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Effect of Different Methods of Icing on the Quality of Squid and Cuttlefish During Storage

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Quality changes in squid (*Loligo duvauceli*) and cuttlefish (*Sepia pharaonis*), stored under various conditions, viz; (i) direct icing (ii) indirect icing, and (iii) in a mixture of ice and salt at 3% level were evaluated by chemical and sensory methods. The rate of nucleotide degradation was slow for samples kept in the mixture of ice and salt. K-values of samples kept out of direct contact with ice increased at a faster rate than the other two. However, K-values and nucleotide related products showed significant correlation with sensory scores, under these conditions. Total volatile base nitrogen and trimethyl amine nitrogen exhibited a steady and faster rise for indirectly iced samples. There was a significant loss of soluble nitrogenous components and salt in directly iced samples while there was very little loss (<10%) in samples chilled by the other two methods during two weeks period. The study indicated that indirect icing preserves most of the nutrients in squid and cuttlefish, but with shorter shelf life, while chilling in a mixture of salt and ice gave a product of better quality.

Key words: Squid, cuttlefish, water extractable nitrogen, non-protein nitrogen, K-value, volatile base nitrogen, trimethyl amine nitrogen, nucleotide, sensory properties

Frozen cephalopods, particularly cuttlefish and squid, form a major component in the marine products export of India. During 1997-98, 72,353 tonnes of frozen squid and cuttlefish, valued at 594.30 million rupees were exported to different countries. Squid and cuttlefish are highly perishable and hence deteriorate rapidly if not properly preserved. The major markets for these products are Japan and some of the European countries, where they are a highly favoured seafood delicacy. High quality products are required for these markets. The pre-freezing ice storage and several washing steps cause leaching of significant quantity of soluble components. The leaching process, if allowed to continue, may result in significant loss of flavour and mineral nutrients. Under tropical conditions, melting of ice and leaching is quite high and hence alternative methods of icing are needed. It has been reported that squids should not be iced directly, even for short periods of storage (Komai, 1959). Some studies pertaining to the ice-storage characteristics of squid and cuttlefish, have been made (Joseph & Perigreen, 1988, Bykowski *et al.*, 1990). Raghunath (1984) observed that total water extractable nitrogen (WEN) and non-protein nitrogen (NPN) fractions decreased considerably in dressed squid mantles stored in a system of crushed ice and melt water. Lakshmanan *et al.* (1996) also observed a similar loss in cuttlefish and squid, stored in ice. Thus, a significant quantity of soluble components are lost during direct icing. The present study examines the advantages of various chilling methods on the quality and shelf life of squid and cuttlefish in order to recommend a better method of chilled storage.

Materials and Methods

Fresh samples of cuttlefish (*Sepia pharaonis*) 20.7 ± 2.68 cm in length and 143.8 ± 25.3 g in weight and squid (*Loligo duvauceli*) of 22.3 ± 2.2 cm in length and 96.0 ± 18.5 g in weight, collected from the

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Fisheries Harbour, Cochin, immediately after landing, were used for the study. Raw squid and cuttlefish appeared very fresh as revealed by the sheen, general appearance and odour. The samples were transported to the laboratory in ice in insulated boxes, within half an hour and iced using three different methods: (i) cuttlefish/squid directly in ice (DI), (ii) indirectly in ice (IDI) (ie., Samples separated from ice by a barrier of thin polythene sheets) and (iii) kept directly in a mixture of ice and salt at 3% level (I+S). All samples were chilled whole and kept in insulated boxes at ambient conditions. Two storage trials were carried out in each case. Chilled squid/cuttlefish were sampled in triplicate every two days. Temperature of the various chilling media was monitored at different points using a freezer temperature monitor, (temperature range -50 to +50°C) with an accuracy of ±0.1°C. Melt water and ice were removed every 24 h and replenished with fresh ice and salt. The quality was evaluated by measuring nucleotide based products (viz., adenosine monophosphate, inosine, monophosphate, inosine, Hx), K-value, moisture, volatile bases, pH, soluble nitrogen, non-protein nitrogen, salt content and sensory assessment.

Moisture, pH, water extractable nitrogen (WEN), non-protein nitrogen (NPN) and salt content in the muscle of cephalopods were determined following AOAC (1990) methods. Nucleotides and related compounds in the muscle were determined following the method of Ryder (1985) using High Performance Liquid Chromatography. Extraction of nucleotides from the muscle was done using 0.6 M perchloric acid at 0°C and neutralized using 1 M KOH. Solution was filtered through a Millipore syringe filter of pore size 0.45 µm. Nucleotide standards and potassium phosphates were obtained from Sigma Chemicals Company. A Hewlett Packard HPLC (model 1090) with ODS Hypersil column 5µ (250 mm x 4.6 mm) was used for the separation of nucleotides. Operating pressures was 100-110 bar and column temperature was 30 ± 1°C (ambient). The mobile phase comprised of 0.06 M K_2HPO_4 and 0.04 µM KH_2PO_4 at pH 6.5-6.8. The flow rate was 1 ml. min^{-1} and the eluate was monitored at 254 nm. The detector response for each of the six nucleotides found in the fish muscle was calibrated daily by injecting standard reference compounds. K-value was computed from the results as described by Saito *et al.* (1959). Protein, fat and salt content were determined as per the AOAC (1990) methods. The volatile bases, viz., total volatile base nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) were estimated by the micro-diffusion method of Conway (1962) using TCA extract of the muscle. Total bacterial count (TPC) was estimated by the pour plate method (FDA, 1978).

The sensory evaluation was carried out by a trained panel of six members on raw and steamed samples by assessment of general appearance, colour, odour, texture and flavour (in the case of cooked samples) and an overall score was given on a 10 point hedonic scale. Cooked flavour score of 4 was taken as the limit of acceptability. The scoring was done as described earlier by Lakshmanan *et al.* (1993) following the guidelines of Torry Research Note 77 on squid quality (Stroud, 1978).

Results and Discussion

The initial moisture level, protein content, fat and total nucleotides in squid and cuttlefish were respectively 79.5 ± 0.6 (%), 17.14 ± 0.35 (%), 2.01 ± 0.2 (%) 6.89 µmol.g^{-1} and 80 ± 0.4 (%), 16.68 ± 0.2 (%), 1.68 ± 0.2(%) and 7.56 µmol.g^{-1}. The temperatures of squid and cuttlefish in the three treatments varied between 0.2 and 0.5°C in DI; 0.4 and 0.9°C in IDI, 0 and -3.5°C in I+S, during the entire storage period. The minimum temperature observed (ie. -3.5°C), immediately after putting the ice salt mixture in the I+S sample agreed well with the standard value of - 3.48°C (Anon, 1959) and no partial freezing of the samples could be

Table 1. Changes in the sensory characteristics of squid and cuttlefish under various chilling methods and the overall average sensory scores (in parenthesis)

Storage time (days)	Whole squid			Whole cuttle fish		
	DI	IDI	I+S	DI	IDI	I+S
0	Fresh seaweed odour, sheen and glossy appearance. Off white colour for skinned sample. Elastic and firm texture	As for DI	As for DI	Fresh seaweedy odour. Sheen and glossy appear Elastic and firm texture	As for DI	As for DI
	(9.3)	(9.3)	(9.3)	(9.2)	(9.2)	(9.2)
2	Fresh squid odour; sheen & glossy; firm texture	As for DI	As for DI	Fresh squid odour. Sheen and glossy. Elastic and firm	As for DI	As for DI
	(8.4)	(8.5)	(8.8)	(8.4)	(8.4)	(8.5)
4	Squid odour Sl. loss of sheen appearance. Elastic and firm. White colour.	Sl. loss of squid odour. Loss of natural bloom. Pink discolouration Sl. flabby.	Good sheen, glossy and off white colour for skinned sample. Elastic and firm texture.	Squid odour. Loss of natural bloom. Elastic and firm.	Sl. loss of squid odour Loss of sheen and bloom. Elastic and firm.	Characteristic squid odour. Sl. sheen and glossy. White colour for peeled sample. Elastic and firm.
	(7.6)	(7.7)	(8.4)	(7.4)	(7.6)	(8.0)
6	Sl. loss of squid odour and bloom. Firm texture.	Sl. cabbage odour and loss of natural bloom. Sl. pink discolouration on peeling.	Squid odour, sheen and glossy. No discoloured pieces. Firm texture.	Sl. loss of fresh squid odour and bloom. Sl. yellow and firm texture.	Sl. cabbage odour. Loss of bloom. yellow/pink coloured pieces. Firm texture.	Squid odour. Sheen and glossy. Elastic and firm.
	(6.4)	(6.6)	(7.5)	(6.9)	(6.7)	(7.4)
8	Sl. squid odour loss of bloom and bruised pieces. Sl. flabby. Sl. Pink in colour.	Cabbage odour and sl. pinkish appear. Sl. flabby.	Squid odour. Sl. loss of sheen and glossy appearance white colour for peeled meat. Firm texture.	Sl. cabbage odour. Loss of bloom. Sl. flabby pink/yellow colour to peeled sample.	Sl. putrid odour. Loss of bloom. Flabby pink/yellow discolouration	Sl. loss of squid odour, sheen and glossy appearance. Firm texture. White meat.
	(5.3)	(5.5)	(6.9)	(5.3)	(5.1)	(6.4)
12	Off odour, flabby and poor appearance. Pink colour.	Putrid odour, slimy and flabby. Colour turned pink.	Loss of sheen. Sl. cabbage odour. White meat and firm texture.	Off odour, flabby and poor appearance.	Putrid odour. Discolouration Flabby texture.	Sl. off odour. Loss of sheen and glossy Sl. firm texture.
	(4.2)	(3.4)	(4.8)	(4.0)	(3.4)	(4.5)

DI: Directly in ice; IDI: Indirectly in ice; I+S: Ice + Salt

observed in this case. The variation in ambient temperature during the period was from 27 to 33° C.

The organoleptic evaluation of the samples and the overall average taste panel score are presented in Table 1. The total sensory score is the mean of all the sensory characteristics. Squid and cuttlefish retained prime quality up to four days in I+S medium as shown by fresh seaweedy odour, sheen and glossy appearance, firm texture, characteristic white colour, sweet flavour and soft, firm and juicy texture of the skinned material. The other two samples could retain the prime freshness only for two to three days. The prime condition of the samples was associated with low K-value (30%). Based on sensory assessment, squid and cuttlefish kept under DI and IDI conditions remained good up to 6 days, while that kept in I+S medium retained good quality up to 8 days. IDI samples had better flavour than DI samples, as these samples could retain higher levels of WEN and flavour compounds like IMP. As storage progressed, loss of bloom, flabby texture and discolouration were observed in these samples with varying intensity. Thus, towards the end of the storage period, the body colour of both squid and cuttlefish in IDI medium became tinged with red. DI samples had lost its flavour and characteristic colour after seven days. However, the samples kept in DI and IDI were acceptable up to 12 and 10 days, respectively (Table 1). Samples kept in I+S medium retained characteristic white colour, soft and firm texture up to 8 days. On the 10th day they were fair to good, but were unacceptable after 12 days.

Table 2. Changes in the moisture content of squid and cuttlefish during chilled storage under different conditions

Storage time (days)	Whole squid			Whole cuttlefish		
	DI	IDI	I+S	DI	IDI	I+S
0	80.20	80.20	80.20	80.13	80.13	80.13
2	83.40	80.90	82.60	83.18	80.74	82.81
4	85.00	80.48	82.20	84.70	81.88	83.20
6	85.30	80.69	84.50	85.85	81.00	84.80
8	86.10	81.15	86.12	86.55	82.00	86.12
12	86.45	82.02	85.90	86.85	81.81	86.58
14	86.55	82.66	86.10	87.20	83.20	86.10

DI: Directly in ice; IDI: Indirectly in ice; I+S: Ice + Salt

Table 3. Changes in the WEN values (mg/100 g) of squid and cuttlefish during chilled storage under different conditions

Storage time (days)	Whole squid			Whole cuttlefish		
	DI	IDI	I+S	DI	IDI	I+S
0	809	809	809	1057	1057	1057
2	638	790	878	938	1001	1362
4	464	763	851	710	1155	1468
6	358	784	790	690	1184	1053
8	318	752	763	720	1064	1075
10	290	774	404	661	1168	1009
12	N.D.	N.D.	N.D.	728	1082	952
14	252	718	638	560	1068	938

DI: Directly in ice; IDI: Indirectly in ice; I+S: Ice + Salt

The results of the study indicated that, in general, the spoilage rate, loss of soluble components and salt content differed greatly in samples kept in the three storage media, for the two species of cephalopods. Moisture levels varied slightly in IDI samples while it increased significantly in DI and I+S samples during the two weeks (Table 2). The loss in total solids (TS) were greatest in DI samples; the values being 32.6% in squid and 35.6% in cuttlefish during the 2 weeks study period. The samples kept in I+S medium also lost significant quantity of dry matter

Table 4. Changes in the NPN (mg/100 g) values of squid and cuttlefish during chilled storage under different conditions

Storage time (days)	Whole squid			Whole cuttlefish		
	DI	IDI	I+S	DI	IDI	I+S
0	650	650	650	708	708	708
2	512	620	576	634	672	645
4	371	587	418	462	637	504
8	169	560	284	255	609	385
12	124	542	210	204	576	340
14	133	529	168	178	545	306

DI: Directly in ice; IDI: Indirectly in ice; I+S: Ice + Salt

while the loss of solids in IDI samples was 12.42% in squid) and 15.5% in cuttlefish, respectively.

The variation in the WEN and NPN values of squid and cuttlefish during chill storage in the three media are presented in Tables 3 and 4. In squid, WEN declined from an initial value of 809 mg/100g to 252, 718 and 638 mg/100g respectively in DI, IDI and I+S, at the end of the storage period; making loss percentages of 68.85, 11.25 and 21.14 in the three samples. A similar pattern of variation of WEN was also observed in cuttlefish, the loss being 47% (DI), 0% (IDI) and 11.25% (I+S). The same pattern was followed in the variation of NPN also (Table 4). Thus, in DI samples, NPN values were reduced by 79.5% in squid and 74.9% in cuttlefish and the percentage loss of this component in samples kept on I+S media were 74.2% (for squid) and 56.8% (for cuttlefish). However, samples kept out of direct contact with ice had retained about 80% of the NPN fractions. The greater loss of WEN and NPN in directly iced samples can be attributed to leaching of these components during storage. Berg (1974) also reported considerable loss of solids in squids due to leaching in contact with water. Joseph *et al.* (1977) noticed that NPN levels in iced squid reduced over five days to 103 mg/100g from an initial level of 682.3 mg/100g. Lakshmanan *et al.* (1993) also observed lower levels of NPN and WEN values in certain commercial samples of squid and cuttlefish. However, it should be noted that in indirectly iced samples, loss of these soluble components was comparatively low.

Table 5. Changes in the levels of salt content (g/100) g in squid and cuttlefish muscle during chilled storage under different conditions

Storage time (days)	Whole squid			Whole cuttlefish		
	DI	IDI	I+S	DI	IDI	I+S
0	0.481	0.481	0.481	0.521	0.521	0.521
2	0.342	0.450	0.841	0.280	0.503	2.140
4	0.233	0.490	1.580	0.193	0.461	2.510
6	0.145	0.530	2.191	0.120	0.480	2.523
8	0.110	0.500	2.630	0.093	0.476	2.600
10	0.060	0.441	2.500	0.070	0.480	2.550
12	0.042	0.450	2.600	0.054	0.430	2.700
14	0.0125	0.460	2.780	0.021	0.440	2.680

DI: Directly in ice; IDI: Indirectly in ice; I+S: Ice + Salt

Table 6. Changes in the levels of nucleotides in squid and cuttlefish muscle during chilled storage under different conditions

Storage time (days)	Nucleotides (μ mol.g^{-1})	Whole squid			Whole cuttlefish		
		DI	IDI	I+S	DI	IDI	I+S
0	AMP	3.20	3.20	3.20	3.82	3.82	3.82
	IMP	4.10	4.10	4.10	2.60	2.60	2.60
	Ino	0.27	0.27	0.27	0.35	0.35	0.35
	Hx	0.32	0.32	0.32	0.24	0.24	0.24
2	AMP	2.54	2.80	4.34	2.54	1.90	3.74
	IMP	3.05	2.90	3.85	2.03	2.44	2.60
	Ino	0.54	0.61	0.62	0.32	0.43	0.55
	Hx	0.90	1.27	1.12	0.78	1.36	0.67
4	AMP	1.80	2.10	3.20	1.90	2.06	2.24
	IMP	2.11	1.48	2.85	1.86	1.40	2.30
	Ino	0.41	0.67	0.34	Nil	0.30	0.40
	Hx	1.56	2.03	1.70	1.74	2.10	1.48
8	AMP	0.60	0.88	1.78	0.84	1.22	1.60
	IMP	0.58	0.64	1.15	0.95	0.83	1.20
	Ino	0.32	Nil	0.41	Nil	Nil	0.13
	Hx	2.48	3.58	2.03	2.12	2.60	1.84
12	AMP	0.05	0.23	0.60	0.24	0.36	0.78
	IMP	0.16	0.41	0.62	0.22	0.48	0.58
	Ino	Nil	Nil	Nil	Nil	Nil	Nil
	Hx	1.79	4.22	2.56	2.70	3.52	2.44

DI: Directly in ice; IDI: Indirectly in ice; I+S: Ice + Salt

A major drain in salt content was observed in directly iced squid and cuttlefish (Table 5). The salt content decreased rapidly in directly iced samples and was reduced to a very low level towards the 14th day of storage. IDI samples retained >95% of the salt. The salt level in squid and cuttlefish kept in ice-salt mixture increased steadily and reached 2.5-2.8% in two weeks. The results indicated that if squid and cuttlefish are indirectly iced, much of the nutrients in squid and cuttlefish could be preserved for further processing.

The nucleotide degradation pattern was similar for the two species. However, the rate of degradation differed in the three types of storage. Thus, the nucleotide degradation

Table 7. Changes in the TVB-N (mg/100 g) values of squid and cuttlefish during chilled storage under different conditions

Storage time (days)	Whole squid			Whole cuttlefish		
	DI	IDI	I+S	DI	IDI	I+S
0	11.82	11.82	11.82	13.44	13.44	13.44
2	14.56	18.80	16.80	17.54	19.65	16.67
4	22.40	23.74	18.40	24.64	26.35	22.40
8	26.64	28.56	22.60	23.56	30.85	25.12
12	28.30	38.40	23.50	25.33	38.60	28.34
14	27.60	41.20	28.35	31.60	44.80	31.70

DI: Directly in ice; IDI: Indirectly in ice; I+S: Ice + Salt

proceeded steadily and at a greater velocity in IDI samples as revealed by the comparatively higher levels of Hx (Table 6). The rates of nucleotide breakdown were slow in samples kept in I+S medium. The initial level of ATP was quite insignificant in squid but ADP level remained at around 0.5 $\mu mol.g^{-1}$ throughout storage, for both species (Lakshmanan *et al.* 1996; Yamanaka & Shimada 1996). In cuttlefish, AMP level was invariably higher than IMP level, while in squid, concentration of IMP was higher than that of AMP. The accumulation of AMP may be attributed to the low AMP deaminase activity in these species. Significant quantities of AMP and IMP were present towards the 8th day of storage in all samples. Inosine concentrations were found to be low in these species, particularly in cuttlefish where it totally disappeared in DI samples in four days and after eight days in IDI samples. This may be due to the rapid conversion of inosine to hypoxanthine (Kassemsarn *et al.* 1963; Fraser & Simpson 1968). There was high build-up of hypoxanthine from the 2nd day of storage, for both the species and the value exceeded 2 $\mu mol.g^{-1}$ in IDI samples at the end of four days and after eight days in other samples. Higher levels of Hx in the muscle reduced the flavour scores in these samples.

Table 8. Changes in TMA-N values (mg/100 g) of squid and cuttlefish during chilled storage under different conditions

Storage time (days)	Whole squid			Whole cuttlefish		
	DI	IDI	I+S	DI	IDI	I+S
0	3.5	3.5	3.5	2.8	2.8	2.8
2	4.2	4.2	3.5	3.5	2.8	2.8
4	4.2	4.9	4.2	4.2	3.5	3.5
6	3.5	4.9	4.2	3.5	4.2	4.2
8	4.2	5.6	4.9	4.2	5.6	4.2
10	4.9	6.0	4.2	5.6	6.3	3.5
12	5.6	6.3	4.9	4.2	6.3	5.6
14	4.2	6.3	5.6	5.6	7.0	5.6

DI: Directly in ice; IDI: Indirectly in ice; I+S: Ice + Salt

Sakaguchi *et al.* (1990), while studying the ice-storage characteristics of the oyster *Crassostrea gigas*, observed the accumulation of AMP and IMP in its adductor muscle. This was supported by the finding that the muscle tissue of bivalves and gastropods have much lower AMP deaminase activity than that of fish and mammals (Fujisawa & Yoshino, 1985, 1987). Sakaguchi *et al.* (1990) proposed that two pathways are operating in ATP degradation: one involves the direct deamination of AMP to IMP, while the second involves the dephosphorylation of AMP to adenosine and then to inosine and hypoxanthine. In squid and cuttlefish, it is presumed that both the above pathways are

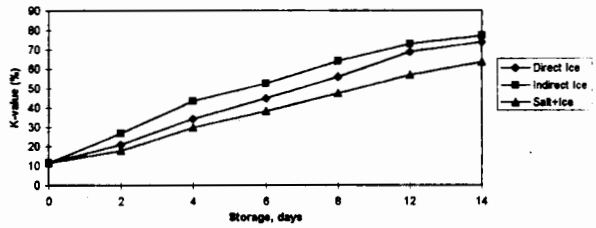

Fig. 1a. Changes in the levels of K-value (%) in squid muscle during chill storage under different conditions.

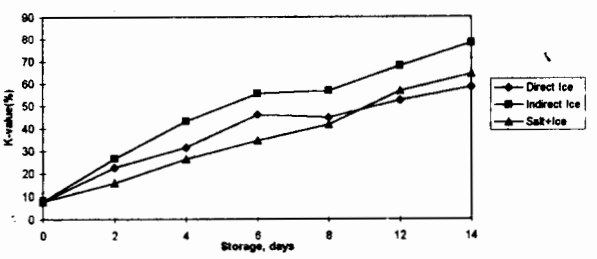

Fig. 1b. Changes in the levels of K-value (%) in cuttle fish muscle during chill storage under different conditions.

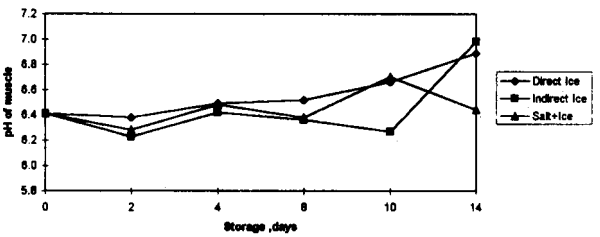

Fig. 2a. Changes in the pH of chill stored squid under different conditions.

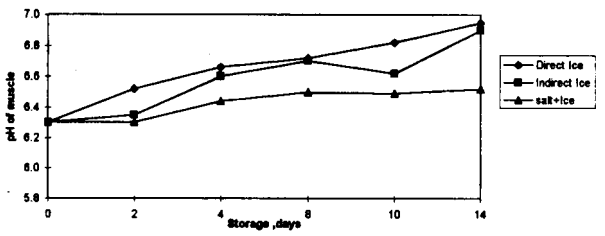

Fig. 2b. Changes in the pH of chill stored cuttle fish under different conditions.

operating as AMP and IMP concentrations ran parallel during chilled storage.

The initial K-value in squid was 11.1% and in cuttlefish, the value was 7.67%. The change in K-value during chilled storage in the three samples is illustrated in Fig.1 a &b. It can be seen that the rate of increase of K-value was always faster in IDI sample, and slowest in I+S sample. K-value of IDI sample reached 52.50% and 55.70% within 6 days period, while it took 12 days to reach that level in I+S samples. Samples with K-value <30% were found to retain prime freshness as revealed by sensory characteristics. The rate of increase of K-value was slow in I+S samples owing to the low temperature condition. Towards the end of storage period, K-value reached around 78% in IDI samples, while lower values were observed in the other samples. K-value of squid and cuttlefish kept in the three media increased linearly with time and exhibited a negative correlation with sensory score.

It was found that K-value of 50 and 55% respectively can be considered as limits for good quality squid and cuttlefish and samples with values in the range of 60-65% were unacceptable.

The changes in TVB-N and TMA-N values are presented in Tables 7 and 8. TVB-N and TMA-N levels increased steadily and at a greater rate for both the samples kept out of direct contact with ice. TVB-N values reached 41.20 mg/100g in squid and 44.80 mg/100g in cuttlefish, kept out of direct contact with ice during 2 weeks period. However, the rate of increase of TVB-N was found to be slow for I+S samples. The rate of increase of TVB-N in directly iced samples was erratic, owing to the leaching effect. Woyewoda & Ke (1980) suggested 30-40 mg/100g TVB-N and 3-10mg/100g TMA-N as the limit of acceptability for squid. In the present study, the TVB-N values approximately indicated the acceptability in IDI and I+S samples. However, in DI samples, TVB-N values did not indicate the acceptability, probably owing to the leaching effect.

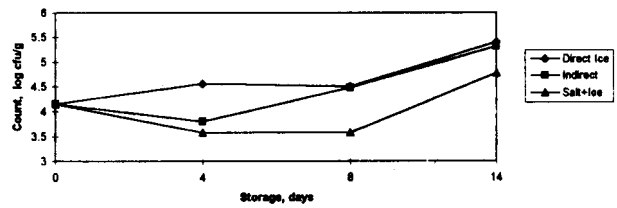

Fig. 3a. Changes in the levels of TPC in squid during chill storage under different conditions.

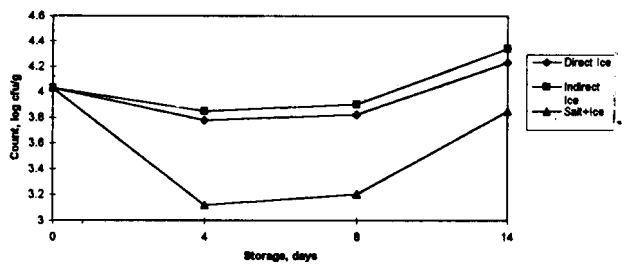

Fig. 3b. Changes in the levels of TPC in cuttle fish during chill storage under different conditions.

Variations in pH of muscle of squid and cuttlefish kept in different chilled conditions are presented in Fig.2 a & b. The pH of muscle increased to around 7.0 in DI and IDI samples from an initial value of 6.41. However, in samples kept in I+S media, pH

did not vary significantly, indicating a slower spoilage rate.

The changes in the total plate count (TPC) of whole squid and cuttlefish during chilled storage under different conditions are presented in Fig. 3a & b. The initial bacterial load of squid and cuttlefish, respectively, were $4.14x10^4$ and $4.02x10^4$ cfu.g^{-1}. Increase in the bacterial load of I+S samples was lower compared to that in the other two samples. The count did not reach 10.7 cfu.g^{-1}, the maximum microbiological limit set by ICMSF for Foods (ICMSF, 1978) in any of the samples during the storage period. The comparatively low bacterial count may be attributed to a washing effect of ice melt water.

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