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ORIGINAL ARTICLE

# Quality changes in squid (*Loligo duvaucelli*) tubes chilled with dry ice and water ice

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**Abstract** Squid tubes were packed with 100% (w/w of squid) dry ice (PI), 20% dry ice and 50% water ice (PII) and 50% water ice (PIII) in polyethylene bags and store in thermocole boxes at room temperature (32 ± 2°C) for 24 h. Quality changes curing storage were studied. Lowest temperature of -30.3°C was attained in PI while it was 15–16°C in PII and PIII at 1 h of storage. The gas compositions in packages initially were 21% $O_2$, 0.4% $CO_2$ and 78.1% $N_2$ in PI, PII and PIII, respectively. During storage for 24 h highest level of 82.5% $CO_2$ was noticed in PII. Fresh squid tubes had bacterial flora of *Hafnia, Pseudomonas, Bacillus, Flavobacterium* and *Alcaligens. Hafnia* constituted 74% of the flora. *Alcaligenes* (47%), *Alteromonas* (30%) and *Alcaligenes* (56%) were dominant in squid tubes stored in 100% dry ice, in the combination package, and in 100% water ice, respectively. Increase in total volatile base nitrogen and trimethylamine nitrogen, no definite trend in free fatty acid values in all packages while increase in pH in PI and PIII and no consistent changes in PI were noticed during storage for 24 h. The PI had lowest bacterial counts and PIII the highest. Squids stored in PI and PII were sensorily acceptable after 24 and 18 h, respectively.

**Keywords** Squid · Dry ice · Water ice · Bacteriological · Biochemical · Sensory quality

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## Introduction

Cephalopods constitute an important part of the marine resource and most suitable for human consumption. Squid belonging to the genus, *Loligo* is generally regarded as more valuable in the world markets (Sikorski and Kolodjska 1986). The appearance of meat depends on its environment especially the temperature and type of illumination (MacDougall 1985). Chilling of squids with water ice have some disadvantages such as more drip loss, textual toughness, nutrient loss and decreased protein extractability (Putro 1989). Frozen cephalopods particularly cuttlefish and squid, form a major component in the marine products export of India. The most common chilling medium is water ice. Water ice is used at 100% level and even more under tropical conditions (Lima dos Santos et al. 1981) resulting in exorbitant cost of air transportation, besides the problems of melting of ice and leaching and hence alternative methods of icing are needed (Prafulla et al. 2000). Dry ice has currently gained popularity in India as a novel freezing medium for the rapid transportation of fresh fish by air (Jeyasekaran et al. 2004a). Since squid is one of the important fish varieties that is chilled and exported from India, the present study was undertaken to find out the effect of chilling with dry ice and water ice on its quality.

## Materials and methods

Squid (*Loligo duvaucelli*) were procured from a fish landing center of near the laboratory is located. Time interval between harvesting and arrival of fish at the lending center was 6-8 h and during this period they were iced. Whole squid had an average length of 33 cm and weight of 96 g. They were immediately brought to the laboratory in insulated containers, washed with potable water and tubes were prepared. The tubes had an average length and weight of 18 cm and 72 g, respectively. The tubes were divided into 3 lots. Each lot had a weight of about 20 kg, and the number of tubes in each lot was 280. Each lot was again divided into 10 packs. First lot was packed with 100% dry ice (PI) (Thermosafe Dry ice Machine, USA), second lot with a combination of 20% dry ice and 50 % water ice (PII) (Sasi et al. 2000) and the third with 100 % water ice (PIII) (Ziegra Flake ice Maker, Germany), which served as control (Lima dos Santos et al. 1981). Packages were wrapped in polythene bags, placed in conical shaped thermocole boxes and sealed airtight with cellophane tape. Thermocole boxes were stored at room temperature (32 ± 2°C). One pack from each lot was periodically analyzed in triplicate for quality.

*Physical parameters:* Changes in temperature of all the packages were recorded by using ultrafreezer temperature probe (Consort Model T 852, Belgium). The changes in gas composition of samples stored in different packages were measured by gas analyzer (PBI Dansensor CheckMate 9900, Denmark). pH was determined by pH meter (Digisun Electronics Digital pH Meter 707, India) by taking 10 g of homogenized sample in 100 ml distilled water.

*Biochemical parameters:* Total volatile base nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) contents were determined by the Conway micro-diffusion method (Cobb et al. 1973). Free fatty acid (FFA) content (Pearson 1968) and Hypoxanthine (Hx) content (Howgate 1982) were also estimated.

*Bacteriological quality:* Total bacterial load, psychrophiles, lactics, coliforms and anaerobic sulphite reducers were determined using the media obtained from Hi-media Mumbai, India. Squid tube muscle was cut using sterile knife and forceps, pooled together and mixed by cutting into very small pieces. Sample (25 g) was homogenized using 225 ml physiological saline and serial decimal dilutions of each homogenate were carried out with the same diluent for the respective bacteriological analysis (APHA 2001).

*Sensory evaluation:* Overall sensory quality based on general appearance including colour, odour and texture of squid tubes was assessed by a panel of 6 experienced members of our faculty on the basis of 10-point scale (10-9 excellent, 8-7 good, 6-5 fair and acceptable, 4-3 poor and 2-1 very poor) (Kreuzer 1984).

*Statistical analysis:* Experiments were carried out in triplicate. 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

*Physical parameters:* Immediately after packaging, the temperatures recorded in PI, PII and PIII were 30.2, 15.8 and 15.4°C, respectively (results not shown). At that time, room temperature was 32.4°C. Lowest temperature of –30.3°C was observed in PI at the 1 h and the subzero temperatures were maintained until 6 h of storage, whereas in PII and PIII, the lowest temperature recorded was 7.4 and 7.0°C, respectively at 3 h and 5 h of storage. After that, it increased gradually to 31–32°C in 24 h in all 3 packages (results not shown).

The atmospheric gas composition was 21.5% $O_2$, 0.4% $CO_2$ and 78.1% $N_2$. After 1 h of packing, the corresponding concentrations were 6.3%, 68.6% and 25.1% in PI, 3.71%, 82.5% and 13.8% in PII and 20.8%, 0.7% and 78.5% in PIII (Fig. 1). Highest level of about 82% $CO_2$ was noticed on the 6 h in PI and 1 h in PII, which was due to packing of squid tubes in dry ice. On the other hand, slight variation was observed in PIII throughout the storage period. The longer shelf life obtained in dry ice packed squid tubes (see later)

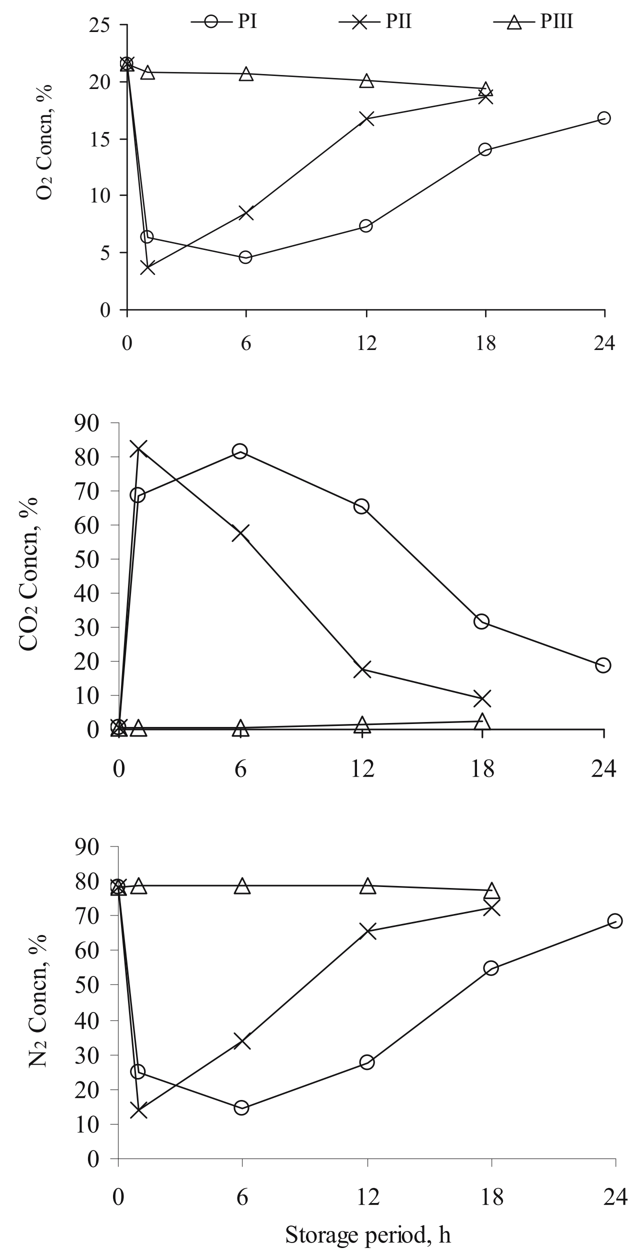


**Fig. 1** Changes in gas concentration during storage of squids in 3 different packages (n=3)

might be due to high content of $CO_2$ in such packages (Clark and Lentz 1969).

*Biochemical changes:* Fresh squid tubes had a TVB-N content of 11.2 mg/100 g, which increased gradually to 19.9, 20.6 and 52.8 mg/100 g at the end of the storage period in PI, PII and PIII, respectively (Fig. 2). Moral et al. (1983) reported an increase in TVB-N content in squid (*Loligo vulgaris*) during its storage in ice. Prafulla et al. (2000) observed a higher TVB-N content of 31.6 mg/100 g in whole iced cuttlefish at the end of storage period. It has also been reported that the rejection limit for TVB-N in fish varied with species and processing condition (Dalgaard 2000).

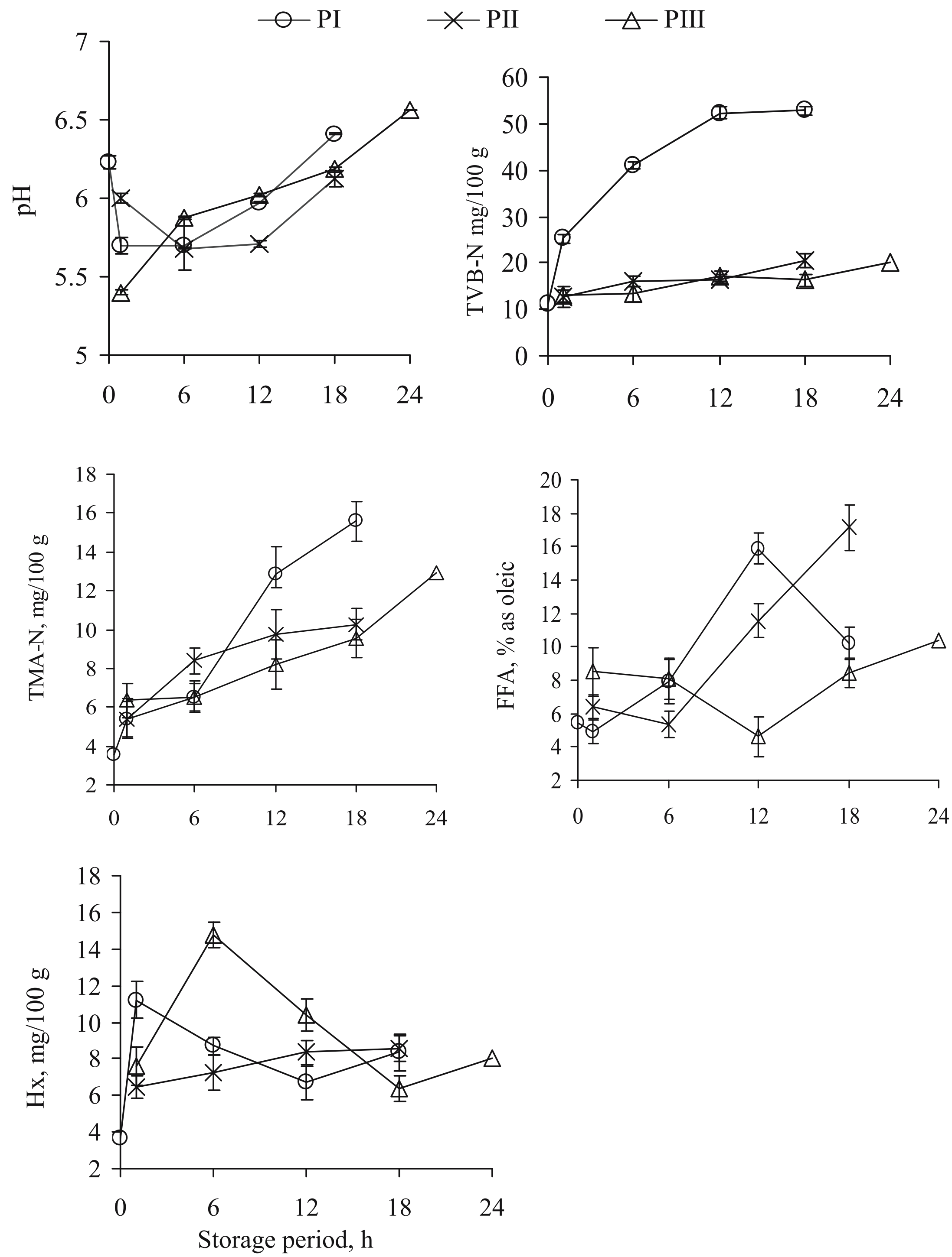


**Fig. 2** Chemical changes in squids stored under different packages (n=3). TVB: Total volatile base nitrogen; TMA-N: Trimethylamine nitrogen; FFA: Free fatty acids; Hx: Hypoxanthine

Initial TMA-N content was 3.5 mg/100 g, which increased gradually in all 3 packages (Fig. 2) similar to TVB–N values. Ruiz-Capillas et al. (2002) found low levels of TMA-N contents (less than 1 mg/100 g) in ice stored volador (*Illex coindetii*), pota (*Todaropsis eblanae*) and octopus (*Eledone cirrhosa*). Prafulla et al. (2000) also observed a TMA-N content of 5.6 mg/100 g in whole cuttlefish at the end of storage period in ice. However, Paarup et al. (2002b) observed very high level of TMA-N content (above 40 mg/100 g) at the end of storage. Rate of increase in TMA-N in marine fish varied considerably from species to species (Huss 1988).

Initially the fresh tubes had FFA content of 5.4 % as oleic acid. During storage, the FFA content did not show any consistent trend in all 3 packages (Fig. 2). Garg and Stephen (1982) found FFA content of 1.65% in kati (*Pellona* sp.) stored in ice for 13 days. However, Kyrana and Lougovois (2002) reported FFA content of 2.73% in farm-raised European seabass (*Dicentrachus labrax*), which was stored in melting ice for 22 days.

Initial hypoxanthine level was 3.7 mg/100 g and it did not show any consistent trend throughout the storage period in all 3 packages (Fig. 2). However, Prafulla et al. (2000) observed an increasing trend of hypoxanthine content in whole cuttlefish stored in ice.

Fresh squid tubes had an initial pH value of 6.2. At 1 h, it decreased to 5.4, 6.0 and 5.7 in PI, PII and PIII, respectively (Fig. 2). On further storage, it was in the increasing trend in PI and PIII, whereas it did not show any consistent trend in PII. Paarup et al. (2002b) reported that the pH ranged from 6.8 to 7.8 in squid mantle (*Todaropsis eblanae*) stored at 4°C. Prafulla et al. (2000) observed that the pH of squid and cuttlefish muscle stored in ice did not vary significantly. The pH value depends on many factors, such as the time that has

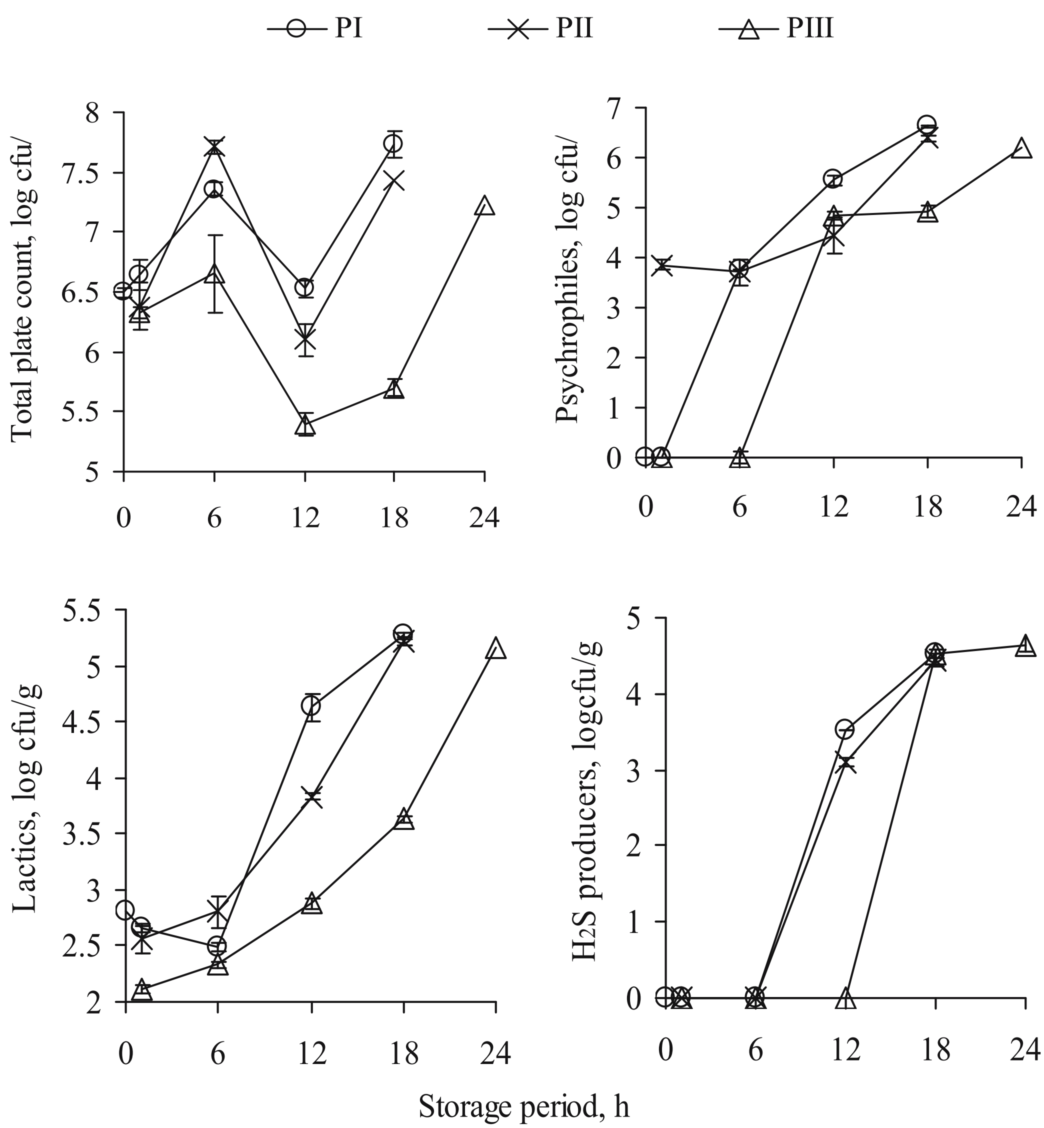


**Fig. 3** Changes in microbial quality of squids during storage under different packages (n=3)

elapsed since the capture, storage temperature and physiological state of animal (Moral 1987).

*Bacteriological quality:* The initial load of 6 log cfu/g was maintained till 1 h in PII and PIII and increased by a log in 6 h and decreased by a log in 12 h and again increased by a log to 7 log cfu/g, whereas in PI, the initial count decreased by a log and this was maintained until 18 h of storage (Fig. 3). The load further increased to 7 log cfu/g at 24 h. Paarup et al. (2002b) reported that the aerobic plate count of squid mantles stored at 4°C increased from an initial level of 4 to 7 log cfu/g, when it was sensorially rejected. Pires and Barbosa (2004) found a bacterial load of 5–6 log cfu/cm$^2$ in octopus (*Octopus vulgaris*) stored in crushed ice at the point of rejection. Prafulla et al. (2000) observed that the total plate count did not reach 7 log cfu/g.

Psychrophiles appeared only on the 1 h (3 log cfu/g) in PII, 6 h in PIII (3 log cfu/g) and 12 h (4 log cfu/g) in PI (Fig. 3). On storage, the count gradually raised to 5 log and 6 log cfu/g in PI and PII, respectively at the end of storage period. In PIII, the count drastically increased from 3 to 5 log cfu/g at 12 h and finally reached to 6 log cfu/g. Lapa-Guimaraes et al. (2002) also observed a psychotropic bacterial count of 6 log cfu/g in squid (*Loligo plei*) after 16 days of storage in ice.

Initial lactics count was 2 log cfu/g. Up to 6 h of storage, the count was maintained at the same level in all 3 packages and after that it gradually increased to 5 log cfu/g in PII, whereas in PI, no change in lactics count till 12 h and then increased and reached to 5 log cfu/g at the end of storage period (Fig. 3). Paarup et al. (2002b) also detected lactic acid bacteria in squid mantle (*Todaropsis eblanae*), but in low numbers (2 log cfu/g) throughout the storage period at 4$^0$C.

$H_2S$ producers were found only at 12 h with a load of 4 log cfu/g in PII and PIII, which increased by a log at 18 h in both (Fig. 3). On the other hand, in PI, the count appeared only on the 18 h (4 log cfu/g), which was maintained up to 24 h. Civera et al. (1999) reported that counts of specific spoilage indicators ($H_2S$ producer) were appropriate to determine the early stage of deterioration in cephalopods during iced storage.

Fresh tubes had a coliform count of 2 MPN/g, which maintained on the 1 h in PI, whereas it was found to be less than 1.0 MPN in PII and PIII (results not shown). At 18 h, the coliform was 5, 40 and 33 MPN/g in PI, PII and PIII, respectively. Coliforms attained a higher level of 1600 MPN/g at the end of storage period in all 3 packages. Pires and Barbosa (2004) reported that total enterobacterial count particularly coliforms was less than 10$^2$ cfu/cm$^2$ in iced octopus (*Octopus vulgaris*). Paarup et al. (2002b) also observed the same result in squid mantle (*Todaropsis eblanae*) stored at 4°C. Ramachandran et al. (1990) found the presence of coliforms particularly *E. coli* in immediately iced Chinese herring, *Hilsa toli* at 2.60 × 10$^2$/g: however, it did not survive after 2 days of storage. The initial total anaerobic sulphite reducers were 25 MPN /g (results not shown). On further storage, they were not showing any consistent trend in all the three packages. The exact reason for the inconsistent counts of anaerobic sulfite reducers in ice-stored fish was not known; however, the partial aerobic and anaerobic conditions prevailing during iced storage could have attributed to cause such differences (Jeyasekaran et al. 2004b).

Squid tubes used in this study carried a bacterial flora of *Hafnia, Pseudomonas, Bacillus, Flavobacterium* and *Alcaligenes*. *Hafnia* constituted about 74% of the initial flora. Paarup et al. (2002a) isolated a heterogeneous group of Gram-negative bacteria in fresh squid. *Alcaligenes* (47%) was the dominant species in the tubes stored in PI followed by *Acinetobacter, Alteromonas, Plesiomonas, Flavobacterium, Micrococcus* and *Hafnia*. There was a shift in the bacterial flora when the tubes were stored in different ice packages. *Alcaligenes*, which was very low in numbers (3%) in the fresh squid tubes, became the dominant flora in dry ice stored tubes. Paarup et al. (2002a) also found that dominating flora in squid stored at 4°C included motile, Gram-negative rods. In the squid tubes stored in PII, *Alteromonas* was found to be in high numbers (30%) followed by *Micrococcus, Hafnia*, *Alcaligenes, Escherichia, Staphylococcus, Aeromonas, Flavobacterium, Acinetobacter, Vibrio, Plesiomonas* and *Bacillus*. Earlier investigators also reported that certain gram-negative bacteria are the main cause of spoilage, especially *Shewanella* (formerly *Alteromonas /Pseudomonas*) *putrefaciens* was the most important fish spoilage bacteria of marine fish stored at 0$^0$C (Hobbs and Hodgkiss 1982, Gram et al. 1987). *Alcaligenes* (56%) was the dominant species in the case of tubes stored in PIII, followed by *Hafnia, Alteromonas, Bacillus, Pseudomonas, Vibrio, Acinetobacter, Staphylococcus* and *Micrococcus*. However, Surendran et al. (1985) observed that *Acinetobacter* was the dominant spoilage flora in ice stored shrimps, *Penaeus indicus, Metapenaeus dobsoni* and *M. affinis*. Paarup et al. (2002b) also reported that *Vibrio* and *Aeromonas* were in low levels in gutted squid stored in ice at the end of storage.

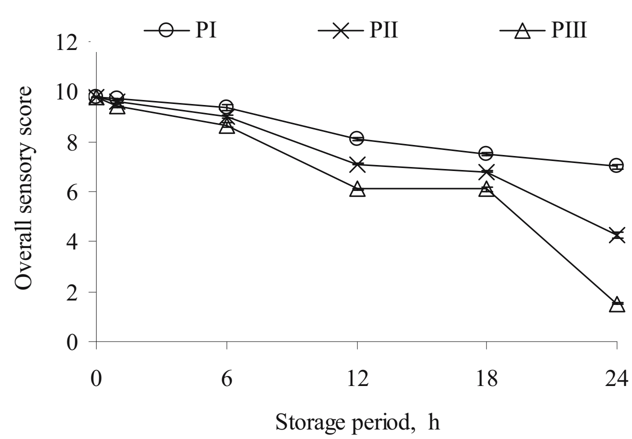


**Fig. 4** Changes in overall sensory scores of squids during storage under different packages (n=6 panelists)

*Sensory quality:* Squid tubes had fresh meat and seaweedy odor, bright white in color with shiny appearance and firm texture and were considered as excellent with the overall score of 9.8. No remarkable change was observed in packages PII and PIII at 6 h of storage and exhibited slight sulphide odor at 12 h, but its texture and color were good (Fig. 4). This condition was maintained at 18 h in PII, whereas, in PIII, a slight loss of textural stiffness was observed with meat color became pale yellow having a score of 6.5. On the 24 h of storage, PII and PIII were sensorially unacceptable with putrid odor and mushy texture. On the other hand, PI was in frozen condition at 1 h of storage, which remained normal only at 12 h. At 18 h of storage, slight bleach in color was observed and discoloration and slight sulfide odor were noticed at 24 h with a score of 7.0. Paarup et al. (2002a) reported that the mantles from whole and gutted squid (*Todaropsis eblanae*) stored in ice were rejected sensorially after 10 and 12 days of storage, respectively. Strong ammoniacal off-odors were also detected by Paarup et al. (2002b) in the squid mantles stored at 4°C by the end of storage life. Prafulla et al. (2000) also observed that the whole cuttlefish was in acceptable condition up to 12 days in ice. Civera et al. (1999) reported that common cuttlefish (*Sepia officinalis*), musky octopus (*Eledone moschata*) and broadtail squid (*Illex coindetii*) showed decreasing sensory quality after 7 days of storage at 1–2°C, being rejected at the 10 day of storage. The variation in the shelf life observed by different workers for cephalopods may be due to the difference in the storage temperatures,the species,form (whole/ gutted/ mantles/ fillets) of cephalopods and the method of icing (Pires and Barbosa 2004). Since reicing was not done in our study, the squid tubes were sensorially unacceptable on the 24 h of storage.

## Conclusion

The results showed that the squid (*Loligo duvaucelli*) tubes stored in 100% dry ice had longer shelf life and better quality when compared to their storage in water ice (100%) as well as in the combination package of dry ice (20%) and water ice (50%) on the basis of important quality indicators. But, the use of dry ice alone for packing chilled fish is very expensive and at times results in freezing. Even though the results indicated that the squid tubes stored in the combination package of dry ice (20%) and water ice (50%) and in only water ice (100%) had same shelf life, the quality of squid tubes stored in combination package were certainly better than that of water ice stored tubes. The seafood industries that are involved in the export of chilled fish and shellfish by air may adopt the combination of dry ice and water ice at the level of 20%:50% for short-term transport of chilled squid tubes by air.

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