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Quality changes in Bombayduck *Harpodon nehereus* stored in slurry ice and flake ice: A comparative study

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ABSTRACT

The effect of slurry ice on quality changes of Bombayduck (*Harpodon nehereus*) was evaluated in comparison with conventional flake ice. Biochemical, microbiological and sensory analyses were carried out upto 16 days. Total volatile base nitrogen (TVB-N), trimethylamine (TMA) and pH were found to be lower in fish stored under slurry ice (SI). Similarly, lower peroxide value (PV) of 19.25±1.1 meq O_2 kg^{-1} was recorded for SI on 16th day of storage, while fish stored in flake ice (FI) recorded PV of 20.15±0.9 meq. O_2 kg^{-1} on 13th day. TBA values were found to be higher in SI than FI. However, all the samples had TBA value within the limit of acceptability. Salt content ranged between 0.15-0.28 and 0.15-2.2% in FI and SI respectively.Total plate count (TPC), psychrophilic bacterial count, *Pseudomonas* spp. and H_2S producers showed an increasing trend during storage. In the present study, psychrophilic bacterial count served as a good microbiological quality indicator than TPC. Results revealed that slurry ice was able to control both enzymatic and microbial activity in fish during storage period as indicated by the extended shelf life of Bombayduck upto 15 days of storage as compared to 12 days recorded for control samples.

Keywords: Bombayduck, Flake ice, *Harpodon nehereus*, Slurry ice, Shelf life

Bombayduck (*Harpodon nehereus*) is one of the major marine species abundant along the north-west coast of India. It is consumed domestically in fresh and dried form (Balli *et al*., 2011). Bombayduck occupied 4th position after the Indian oilsardine, mackerel and ribbon fish catch in all India marine fish landings during 2017 (CMFRI, 2018). Fishing season for Bombayduck commences from September to January along the north-west coast of India. During the peak season, a large quantity of catch are sundried and exported. Due to high moisture content (89%), it is prone to spoilage by enzymatic and bacteriological activity. Therefore, once the fish is caught, it should be immediately kept under chilled or refrigerated condition in order to preserve the quality of fish. Traditionally block ice or flake ice is used for preservation of fish.

There is a demand for alternate icing system for preservation of seafood. It has been reported that slurry ice could maintain the quality of fish onboard as compared to block ice (Rodriguez *et al*., 2006; Mugica *et al*., 2008). Slurry ice is the mixture of ice-water with brine solution, seawater or glycol. It is also known as liquid ice or flow ice. Slurry ice provides rapid chilling due to large heat transfer surface area facilitated by the presence of numerous ice micro-crystals and it also reduces physical damage to seafood as it covers the fish surface completely which reduces dehydration and oxidation. Slurry ice units can be fixed onboard and ice can be made as per requirement during fishing voyage. Slurry ice system can be operated either by diesel engine or through electrical power. Several authors have reported that fish and shellfish stored under slurry ice had extended shelf life (Mugica *et al*., 2008; Zhang *et al*., 2015; Narasimha Murthy *et al*., 2017; Jeyakumari *et al*., 2018). They also found that the use of slurry ice instead of traditional ice decreased bacterial counts and reduced the production of off-flavours in fish. Several studies have reported the quality of Bombayduck dried under different conditions and storage systems. Jeyakumari *et al*. (2017) studied the possibilities of better utilisation of Bombayduck for the development of battered and breaded fish fingers. However, very few studies have been carried out on effect of icing and freezing on Bombayduck (Reza *et al*., 2009; Parvathy *et al*., 2016). Hence, the present study was aimed to evaluate the biochemical, microbiological and sensory quality of Bombayduck preserved in slurry ice in comparison with fish preserved in conventional flake ice.

Fresh Bombayduck (200-250 g size; 20±0.5 cm) was procured from local fish market at Vashi, Navi Mumbai and brought in ice (fish to ice ratio = 1:1) to the fish processing laboratory. Two sets of fish samples were prepared and the first set FI was stored in flake ice and the second set (SI) was stored under slurry ice. The ratio of fish to flake ice/slurry ice was maintained at 1:1. Slurry ice was prepared from filtered seawater (salinity: 3.5%) using ICEFLOW (Chirag, Navi Mumbai, India) machine, which comprised BOCK F4 compressor (1800 rpm), wherein R-22 was used as a refrigerant. Flake ice was prepared from potable water using lab scale flake ice machine (BANWAY-IRC, New Delhi, India). During storage period, required ice was added to compensate the melted ice. The fish samples were taken for analysis at known intervals (two days interval for biochemical quality analysis; microbial and sensory analysis were done at two days interval up to 10 days and thereafter sampling was done daily) until overall acceptability rejection was reached.

Proximate composition, peroxide value (PV) and salt content were estimated according to AOAC (2012). pH of fish homogenate was determined using digital pH meter (Cyberscan 510, Eutech Instruments, Singapore) according to AOAC (2012). Total volatile base nitrogen (TVB-N) and trimethylamine (TMA) content was evaluated as described by Conway (1950). Thiobarbituric acid (TBA) value was estimated as per Tarladgis *et al.* (1960).

Sensory evaluation was done by trained panelists in fish processing, following the method of Meilgaard *et al.* (1999). A score of seven to nine was considered as acceptable while score below five was considered unacceptable.

Total plate count (TPC) and psychrophilic bacterial count was evaluated as per FAO (1992). *Pseudomonas* species count was determined according to Mead and Adams (1977). Hydrogen sulphide (H_2S) producing bacteria were enumerated as described by Koneman *et al.* (1992) with slight modification. Serially diluted fish tissue homogenate samples were spread over preset peptone iron agar (PIA) plates and incubated at 22°C. After 5 days of incubation, black coloured colonies were considered as H_2S producing bacteria.

The data obtained were analysed by one way analysis of variance (ANOVA) using SPSS software version 16.0 (SPSS Inc, Chicago, Illinois, USA). All mean separations were tested at a significance level of 5%.

Fresh Bombayduck had 88.92±0.20% moisture, 9.65±0.15% protein, 0.85±0.02% fat and 0.92±0.01% ash. The present results are in accordance with earlier reports for fresh Bombayduck (Chakrabarti, 2010; Jeyakumari *et al*., 2017). In general, both FI and SI showed increasing trend in moisture content during storage. Moisture content of FI increased from 88.93±0.25% to 91.46±0.35%. Similarly, moisture content of SI increased from 88.93±0.30% to 89.26± 0.28% (Fig. 1a). Initial pH of fish was 6.70 and showed gradual increase during storage. Lower pH values were observed for SI than FI (Fig. 1b). Results were in accordance with previous reports for fish stored under slurry and flake ice (Rodriguez *et al*., 2006; Jeyakumari *et al*., 2018). Salt content was slightly higher in SI than FI samples. This might be due to the salt content in slurry resulting in uptake of salt in fish during storage. It has been reported that slow absorption of salt does occur into the fish stored under slurry ice (Losada *et al*., 2005; Aubourg *et al*., 2007).

Total volatile base nitrogen (TVB-N) is used to measure the level of spoilage in fish stored under ice. Initial TVB-N content in fish was 4.56±0.15 mg% which showed an increasing trend during storage. It has been reported that TVB-N and TMA values for freshly caught fish ranged from 5-20 mg% and 0.5-2.0 mg%, respectively and these values increased as spoilage progressed (Connell, 1995). Results indicated that fish used for the study was fresh and TVB-N content was comparatively

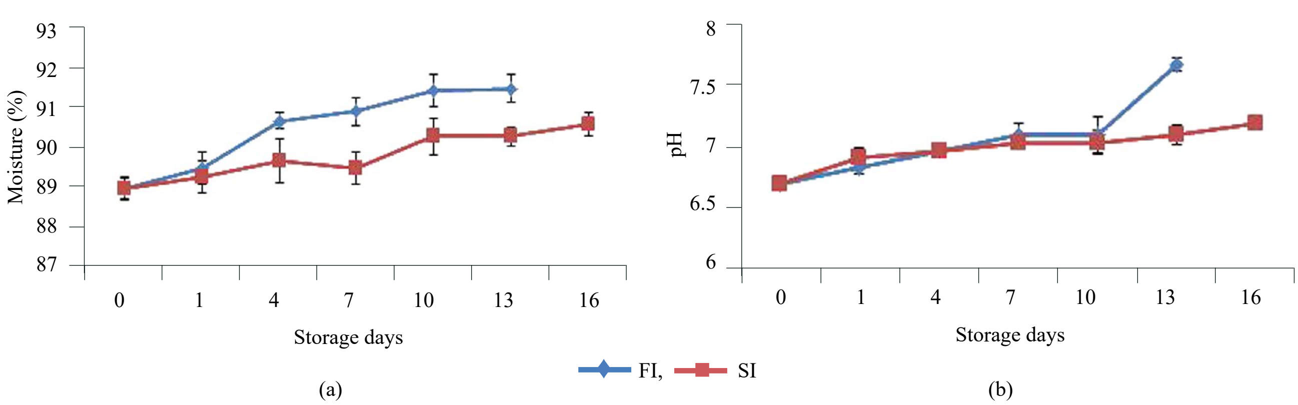

Fig. 1. (a) Moisture content and (b) pH in Bombayduck stored in slurry ice (SI) and flake ice (FI)

lower in fish stored under slurry ice. However, TVB-N content was within the acceptable limit in both the samples during storage (Fig. 2a). Jeyakumari *et al*. (2018) found comparable results for croaker fish preserved in slurry ice. The formation of trimethylamine (TMA) during iced storage of fish occurs mainly due to reduction of trimethylamine oxide in fish by microbial activity (Aubourg *et al*., 2007). The level of TMA is used as spoilage index for marine fish. It was observed that TMA content was nil upto 4th day and showed gradual increase during storage. Similar to TVB-N, trimethylamine content was also within acceptable limit during storage. There was no significant difference ($p>0.05$) in TMA content in both systems. Results indicated that only limited growth of TMA-producing bacteria occurred in the fish samples under SI and FI. Campos *et al*. (2006) noticed parallel results for farmed turbot kept under slurry ice. Overall, the results for TVB-N and TMA content in SI and FI were found to be poor indicators of spoilage and did not show substantial increase even when fish was considered as spoiled according to the sensory evaluation. Results were in agreement with previous reports for freshwater fish (Chytiri *et al*., 2004; Nath and Majumder, 2017).

Peroxide value (PV) measure the primary oxidation levels of fish and fishery products. Peroxide value of fresh fish used for the study was found to be nil and a gradual increase was noticed in both samples, which reached 20.15 meq O_2 kg^{-1} on 13th day for FI, whereas, SI sample recorded PV of 19.25 meq O_2 kg^{-1} on 16th day (Fig. 2b), The difference in PV between the SI and FI samples were found to be statistically significant ($p<0.05$). Rodriguez *et al*. (2006) observed similar results for farmed turbot stored in slurry ice. Thiobarbituric acid (TBA) value is used to measure the secondary lipid oxidation products. It has been reported that TBA values of 1-2 mg of MDA kg^{-1} is considered as acceptable beyond which fish will usually develop an objectionable odour (Connell, 1995). However, fish can be consumed up to 5-8 mg MDA kg^{-1} of TBA content (Adenike, 2014). In the present study, SI samples showed significantly higher ($p<0.05$) TBA values than the FI samples (Fig. 2c). Results indicated that both SI and FI samples had an acceptable limit of TBA values during storage period.

Fresh Bombayduck had an initial total plate count (TPC) of 3.2 log cfu g^{-1}. The lower TPC on initial day

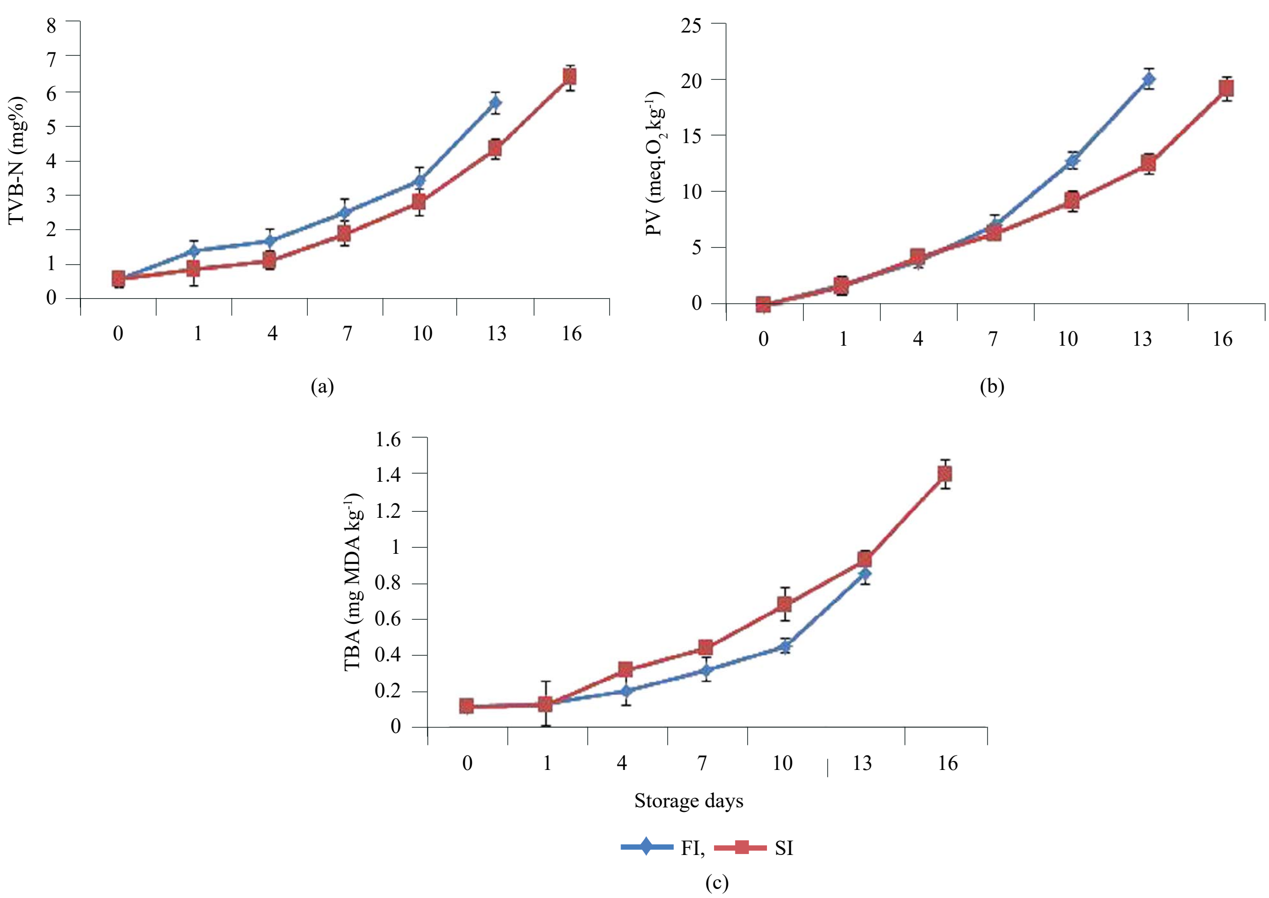

Fig. 2. (a) TVB-N, (b) PV and (c) TBA levels in Bombayduck stored in slurry ice (SI) and flake ice (FI)

indicated that fish used for the study was fresh and of good quality. TPC showed increasing trend during storage and reached 5.09 log_{10} cfu g^{-1} in FI samples on the day of rejection (13^{th} day) while SI samples recorded 4.95 log_{10} cfu g^{-1} on the day of rejection (16^{th} day) (Fig. 3a). Significant difference ($p<0.05$) was noticed in TPC between the SI and FI samples. Results indicated that TPC was within the limit of acceptability (7 log cfu g^{-1}) during storage (ICMSF, 1998) and comparatively lower microbial growth in slurry ice which are in agreement with previous reports (Rodriguez *et al*., 2006; Mugica *et al*., 2008). In general, TPC and psychrophilic bacterial count are used to assess the bacteriological quality of fish stored under ice. Psychrophilic bacteria grow rapidly at or below 7.2°C. In the present study, psychrophilic bacteria showed gradual increase during storage and reached 6.9 log cfu g^{-1} and 7.39 log cfu g^{-1} on the 12^{th} and 13^{th} day respectively for FI samples, whereas it was 6.5 log cfu g^{-1} and 7.27 log cfu g^{-1} on the 15^{th} and 16^{th} day respectively for fish stored under-slurry ice (Fig. 3b). Psychrophilic count showed significant difference ($p<0.05$) between the SI and FI samples. Results indicated that psychrophilic bacterial count can be considered as a good microbiological quality indicator than TPC for Bombayduck stored under ice. Similar results were also observed for cobia fish stick and seabass stored under refrigerated condition (Kilinc *et al*., 2007; Gonçalves and Santos, 2018).

Pseudomonas spp. form specific spoilage microorganism of fish and shellfish stored in ice or under refrigerated condition (Akintola and Bakare, 2011). Initial *Pseudomonas* spp. count in SI was 2.94 log cfu g^{-1} and it increased to 5.2 log cfu g^{-1} on the day of rejection. Whereas, FI had *Pseudomonas* spp. count of 6.13 log cfu g^{-1} on the day of rejection (Fig. 3c). Jeyakumari *et al*. (2018) reported that *Pseudomonas* spp. served as a better microbial spoilage indicator for croaker fish stored under slurry ice than aerobic plate count. However, in the present study *Pseudomonas* spp. count did not cross 7 log cfu g^{-1} on the day of rejection (Mai and Huynh, 2017; Zaid *et al*., 2014). Previous researchers reported that fish stored in slurry ice had a lower enzymatic and microbial activity (Campos *et al*., 2006; Rodriguez *et al*., 2006). H_2S forming bacteria are also classified as spoilage bacteria of fish stored in iced condition. Similar to *Pseudomonas* spp., H_2S producer count also was found to be lower in SI (5.8 log cfu g^{-1} on 13^{th} day) as compared to FI (5.9 log cfu g^{-1} on 13^{th} day) during storage (Fig. 3d).

The raw material used for the study were fresh with species-specific taste, natural flavour and odour. It was

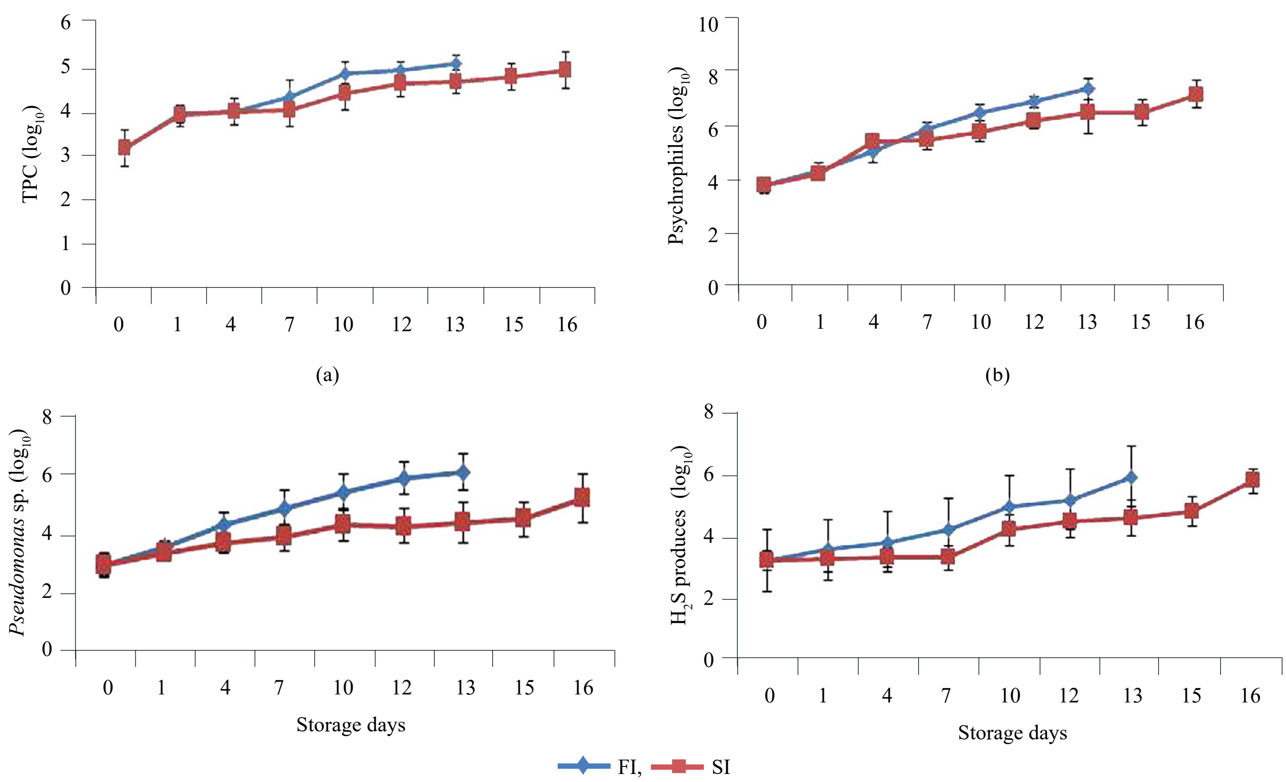

Fig. 3. (a) TPC, (b) Psychrophilic count, (c) *Psudomonas* spp. count and (d) H_2S producing bacterial count in Bombayduck stored in slurry ice (SI) and flake ice (FI)

observed that Bombayduck stored in slurry ice retained good quality up to 10th day and was rejected on 16th day. FI samples retained good quality upto 7th day and were rejected on 13th day (Fig. 4). During rejection, fish showed noticeable signs of spoilage, such as bleached gills and belly cavity and unpleasant smell. Results coincided with increase in psychrophilic count in both systems. Reza *et al*. (2009) also observed comparable results for Bombayduck preserved under flake ice. Sensory analysis revealed that SI had a higher score for overall acceptability. Several authors reported that improvement of sensory attributes can be achieved by employing slurry ice (Lasada *et al*., 2005; Jeyakumari *et al*., 2018).

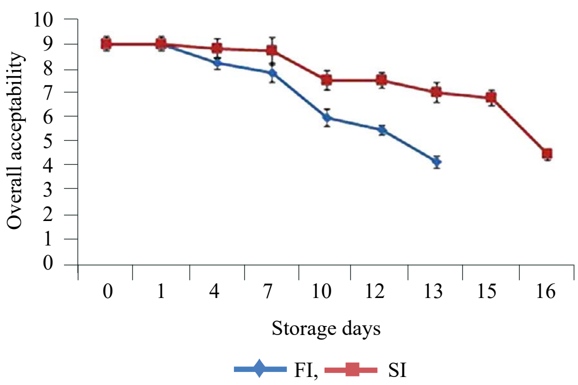

Fig. 4. Overall acceptability of Bombayduck stored in slurry ice (SI) and flake ice (FI)

The effect of slurry ice on the quality of Bombayduck was studied in comparison with flake ice. Biochemical quality parameters such as pH, TVB-N, TMA and PV were found to be lower in SI samples. Microbial analysis revealed that SI had lower total plate count, psychrophilic count, *Pseudomonas* spp. and H_2S forming bacteria. Moreover, except psychrophilic count, others (total plate count, *Pseudomonas* spp. and H_2S forming bacteria) had not crossed the 7 log cfu g^{-1} limit during the storage period. Sensory analysis revealed that SI had a higher score for overall acceptability. Results revealed that slurry ice could control both enzymatic and microbial activity in fish during storage period as indicated by extension of shelf life of Bombayduck upto 15 days as compared to 12 days for the control.

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