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Spoilage Patterns and Organoleptic Acceptability of *Sardinella longiceps* Stored at Four Different Temperatures

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Abstract

Spoilage patterns of *Sardinella longiceps* stored at 26°C, 6°C, 0°C and -17°C were examined by monitoring some biochemical and microbiological parameters as well as by conducting organoleptic assessment. The shelf life of the fish after landing under poor preservation conditions was found to be 7 hours, 3.5 days, 7 days and over 50 days, respectively, for above storage temperatures as judged by a taste panel comprised of 5 individuals.

Statistically significant linear correlations were found between taste panel score and storage time for 26°C ($r=-0.956$, $p< 0.001$), 6°C ($r=-0.835$, $p<0.01$) and -17°C ($r=-0.726$, $p<0.05$) temperatures. In general, the taste panel score did not show a simple, temperature independent relationship with any of the biochemical and microbiological parameters (i.e. trimethylamine, total volatile nitrogen, pH and total bacterial count) studied. Therefore, above parameters may not be taken individually as indicators for testing the quality of *S. longiceps*.

Introduction

Sardinella longiceps, commonly known as Indian oil sardine, occurs mostly in the central indo-pacific region. This bony fish which belongs to the family Clupeidae is a popular food fish specially among low income generating families of the South East Asian countries including Sri Lanka.

The annual production of marine fish in Sri Lanka is about 170,000 metric tons of which about 45% comes from various sardine species including Indian oil sardine (Anon. 1986). Generally, one day fishing trips are made to catch sardines and the day's harvest is brought ashore within 12 hours of catching, usually without chilling or under poor chilling conditions. It is also observed that adequate measures are not taken to control spoilage of fish at landing sites, during transport to the markets and while kept in retail markets for sale. This will lead to deterioration of the quality of fish which inevitably causes a reduction in the shelf life and a decline in consumer acceptability.

Effect of some preservation methods on storage of sardine have been studied in literature. *S. longiceps* stored at ambient temperatures of 25-28°C on deck and iced after landing produced more trimethylamine and other volatile bases than that stored in chilled seawater on deck and iced at landing (Krishnakumar et al. 1985). The fish subjected to the former treatment were organoleptically acceptable only for five days whereas the latter method increased the acceptable period up to seven days (Krishnakumar 1986). Furthermore, it has been shown that delayed icing can cause considerable quality deterioration and a reduction of shelf life during subsequent frozen storage (Dora & Hiremath 1987).

Most of the storage studies reported in literature on sardine have been concentrated on two preservation methods; icing and storage in chilled/refrigerated sea water. The present study

examines spoilage patterns of *S. longiceps* at four different temperatures that are commonly utilized in day to day life. The four temperatures selected were 26°C, 6°C, 0°C and -17°C which simulated storage at ambient temperature, in the chilling compartment of domestic refrigerator, in ice and in the deep freezer, respectively. Variations in some biochemical and microbiological parameters as well as in the organoleptic acceptability during storage of *S. longiceps* were measured in order to determine the shelf life of the fish at each temperature. The biochemical and microbiological parameters monitored were pH, total volatile nitrogen (TVN), trimethylamine (TMA) and total bacterial count (TBC). It was also investigated whether the above biochemical/microbiological parameters could substitute for skill dependent sensory evaluation in testing fish quality.

Materials and Method

S. longiceps was collected from the Negombo fish landing site immediately after landing and transported to the laboratory at the National Aquatic Resources Research and Development Agency in ice except for storage trials at ambient temperature for which fish were brought un-iced.

Storage studies at 26°C were conducted by keeping a portion of fish at ambient temperature. For experiments conducted at 0°C, another portion of fish was stored in an insulated box having a hole in the bottom to facilitate drainage of melting ice. Alternative layers of fish and ice were packed in 1:1 ratio, and, whenever necessary, melted ice was replenished with fresh ice. Two more lots were stored, respectively, in the chilling compartment (6°C) of a domestic type refrigerator and in a deep freezer (-17°C).

Samples were drawn from each of the four lots periodically for biochemical, microbiological and organoleptic analysis. Each sample consisted of 25 g of fish muscle collected from 15-18 randomly selected sardines. The sample was homogenized in a blender with 75 ml distilled water and made up to 100 ml in a volumetric flask. Appropriate portions of the preparation were used for biochemical and microbiological analysis. TMA, TVN, TBC and pH were determined by standard methods (Horwitz 1980; Speck 1984).

For organoleptic analysis, degutted, washed sardines were sealed in polythene bags and heated at 90°C for 10 minutes after immersing in a water bath. The flavour of the cooked sardine was tested by a taste panel comprised of five individuals trained in sensory evaluation of fish and fish products and a score was given by each panel member according to the score sheet shown in Table 1. The average taste panel score was taken as a measure of organoleptic acceptability of the fish.

Table 1. Score sheet used by the taste panel for organoleptic assessment of *S. longiceps*

Flavour of cooked sardine	Score
Fresh or sweet	10
Neutral taste	8
Off flavour	6
Old taste	4
Rancid	2
Stale or putrid	0

Results and Discussion

Total volatile nitrogen (TVN) and Trimethylamine (TMA)

Production of TVN and TMA in fish muscle at different storage temperatures is shown in Fig. 1. A rapid increase in TVN and a moderate rise in TMA with storage time were observed at both 26°C and 6°C storage temperatures. At 26°C, TVN content showed a three

fold increase within 24 hours of storage reaching a value of 67 mg N/100 g whereas TMA indicated about 15 fold increase during the same storage period approaching a value of 15 mg N/100g. Comparatively, at 6°C, it took 7-8 days to produce similar levels of TVN and TMA in sardine. The rates of formation of TVN and TMA were 6.7 and 5.4 times higher at 26°C than at 6°C which show the effect of temperature on biochemical reactions assisted by microbiological processes. However, at both temperatures, the rate of increase of TVN is much greater than that of TMA.

TVN and TMA contents of sardine stored in ice (0°C) showed a random fluctuation. This may be due to repeated bacterial contamination of sardine by fresh ice which was used to replenish melted ice. The ice was found to contain a bacterial load of about 10^4/g. Replenishment with ice may have introduced different loads of bacteria at different times causing changes in biochemical/microbiological processes already undergoing in the fish. However, in general, TVN content of sardine varied between 10-27 mg N/100 g while TMA level fluctuated within 0.2-7 mg N/100 g during 17 days of storage at 0°C. Krishnakumar et al. (1985) also reported that TMA content of *S. longiceps* stored in ice remained within 10 mg N/100g during a nine day storage period. Both TVN and TMA contents of fish stored at -17°C temperature were more or less similar to those obtained prior to storage (i.e., 21 and 3 mg N/100 g respectively) for a period up to 50 days at which point the study was terminated. It appears that the spoilage process of sardine can almost be completely suppressed for at least 50 days by storing at -17°C.

Connell (1975) suggested that 10-15 mg N TMA/100g and 30-35 mg N TVN /100g of fish be regarded as limits of acceptability for human consumption. According to the present results, sardine stored at 26°C exceeded above levels at 9-24 hours and 5-6 hours respectively, while at 6°C, same levels of TMA and TVN were observed within 5-6 days and 3-3.5 days, respectively. At both temperatures, TVN content in fish muscle exceeded the suggested acceptability limit at a shorter duration than TMA surpassed its limit. However, the fish stored in ice and at -17°C were well within the acceptable limits of nitrogen bases throughout the trial period (i.e. 17 days and 50 days, respectively). Significant differences between TVN and TMA contents which extended from 2 to 8 fold were noticed at all four storage temperatures. This suggests that, in addition to TMA, which is known to be produced due to reduction of trimethylamine oxide by bacteria, some other unidentified volatile bases are formed during spoilage of sardine. In general, difference between TVN and TMA is attributed to formation of ammonia. The higher rate of TVN formation compared to that of TMA at 26°C and 6°C suggests that the development of these unidentified bases in sardine muscle occurred at a faster rate than TMA as storage period continued.

pH

Sardinella longiceps stored at 26°C, 0°C and -17°C exhibited a rather similar pattern of change in pH during storage (Fig. 2). In all these cases, the initial pH values of fish muscle which were in the range of 6.2 to 6.6 decreased slightly within the first 1-2 days of storage and then increased rapidly during early stage of spoilage, reaching corresponding maxima at 7.4, 7.1 and 7.0 after storage periods of 13 h, 2 days and 7 days, respectively. Thereafter, the pH decreased at different rates as spoilage continued. The pH of iced fish declined rapidly to 6.3-6.5 within 6 days and appeared to have stabilized in the same pH region for the rest of the storage period. The slowest rate of decrease was observed in fish kept in the deep freezer, even though the pH continuously declined during 50 day observation period. The pH value of the sardine stored at 6°C showed a continuous upward trend after the initial decrease within the first day of storage. The

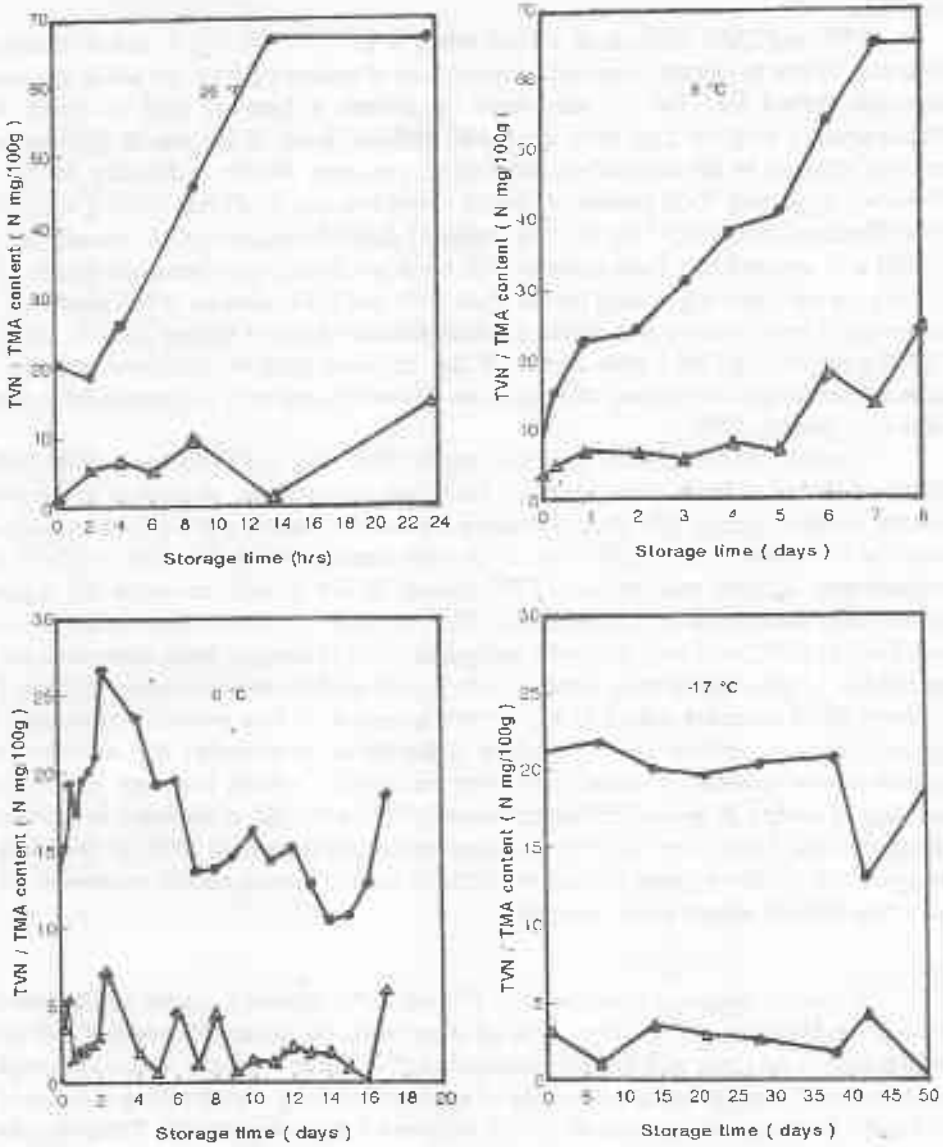

Fig. 1. Variation of total volatile nitrogen (TVN; black circles) and trimethylamine (TMA; triangles) in *Sardinells longiceps* fish muscle with storage time.

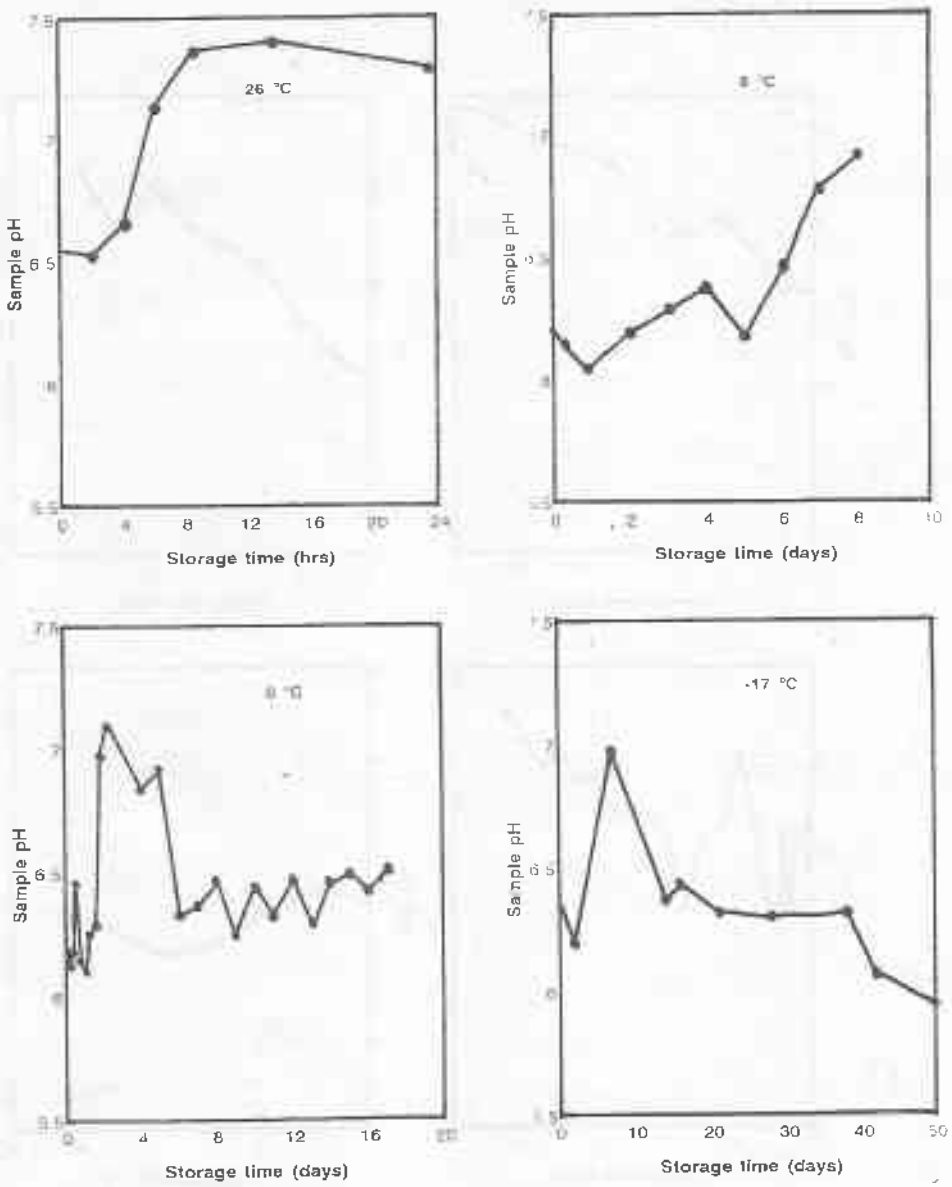

Fig. 2. Variation of pH in *Sardinells longiceps* fish muscle with storage time.

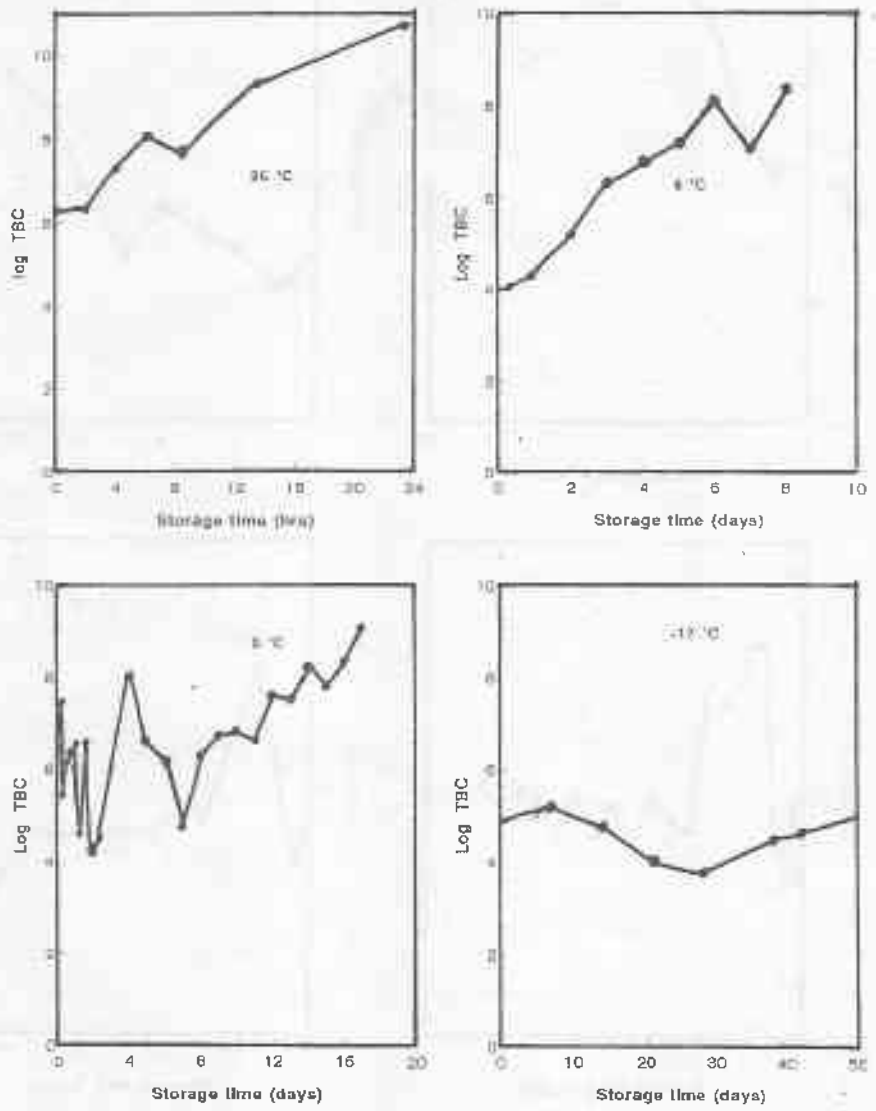

Fig. 3. Variation of total bacterial count (TBC) in *Sardinella longiceps* with storage time

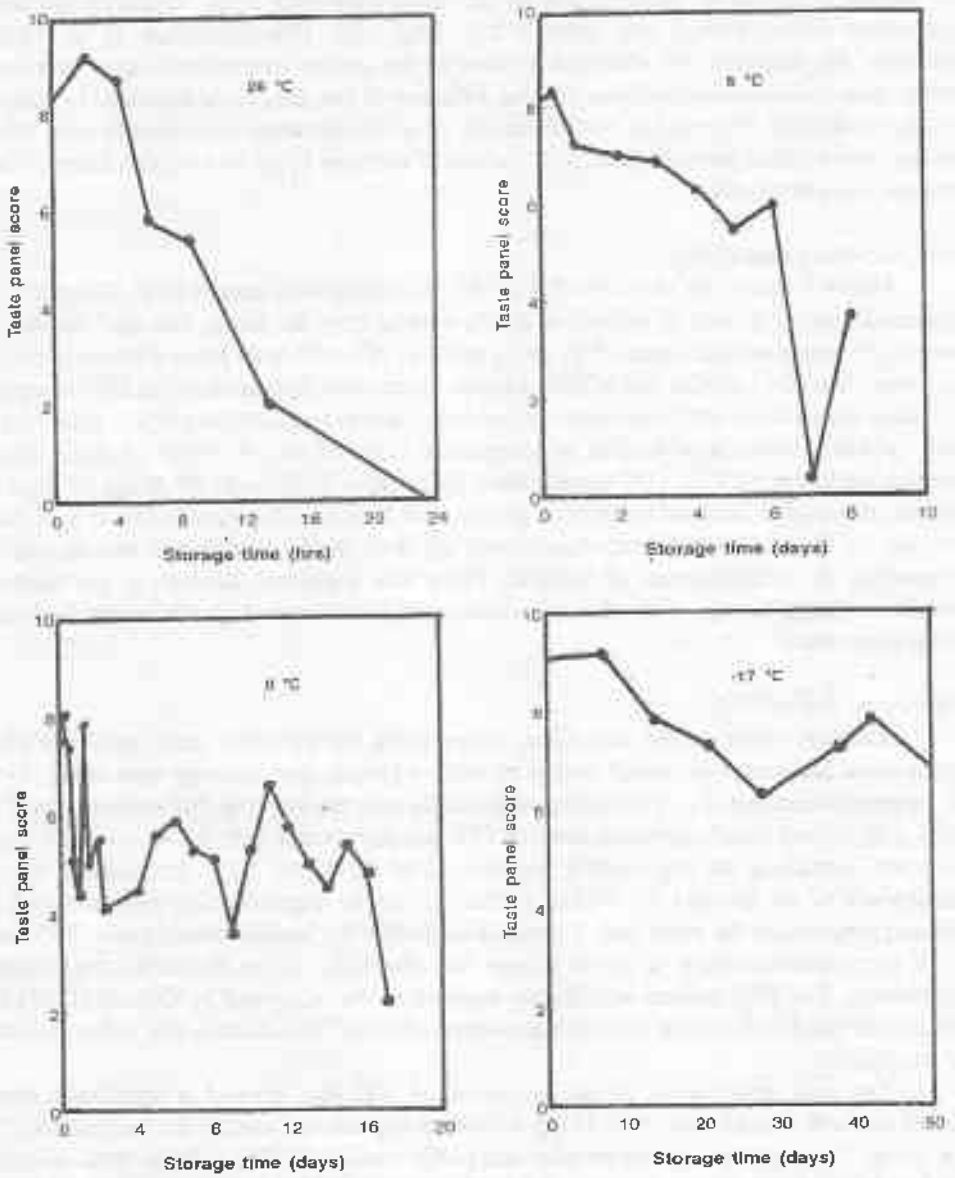

Fig. 4. Variation of taste panel score of *Sardinella longiceps* fish muscle with storage time.

initial decrease in pH in fish muscle during storage may be attributed to formation of lactic acid due to break down of glycogen whereas the subsequent rapid increase in pH may have taken place as a result of the formation of volatile nitrogen bases by microbial action (Love 1980). Oil sardine is known to be very rich in polyunsaturated fatty acids. Oxidation of these compounds during storage can produce free fatty acids (Viswanathanair et al. 1978). Therefore, the decline in pH observed at some of the storage temperatures upon continued storage may probably have been due to some influence of free fatty acids that could be formed by lipid hydrolysis. Since pH of the fish muscle only slightly exceeded neutrality even when spoilage was evident, particularly at 26°C, the use of variation in pH as a reliable index of fish spoilage is not satisfactory.

Total Bacterial Count (TBC)

Figure 3 shows the variation of log TBC of sardine with storage time. The effect of temperature on the growth of bacteria is clearly evident from the graph. The total number of bacteria present in sardine increased by about $3x10^4$ at 26°C within 24 hours whereas at 6°C, 8 days were taken for a similar rise in TBC. Despite the random fluctuation of log TBC observed in sardine stored in ice which probably had occurred due to replenishment of ice, there was a clear growth of bacteria as evident by an increase from 10^5 to 10^9 within 17 days. Under freezing conditions (-17°C), TBC varied within the range of $5x10^3$ to $2x10^5$ during 50 days of storage, indicating a decrease in bacterial growth until 28th day of storage followed by a slow increase. Of the four storage temperatures, only the deep freezer temperature was capable of suppressing the multiplication of bacteria. There was a gradual increase in the bacteria population during storage at all other temperatures and the bacterial growth accelerated with rising temperature.

Taste Panel Score (TPS)

Average values of the individual scores given by the taste panel members after organoleptic assessment of cooked sardine muscle are plotted against storage time in Fig. 4. At all temperatures except 0°C, TPS decreased gradually with storage time. For sardines stored at 26°C, a significant linear correlation between TPS and storage time ($r = -0.956$; $p < 0.001$) was observed. Assuming an organoleptic rejection level of 6 (i.e. 60% acceptability which corresponds to off flavour) for cooked sardine, it can be suggested that sardine stored at ambient temperature for more than 7 hours is unsuitable for human consumption. TVN and TMA values corresponding to above storage time (from Fig. 1) are 39 and 6.4 mg N/100g, respectively. The TVN content was slightly higher than that suggested by Connell (1975) for rejection of fish based on total volatile base content whereas TMA content was within the limit of acceptability.

The taste panel score of sardine stored at 6°C also showed a significant linear correlation with storage time ($r=-0.835$; $p < 0.01$). It appears that sardine can be stored at 6°C for about 3.5 days in organoleptically acceptable condition. The volatile base contents produced during this storage time (i.e., 6.4 mg N/100g and 35 mg N/100g of TMA and TVN, respectively) were within the limits suggested by Connell (1975).

Even though the organoleptic assessment of sardine stored in ice did not yield a significant linear correlation with storage time due to random error introduced by poor quality ice contaminated with bacteria, some obvious trends were noticed. During the first two days of storage, TPS decreased drastically from 8 to 4.5 and, thereafter, fluctuated between 3.6 and 6.6 for about 16 days. Unlike at other temperatures, the safe storage period of sardine in ice cannot be deduced directly due to wide fluctuation of taste panel score. However, average taste panel score indicates that the fish can be preserved in ice in acceptable quality for 7 days if melting ice is allowed to drain and is replenished with fresh ice. However this may lead to large losses of cell sap resulting to nutrient losses and therefore can not be recommended. Storage in ice is

normally accepted for transport. A previous study has reported that *S. longiceps* landed un-iced could be preserved in ice for five days in organoleptically acceptable quality (Krishnakumar et al. 1986). At -17°C, a significant linear correlation was found between TPS of sardine and storage time (r=-

0.726; $p<0.05$). When the experiment was terminated on the 50th day of frozen storage, TPS still remained over 6. If the linear relationship is assumed to be valid for longer storage time, the shelf life at -17°C can be estimated to be 65 days.

Taste panel score vs measured parameters

Table 2 presents the data of the correlations between the taste panel score and the measured biochemical/microbiological parameters for different storage temperatures. The data indicate that at 26°C and 6°C, sensory evaluation followed a statistically significant ($p<0.05$) linear correlation with TVN, log TBC and pH. However, none of the measured parameters showed a simple correlation with the taste panel score at other two temperatures. These results therefore, suggest that there is no simple relationship between any of the measured biochemical/microbiological parameters and the sensory evaluation that can be commonly applicable to all temperatures. As such, any of the biochemical and microbiological parameters studied may not be considered as a reliable substitute to the traditional sensory method for testing the quality of fish during storage.

Table 2. Correlations between taste panel score and measured parameters at different storage temperatures. n = sample size, r = linear correlation coefficient (r denoted by asterisks are significant at 5% level).

Parameter	Storage temperature °C	n	r
TVN	26	7	-0.973*
	6	10	-0.875*
	0	24	-0.156
	-17	8	0.189
TMA	26	7	-0.479
	6	10	-0.626
	0	24	-0.0531
	-17	8	0.139
log TBC	26	7	-0.964*
	6	10	-0.657*
	0	24	-0.420
	-17	8	0.670
pH	26	7	-0.851*
	6	10	-0.826*
	0	24	-0.315
	-17	8	0.639

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