CHAPTER - 3

FACTORS AFFECTING THE VELOCITY OF SALT PENETRATION DURING SALTING PRESERVATION IN RELATION TO BIO-CHEMICAL QUALITY CHANGES OF HILSA FISH, HILSA ILISHA (HAM-BUCH)

INTRODUCTION

Hilsa fish, Hilsa ilisha (Ham-Buch) belonging to the family clupeidae are abundantly found in coastal water in the vicinity of the mouth of the river Narmada (Joseph, 1967). During the fishing seasons, most of the hilsa fish are salted and rest being locally utilized in the fresh condition (Jhingran, 1977). Salting of fish is likely to increase the importance since it seems that in many cases the distribution and marketing costs for salting fish may compare favourably with those for large scale distribution of iced fish. The fish technologists have realized that the high technology developed for use in marine and fresh water fish preservation may not be economical in the developing country.

The preservation of fish by salting remains of considerable importance to the developing countries of the world. It is usually a very simple process, requiring a minimum of capital investment and therefore can provide a cheap source of high-quality protein. It was, therefore, considered desirable to conduct a thorough study of the salting process of hilsa fish in relation to biochemical changes.

The salting rate is defined as the amount of salt which has penetrated into the fish during a relatively short period of time. This rate of penetration depends upon the following factors : Chemical composition, specific surface and shape of the fish body, concentration and temperature of the brine, salting method and chemical composition of the salt used. The effects of these factors on the velocity of the salt penetration either directly or indirectly is discussed by Berezan (1951); Semenov (1952); Voskresensky (1958) and Wistrich, et al., (1959). Investigation elsewhere on herring and other species indicate that uptake of salt is influenced by the mode of application of salt and some other factors. Tressler and Lemon (1951) stated that salt penetration increased with increase in temperature Beatty and Fouger (1957) and Crean (1961) demonstrated that temperature had an influential effect on salt-water changes, though it was little. The rate of ripening of salted fish depends on (1) the original chemical composition of the raw fish (2) the salt composition employed (3) the temperature (4) the brine composition and (5) the amount of salt in the fish tissues. Information is very scanty as to the actual mechanism whereby these various factors exert their influence (Voskresensky, 1958).

The size and nature of the fish body exert a great influence upon the salting rate, mostly delaying the motion of salt molecules into the fish, for this reason, the speed of salt uptake is usually considerably less in

non-stirred brines than during salting with dry salt or in circulating brine (Voskresensky, 1958). During preservation and subsequent storage, salt retard the enzymatic and bacterial spoilage of fish. During salting the peculiar flavours of the products are in fact due to activity of some of the surviving micro-organisms. Due to long term storage at low temperature, the product may become salty and rancid.

The movement of the salt molecules from the brine into the fish takes place through a layer of salt-solution covering formed around the fish and having a concentration below that of the brine. This layer is formed because water diffuses from the fish, in the beginning of the process, with greater speed than salt molecules from the brine enter into the fish. Lightly salted fish develops a cured flavour but its storage life is shorter because low concentration of brine cannot withdraw sufficient amount of water from the tissue of the fish and if the salt concentration is lower than the concentration of tissue fluid, water from the brine finds its way into the tissue. So, brine with low concentration cannot stop spoilage at ordinary temperature. However, the uptake of salt increases with the increase of salt concentration. Overall, the experiment deals with few factors affecting the rate of penetration of salt during storage at 0°C and 8°C temperature. The first part of experiment deals with the factors affecting the salt penetrations and the second part with the bio-chemical quality

analysis of different salt-treated hilsa fish during short-term storage at refrigerated temperature at 0°C and 8°C respectively.

MATERIALS AND METHODS

Iced hilsa fish, Hilsa ilisha (Ham-Buch) were collected from local market. The fish were organoleptically fresh and of uniform size (30 cm average). After procurement the samples were gutted and washed. Ventral and dorsal muscles were uniformly sliced, having thickness of 1 cm and 3 cms.

BRINING

Five percent and saturated solution of common salt, sodium chloride, were prepared in a glass container. Fish slices were dipped and kept immersed in the solution at room temperature ± 32°C before using the dry salt.

DRY SALTING

After brining the fish slices were mixed thoroughly with different proportion of salt. The slices were then placed in large glass containers. The samples were kept at 0°C and 8°C temperature respectively. The samples were taken out at different time intervals for determination of salt contents, pH, peroxide value, acid value and free fatty acid. About twenty slices of fishes for individual batches were thoroughly mixed with different proportion

of salt-fish ratio. The batches are as follows :

Batch-1 : One hour dipping in saturated salt solution after which the fish samples were covered with common salt (Nacl).

Batch-2 : Without salt.

Batch-3 : One hour dipping in saturated salt solution, after which the fish samples were covered with salt, using fish salt ratio 5:1.

Batch-4 : One hour dipping in 5% salt solution after which the fish samples were covered with salt.

DETERMINATION OF SALT CONTENTS

The fish slices were quickly washed under the tap water for few minutes (2-3 minutes approximately) to remove the surface salt. The samples were then thoroughly macerated and salt was extracted with distilled water in a conical flask by occasional shaking and keeping it overnight at 8°C temperature. The contents were then filtered and the salt contents in the filtrate was determined by titrating it against a standard silver nitrate solution (Mohr, 1959).

pH VALUES

Fish muscle (2 gm) was homogenized in 10 ml distilled water. The pH was measured at room temperature with a pH

meter using a glass electrode. Each determination was performed in duplicate (Poulter et al., 1978).

PEROXIDE VALUE

The peroxide content of the extracted lipids was determined using potassium iodate and sodium thiosulfate (Pearson, 1976) and expressed as milli equivalent/kg lipid.

ACID VALUE AND FREE FATTY ACID

The acid value of the extracted lipids was determined according to the method of Pearson (1976). The result is expressed as the percentage of free acidity. The free fatty acid is calculated as oleic acid (1 ml 0.1M sodium hydroxide ≡ 0.0282 g oleic acid) in which case the acid value = 2 X FFA (Pearson, 1976).

STATISTICAL METHODS

Co-relation co-efficient and regression analyses was carried out according to the method of Mian and Miyan, (1984). The results of co-relation coefficient and regression analyses among different salt-treated samples are shown in Table-4.

RESULTS AND DISCUSSION

The highest salt content was found in the dorsal

portion of the fish muscle and lowest salt content was found in the ventral portion of the fish muscle stored at 0°C and 8°C temperature. The salt uptake was always less in ventral portion as compared to the dorsal portion (Table-1). The salt uptake gradually increase with the increase of storage period. There were significant differences in salt uptake at two different temperature, 1 cm thick fish sample contained higher percentage of salt, the 3 cm thick samples contained lower percentage of salt (Table-1). The salt uptake is faster at higher temperature but at same temperature, length of dipping have little effect on salt uptake. At the same storage temperature and same concentration of salt, the penetration of salt depends upon the muscle thickness (Table 1). During the initial storage period, penetration of salt proceeds at a rapid rate and thereafter, it slows down. Salt uptake is influenced by the content of the fish. The data indicated that salt uptake become progressively slower in ventral portion as compared to dorsal portion of the fish muscles (Table-1). The fat content in the ventral portion of hilsa fish acts as a barrier to the entry of the salt within the muscles. Milligan (1965) reported that low rate of salt penetration may possibly be due to the fatty layer immediately beneath the skin. The higher the temperature, greater was the uptake of salt but differences were not so distinct. The salt uptake was most rapid in the first stage of salting process. After four days of storage, the salt content was almost constant (Table-1). When muscle is

TABLE-1 : SALT UPTAKE AND pH VALUES IN VENTRAL AND DORSAL FISH MUSCLE OF HILSA ILISHA DURING STORAGE AT 0°C AND 8°C TEMPERATURE

SALT-TREATED SAMPLES : BATCH-I

Storage period (days)	Muscles	Thickness (1 cm) Salt contents(%)		Thickness (3 cm) Salt contents(%)		Thickness (1 cm) pH values		Thickness (3 cm) pH values	
		0°C	8°C	0°C	8°C	0°C	8°C	0°C	8°C
1	Ventral	4.5	6.6	2.25	2.38	6.0	6.1	6.5	5.8
1	Dorsal	4.7	7.4	2.38	3.87	6.2	6.3	5.6	6.8
2	Ventral	8.3	8.5	3.75	4.63	6.3	6.4	6.4	6.7
2	Dorsal	9.4	9.4	4.38	4.88	6.4	6.5	6.3	5.8
3	Ventral	7.6	7.4	6.25	5.00	6.4	6.9	6.8	6.6
3	Dorsal	11.1	9.7	6.62	6.87	7.2	7.5	6.7	6.7
4	Ventral	10.3	11.0	6.88	7.75	7.0	7.3	6.7	6.0
4	Dorsal	11.2	13.2	7.25	8.5	7.4	7.9	6.9	5.8
5	Ventral	14.2	13.2	8.75	9.38	7.2	8.4	6.5	7.4
5	Dorsal	13.0	14.4	8.08	9.8	7.8	8.6	6.6	6.3
6	Ventral	16.0	17.8						
6	Dorsal	16.8	20.3						

kept in salt and allowed to equilibriate with it, either it gains or looses water. At low and intermediate salt concentrations water is transferred from the brine to the muscle and the muscle swells. At higher salt concentrations beyond a certain point, water is transferred from the muscle to the brine in which case salt penetration is high enough (Hamm, 1960). Salt penetration increase with the increasing temperature and muscle thickness. The distribution of salt uptake in muscles of fishes will increase with decreasing water content by the action of higher proportion of salt and will increase with increasing temperature (Table-1). Both the sodium, chloride ions and the muscle proteins have a high affinity for water (Hamm, 1960). He also reported that fish muscle immersed in brine at low concentration would gain water and swell but when immersed in higher concentration lose water.

Maximum uptake of salt at the initial stage has been noticed, after which it occurs at a much slower rate. When the amount of salt in the flesh rises above about 9%, certain irreversible changes occur. Considering all the results of salt uptake, as shown in Table-1, it is evident that there is less uptake of salt 2.25% (ventral muscle at 0°C, of 3 cm thickness) at initial storage day. The highest uptake of salt 20.3% (dorsal muscle at 8°C of 1 cm thickness) was observed during the end of storage period. Perhaps subcutaneous layer or fat plays role in uptake in

heavy salted fish, residual water is completely saturated with salt. Penetration of salt depends upon its concentration and length of dipping time and the muscle thickness.

Fougeres (1952) reported that under the influence of higher temperature structural arrangement of the protein molecules has been changed, whereby the protein is rendered insoluble, the soluble protein is gradually reduced and reaches a constant level. When salted for a shorter period the skin can not be torn from the flesh. It has also been observed that under the conditions of the experiment, the salt content in the fish muscle increased with the thickness of the fish samples, anatomical portions and length of period in dipping. The results are in agreement with the findings of Berezen (1948) who reported that salt uptake in the fish muscles was dependent on the concentration of salt. During salting process some physico-chemical changes of the fish muscle are brought about by combined action of salt and enzymes as a result of which protein breaks into peptides, amino acids, amine etc. that go into solution into the soaking media (Borgstrom, 1965). During the final stage of salting an increase in weight takes place due to movement of water from the brine into the fish slices (Kleymenov, 1952). Akiba (1955) reported that at any rate of salt penetration, myosin and actomyosin in the presence of excess salt are deprived of bound water. In the final stage of the salt-

ing, absorption of water from the brine by the fish tissue takes place at the expense of complex bodies formed of sodium chloride and protein. Investigation at Torry Research Station (1962) also revealed that fish muscle when immersed in brine gained or lost water from or to the brine depending upon the concentration of brine. Hamm (1960) reported that both sodium chloride ions and the muscle protein have a high affinity for water. At high salt concentration however the sodium and chloride ions must complete with protein molecules for the relatively small amount of water present. Since electrolyte ions generally attract water molecules more strongly than do the protein molecules, the former rob the latter of their hydration water. As a result protein comes out of solution This is the salting out effect, which is exhibited by many proteins including muscle proteins.

pH VALUES

The pH values of stored salted hilsa fish samples under the present study are shown in Table-1. Initially the pH values of the fish muscle varies considerably. The changes in the muscle pH of the fish studied here shows correlation with the percentage of salt uptake (Table-1). Varela and Wojciech (1956) and Wojciech and Varela (1958) have reported that the pH varied initially and that it showed no real or significant correlation with the onset of spoilage in fresh fish and salted fish (e.g. herring,

carp, pike and rock). Charnley and Goard (1942); Misesicordia (1954) and Montefredine (1955) concluded that pH was a reasonably reliable indicator of the degree of freshness. In the present study the maximum value of pH noticed is 8.6 and minimum 5.6. Higher or lower pH value depends on storage temperature, thickness of the particular portion of the sample and length of storage (Table-1). Amlacher (1961) reported that when fish death occurs, the pH values is generally around 7.0. At the end of rigor pH value (7.0) was obtained for coach and 7.1 for lake perch. There was significant differences of pH values of 3 cm and 1 cm thick samples, 0°C and 8°C storage samples (Table-1). Misericordia (1954) reported that spoilage ran parallel to the increase of pH of the muscle tissue during storage. Observations during present study find support in the work of above cited researchers.

PEROXIDE VALUES

There were significant differences of peroxide values between 8°C and 0°C storage samples. The peroxide values of the samples stored at 8°C temperature increased rapidly during the initial period of storage and then declined, whereas at 0°C there was a slight increase and decrease of peroxide value over the storage period. Peroxides are intermediate fat breakdown products and hence they accumulate in the early stage of oxidation rancidity and are then brokendown. During storage the peroxide

TABLE-2.1 : PEROXIDE VALUES IN MILLI EQUIVALENT/KG OF DIFFERENT SALT-TREATED SAMPLES DURING STORAGE AT $0^{o}C$ AND $8^{o}C$ TEMPERATURE

Storage period (days)	BATCH-1		BATCH-3		BATCH-4	
	Storage Temperature		Storage Temperature		Storage Temperature	
	($0^{o}C$)	($8^{o}C$)	($0^{o}C$)	($8^{o}C$)	($0^{o}C$)	($8^{o}C$)
1	17.2	17.4	41.2	52.8	21.6	33.6
2	12.0	13.6	42.8	44.8	32.6	44.0
3	18.0	18.4	26.4	28.2	29.4	38.4
4	29.6	39.3	45.4	72.7	16.0	21.6
5	52.0	54.5	31.0	35.0	30.2	48.8
6	48.0	47.9			12.0	23.13
7					15.59	26.20

values of the salted samples fluctuate (increase/decrease) in both 0°C and 8°C stored samples (Table-2.1). During the initial storage period there was a steady build up of peroxide in the hilsa fish muscle and this must be a factor contributing to the decrease in taste acceptability. According to Pearson (1970) just spoilage values of peroxide are 10-20 milli equivalents per kg fat, depending on the type of the fat. This is the range of values at which the fat itself tastes rancid. The pattern of changes in peroxide values obtained in salted samples studied here suggests that fish samples stored at 0°C temperature showed low peroxide value whereas in those stored at 8°C the development of peroxide was much higher. The peroxide values of the oil present in the fish increases at a greater rate than the value of extracted herring oil (Banks, 1938a). Similarly in the storage of sardine and extracted sardine oil at -15°C the peroxide value of the oil present in the fish increases more rapidly than the value of extracted sardine oil. Moreover, the acidity of the sardine oil in the fish increases rapidly with the duration of storage, whereas the value of extracted sardine oil scarcely changes (Hashimato et al., 1946).

ACID AND FREE FATTY ACID

The acid and free fatty acid values are shown in Tables 2 and 3. The acid values of the samples stored at 8°C temperature increased rapidly during the initial stage

TABLE-2 : ACID VALUES OF DIFFERENT SALT-TREATED SAMPLES DURING STORAGE AT 0°C AND 8°C TEMPERATURES

Storage days	BATCH-1		BATCH-2		BATCH-3		Batch-4	
	Storage Temp.		Storage Temp.		Storage Temp.		Storage Temp.	
	0°C	8°C	0°C	8°C	0°C	8°C	0°C	8°C
1	2.24	2.80	3.93	5.61	2.24	2.55	1.68	3.36
2	3.36	4.49	4.00	5.61	2.81	3.93	1.72	3.37
3	6.17	6.73	6.17	9.53	4.48	5.61	3.92	4.49
4	5.61	7.29	7.49	10.04	8.98	10.65	6.98	8.00
5	7.85	8.97	9.24	14.48	9.53	11.78	8.30	9.37
6	7.95	11.78	11.10	16.26			12.90	15.14
7			14.95	19.07			16.63	15.67

TABLE-4 : CO-RELATION CO-EFFICIENT AND REGRESSION ANALYSES AMONG DIFFERENT SALT-TREATED PAIR OF SAMPLES (Functional relationship between X and Y)

Relationship between X	Y	Correlation co-efficient (r)	Intercept (a)	Slope (b)
Ventral, 0°C, 1 cm	Ventral, 0°C, 3 cm	0.95	0.25	0.50
Dorsal, 0°C, 1 cm	Dorsal, 0°C, 3 cm	0.81	1.02	0.41
Dorsal, 8°C, 1 cm	Dorsal, 8°C, 3 cm	0.91	-2.15	0.72
Ventral, 8°C, 1 cm	Ventral, 8°C, 3 cm	0.70	1.54	0.39
0°C, 1 cm	8°C, 1 cm	1.00	-0.92	1.18
0°C, 3 cm	8°C, 3 cm	0.97	0.47	1.07
Ventral, 0°C, 1 cm	Dorsal, 0°C, 1 cm	0.94	10.93	0.01
Ventral, 0°C, 3 cm	Dorsal, 0°C, 3 cm	0.98	0.81	0.88
Ventral, 3 cm, 8°C	Dorsal, 3 cm, 8°C	0.91	0.94	0.87

The relationship may be expressed as $Y = a + bx$, where X and Y are the pair of samples.

The values of X and Y (Table-1)

of storage and then showed gradual decrease, whereas in storage sample at 0°C, the increase of acid value was less as compared to the 8°C storage samples over the storage period. However, the pattern of changes in acid values obtained in the different salt-treated samples and without salt studied here suggests that those stored at 8°C temperature showed rapid increase of acid values whereas, in the similar samples stored at 0°C temperature, the development of acid values was much slower. The salt concentration, storage temperature and length of storage days had little effect on the development of acid values. The impurities in the salt for use in salt-curing appear to accelerate to oxidative deterioration of oils in fish flesh. In herrings stored at 2-4°C without salt, the acid value of oil in flesh after 2 days of storage, doubles the value in the original fish, after 4 days about 3 times and after 6 days about 4 times (Charnley, 1945). Therefore, the fish are subjected to refrigeration, the duration of storage during which the fish remain in a favourable state. Luijpen (1954 b) reported that the acid value of salted herring, the fat contents showed no direct relation to the storage period or temperature. Ona, (1935) showed that fatty acids increased during storage, in sardines and mackerel at low temperatures. Dyer and Fransier, (1959) investigated the lipid hydrolysis and its relation to protein changes in cod during storage at low temperature. Rockwood et al., (1947) studied the hydrolytic changes

TABLE-3 : FREE FATTY ACID (FFA) OF DIFFERENT SALT TREATED HILSA FISH SAMPLES (HILSA ILISHA) DURING STORAGE AT 0°C AND 8°C TEMPERATURE

Storage period (days)	BATCH-1		BATCH-2		BATCH-3		BATCH-4	
	Storage Temp.		Storage Temp.		Storage Temp.		Storage Temp.	
	0°C	8°C	0°C	8°C	0°C	8°C	0°C	8°C
1	1.92	1.4	1.97	2.81	1.12	1.28	0.84	1.68
2	1.68	2.25	2.0	2.81	1.41	1.97	0.86	1.69
3	3.09	3.37	3.09	4.77	2.24	2.81	1.96	2.25
4	2.81	3.65	3.75	5.02	4.49	5.33	3.49	4.0
5	3.93	4.49	4.62	7.24	4.17	5.89	4.15	4.69
6	3.98	5.89	5.55	8.13			6.45	7.57
7	-	-	7.48	9.54			8.32	7.84

leading to free fatty acids. Fish stored at 0°C and 8°C temperature with and without protection of salt showed the increase in free fatty acid (Table-3). Fish samples storage at 8°C temperature after salting resulted in the increase in FFA. The increase in FFA began after the fish were placed in storage. The FFA continued to increase at the end of storage period. The increase in FFA did not begin immediately. Fish stored at 0°C the rate of lipid hydrolysis was much slower upto the end of storage. Salted fish samples stored in 8°C showed a gradual increase in the rate of lipid hydrolysis (Table-3). Overall, the free fatty acid values increased from 0.84% to 9.54% (expressed as % of oleic acid of the extracted fat) in the different salt-treated samples at two different storage temperature. Dyer and Fransier (1959) reported that the lipid hydrolysis as measured by free fatty acid formation. Free fatty acid formation appears to be related to actomysin stability (Dyer, 1957).

From the present experiment it can be concluded that at similar storage temperature and salt concentration, salt penetration depends on thickness of fish. Salt uptake is faster at higher temperature. Rate of salt penetration is rapid initially and it is influenced by the content of fish. Fat acts as barrier to the entry of salt. Increase in salt content depends on thickness, anatonical portions and temperature.

pH value changes depend on thickness of muscle and length of storage.

Peroxide values, acid and free fatty acids depends on temperature. Fluctuations are more at higher temperature.