Preservation of Indian Oil Sardine (*Sardinella longiceps*) in Ice and Chilled Seawater. Part II - Changes During Storage with Particular Reference to Salt Penetration and Lipid Deterioration During C S W Holding*

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Oil sardines in prime condition were chilled onboard. Two lots were chilled in CSW (samples C & CI), one lot ice (sample I) and a fourth lot was left un-iced on deck (sample AI). Sample AI was iced after landing and sample CI was taken out of the chilled seawater and iced. All the four samples were kept in a chilled room for storage studies. Sample C, chilled and stored in CSW, recorded a gradual gain in weight and an increase in salt content of the muscle. Presence of salt did not seem to cause any excessive protein denaturation. Salt extractability decreased at a gradual rate in all cases. Presence of salt seemed to wield no noticeable influence on lipid hydrolysis and subsequent peroxidation. Results of chemical and sensory evaluations highlight this. Holding sardines in CSW gave a product of excellent quality for the first four to five days of storage. Beyond the fifth day of storage quality deteriorated rapidly and there was no noticeable superiority for this sample (sample C) over the onboard iced fish. This was evident in the sensory evaluation as well. However, a storage life of five days in a readily acceptable state is sufficient for the fish to be disposed in the market at a premium sale price over other landings of the same species.

The concentration of salt in the holding medium, its penetration into fish flesh, the gain in weight to fish, problems of protein denaturation, rancidity development and sensory acceptability of fish during its storage in refrigerated seawater (RSW), has been extensively studied. The contributions of MacLeod *et. al.* 1960; Roaeh *et. al.* 1961; Meyboom & Van Pel, 1965; Peters *et al.*, 1965; Perigreen *et al.*, 1975; Hiltz *et al.*, 1976 and Boyd *et al.*, 1978 are noteworthy in this context. However, no effort has gone into studying these aspects of preservation during storage of fish in CSW, where dilution would contribute a great deal towards off-setting the adverse effects of seawater holding. The present paper discusses certain changes occuring during the storage of Indian oil sardine (*Sardinella longiceps*) in chilled seawater (CSW) with particular reference to weight changes, salt penetration into the meat and rancidity development.

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Material and Methods

Live oil sardines procured from a purse-seiner was chilled onboard. The CSW medium, prepared with one part seawater (3–4% salinity) and 2.5 parts crushed ice was used for chilling two lots of fish (samples C and CI). However, samples CI was removed from the medium and iced after landing. Sample C was held in the CSW medium without effecting any change to it. A third lot was iced onboard and held as such (sample I). A fourth lot held unpreserved onboard was iced after landing (sample AI). Insulated, galvanised fish boxes were used for storing the fish samples. A 1:1 ratio was maintained for either fish to CSW or fish to ice, as the case may be. All the four samples were stored in a chilled storage at 1 ± 1°C.

One hundred fish were randomly picked and the average length and weight determined. A recording of the temperatures for all the four samples C, CI, I and AI was done from the time of chilling, at hourly intervals. Forty fish, picked randomly, were wrapped in a close-meshed net and left

immersed in the CSW medium. Every day this was taken out, drained for 10 min inside the chilled room and the weight determined. The pH of the meat blend (10 g mince in 50 ml distilled water) was directly recorded using a combined electrode pH meter (Horiba). NaCl in the meat was estimated by the method described in A O A C (1970). Salt extractability of the fish samples was detremined using the method of Dyer *et al.* (1950).

Lipids were extracted using chloroform and methanol (Bligh & Dyer, 1959). Peroxide values (PV)were colorimetrically determined using the modified method of Loftus Hill & Thiel (1943). The colour intensity was measured at 520–580 manometers. The method described by Olley & Lovern (1960) was adopted in estimating the free fatty acid (FFA) content of the extracted lipids. FFA is expressed in per cent total lipids as oleic acid. Representative samples from the four lots of sardines were dressed, cleaned, steamed and presented to a panel of 15 trained judges for estimating overall acceptability, flavour, texture and rancidity. The dissolved oxygen in the CSW medium was determined using a modified Winkler's method (alum flocculation) as described in APHA (1965). For dissolved sulphides the method described in APHA (1965) was used.

Results and Discussion

Cooling rates of the two different methods of chilling namely, icing and CSW, are presented in Fig. 1. CSW could reach a temperature level of 1.9°C in half the time taken by ice. Holding sardines in seawater resulted in the fish steadily gaining weight

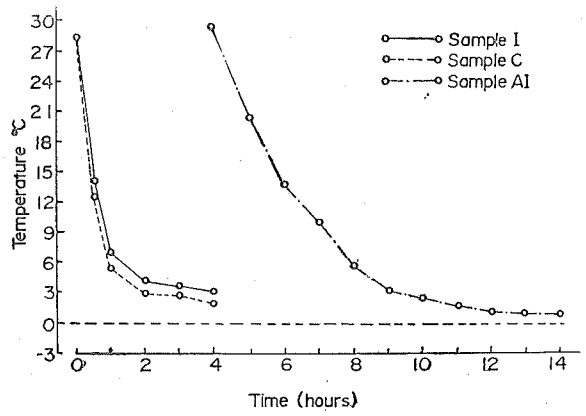

Fig. 1. Cooling rates of different methods of chilling viz. icing, CSW and delayed icing.

during storage (Table 1). While the fish showed a 1.10% weight gain during the first 24 h, the total gain for the entire storage period was 4.6%. The increase was rapid during early storage. The pH of the meat

Table 1. *Change in weight of sardine during storage in CSW*

Storage period (days)	Weight in g (40 nos.)	Weight increase %
0	1730	1.10
1	1759	1.10
2	1780	2.89
3	1790	3.47
4	1795	3.76
5	1800	4.05
6	1803	4.22
7	1805	4.34
8	1810	4.62
9	1810	4.62

recorded an increase for all the treatments. Initial values in the range of 5.8 to 6 gradually rose to 6.6 to 6.8 by the seventh day and then remained constant (Table 2). Change in the salt content of the fish muscle during the storage period is presented in Table 3 which shows an increase in sample C from 0.28 to 0.45% in 9 days. The increase was rapid during the first five days of storage. All the other treatments exhibited a marginal reduction in salt content during the storage period. The salt soluble nitroger as a percentage of total nitrogen showed a

Table 2. *pH of sardine muscle during chilled storage*

Storage period	Samples C	CI	I	AI
0	6.00	5.95	6.00	5.80
1	6.05	5.95	5.10	5.85
2	6.20	6.20	6.20	6.30
3	6.20	6.30	6.20	6.40
4	6.30	6.30	6.25	6.55
5	6.35	6.40	6.35	6.65
6	6.50	6.65	6.55	6.75
7	6.75	6.80	6.70	6.80
8	6.75	6.85	6.75	6.90
9	6.80	6.85	6.75	6.90

decrease in extractability for all the treatment (Fig. 2). Samples AI and CI exhibited a more pronounced actomyosin insolubilization. However, the values showed a

Table 3. *Organoleptic evaluation of sardine (cooked) during chilled storage*

Storage period (days)	Attributes of quality: Texture				Flavour				Overall acceptability				Rancidity			
	C	CI	I	AI	C	CI	I	AI	C	CI	I	AI	C	CI	I	AI
1	9.1	8.9	8.9	8.4	8.9	8.9	8.5	7.8	10	10	10	8.8	Ab	Ab	Ab	Ab
3	7.6	7.3	7.5	6.5	7.6	7.4	7.4	6.9	8.7	8.5	8.6	6.5	Ab	Ab	Ab	Sl
5	7.2	6.7	7.0	5.8	7.4	6.3	6.8	5.6	8.0	6.7	7.5	4.0	Ab	Sl	Ab	St
7	5.0	4.3	4.8	2.8	5.6	4.2	4.4	2.4	7.5	4.0	6.8	2.6	Sl	St	Sl	St
9	4.7	2.6	3.7	0	4.1	2.4	3.5	0	4.0	2.5	3.6	0	St	St	St	St

Scale

Excellent	10
Good	8
Fair	6
Borderline	4
Bad	2
Putrified	0

Ab = Absent
Sl = Slight
St = Strong

C = Chilled seawater preserved
CI = Chilled seawater – iced
I = Iced
AI = Ambient temperature – iced

gradual but steady decline for sample C, during the first five days of storage. The pattern of change of FFA content in all the

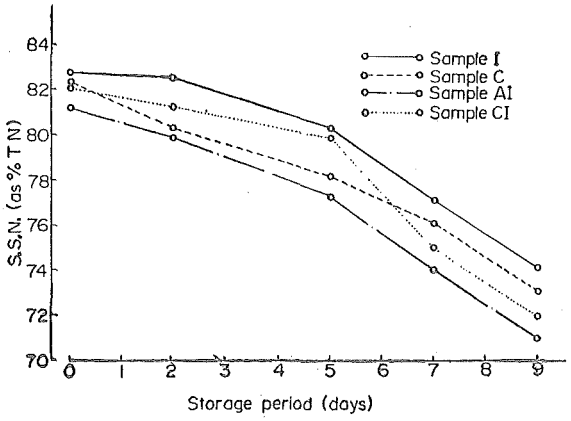

Fig. 2. Change in salt soluble nitrogen content in sardine muscle during chilled storage.

samples, during the course of the experiment, is given in Fig. 3. Free fatty acids exhibited an increasing trend. Sample C, held in CSW, showed the least initial day value and continued to remain least throughout the storage. Peroxide value in all the four samples showed a steady increase during storage. Sample C showed the least increase while AI exhibited the highest (Fig. 4).

The detection of rancidity and the overall acceptability scores of the samples are presented in Table 3. Sample AI was sensorily unacceptable after the fifth, CI after the seventh, and samples C and I by the ninth

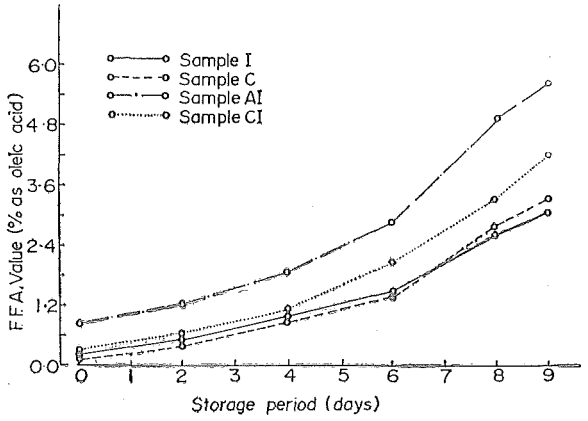

Fig. 3. Change in free fatty acid content in sardine muscle during chilled storage.

day, sample CI by the fifth day and samples C and I by the seventh day. Changes in the CSW medium during storage of sardine

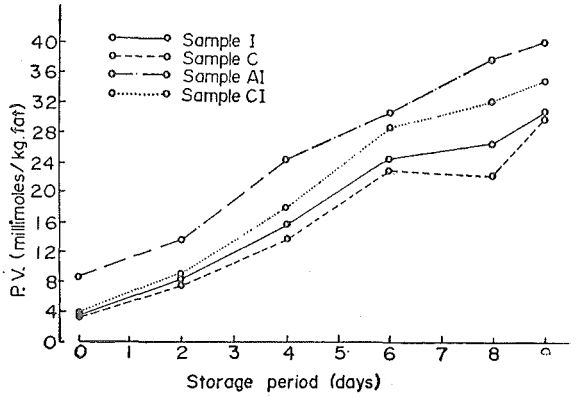

Fig. 4. Change in peroxide value in sardine muscle during chilled storage.

is presented in Table 4. Dissolved oxygen declined from a zero day value of 5.85 ml/litre to 0 ml/litre in four days. Sulphides, in traces for the first four days, increased steeply to 54.49 p.p.m by the ninth day. The bacterial load in the CSW followed an increasing trend.

A cooling efficiency which was roughly double that of icing was achieved by using CSW (Fig. 1). This efficiency could be enhanced several fold if a compressed-air-injection system is used in the CSW hold. Such a method improves circulation and melting rate of ice in the seawater. The temperature of herring preserved in a similar ice-air-seawater mixture, was lowered from

Table 4. *Changes in the chilled seawater during holding of sardine*

Storage period (days)	Dissolved oxygen (ml/l)	Dissolved sulphides (p.p.m.)	Log bacterial count (per ml)
0	5.85	Nil	3.48
1	2.85	0.85	3.79
2	1.40	0.98	—
3	0.40	5.25	—
4	Nil	18.76	3.91
5	Nil	23.62	—
6	Nil	29.52	—
7	Nil	34.08	4.50
8	Nil	46.01	—
9	Nil	54.49	5.64

– = no observation

13°C to 0°C in 60 min (Hansen *et al.*, 1980). The delay involved in icing sample AI resulted in the deterioration of initial quality, as evidenced by the high zero day values of FFA and PV. The CSW, as opposed to ice, besides having a better cooling rate could also lower the temperature of fish below 0°C. A temperature difference of 0.6°C was observed between samples C and I the former showing the lower value. At temperature as low as 0°C, a difference of 0.5 to 1°C can change the shelf life substantially (Spencer & Baines, 1964).

The weight gain observed for sardines in this study (Table 1) was relatively less when compared to results obtained elsewhere. (Meyboom & Van Pel, 1965). The period of storage, the strength of seawater, fat content of the fish as well as the product style-round, eviscerated or filleted-can have varying degrees of influence on the weight gain in fish. Round, fatty fish stored for a week or two in diluted seawater shows lower weight gain when compared to eviscerated or filleted, lean, ground fish stored for over two weeks in full strength seawater (Roach, 1965; Roach *et al.*, 1961).

There was no noticeable difference between the pH values of the samples for the same day (Table 2). The fact that pH values never exceeded neutrality even when spoilage was evident, may be owing to the accumulation of free fatty acids in the muscle as a result of lipid hydrolysis. The credibility of using variation in muscle pH as a reliable index of fish spoilage is questionable (Nair & Lahiry, 1960).

Clupeids are observed to have a salt content of 0.2 to 0.3 as (NaCl) in the muscle (Namboodiry, 1980). Upon storage in ice, fish muscle loses sodium and potassium ions, thereby exhibiting a loss in total salt content (Hiltz *et al.*, 1976). However, fish held in CSW tends to absorb salts into fish muscle during storage in seawater which depends on the strength of seawater, holding time, the species, its fat content as well as the product style-round, eviscerated or filleted (Macleod *et al.*, 1960). With a 3:1 ratio of fish to full strength seawater, the average uptake of salts is unlikely to exceed one fourth that of the seawater itself (Roach *et al.*, 1961). During the present study change in salt content (Table 3) was noticeably higher during the early days of storage as reported by earlier workers (Meyboom & Van Pel, 1965; Perigreen *et al.*, 1975). Increase in the salt content of fish muscle can have adverse effects on the storage life of the fish. However, the final salt concentration in the fish muscle of sample C did not exceed the threshold of 0.50% total salts, which was suggested as the maximum desirable saltiness sensorily (Boyd *et al.*, 1978)

Peroxide value (PV) exhibited an increasing trend during storage (Fig. 4). The values highlight the importance of immediate chilling. A very high zero day value for sample AI as compared to the low values

for the rest of the treatments clearly illustrate this.

There appears to be no distinct advantage for seawater chilling over onboard icing beyond the fifth day of storage. It is likely that the advantages resultant from seawater chilling were nullified by the problems caused by the presence of salt in the holding medium, as storage proceeds. Acceleration of rancidity could be caused by the presence of dissolved oxygen, ozone, salts or metals in the holding medium resulting in induced rancidity (Peters *et al.,* 1965). However, the induction of rancidity triggered by these factors, singly or in combination, is likely to be stifled as a result of a certain amount of dilution inherent in the CSW technique, as compared to the full strength seawater of RSW. Free fatty acid of unfrozen fish muscle is dependent upon the post-mortem holding time, species and the type of muscle (Anderson & Ravesi, 1968). The production of FFA followed a pattern similar to that of PV (Fig. 3). While the data establishes that onboard chilling is imperative in retarding lipid degeneration, it contributes little towards illustrating any distinct superiority for seawater preservation over onboard icing, beyond five days of storage. The rate of increase of FFA and PV is clearly reflected in the flavour scores and rancidity detection during sensory evaluation of the cooked samples.

An inverse relationship between PV and SSN and FFA and SSN, as observed during the present study has been established for frozen fish muscle (Srikar & Hiremath, 1972). Very little information is available on the change in protein extractability during chilled storage. A decreasing trend in protein extractability was observed over a five day period for sardines held in ice (Shenoy & Pillai, 1971). The SSN decrease, as observed during the present study correlates well with the increase in FFA and PV. The rate of decrease in SSN is also reflected in the texture scores of the cooked samples, (Fig. 2). Salt in the holding medium can lead to an accelerated denaturation of proteins. The presence of salt in the holding medium, as in the case of sample C, did not cause excessive denaturation, as evidenced by the gradual decrease in extractability for sample C. The value was comparable to the SSN values obtained for the other treatments. There was no noticeable advantage for any particular treatment, although immediate chilling did result in a lower rate of denaturation as compared to delayed icing.

Sensorily, onboard chilling imparted a distinct superiority to the fish, as compared to that chilled after a delay (Table 4). The role of immediate chilling, using either CSW or ice, in delaying the induction of rancidity seemed quite conclusive, judging by the results of sensory evaluation. Chilling by CSW or ice did not manifest any striking superiority of one method over the other. However, overall acceptability scores did show consistently higher values for CSW preserved sardines as compared to all other treatments, although the difference was only marginal. Chilling the fish in CSW onboard and icing it after landing did not appear to be advantageous since quality deteriorated quickly in this case.

During storage of freshly caught fish in CSW, the blood, slime and proteinaceous matter from the fish body tend to come out into the storage medium, causing a depletion of the dissolved oxygen due to bacterial action and consumption of oxygen for oxidative chemical reactions. Under anoxic conditions, sulphur containing proteinaceous matter liberate sulphides into the medium due to the action of hydrogen sulphide producing anaerobes.Herrings stored in CSW at 0°C showed oxygen depletion and subsequent sulphide production (Hansen *et al.,* 1974).

During the present study, a heavy load of organic matter and the anoxic condition of the medium could have been the causes for high sulphide production, despite a low storage temperature (Table 5). However, the use of iodimetric titrations to determine the spoilage products, particularly sulphides, during storage of fresh fish, is open to criticism since an uptake of iodine by proteins, mucous and lipids is possible without any relation to spoilage development (Farber, 1965). The bacterial load in the CSW followed a pattern comparable to the increase in bacterial load of the fish sample held in it (Krishnakumar *et al.,* 1985).

Although CSW mode of preservation could keep the sardines in prime condition for the first five days, there was no marked improvement in overall shelf life when compared to the onboard iced fish. Despite the presence of salt in the holding medium, the CSW held sardines did not show any excessive protein denaturation lipid hydrolysis or peroxidation. Dilution of the seawater in CSW can be linked to this observation. Generally fresh oil sardines are utilised retained, processed or reduced-within a day or two of capture. Thus a five day life in prime state is sufficient for disposal of the fish with premiums on the sale price.

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