Article

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Microbiological quality of Cuttlefish (*Sepia pharaonis*) fillets stored in dry and wet ice

G. Jeyasekaran, R. Jeya Shakila and D. Sukumar

Abstract

Microbiological quality of cuttlefish (*Sepia pharaonis*) fillets stored in three different ice conditions was studied. Fillets stored in wet ice at a ratio of 1:1 (package III) were sensorially acceptable for only 18 h, while that stored in dry ice at 1:1 (package I) and combination of dry ice and wet ice at 1:0.2:0.5 (package II) were in acceptable condition up to 24 h without re-icing and thus there was an extension of shelf life by about 33%. Total bacterial load was 7 $\log_{10}$ cfu/g at the end of the storage period. Total psychrophilic population increased from zero to 7 $\log_{10}$ cfu/g while total lactic acid bacteria from zero to 5 $\log_{10}$ cfu/g. H_2S producers were detected only at 18 h, with a count of 1 $\log_{10}$ cfu/g. Sulphite-reducing Clostridia increased gradually from zero to 110 most probable number count/g. Fresh cuttlefish fillets carried a bacterial flora of *Micrococcus, Planococcus, Streptococcus, Moraxella, Proteus* and *Aeromonas*. *Pseudomonas* was dominant in wet ice pack, while *Aeromonas* was dominant in both the dry ice and combination pack. Immediately after packing, the temperatures recorded in packages I, II and III were 10.5, 1.2 and 3.0 °C, respectively, which drastically decreased in 1 h and then maintained and finally increased gradually. The results indicate that use of combination of dry ice and wet ice is economical and very much useful to seafood industries, as this package considerably reduced the cost of air freight, as well as improved the quality and shelf life of cuttlefish.

Keywords

Cuttlefish, microbiology, sensory, dry ice, wet ice

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INTRODUCTION

Cuttlefish, being highly perishable, deteriorate rapidly at tropical temperatures. Cephalopods undergo very rapid protein degradation after death due to endogenous and bacterial enzymes, which favor proliferation of degenerative flora and rapid decomposition (Vaz-Pires and Barbosa, 2004). Since cephalopods undergo rapid spoilage due to the action of microorganisms and enzymes resulting in short shelf life, there is a need for extending the shelf life of this species. Proper icing keeps the product in acceptable condition for reasonable periods. Direct icing is not considered a suitable method even for the short-term preservation of cephalopods, as it resulted in decrease of total water extractable nitrogen (WEN) and non-protein nitrogen (NPN) fractions (Raghunath, 1984). Melting of ice and leaching is quite high under tropical conditions that are prevailing in India. Hence, alternative method of preservation is essential to overcome these problems, so as to improve the quality of fresh fish, which can retain their original flavor, color and texture for long period. Prafulla et al. (2000) studied the effect of indirect icing on the quality of squid and cuttlefish in comparison with direct icing method.

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Dry ice (solid carbon dioxide) has recently gained popularity in India as a novel-chilling medium for the rapid transportation of fresh fish by air, as the application of dry ice in fresh fish preservation is new. However, a significant number of seafood exporters use dry ice in combination with wet ice unscientifically for fresh fish transportation. In this regard, application of dry ice along with wet ice at the ratio of 1:0.2:0.5 is found to be effective for preserving fresh fish (Jeyasekaran et al., 2004) by reducing the temperature rapidly and also more economical. Dry ice has certain advantages viz., it has bacteriostatic effect and it acts as an insulant enveloping the fish upon evaporation (Putro, 1989). It is also used as coolant in the present trends of shipping of fresh seafood (Schoemaker, 1990). Jeyasekaran et al. (2010) reported the quality changes in squid (*Loligo duvaucelli*) tubes chilled with dry ice and water ice. Since the impact of different iced storage conditions on the microbiological quality of cephalopods has not been thoroughly explored (Lapa-Guimaraes et al., 2002) and cuttlefish is one of the important fish varieties exported from India in chilled condition, the present study was conducted to find out the influence of preservative effect of dry and wet ice on the microbiological quality of cuttlefish (*Sepia pharaonis*) fillets in relation to their storage life.

MATERIALS AND METHODS

Sampling

Cuttlefish (*Sepia pharaonis*) were procured from fish landing center of Tuticorin, India. Time interval between harvesting and the arrival of fish at the landing center was 6–8 h and during this period they were iced. They were immediately brought to the laboratory in insulated containers. Fillets were prepared by removing their head, shell, gut and ink sac and washed in tap water. Fillets had an average length of 11 cm and weight of 100 g. They were divided into three lots; each had a weight of about 14 kg and the number of packs in each lot was seven. First lot was packed with dry ice (Thermosafe Dry ice Machine, USA) at the ratio of 1:1 (wt/wt), second lot with a combination of dry ice and wet ice (Jeyasekaran et al., 2006; Sasi et al., 2000) at a ratio of 1:0.2:0.5 and the third lot with wet ice (Ziegra Flake ice Maker, Germany) at a ratio of 1:1 (Lima dos Santos et al., 1981), which served as control and were designated as packages I, II and III, respectively. The fillets were packed separately in bags before being stored in ice. Gloves were worn during handling of ice and fillets. Care was taken to avoid direct contact of fillets with wet ice and dry ice. Re-icing was not done during the course of experiment. Packages were wrapped in Polypropylene bags (200 gauge), placed in conical shaped styrofoam boxes and sealed with cellophane tape. Styrofoam boxes were stored at room temperature ($33 \pm 2\,^{\circ}C$). One pack from each lot was periodically analyzed in triplicate for sensory, bacteriological and physical quality until they were organoleptically unacceptable.

Methods

Sensory evaluation. Sensory characteristics and overall acceptability of cuttlefish fillets were assessed by a panel of six experienced members of the Faculty of Fish Processing Technology of Fisheries College and Research Institute on the basis of ten point scale on each sampling. The sensory characteristics studied included appearance (inclusive of color), odor and texture. The scores were given in the decreasing order scale with 10-9 for excellent, 8-7 for good, 6-5 for fair and acceptable, 4-3 for poor and 2-1 for very poor. The scoring system adopted (Table 1) in this study was a modification of the systems given in the FAO document (Kreuzer, 1984) following the guidelines of Stroud (1978). The mean of the scores given by the panel represented the overall sensory quality and a score of ≤ 4 constituted unacceptable and shelf life failure.

Bacteriological analysis. Bacteriological analyses carried out in this study included total bacterial load, total psychrophilic count, total lactic acid bacteria, total H_2S producers, total coliforms and total sulphite-reducing Clostridia. All the media used in this study were obtained from Hi-Media Laboratories, Mumbai, India. Muscle from different regions of cuttlefish fillet was cut into small pieces using sterile knife and forceps and 25 g was taken from this mixed pool for analysis. The sample was homogenized using 225 mL sterile physiological saline (0.85%) and serial decimal dilutions of each homogenate were carried out with the same diluent for the respective bacteriological analysis (APHA, 2001). Appropriate dilutions were spread plated onto Trypticase Soy Agar (TSA) for the enumeration of total bacterial load and total psychrophilic count. The plates were incubated at $37\,^{\circ}C$ for 24 h for the enumeration of total bacterial load, whereas they were incubated under refrigerated condition ($5\,^{\circ}C$) for 7 days for the enumeration of psychrophiles. The colonies from total plate count plates (<300 colonies) of packages I, II and III at each storage time were isolated and identified by various biochemical tests (Balows et al., 1992; LeChevallier et al., 1980). Representative colonies were chosen from each treatment for the identification. Double-layer pour plate technique (USFDA, 2001) was followed for the enumeration of total lactic acid bacteria and simple pour plate technique was carried out for total H_2S producers using deMan Ragosa Sharpe (MRS) Lactobacillus Agar (deMan et al., 1960)

Table 1. Scoring system for evaluating sensory quality of cuttlefish (*Sepia pharaonis*) fillets stored in dry ice and wet ice

Sensory characteristics	Numerical scoring				
	10-9 (Excellent)	8-7 (Good)	6-5 (Fair)	4-3 (Poor)	2-1 (Very poor)
Appearance	Good sheen, shiny, white	Slight loss of sheen, shiny, loss of white color	Slightly sheen, creamy to yellowish	No sheen, bleached, yellow to light brown	Completely bleached, pinkish brown
Odor	Fresh shellfish meat odor	Loss of fresh shellfish meat odor, slightly cooked cabbage like odor	Stronger cooked cabbage like odor, slightly stale, slightly musty	Unpleasant, slightly ammoniacal odor, slightly sour, musty	Strong ammoniacal, putrid odor, very foul smelling
Texture	Firm and smooth	Firm, rubbery, slightly tough	Sticky and gluey	Slightly mushy and slimy	Thickly mushy and slimy

and Peptone Iron Agar (PIA), respectively. The plates were incubated under microaerophilic condition for 48 h at room temperature for lactic acid bacteria and only at room temperature for 48 h for H_2S producers. The numbers of typical colonies appearing on the plates were counted and expressed as cfu/g. Most probable number (MPN) technique was followed for the enumeration of total coliforms and total sulphite-reducing Clostridia using Lauryl Sulphate Tryptose Broth and Differential Reinforced Clostridial Medium (DRCM), respectively (Jeyasekaran, 1996; Surendran et al., 2003). For the enumeration of total sulphite-reducing clostridia, the inoculated tubes were overlaid with sterile liquid paraffin and incubated in a water bath at 37 °C for 4 days. The tubes exhibiting black precipitate were counted as positive and expressed as MPN count/g. For total coliforms, the tubes were incubated at 37 °C for 24 h and the tubes showing acid and gas production was counted as positive and expressed as MPN count/g (APHA, 2001).

Physical evaluation. Physical parameters studied included pH, temperature and gas composition. The pH was determined by using pH meter (Digisun Electronics Digital pH Meter 707, India) by taking 10 g of homogenized sample in 100 mL of distilled water. Changes in cold spot temperature of different ice packages were recorded by using Ultrafreezer temperature probe (Consort Model T 852, Belgium) by placing the thermocouple in the hottest inner part of the fillets. Gas composition of different packaging environment was measured by gas analyzer (PBI Dansensor CheckMate 9900, Denmark), as gases inside the package affect the quality and shelf life of products.

Statistical analysis. Analysis of variance (ANOVA) was performed using standard statistical package (SPSS 10.0) to examine whether any significant difference exists between different treatments, with respect to the different fish quality characteristics at 5% level.

RESULTS AND DISCUSSION

Sensory evaluation

The changes in the sensory scores of cuttlefish fillets stored in three different packaging conditions are shown in Figure 1. Raw cuttlefish fillets exhibited fresh shellfish odor, were white in color with elastic firm texture and was considered as excellent with the overall score of 9.8. No remarkable change was observed in package III, while the dry ice contacted fish surface became frozen in package II in 1 hour, whereas the cuttlefish in package I was completely frozen due to drastic fall in temperature. No characteristic change was noticed up to 12 h of storage in all the

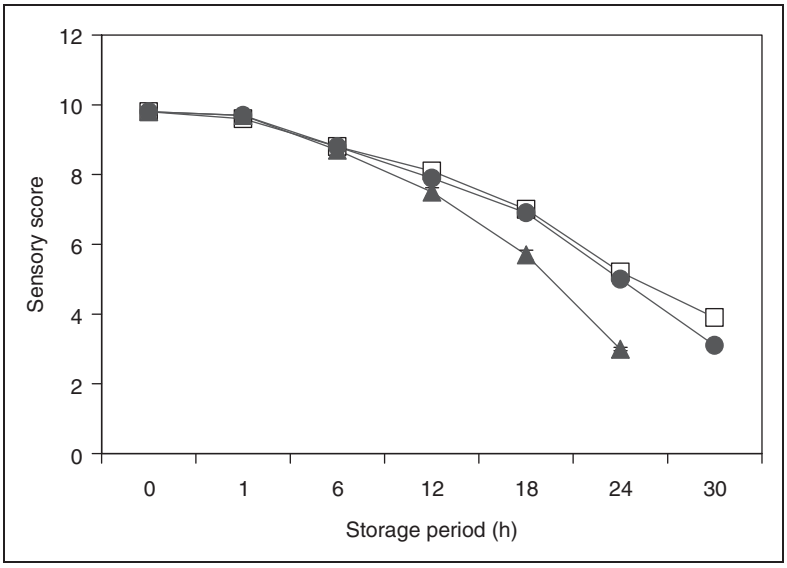

Figure 1. Changes in the sensory score of cuttlefish (*Sepia pharaonis*) fillets stored in: (□) dry ice (1:1 ratio), (•) dry ice and wet ice (1:0.2:0.5 ratio), and (▲) wet ice (1:1 ratio).

three packages. Ke et al. (1991) found that the fresh squid could be stored for longer in non-contact ice than in contact storage in chilled seawater (CSW). At 18 h, package III exhibited slight musty odor, color became milky white and slightly lost its texture with a sensory score of 5.7, whereas, packages I and II exhibited slight cooked meat odor, but, the texture and color was similar to that of fresh fillets. At 24 h, package III was sensorially spoiled with strong ammoniacal and faecal odor and the meat color became slight yellowish, while the same sensory characteristics was observed in packages I and II only at 30 h. It has been earlier reported that the appearance of an offensive putrid smell, change in color and soft or flabby texture, are critical parameters used to determine the sensory quality (Lakshmanan et al., 1993; Ohashi et al., 1991; Yamanaka et al., 1987). A negative relationship was observed between the sensory scores and the various bacterial counts in this dry and wet ice storage studies on cuttlefish fillets. Jeyasekaran et al. (2010) have reported that the squid tubes stored in a combination of dry ice and wet ice had a shelf life of 18 h based on the sensory analysis.

Bacteriological analysis

Total bacterial load. The changes in total bacterial load of cuttlefish fillets stored in three different packaging conditions are shown in Figure 2. Initial total bacterial load of fresh fillets was 5 $\log_{10}$ cfu/g, which reduced by a log in package III and two logs in packages I and II at 1 h. At 6 h, the load further reduced by a log in packages I and III, whereas, it remained 3 $\log_{10}$ cfu/g in package II. The drastic reduction in bacterial load might be due to cold shock. On further storage, the count increased gradually and reached a level of 7 $\log_{10}$ cfu/g in all the three packages at the end of storage. Similar bacterial count was also observed by earlier workers (Jeyasekaran et al., 2010; Paarup et al., 2002a; Prafulla et al., 2000). Huss et al. (1997) reported that the common number of spoilage bacterial count at the point of rejection of fish product was from 7 to 9 $\log_{10}$ cfu/g. A significant difference ($p < 0.05$) was observed in the total bacterial load of cuttlefish fillets stored in three different packages of ice. It has been observed that the difference in the total bacterial load observed among the dry ice stored, wet ice stored and combined ice stored cuttlefish fillets was statistically significant ($p < 0.05$).

Bacterial flora. Fresh cuttlefish fillets used in this study carried a bacterial flora of *Micrococcus, Planococcus, Streptococcus, Moraxella, Proteus* and *Aeromonas* (Table 2). *Micrococcus* constituted about 90% of the flora. *Aeromonas* was the dominant microflora in packages I and II with varying percentage. Callow (1932) suggested that displacement of oxygen by CO_2 inhibits the growth of aerobic microorganisms and results in shift of dominant flora. A shift in the dominant flora was also observed in the present study. *Aeromonas* constituted about 20% in package I followed by *Planococcus, Bacillus*, *Pseudomonas, Corynebacterium, Staphylococcus, Serratia, Micrococcus, Moraxella* and *Acinetobacter* (Table 2). But, in package II, *Aeromonas* constituted about 33% followed by *Acinetobacter, Pseudomonas, Planococcus, Corynebacterium, Proteus, Bacillus, Micrococcus* and *Escherichia* (Table 2). *Pseudomonas* was the dominant flora in package

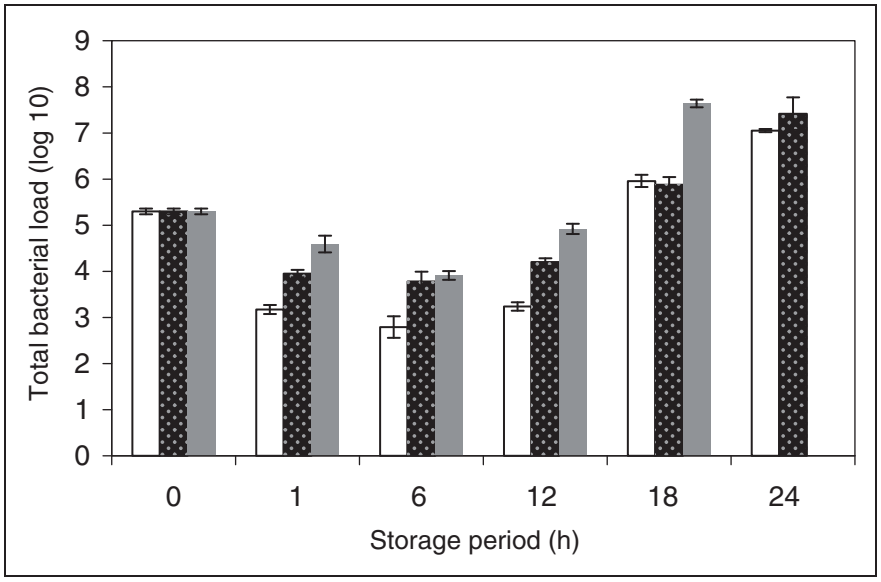

Figure 2. Changes in total bacterial load (cfu/g) of cuttlefish (*Sepia pharaonis*) fillets stored in: (□) dry ice (1:1 ratio), (▮) dry ice and wet ice (1:0.2:0.5 ratio), and (▮) wet ice (1:1 ratio).

Table 2. Bacterial flora (%) associated with raw, dry ice stored, combination of dry and wet ice stored and wet ice stored cuttlefish (*Sepia pharaonis*) fillets. Package I, fish:dry ice (1:1 ratio); Package II, fish:dry ice:wet ice (1:0.2:0.5 ratio); Package III, fish:wet ice (1:1 ratio)

Genus	Raw cuttlefish	Package I	Package II	Package III
Micrococcus	90	5	2	11
Pseudomonas	–	14	20	41
Aeromonas	2	20	33	1
Staphylococcus	–	8	–	29
Acinetobacter	–	2	22	7
Planococcus	2	17	10	2
Corynebacterium	–	13	5	2
Enterobacter	–	–	–	4
Bacillus	–	15	2	–
Moraxella	2	2	–	1
Streptococcus	2	–	–	–
Proteus	2	–	5	–
Escherichia	–	–	1	–
Serratia	–	4	–	2
Total	100	100	100	100

III, which constituted about 41% of the total flora followed by *Staphylococcus, Micrococcus, Acinetobacter, Enterobacter*, *Corynebacterium, Palonococcus, Serratia, Aeromonas* and *Moraxella* (Table 2). Paarup et al. (2002b) reported that *Pseudomonas, Shewanella putrefaciens* and *Pseudoalteromonas* were the dominant microflora in spoiled gutted squid stored in iced condition. The present investigation also found that *Pseudomonas* was dominant in ice stored fillets. Earlier studies also reported that gram-negative bacteria are the main cause of spoilage in marine fish stored at 0 °C (Gram et al., 1987; Hobbs and Hodgekiss, 1982). It has also been reported that *Alcaligenes* and *Alteromonas* were dominant in the squid tubes stored in 100% dry ice and in the combination package of dry ice and wet ice, respectively (Jeyasekaran et al., 2010).

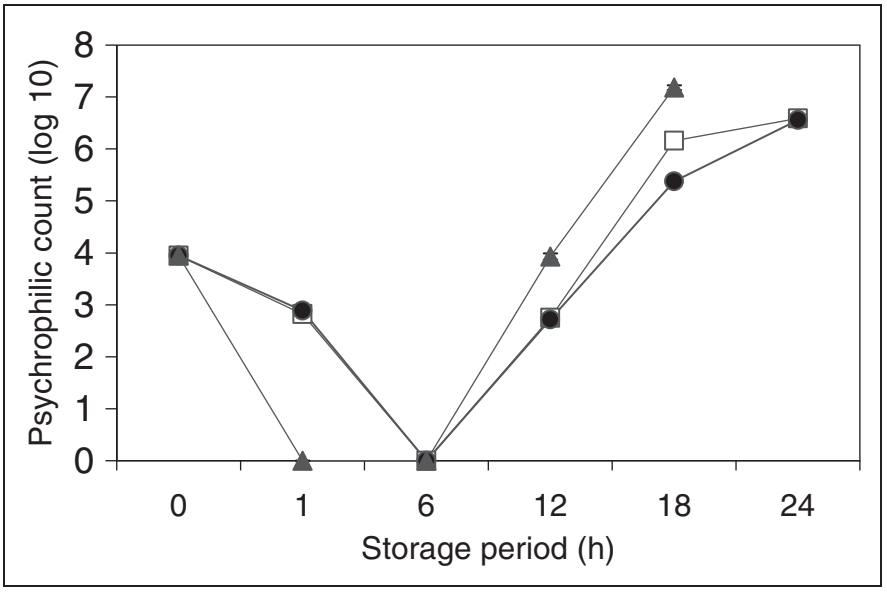

Figure 3. Changes in total psychrophilic count (cfu/g) of cuttlefish (*Sepia pharaonis*) fillets stored in: (□) dry ice (1:1 ratio), (•) dry ice and wet ice (1:0.2:0.5 ratio), and (▲) wet ice (1:1 ratio).

Total psychrophiles

The changes in total psychrophilic count of cuttlefish fillets stored in three different packaging conditions are shown in Figure 3. Fresh fillets exhibited psychrophilic count of 3 $\log_{10}$ cfu/g, which reduced by a log in packages I and II, whereas the population was completely inhibited in package III at 1 h. At 6 h, psychrophilic population was not detected in all the three packages. This might be due to severe cold shock exhibited on certain microorganisms that are present in the tested samples at very low temperature. Paarup et al. (2002b) recommended the use of psychrophilic count as quality index to assess freshness and degradation of cephalopods. On further storage, the count reappeared and increased to 6 $\log_{10}$ cfu/g in packages I and II, and 7 $\log_{10}$ cfu/g in package III at the end of the storage period. A psychrotrophic bacterial count of 6 $\log_{10}$ cfu/g in squid (*L. plei* and *L. duvaucelli*) tubes at the end of storage in ice was also observed (Jeyasekaran et al., 2010; Lapa-Guimaraes et al., 2002).

Total lactics

The changes in total lactic acid bacterial count of cuttlefish fillets stored in three different packaging conditions are given in Figure 4. Initial lactic acid bacterial count was 1 $\log_{10}$ cfu/g, which became zero at 1 h in all the three packages and maintained up to 6 h in package III. In packages I and II, the population reappeared with a low count of 1 $\log_{10}$ cfu/g, which maintained up to 12 h. But in package III, the count was 2 $\log_{10}$ cfu/g at 12 h. On further storage, the count increased and reached 5 $\log_{10}$ cfu/g in all the three packages at the end of the storage period. A significant difference ($p < 0.05$) was observed in the total lactic acid bacterial count of cuttlefish fillets stored in different packages of ice. Sasi et al. (2003) observed a lactic acid bacterial count of 5 $\log_{10}$ cfu/g in seerfish (*Scoberomorus commersonii*) stored in dry ice at the end of storage period and stated that dry ice provided a favorable environment for the growth of lactic acid bacteria. But, Paarup et al. (2002a) detected low numbers (2 $\log_{10}$ cfu/g) of lactic acid bacteria in squid mantle (*Todaropsis eblanae*) throughout the storage period at 4 °C. However, Jeyasekaran et al. (2010) also reported a similar lactics counts at the end of storage in squid tubes stored in dry ice and wet ice.

Total H_2S producers

H_2S producers were detected only at 18 h with a count of 1 and 3 $\log_{10}$ cfu/g in packages II and III, respectively (Figure 5). Dalgaard (2002) reported that the specific spoilage organism grow without a lag phase and produce the metabolites responsible for spoilage. At 24 h, the population was 5 and 3 $\log_{10}$ cfu/g in packages I and II, respectively. Civera et al. (1999) reported that the counts of H_2S producers are appropriate to determine the early stage of deterioration in cephalopods. This might be the reason for the absence of H_2S producing bacteria on the earlier stages of storage.

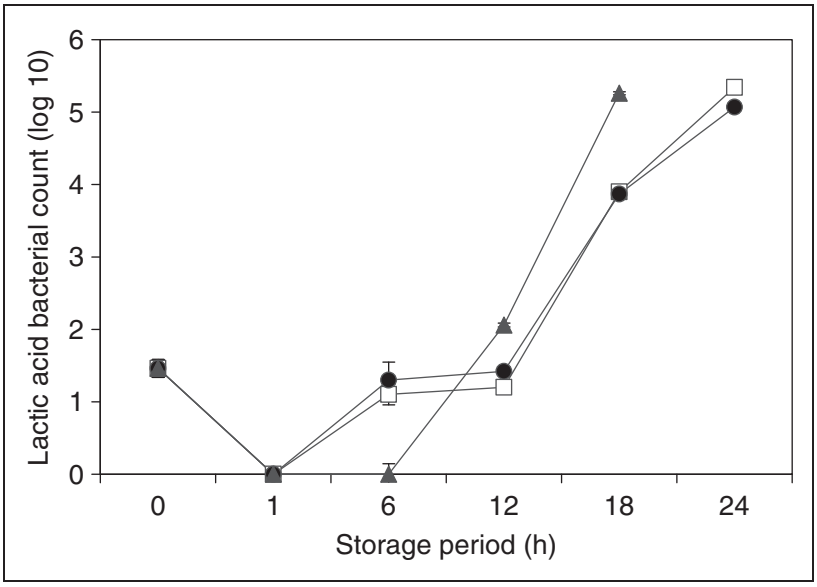

Figure 4. Changes in total lactic acid bacterial count (cfu/g) of cuttlefish (*Sepia pharaonis*) fillets stored in: (□) dry ice (1:1 ratio), (●) dry ice and wet ice (1:0.2:0.5 ratio), and (▲) wet ice (1:1 ratio).

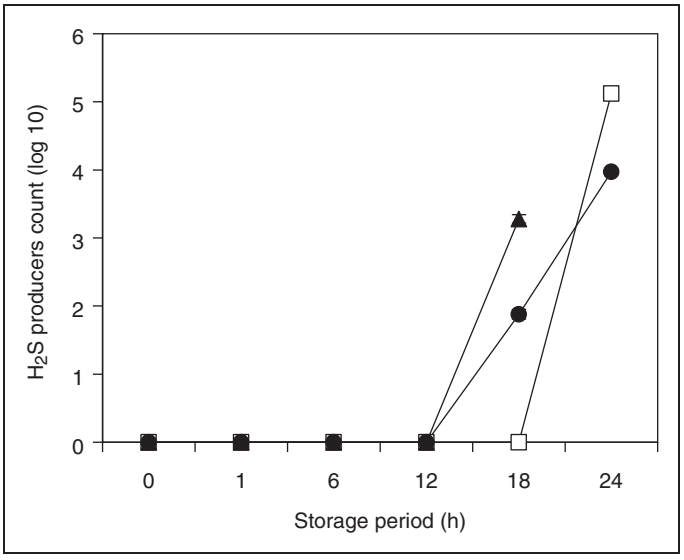

Figure 5. Changes in total H_2S producers count (cfu/g) of cuttlefish (*Sepia pharaonis*) fillets stored in: (□) dry ice (1:1 ratio), (●) dry ice and wet ice (1:0.2:0.5 ratio), and (▲) wet ice (1:1 ratio).

Total coliforms and total sulphite-reducing Clostridia

The changes in total coliforms and total sulphite-reducing Clostridia of cuttlefish fillets stored in three different packaging conditions are presented in Table 3. The initial coliforms count was 14.0 MPN/g, which became zero at 1 h and maintained up to 6 h and 12 h in package I and II, respectively. But in package III, the coliforms population became zero only at 12 h. This might be due to the absence of coliforms in the tested samples at the time of sampling. Vaz-Pires and Barbosa (2004) reported that total enterobacterial count particularly coliforms was less than 2 $\log_{10}$ cfu/cm^2 in iced octopus (*Octopus vulgaris*). On further storage, the count increased and reached a level of 345 MPN/g in packages I and II, while it was 542 MPN/g in package III at the end of the storage period. Initial total sulphite-reducing Clostridia was 0.9 MPN/g, which reduced to 0.4 and 0.7 MPN/g at 1 h in packages II and III, respectively, while they were not detected up to 6 h of storage

Table 3. Changes in total coliforms and total sulphite-reducing Clostridia and pH of cuttlefish (*Sepia pharaonis)* stored in dry ice and wet ice. DC, discontinued; Package I, fish:dry ice (1:1 ratio); Package II, fish:dry ice:wet ice (1:0.2:0.5 ratio); Package III, fish:wet ice (1:1 ratio)

	Total coliforms (MPN/g)			Total sulphite reducing Clostridia (MPN/g)			pH		
Storage period (h)	Package I	Package II	Package III	Package I	Package II	Package III	Package I	Package II	Package III
0	14	14	14	0.9	0.9	0.9	7.08	7.08	7.08
1	0	0	14	0	0.4	0.7	6.64	6.49	6.34
6	0	0	11	0	0	4.5	6.84	6.91	6.98
12	7.0	0	0	2.5	4.5	9.5	6.83	6.90	6.80
18	278	40	542	20	4.5	20	6.87	6.90	7.14
24	345	345	DC	110	110	DC	7.24	7.10	DC

Table 4. Changes in gas composition of cuttlefish (*Sepia pharaonis*) fillets stored in dry ice and wet ice. DC, discontinued; Package I, fish:dry ice (1:1 ratio); Package II, fish:dry ice:wet ice (1:0.2:0.5 ratio); Package III, fish:wet ice (1:1 ratio)

Storage period (h)	Package I			Package II			Package III		
	O_2 (%)	CO_2 (%)	N_2 (%)	O_2 (%)	CO_2 (%)	N_2 (%)	O_2 (%)	CO_2 (%)	N_2 (%)
0	20.7	1.0	78.3	20.7	1.0	78.3	20.7	1.0	78.3
1	0.011	100	0	0.095	100	0	20.9	1.2	77.9
6	0.31	99.8	0	4.96	73.9	21.1	20.2	2.0	77.8
12	7.22	64.6	28.2	14.6	27.5	57.90	20.1	1.5	78.4
18	11.7	37.5	50.8	12.4	38.6	49.0	19.6	1.8	78.6
24	14.7	27.3	58.0	14.6	28.3	57.1	18.3	3.30	78.4
30	15.0	7.3	77.7	18.3	5.6	76.1	DC	DC	DC

in package I. At 6 h, the count became zero in packages II. On further storage, the load increased and reached a level of 110 MPN/g in packages, I and II, while it was 20 MPN/g in package III. This increased count in packages I and II might be due to the anaerobic environment prevailing in those packages.

Physical evaluation

Changes in pH. The changes in the pH of cuttlefish fillets stored in three different packaging conditions are presented in Table 3. Fresh fillets exhibited an initial pH of 7.08. During storage period, the pH of all the three packages was within 7.00. But, it exceeded 7.00 only at the end of storage period. Hebard et al. (1982) reported that the accumulation of alkaline metabolites during microbial spoilage was responsible for increase in the pH to above 7.0 and suggested that pH is more a quality index than other characterization parameters. Prafulla et al. (2000) also observed the pH of muscle increased to around 7.0 in iced squid and cuttlefish samples. It is a fact that the pH depends on many factors, such as the time that has elapsed since the capture, storage temperature and physiological state of animal.

Gas composition. The changes in gas composition of cuttlefish fillets stored in three different ice conditions are shown in Table 4. At 1 hour, CO_2 inside the packages I and II was 100%, while in package III, the percentage of oxygen, carbon dioxide and nitrogen was 20.9, 1.2 and 77.9 respectively. On further storage, CO_2 level in packages I and II was found to be in the decreasing trend. On the other hand, a slight variation in the gas composition was observed in package III throughout the storage period. A similar trend was also observed by Jeyasekaran et al. (2010) in squid tubes stored in dry ice and wet cie. It has been earlier reported that higher content of CO_2 increased the shelf life (Clark and Lentz, 1969; Randell et al., 1999) and this might be the reason for longer shelf life obtained in dry ice stored fillets.

Changes in temperature. Immediately after packaging, the temperatures recorded in packages I, II and III were 10.5, 1.2 and 3.0 °C, respectively (Figure 6).

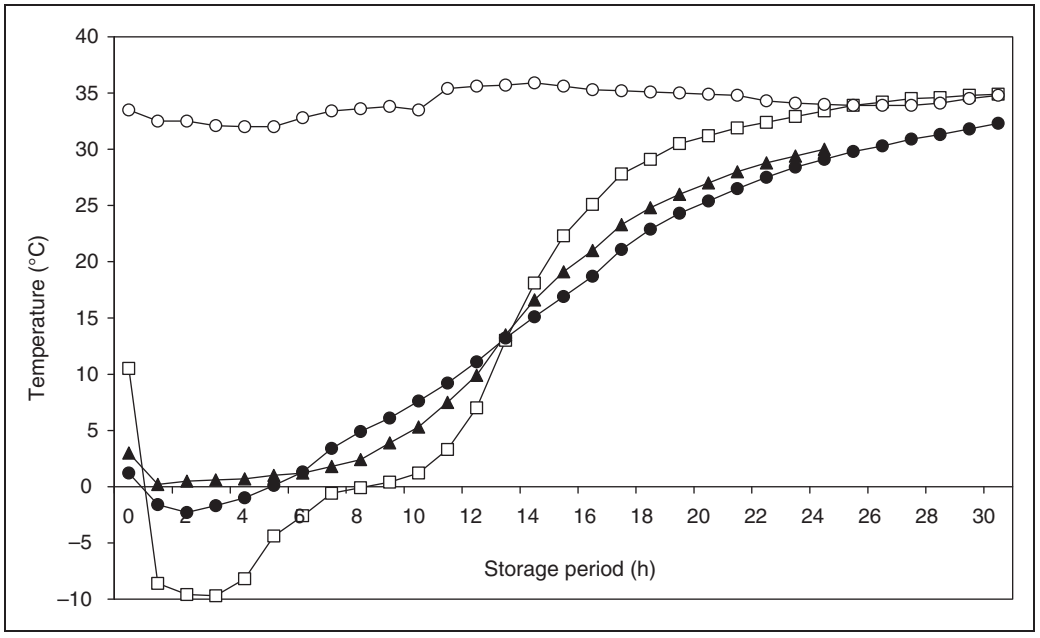

Figure 6. Changes in the temperature profiles of cuttlefish (*Sepia pharaonis*) fillets stored in: (□) dry ice (1:1 ratio), (●) dry ice and wet ice (1:0.2:0.5 ratio), and (▲) wet ice (1:1 ratio) and at room temperature.

At that time, room temperature was 33.5 °C. The temperature became subzero at 1 h, which was maintained up to 8 and 4 h in packages I and II, respectively. However, the lowest temperature recorded in package III was 0.2 °C at 1 h. After that, it increased gradually throughout the storage period. Sivertsvik et al. (2002) stated that the rate of deterioration/spoilage is highly temperature dependent and the results of present investigation also confirmed this statement.

It can be concluded that both dry ice (at the ratio of 1:1) as well as a combination of dry ice and wet ice (in the ratio of 1:0.2:0.5) improved the quality and increased the shelf life of cuttlefish fillets by about 33%, when compared to wet ice alone (in the ratio of 1:1). Since the combination of dry ice and wet ice was more economical than dry ice alone and even the quality of fillets in both the storage conditions was similar, fresh fillets could be chilled in the combined dry ice and wet ice package and transported more effectively by air through out the World to meet the demand of fresh cuttlefish. About 15 to 25% reduction in the cost of air transport of chilled cuttlefish fillets packed with a combination of dry ice and wet ice at the ratio of 1:0.2:0.5 was possible when compared to transport in wet ice, in addition to the elimination of melt ice problem encountered while using flake ice during air shipment.

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REFERENCES

APHA (2001). *Compendium of Methods for the Microbiological Examination of Foods*. Washington DC, USA: American Public Health Association. Balows A, Truper HG, Dworkin M, Harder W and Schleifer KH. (1992). *The Prokaryotes – A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification and Application*, 2nd edn. New York, USA: Springer-Verlag Inc.

Callow EH. (1932). Gas storage of pork and bacon. Part I-Preliminary experiments. *Journal of the Society of Chemical Industry* 51: 116–119.

Civera T, Grassi MA and Pattono D. (1999). Chemical and microbial characteristics of cephalopods during storage in ice. *Industrie Alimentari* 38(384): 933–937.

Clark DS and Lentz CP. (1969). The effect of carbon dioxide and growth of slime producing bacteria on fresh beef. *Canadian Institute of Food Science and Technology Journal* 2(2): 72–75.

Dalgaard P. (2002). Modeling and predicting the shelf-life of seafood. In: Bremner HA (ed.) *Safety and Quality Issues in Fish Processing*. Cambridge, UK: Woodhead Publishing Ltd., 191–219.

deMan JC, Ragosa M and Sharpe M. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Microbiology* 23(1): 130–135.

Gram L, Trolle G and Huss HH. (1987). Detection of specific spoilage bacteria from fish spoiled at low (0 °C) and high

(20 °C) temperature. *International Journal of Food Microbiology* 4(1): 65–72.

Hebard CE, Flick GJ and Martin RE. (1982). Occurrence and significance of trimethylamine oxide and its derivates in fish and shellfish. In: Martin RE, Flick GF and Hebard X (eds) *Chemistry and Biochemistry of Marine Food Products*. Westport, Connecticut, USA: Avi. Publishing Company, 149–304.

Hobbs G and Hodgekiss W. (1982). The bacteriology of fish handling and processing. In: Davies R (ed.) *Developments in Food Microbiology*. London, UK: Applied Science Publishers, 71–117.

Huss HH, Dalgaard P and Gram L. (1997). Microbiology of fish and fish products. In: Luten JB, Borresen T and Oehlenschlager J (eds) *Seafood from Producer to Consumer, Integrated Approach to Quality*. Amsterdam, The Netherlands: Elsevier, 413–430.

Jeyasekaran G. (1996). *Microbiology of Fish and Fishery Products – A Manual*. Chennai, India: Tamil Nadu Veterinary and Animal Sciences University.

Jeyasekaran G, Ganesan P, Anandaraj R, Jeya Shakila R and Sukumar D. (2006). Quantitative and qualitative studies on the bacteriological quality of Indian white shrimp (*Penaeus indicus*) stored in dry ice. *Food Microbiology* 23(6): 526–533.

Jeyasekaran G, Ganesan P, Jeya Shakila R, Makeshwari K and Sukumar D. (2004). Dry ice is a novel chilling medium along with water ice for short-term preservation of fish Emperor breams, lethirnus (*Lethrinus miniatus*). *Innovative Food Science Emerging Technologies* 5(4): 485–493.

Jeyasekaran G, Jeya Shakila R, Sukumar D, Ganesan P and Anandaraj R. (2010). Quality changes in squid (*Loligo duvaucelli*) tubes chilled with dry ice and water ice. *Journal of Food Science and Technology* 47: 401–407.

Ke PJ, Fierheller M and Lomon DW. (1991). Studies on processing technology for Atlantic shortfin squid (*Illex illecebrosus*). *Lebensmittel-Wissenschaft und-Technologie* 24(4): 328–333.

Kreuzer R. (1984). Cephalopods: Handling, processing and products. *FAO Fisheries Technical Paper No. 254*. Rome, Italy: FAO, p. 154.

Lakshmanan PT, Varma PRG and Iyer TSG. (1993). Quality of commercially frozen cephalopod products from India. *Food Control* 4(3): 159–164.

Lapa-Guimaraes J, De-Silva MAA, De-Felicio PE and Guzman EC. (2002). Sensory, color and psychrotrophic bacterial analyses of squids (*Loligo plei*) during storage in ice. *Lebensmittel-Wissenschaft und-Technologie* 35(1): 21–29.

Lechevallier MW, Seidler RJ and Evan TM. (1980). Enumeration and characterization of standard plate count bacteria in chlorinated raw water supplies. *Applied and Environmental Microbiology* 40(5): 922–930.

Lima dos Santos CAM, James D and Teutscher F. (1981). Guidelines for chilled fish storage experiments. *FAO Fisheries Technical Paper No. 210*. Rome, Italy: FAO, p. 22.

Ohashi E, Okamoto M, Ozawa A and Fujita T. (1991). Characterization of common squid using several freshness indicators. *Journal of Food Science* 56(1): 161–163.

Paarup T, Sanchez JA, Moral A, Christensen H, Bisgaard M and Gram L. (2002b). Sensory, chemical and bacteriological changes during storage of iced squid (*Todaropsis eblanae*). *Journal of Applied Microbiology* 92(5): 941–950.

Paarup T, Sanchez JA, Pelaez C and Moral A. (2002a). Sensory, chemical and bacteriological changes in vacuum-packed pressurized squid (Todaropsis eblanae) stored at 4 °C. *International Journal of Food Microbiology* 74(1): 1–12.

Prafulla V, Francis L and Lakshmanan PT. (2000). Effect of different methods of icing on the quality of squid and cuttlefish during storage. *Fishery Technology* 37(2): 81–88.

Putro S. (1989). Dry ice - possible uses in fresh and live fish handling. *INFOFISH International* 4: 24–25.

Raghunath MR. (1984). Soluble nitrogen losses in squids (*Loligo duvauceli*) during storage in slush ice. *Journal of Food Science and Technology* 21(1): 50–52.

Randell K, Hattula T, Skytta E, Sivertsvik M, Bergslien H and Ahvenainen R. (1999). Quality of filleted salmon in various retail packages. *Journal of Food Quality* 22(5): 483–497.

Sasi M, Jeyasekaran G, Shanmugam SA and Jeya Shakila R. (2000). Chilling of fresh fish in wet and dry ice. *Asian Fisheries Science* 13: 375–382.

Sasi M, Jeyasekaran G, Shanmugam SA and Jeya Shakila R. (2003). Evaluation of the quality of seerfish (*Scomberomorus commersonii*) stored in dry ice (solid carbon dioxide). *Journal of Aquatic Food Product Technology* 12(2): 61–72.

Schoemaker R. (1990). Shipping of fresh seafoods. *INFOFISH International* 4: 27–30.

Sivertsvik M, Jeksrud WK and Rosnes JT. (2002). A review of modified atmosphere packaging of fish and fishery products – significance of microbial growth, activities and safety. *International Journal of Food Science and Technology* 37(2): 107–127.

Stroud GD. (1978). Squid. *Torry Advisory Note* No. 77. p. 6.

Surendran PK, Thampuran N, Nambiar VN and Lalitha KV. (2003). *Laboratory Manual on Microbiological Examination of Seafood*. Cochin, India: Central Institute of Fisheries Technology.

USFDA (2001). *Bacteriological Analytical Manual*, 8th edn. Revision A. Gaithersburg, Md: United States Food and Drugs Administration, AOAC International.

Vaz-Pires P and Barbosa A. (2004). Sensory, microbiological, physical and nutritional properties of iced whole common octopus (*Octopus vulgaris*). *Lebensmittel-Wissenschaft und-Technologie* 37(1): 105–114.

Yamanaka H, Shiomi K and Kikuchi T. (1987). Agmatine as a potential index for freshness of common squid (*Todarodes pacificus*). *Journal of Food Science* 52(4): 936–938.