Preservation of *Sardinella longiceps* in Iced and Chilled Seawater. Part I - Changes During Storage With Particular Reference to Bacterial Load and Nitrogenous Compounds*

S. KRISHNAKUMAR, G. G. HIREMATH, N. R. MENON** and H. P. C. SHETTY

University of Agricultural Sciences, College of Fisheries, Mangalore - 575 002

Oil sardines in prime condition were subjected to onboard chilling. Two lots were chilled in CSW (samples C and CI), a third lot was chilled in crushed ice (sample I) and a fourth lot left uniced on deck (Sample AI). Upon landing sample AI was iced and sample CI was removed from the CSW and iced. All the four samples were kept in a chilled room for storage studies. The fish chilled and stored in CSW recorded the least, and the fish subjected to delayed icing, the highest values for all the indices of spoilage namely, free amino nitrogen, trimethylamine (TMA) and total volatile base nitrogen (TVBN). The total psychrophilic bacterial number also showed a similar trend. The organoleptic assessment of the cooked samples revealed C I,CI, AI to be the order of preference throughout the storage. This assessment was found to hold good for the rest of the parameters as well. The CSW held fishes were found to be distinctly superior to the iced ones for the first five days of storage. Such a marked prevalence in quality for five days would suffice for the fish to fetch a premium in the market over other landings of the same fish whether chilled or unchilled. Chillingonboard in CSW and icing the same after landings, did not show encouraging results.

Unlike *refrigerated seawater* (RSW) there is limited literature available on chilled seawater (CSW) as a mode of preservation. The potency of CSW in chilling and preserving freshly caught fish was known as early as 1931. The view that a quicker and more effective temperature reduction, brought about through RSW/CSW chilling, retards bacterial action thereby enhancing the shelf-life has been extensively supported (Roach *et al.* 1961; Spencer & Baines, 1964 and Shewan, 1965). The effects of RSW/CSW holding on the keeping quality of temperate species (demersal/pelagic) have been studied in detail. However, data on tropical species is limited. Information on the pattern of spoilage of oil sardine, during CSW holding, is lacking and the present paper reports the attempt of the authors on ice and CSW preservation of oil sardines.

*Formed part of the M.F.Sc. Thesis (University of Agricultural Sciences, Bangalore) of the first author.

**Present address: School of Marine Sciences, University of Cochin, Cochin-682 016

Materials and Methods

Live oil sardines were procured from the net of a purse seiner operating off Mangalore. The CSW medium was prepared by mixing one part of seawater (of 3.4‰ salinity) with 2.5 parts of crushed ice. Insulated, galvanised MPEDA fish boxes were used for storing the samples. The raw material was divided into four lots of 35 kg each. Two lots were chilled in CSW in the fish boxes, the ratio of fish to CSW being 1:1 by weight. A third lot was preserved in ice, in a fish box. To simulate purse seine fish handling practices, a fourth lot was heaped on deck, exposed to sun and wind, transported in bamboo baskets to the laboratory, iced and stored in a fish box (4 h after capture). All the four samples were plced inside a chilled storage (1 ± 1 °C) for further storage studies. The samples were subjected to treatment as follows:

1. Sample C, chilled in CSW onboard, was further stored as such.

2. Sample CI, also chilled in CSW onboard, was removed from the medium and iced.

3. Sample I, iced onboard, was drained of meltwater and replenished with fresh ice.

4. Sample AI, held uniced onboard, was iced.

Icing was done in alternate layers of fish and ice, in the ratio 1:1 by weight. Daily melting of ice was compensated for with fresh ice.

A daily assessment of the rate of belly bursting was made. One hundred fish were randomly picked from each sample and the percent belly burst determined each day. Moisture, protein nitrogen, non-protein nitrogen, crude fat and ash content in the fish samples were determined by the methods described by AOAC (1970). Free amino nitrogen was determined by the method of Pope & Stevens (1939). The colorimetric method of AOAC (1975) was followed for trimethylamine (TMA) in the muscle. Total volatile base nitrogen (TVBN) was determined by Conways' micro-diffusion method as described by Beatty & Gibbons (1937).

Ten grams of skin and flesh were homogenised with 90 ml saline. Serial dilutions were made and plated in duplicate on nutrient agar (fortified with 3% sodium chloride) by spread plate. The psychrophilic bacterial count was determined after incubation at 20°C.

Representative samples from all the treatments were dressed, cleaned and steamed for 10 min and then presented to a panel of fifteen trained judges. Attributes of quality such as appearance, flavour, odour and texture were judged on the basis of a ten point hedonic scale. The judgement of the panel was valued and average scores taken. Data of the organoleptic assessment was subjected to statistical analysis.

Results

The average values of moisutre, protein, fat and ash of the fish samples are presented in Table 1. A gradual decrease in total nitrogen was observed for all the samples over the storage period. While sample I

Table 1. *Proximate composition of sardine muscles* (as per cent of meat)

Moisture	Protein	Fat	Ash
68.36	19.00	10.30	1.94

exhibited the minimum decrease, sample AI recorded the maximum (Table 2). The non-protein nitrogen as a percentage of total nitrogen exhibited an increasing trend. There was no noticeable difference between treatments. Free amino nitrogen values also recorded an increasing trend. Sample C recorded the least increase (Fig. 1). The total volatile base nitrogen found to increase

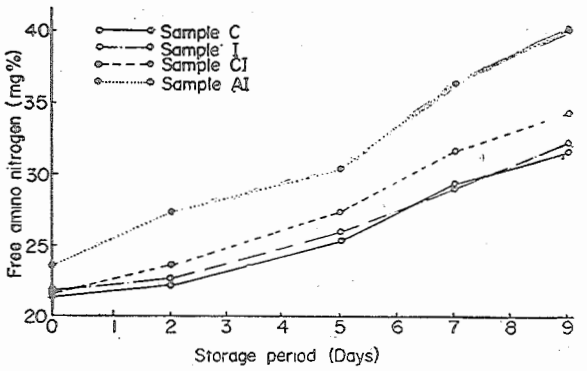

Fig. 1. Changes in free amino nitrogen content of sardine muscle during chilled storage.

steadily. Sample C showing the least increase and sample AI the highest (Fig.2). It is of interest to note here that sample CI recorded a higher rate of increase of TVBN towards the latter half of the storage

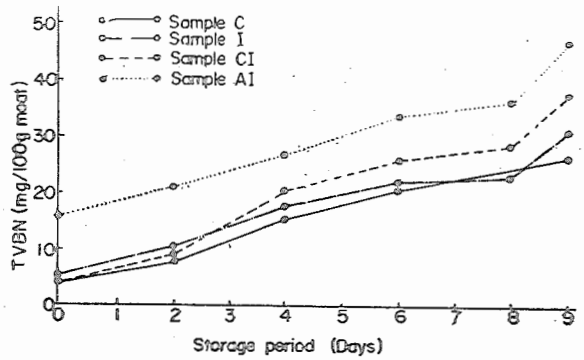

Fig. 2. Changes in volatile base nitrogen content in sardine muscle during chilled storage.

Table 2. *Change in nitrogenous constituents of sardine muscle during chilled storage in ice and CSW*

Total nitrogen (g/100 g meat) C	CI	I	AI	Storage period (days)	NPN (as per cent TN) C	CI	I	AI
3.38	3.38	3.39	3.35	0	9.88	9.88	9.99	10.12
3.35	3.32	3.35	3.29	2	9.85	9.98	9.90	10.18
3.30	3.28	3.29	3.20	5	10.12	10.18	10.08	10.33
3.19	3.15	3.20	3.09	7	10.33	10.43	10.33	10.78
3.09	3.02	3.12	2.98	9	10.58	10.74	10.69	11.09

period. Similar trends were noticed in trimethylamine (Fig.3) as well as total plate

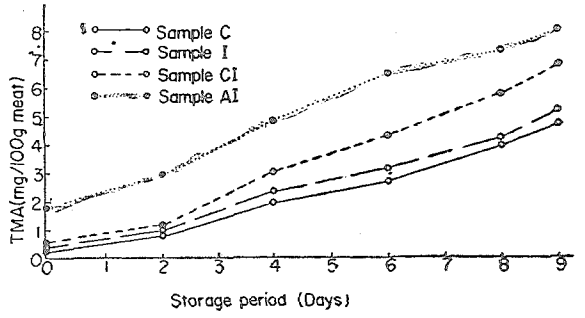

Fig. 3. Changes in trimethylamine content in sardine muscle during chilled storage.

count of psychrophilic bacteria (Fig. 4). The TMA values did not exceed 10% mg in any of the samples. Bacterial count exhibited an approximate three-fold increase.

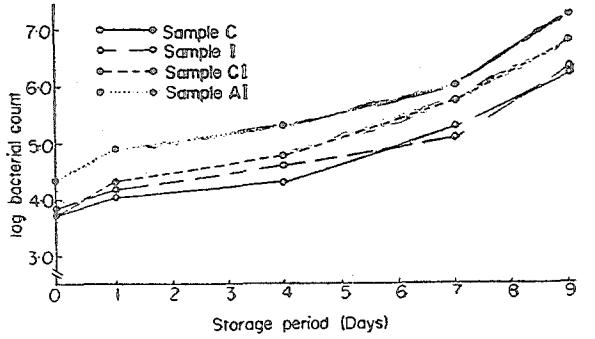

Fig. 4. Changes in bacterial load during chilled storage of oil sardine.

Sardine held in CSW showed a relatively very low belly burst percentage upto the fifth day (11%), while samples, CI, I and AI recorded values over 20% (Table 3). By the ninth day while sample AI had 81% fish with torn bellies, the value was as low as 55% for the CSW held sardine (sample C).

Table 3. *Percentage belly burst during chilled-storage of sardine in ice and CSW*

Storage period, days	Samples C	CI	I	AI
0	0	0	0	4
1	0	0	0	8
2	2	10	4	16
3	3	15	8	20
4	5	19	15	26
5	11	24	20	34
6	24	39	34	52
7	34	40	42	63
8	46	60	51	70
9	55	70	60	81

The appearance, odour, flavour and texture of sample AI became unacceptable after the fifth day, and those of samples CI and I after the seventh day. Sample C remained acceptable in terms of texture

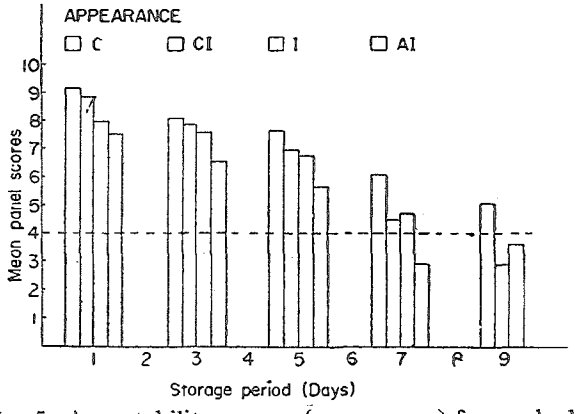

Fig. 5. Acceptability scores (appearance) for cooked oil sardine during chilled storage. (Excellent ≤ 10; Good ≤ 8; Fair ≤ 6; Borderline $= 4$; Bad ≥ 2; Putrified ≥ 0

Table 5. *Analysis of variance for mean panel scores for samples in various treatments*

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-ratio
Between attributes	3	0.327	0.1090	3.2812*
Between samples	3	54.973	18.3243	551.6045*
Between days	4	364.190	91.0475	2740.7435*
Interaction between attributes and samples	9	1.324	0.1471	4.4281*
Interaction between samples and days	12	19.787	1.6489	49.6358*
Interaction between Attributes & days	12	1.083	0.0903	2.7182*
Error	36	1.196	0.0332	
Total	79	442.880	5.6061	

*Significant at 5% level

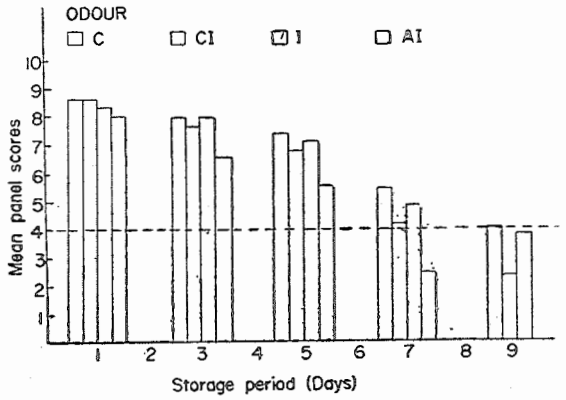

Fig. 6. Acceptability scores (odour) for cocked oil sardine during chilled storage (Excellent $\leq$ 10; Good $\leq$ 8; Fair $\leq$ 6; Borderline = 4; Bad $\geq$ 2; Putrified $\geq$ O)

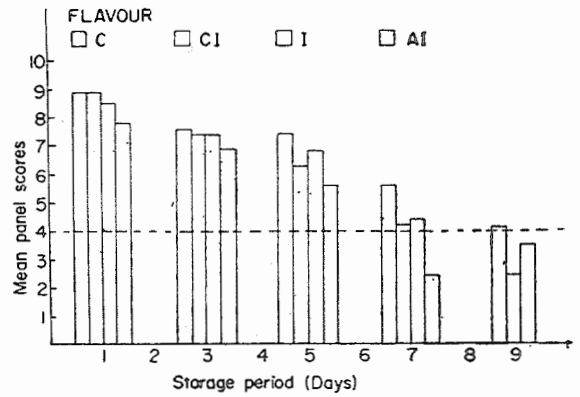

Fig. 7. Acceptability scores (flavour) for cooked oil sardine during chilled storage. (Excellent $\leq$ 10; Good $\leq$ 8; Fair $\leq$ 6; Borderline = 4; Bad $\geq$ 2 Putrified $\geq$ O)

and appearance for the ninth day also. However, the average odour and flavour scores of sample C had declined to the border line of acceptability by the ninth day (Figs. 5, 6, 7 & 8). Analysis of variance of the mean panel scores (Table 4) showed significant differences due to samples, attributes, and days and also between their interaction. The highest significance was

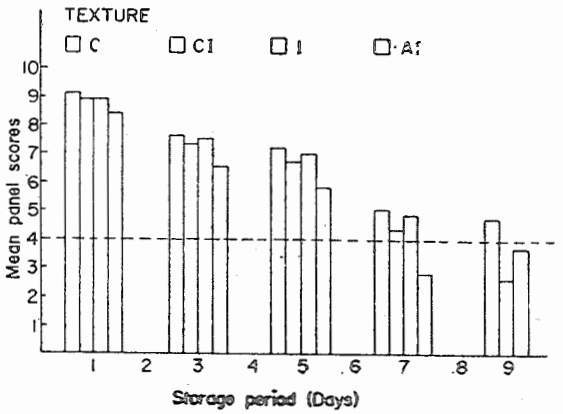

Fig. 8. Acceptability scores (texture) for cooked oil sardine during chilled storage (Excellent $\leq$ 10; Good $\leq$ 8; Fiar $\leq$ 6; Borderline = 4; Bad $\geq$ 2; Putrified $\geq$ O)

due to days followed by samples and attributes. The highest interaction was noticed between samples and days followed by attributes and samples.

Discussion

The marginal decrease in total nitrogen and the increase in non-protein nitrogen

(Table 2) after an initial marginal recuction can be attributed to proteolysis and bacterial action. A similar trend in total nitrogen was observed for sardines held in ice (Shenoy & Pillai, 1971). The free amino nitrogen did not show an initial decline as reported earlier (Govindan, 1971). However, the increase in free amino nitrogen for the first five days was marginal. Beyond the fifth day the rate of increase was noticeably high, though not pronounced (Fig. 1). Leaching losses could have played a significant role in masking the actual rate of production of free amino acids. Increase in free amino acid content towards the later stages of storage can be linked with bacterial proteolytic action (Shewan, 1961).

Volatile bases increased rapidly in samples AI and CI. This increase in TVBN for sample AI can be attributed to its exposure to a high ambient temperature before it was iced. This is further supported by a very high zero day value for samples AI as compared to the other three samples (Fig. 2). Holding temperature had a direct impact on the TVBN production in fish (Hansen *et al.* 1974). The levels of volatile bases recorded for sample C throughout the study establishes the superiority of CSW as mode of preservation. Results obtained by Hiremath *et al.* (1980) do corroborate this. A similar pattern was established for the trimethylamine values of the four samples. While TMA increased rapidly in samples CI and AI, the rate of increase in samples I and C remained relatively low, sample C recording the least increase (Fig. 3). Although spoilage was sensorily evident by the 5th, 7th and 9th days for samples AI, CI and I respectively, the TMA values for no sample exceeded 10 mg%.

Increase in the TMA values can have a direct bearing on the viable count of bacteria (Tarr, 1938). An increase in the viable count of the bacteria during the present study correlates well with the increase in TMA. Holding sardines in CSW and ice for nine days resulted in an approximate three fold increase in their bacterial load (Fig. 4). Similar results were obtained during holding of sardines in RSW for six days (Perigreen *et al.,* 1975). During the present study sardines subjected to delayed icing showed much higher bacterial load. Upto the fourth day bacterial count for CSW held sardines was significantly lower than that of iced ones. This can be attributed to a faster rate of chilling and a lower temperature of storage obtained in CSW (Spencer & Baines, 1964) and also due to the anaerobic environment in CSW, inhibiting the growth of obligate aerobes (Shewan, 1965). However, beyond the fifth day the bacterial load of the CSW held sardine parallels that of the iced one (sample I). This may be due to the microflora released from the gut of the CSW held sardines, as noticed by a peak in the belly burst rate after five days. Relatively higher bacterial load for samples CI and AI may also be attributed to higher belly damage rates observed during the same period.

Holding fish in CSW or RSW has the distinct advantages of preventing undue pressures on the fish, thereby eliminating any physical damage (Eddie & Hopper, 1974). RSW was found to be a better medium in reducing physical damage as compared to ice (Perigreen *et al.,* 1975). However, very low belly burst rates observed during the present study (Table 3) as compared to values obtained elsewhere, may be owing to the fact that chilling of the catch was done within 15 min of capture. Thus there is an emphasis on the importance of immediate and rapid onboard chilling.

Analysis of variance for the mean panel scores of the cooked samples (Table 4) showed significant differences for samples, days, attributes and their interactions. The results of organoleptic analysis of cooked samples (Figs. 5, 6, 7 and 8) reveal that immediate chilling by CSW definitely improve the shelf-life of the fish by atleast 4 to 5 days. The CSW held sardine (sample C) showed a shelf-life of 9 days as compared to 7 days for sample I. Sample C was found to be noticeably superior in appearance and texture based on sensory evaluation

Compared to sample AI, sample CI exhibited lower values of total psychrophilic bacterial count, TVBN and TMA during the first 6 days of the experimental period.

However, towards the latter half of the experiment, the rate of increase of the three parameters in sample CI was significantly higher as compared to the other 3 samples. The fact that sample CI was handled excessively due to the re-icing process could have contributed towards a higher contamination of the fish with the putrefactive types of bacteria.

Onboard chilling of fatty species, like sardine in CSW enhances the shelf-life of the fish by 2 to 4 days. The quality of CSW held fish during the first 5 days of storage, was distinctly superior to the iced samples, including the onboard iced sample. Although there were no distinct advantages beyond the fifth day, for CSW over ice, it is of little significance to fresh fish preservation since the fish is consumed - processed, retailed or reduced - within a day or two of capture. Hence it may be of interest to note here that a five day life in prime condition provides ample time to transport the fish to even distant markets for retailing, thereby creating the possibility of wider markets and premiums on the sale price.

References

AOAC (1970) *Official Methods of Analysis,* 11th edn. Association of Official Agricultural Chemists, Washington, D.C.

AOAC (1975) *Official Methods of Analysis,* 12th edn. Association of Official Agricultural Chemists, Washington, D.C.

Beatty, S.A. & Gibbons, N.E. (1937) *J. Biol. Bd Canada.* **3,** 77

Eddie, G.C. & Hopper, A.G. (1974) *Fishery Products,* (Kreuzer, R. Ed.), p. 69, Fishing News (Books) Ltd., England

Govindan, T.K. (1971) *Indian Fd Packer* **25,**27

Hansen, P., Olson, K.B. & Peterson, T.E. (1974) *Fishery Products,* (Kreuzer, R. Ed.) p. 64, Fishing News (Books) Ltd., England

Hiremath, G.G., Teutscher, P. & Nordheium, A. (1980) *FAO Technical Report, FAO-DANIDA Workshop on Handling of Small Fish in the Arabian Sea,* Mangalore, India

Perigreen, P.A., Pillai, A.S., Surendran, P.K. & Govindan, T.K. (1975) *Fish. Technol.* **12,** 105

Pope, C.G. & Stevens, M.F. (1939) *Biochem. J.* **33,** 1070

Roach, S.W., Harrison, J.S.M. & Tarr, H.L.A. (1961) *Bull. Fish. Res. Bd. Canada,* **126**

Shenoy, A.V. & Pillai, V.K. (1971) *Fish. Technol.* **8,** 37

Shewan, J.M. (1961) *Fish as Food,* Vol. 1. (Borgstrom, G Ed.), p. 484, Academic Press New York

Shewan, J.M. (1965) *Fish Handling and Preservation,* p. 95 O.E.C.D., Paris

Spencer, R. & Baines, C.R. (1964) *Fd. Technol.* **18,** 175

Tarr, H.L.A. (1938) *Nature.* **142,** 1078