 10% collagen. The probable reason for this is the presence of high amount of non-protein nitrogenous matter, particularily urea in shark meat. If some method is developed to remove this non-protein nitrogenous matter from the meat without affecting its functional properties, the acceptability of the product can be increased, which in turn will reduce the task of incorporating collagen in the surimi.

Plate 5.4.1a MODIFIED MINCE CAKE. -STEAMED

Plate 5.4.1b MODIFIED MINCE CAKE -FRIED



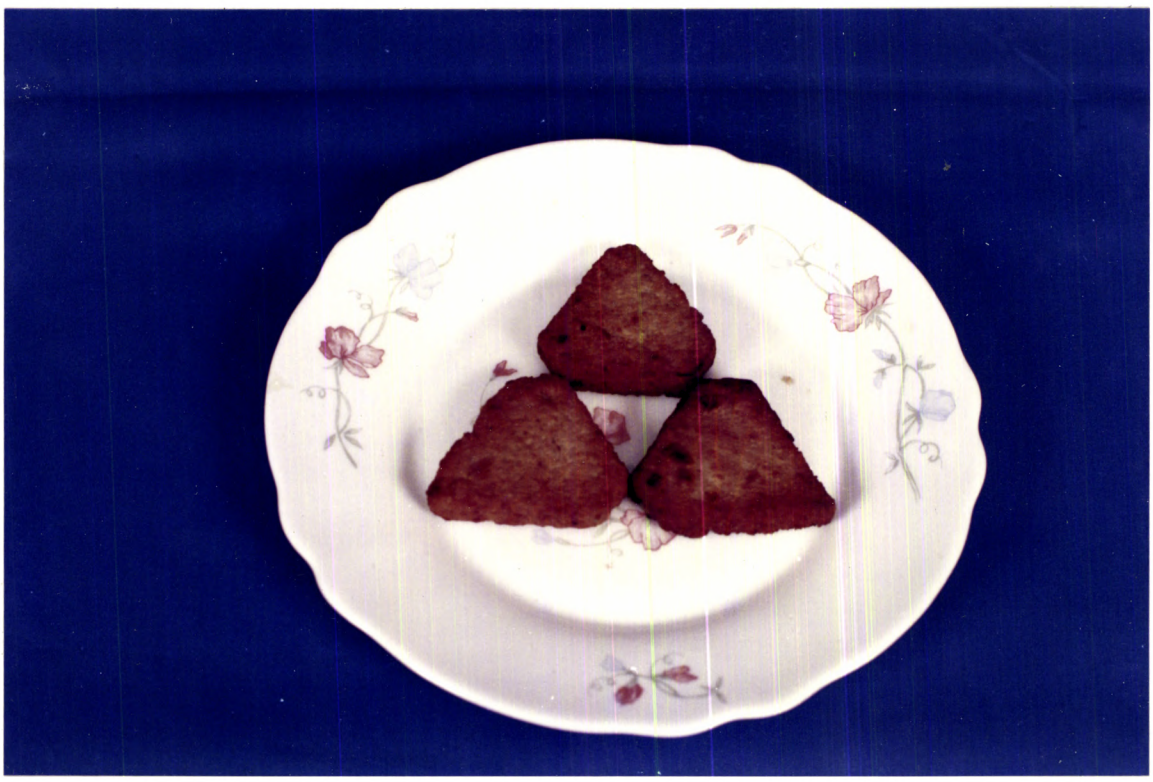
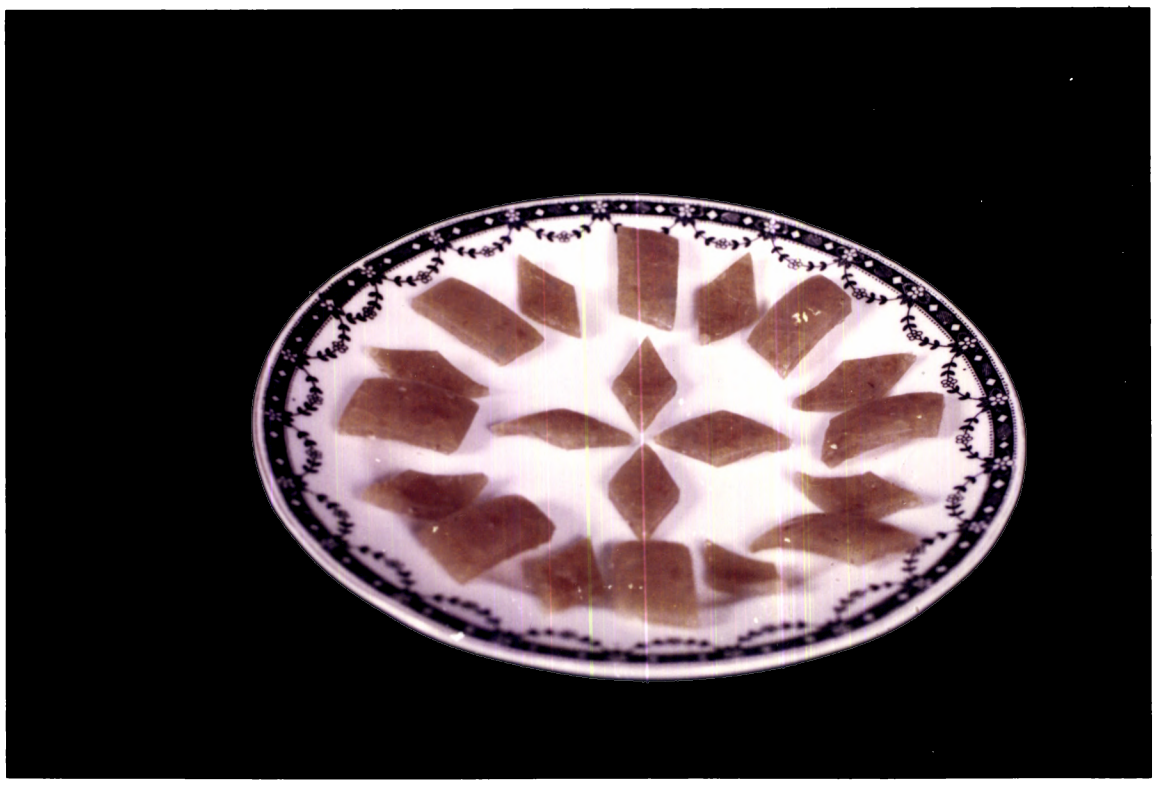


Plate 5.4.2a MOULDED SURIMI SAUSAGE -STEAMED

Plate 5.4.2b. MOULDED SURIMI SAUSAGE -FRIED

Plate 5.4.3a FISH MINCE CRISPS -RAW

Plate 5.4.3b FISH NINCE CRISPS -FRIED



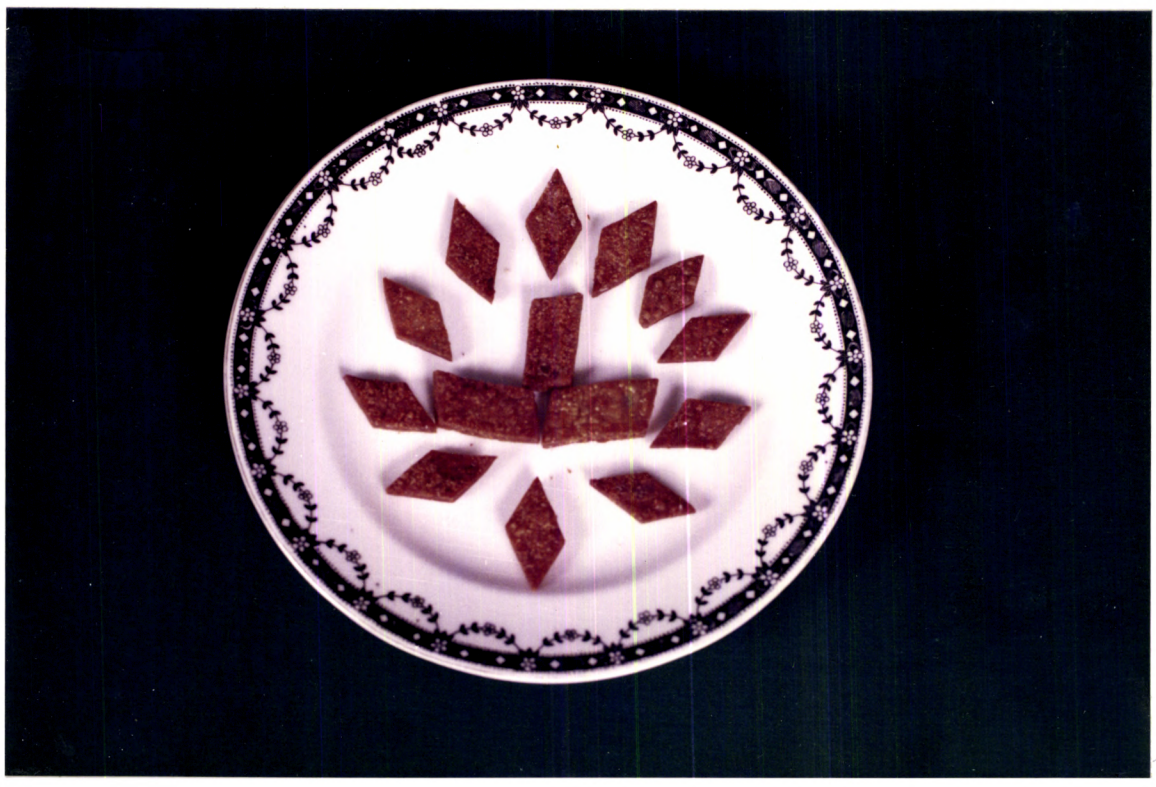


Plate 5.4.4a. FISH MINCE CHIPS -RAW

Plate 5.4.4b FISH MINCE CHIPS -FRIED

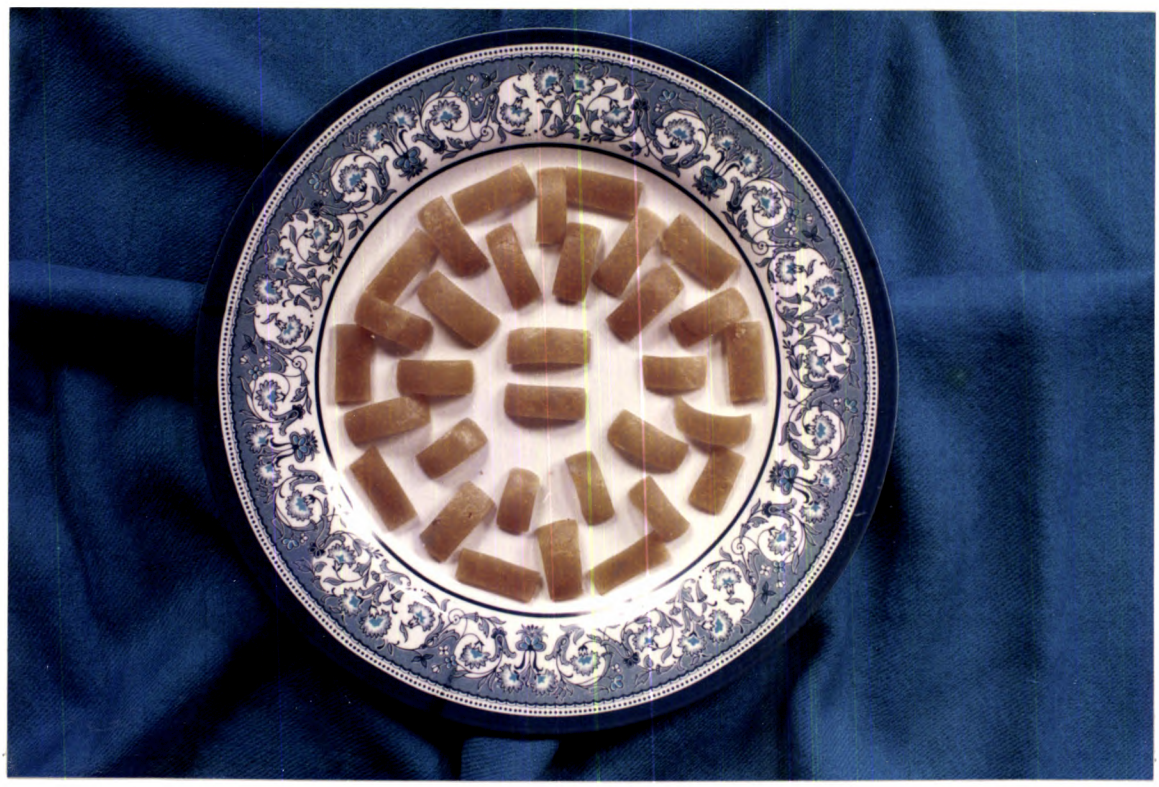
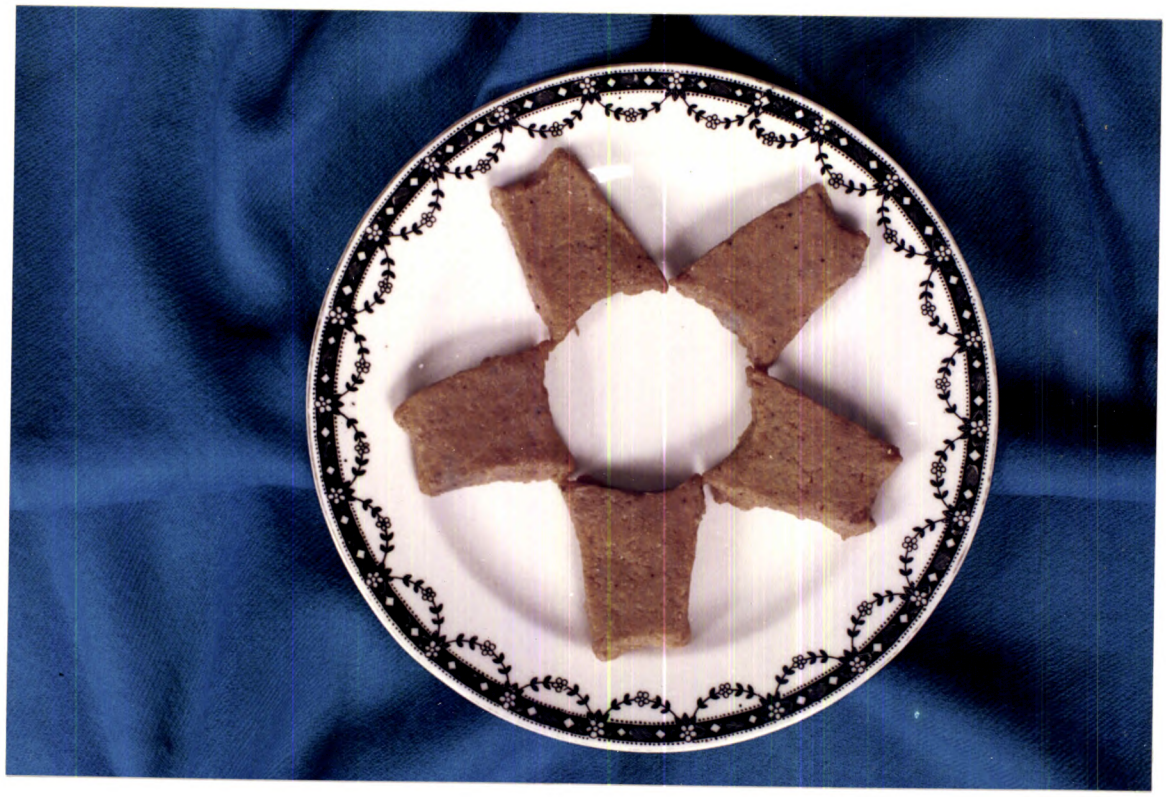


Plate 5.4.5a SAVOURY FISH FINGERS -STEAMED

Plate 5.4.5b SAVOURY FISH FINGERS -FRIED



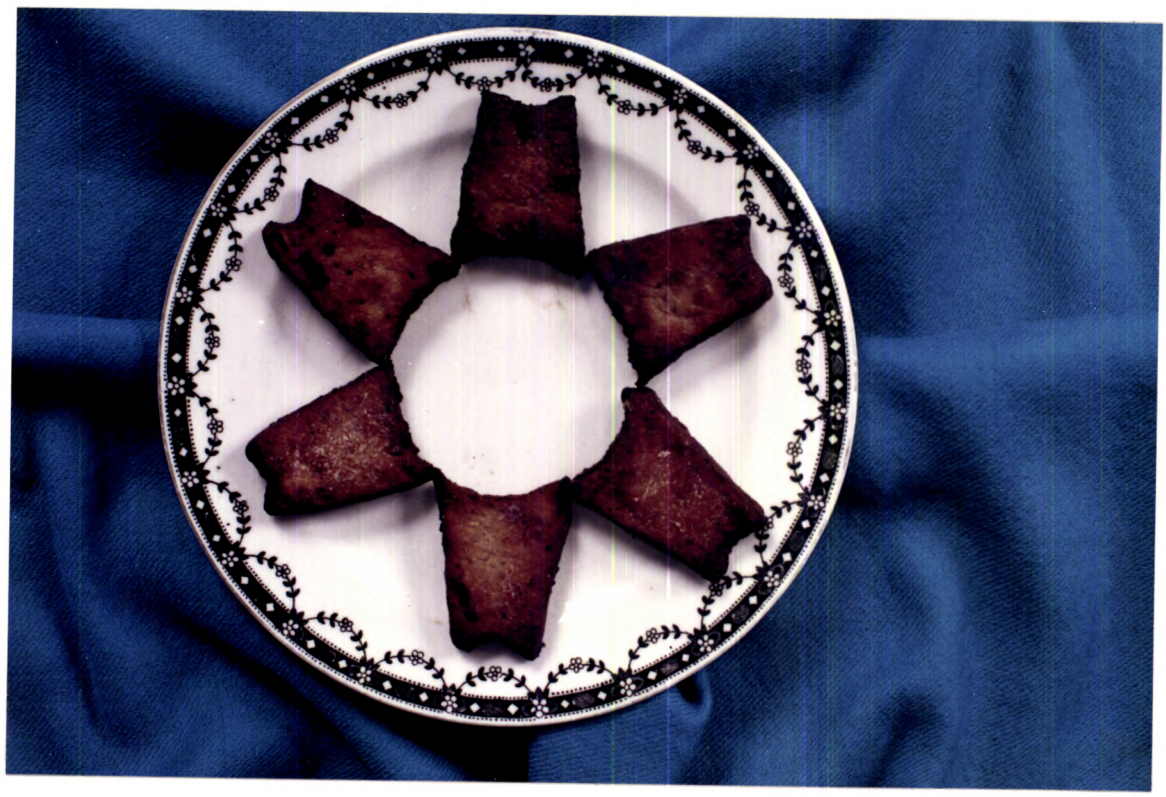


Plate 5.4.6 FISH MINCE PASTE

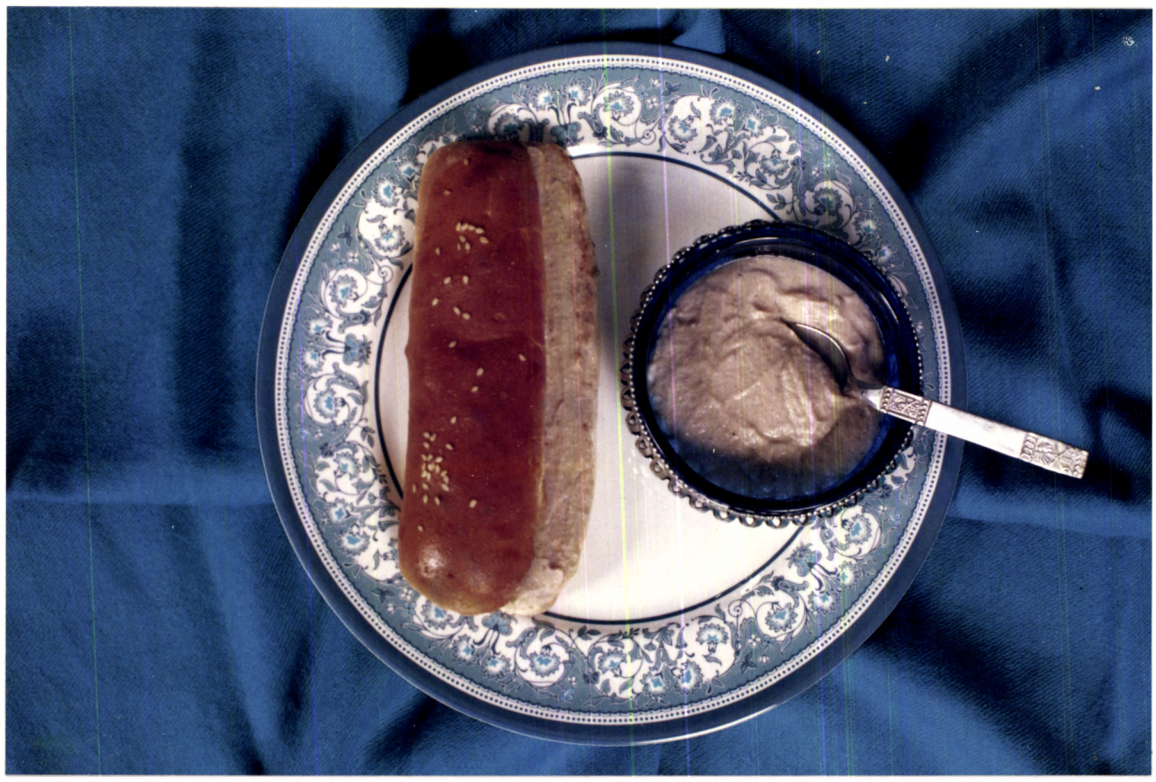


Plate 5.4.7a SHRIMP ANALOG -STEAMED

Plate 5.4.7b SHRIMP ANALOG -FRIED

SUMMARY AND CONCLUSION

1. The proximate composition of twenty commercially important tropical fishes on the west coast of India, namely, *Pampus argentius, Sardinella longiceps, Rastrelliger kanagurta, Cynoglossus semifaciatus, Caranx spp., Trichurus sawala, Anchoviella, Oreochromis mossambicus, Cyprinus carpio, Labeo rohita, Megalops cyprinoides, Barbus spp., Kowala kowal, Nemipterus japonicus, Mugil cephalus, Catla catla, Euthinus affinis, Sillago sihama, Himantura spp. and Scoliodon sorrakowah.* Fishes studied include freshwater, brackishwater and marine fishes. Both teleosts and elasmobranchs were included in the study.

Moisture content of fishes studied vary from 70.86% - 80.54%, protein content between 17.9% - 22.9%, fat content between 0.09% - 7.5%, and ash content 0.9% - 3.14%.

2. Estimation and fractionation of collagen content of these twenty species were done by the method of Sato, 1988. Among fishes studied soluble collagen (ASC) vary between 0.09 to 2.03gm/100gm wet meat, insoluble collagen (ISC) between 0.09 to 1.08gm/100gm meat and total collagen (TC) between 0.3 to 2.99gm/100gm meat.

Percentage of total collagen on the basis of total protein was also worked out and the value ranged between 1.58% to 13.39%.

3. The fishes were classified into three groups based on the percentage of total collagen on total protein, viz. fishes having collagen content upto 5% were grouped as low collagen fishes, 5-10% as medium collagen fishes and above 10% as high collagen fishes.

4. Four species of fishes of freshwater, brackishwater and marine origin were selected for studying the different parameters of surimi. The species selected were common carp, tilapia, nemipterus and shark. The parameters studied can be grouped as physico-chemical characteristics, microbiological and organoleptic characteristics. Physico-chemical characteristics studied were moisture, total protein, non-protein nitrogen, pH, trimethylamine content, total volatile nitrogen content, α-amino nitrogen content, gel strength, percentage expressible moisture and visual contaminants. Microbiological parameters studied were TPC, *E. coli*, *Staphylococcus*, *Salmonella* and *Vibrio cholera*. Appearance, colour, odour, texture and overall acceptability were the sensory characteristics studied. Storage quality was studied for a period of one year.

Shark surimi was found to have some desirable functional properties as far as product development is concerned. The gel strength of shark surimi is more compared to other species. After one year of storage also shark surimi showed acceptable gel strength and percentage expressible water. Though it showed high values for chemical spoilage parameters, its organoleptic and physical qualities suitable for product developement were good. Microbiological parameters were not showing any significant variation between species. The desirable quality characteristics of shark surimi may be due to its high collagen content.

5. From the air bladder of carp collagen of edible grade was prepared by the method of Gallop *et al.*, 1963.

6. To study the role of collagen on the texture and storage quality of surimi of lesser collagen content fishes, pre-determined levels of collagen prepared from the air bladder of common carp was incorporated. The level of collagen incorporated in the surimi were fixed as 5%, 10% and 15% of the total protein content of fish.

7. After incorporation of progressive levels of collagen, different parameters- physico-chemical, organoleptic and microbiological, were studied at different storage intervals. On frozen storage, a decrease in desirable quality characteristics was noted. Statistical analysis was done to find out the optimum level of collagen to be incorporated in the surimi. 10% level of collagen gave better values compared to other levels. When compared to all the quality parameters studied, the characteristics connected with texture gave a better significance statistically in all the species irrespective of the habitat difference.

It was found that all treatments with added collagen had changed the textural properties of surimi. A significant variation at 0.1% level was noted in the sensory attributes-texture and overall acceptability between different levels of collagen and highest significant variation was given by 10% level of collagen. The treatments with 15% level of collagen showed undesirable toughness to the surimi. Treatments with 5% level of collagen showed an increase in texture but a more acceptable level is 10%. When surimi was incorporated with 15% collagen it gave an objectionable taste to the product.

8. Seven speciality products were prepared from the base material- a) Prepared from surimi of common carp with 10% level of collagen and b) Shark surimi without collagen. The sensory evaluation of these products revealed that overall acceptability of products prepared from shark surimi is less compared to the products prepared from common carp.

9. For a consumer or producer of minced based products, the data showing collagen content in the muscle of fish is very important. Since there is a direct relationship between collagen content and texture of meat, to produce products from surimi of desired texture, the data showing the content of collagen in fish serves as a reference to select suitable species for a particular texture.

10. The air bladder of fishes can be used for modifying texture during the commercial production of minced based products with high consumer acceptability. These products can contribute high export earnings for the country which will also indirectly increase the demand and thereby the price of air bladder of fishes. The present study has revealed that the air bladder of common carp (*Cyprinus carpio)* is highly effective for the

preparation of collagen. Commercial application of the air bladder of these fishes for the production of mince based products can give a better price stability for these cultured fishes.

11. Further work need to be done to demonstrate the rheological changes as a valuable approach to understand gelation of fish proteins with the optimum level of collagen incorporated to various species of low collagen fishes. The thermal setting process as a function of rheological properties are to be studied.

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ANNEXURE I

EVALUATION OF ORGANOLEPTIC QUALITY OF MINCE BASED MEAT PRODUCT, SURIMI

Name of Judge Date:

Samples of mince based meat products, are presented . Kindly examine the products for organoleptic qualities. Indicate the quality of each sample by putting a tick mark (√) against the appropriate description.

Appearance

Sample	Excellent	Very good	Good	Neither good nor bad	Bad	Very bad	Extremely bad
Raw							
Cooked							
Raw							
Cooked							
Raw							
Cooked							

Colour

Sample	Excellent	Very good	Good	Neither good nor bad	Bad	Very bad	Extremely bad
Raw							
Cooked							
Raw							
Cooked							
Raw							
Cooked							

Odour

Sample	Excellent	Very good	Good	Neither good nor bad	Bad	Very bad	Extremely bad
Raw							
Cooked							
Raw							
Cooked							
Raw							
Cooked							

Texture

Sample	Excellent	Very good	Good	Neither good nor bad	Bad	Very bad	Extreeely bad
Raw							
Cooked							
Raw							
Cooked							
Raw							
Cooked							

Overall acceptability

Sample	Excellent	Very good	Good	Neither good nor bad	Bad	Very bad	Extreeely bad
Raw							
Cooked							
Raw							
Cooked							
Raw							
Cooked							