



Comparison of Flake ice and Gel ice in the Preservation of *Lethrinus lentjan* (Lacepede, 1802) Fillets

Femeena Hassan* and C. G. Joshy

ICAR-Central Institute of Fisheries Technology, P. O. Matsyapuri, Cochin - 682 029, India

Abstract

Effectiveness of different icing, gel ice and flake ice in extending the shelf life of fish fillets (*Lethrinus lentjan*) (Lacepede, 1802) was evaluated. Samples were taken from preserved samples at two days intervals for organoleptic, biochemical and microbiological analysis up to 16 days of storage. Based on t-test, the biochemical variables viz., pH, Total Volatile Base Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N), ThioBarbituric Acid Value (TBA) Free Fatty Acid (FFA) and Peroxide value (PV) showed a significant difference between two methods of icing at. Organoleptic scores for appearance colour, odour, texture and overall acceptability were tested. The results revealed that there was no significant difference between two methods of icing at 5% level of significance during storage, but the significant difference noticed at 10% level of significance. Based on the biochemical and organoleptic analysis, it is inferred that gel icing was better than flake ice storage for storage up to 8 days. Simple linear regression model (significant R^2 value) to the experimental data of both type of icing to quantify the rate of change which indicated a significant increase in the quality indices as the storage days increased. On the other hand the rate of increase of biochemical quality indices was minimum for samples stored in flake ice.

Keywords: Gel ice, flake ice, quality attributes, shelf life

Introduction

Post mortem changes leading to spoilage is associated with the breakdown of the cellular structure

and biochemical reactions, as well as due to the growth of microorganisms that are either naturally associated with the fish, or due to contamination during handling (Ehira & Uchiyama, 1987). In spite of availability of cold-chain and better transport facilities, distribution of fresh fishery products still remains as a problem (Gram & Huss, 1996; Ward & Baj, 1988). The one factor, which has profound influence on the quality of the fish, is temperature. Being highly perishable, it is essential to cool fish as quickly as possible to a low temperature just above freezing point (Hansen & Jensen, 1982). The common and simplest method of cooling of fish is icing (Govindan, 1985) and this is widely accepted as the most economical and readily available method (Jain et al., 2005). Ice keeps the chilled fish moist and glossy and prevents the dehydration (Graham et al., 1992) and helps to delay, reduce or inhibit the microbial and enzymatic spoilage (Gram, 1992).

Different cooling techniques have been used commercially to reduce the spoilage of fish and to increase shelf life. Dry ice is one material used as coolant for shipping of fresh seafood (Schoemaker, 1990). The effect of super chilling with CO_2 snow was reported by Leblanc & Leblanc (1992). Dry ice has recently gained popularity as a novel and innovative chilling medium for the rapid transportation of fresh fish by air. Most often dry ice in combination with water ice used blindly without any scientific basis for fresh fish transportation. The potential hazards of dry ice during transportation include possible explosion, suffocation and contact hazards (Russ, 2003). The disadvantages of using water ice are more drip loss, textural toughness, nutrient loss and decreased protein extractability of fishes (Putro, 1989). Slurry ice, also known as fluid ice, slush ice, liquid ice or flow ice, has been reported to be a promising technique for the preservation of aquatic food products in an ice-water suspension at subzero temperature (Rodríguez et al., 2003). Ice slurry has high energy storage

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*E-mail: femeenahassan@rediffmail.com

density because of the latent heat of fusion of its ice crystals. It also has a fast cooling rate due to the large heat transfer surface area created by its numerous particles. Super chilling techniques are especially useful when fishing grounds are so far from ports and the transport of live fish (Price et al., 1991). However, the negative effects of super chilling on sensory quality limit practical application of the technique (Aune, 2003). With crushed ice there is always the risk of physical damage for fish. The main negative aspect related to quality loss in slurry ice corresponded to the appearance of eyes and gills. Using slurry ice during transportation did not extend the shelf life of fish stored at 4°C (Taliadourou et al., 2003). However traditional cooling in flake-ice is insufficient to guarantee a high quality of the fish material landed and sold. Since, the existing methods have many limitations pertaining to quality preservation, there is always a need for an alternative method of chilling fresh fish to retain high quality of fresh fish. Gel ice is another concept sparingly used in the industry.

In the present study, a comparative evaluation on the quality of pink ear emperor (*Lethrinus lentjan*) fillets stored in flake ice and gel ice is carried out.

Materials and Methods

Freshly caught fish *L. lentjan* of average size range 550-600 g procured from Kochi Fisheries harbor were used for the study. The fish immediately after procurement were brought to the laboratory in iced condition within 30 min. Upon reaching the laboratory the samples were deiced and washed in potable water. Then fillets were prepared and each fillet was wrapped in polythene sheet. The fillets were divided into two batches. For one batch flake icing was done and for other batch gel icing was done. Both batches of fish were kept in chilled storage (at or below 4°C). The fillets iced with flake ice were re-iced every day after removing ice of the previous day during the storage period. Samples were drawn every alternate day for evaluating quality indices.

Flake ice was prepared with an Icematic F100 Compact device (CASTELMAC SPA, Castelfranco, Italy). Gel ice was procured from a processing facility where it was prepared using an imported machine GELPACK MODEL08, REG DESIGN.

The sensory attributes of the fillets stored in both icing methods were evaluated by a panel of ten

trained panelists for a storage period 16 days. The sensory evaluation was carried out by using a 9 point hedonic scale, as described by Peryam & Pilgrims (1957) by giving numerical values of 1 (dislike extremely) to 9 (like extremely). Panelists indicated their rating for each sample by choosing the appropriate numerical score. The sensory attributes assessed included the appearance, flavour, colour, texture and overall acceptability. The limit of acceptability was 4.

Total Volatile Base Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N) were determined by the Conway micro diffusion (Beatty & Gibbons, 1937) and expressed as mg TVB-N 100 g⁻¹ meat and TMA-N 100 g⁻¹ meat respectively. The pH was determined by homogenizing 10 g fish fillet in 100 ml distilled water and measured using a pH meter. Peroxide value (PV) was determined according to Jacobs (1958) and expressed in milli equivalents of peroxide oxygen per kilogram of fat. Free Fatty Acid (FFA) were determined as per Takagi et al. (1984) and expressed as percentage of oleic acid. ThioBarbituric Acid Value (TBA) was determined in fish fillets to evaluate the oxidation stability during storage period, using AOAC (2006) method and the results expressed as milligrams of malonaldehyde per kg flesh.

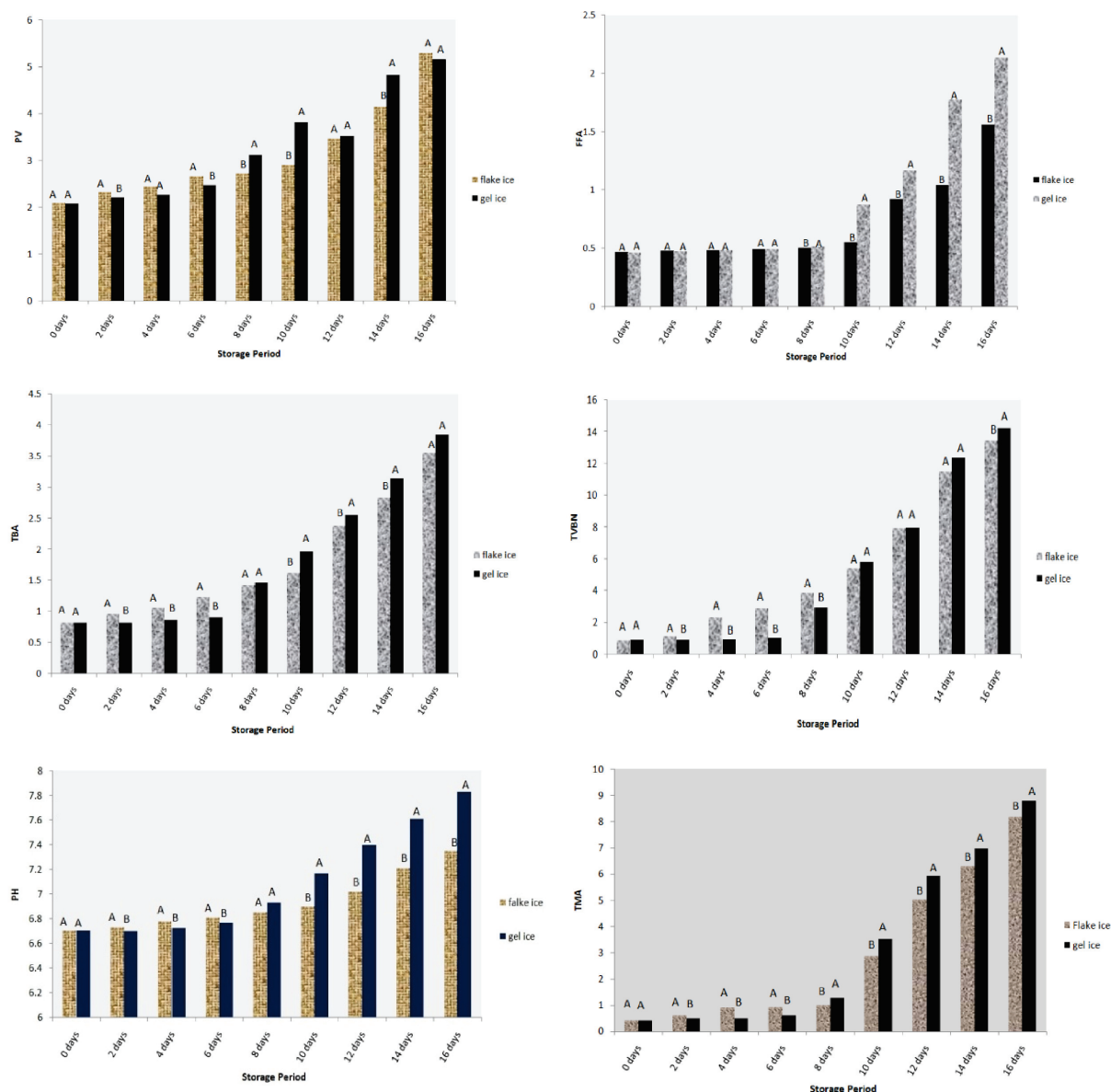
Aerobic plate count (Total plate count) in the sample was determined as per USFDA (USFDA, 2001).

The data were analyzed using test for biochemical variables and Mann-Whitney U test for organoleptic variables. One way analysis of variance was performed separately for flake and gel icing methods to compare the effect during storage period. Simple linear regression of the form $y=a+bx$, where y =dependant variable and x =days, was fitted to the biochemical variables of both icing methods to find rate of change as storage days advances. All the statistical analysis was carried out using SAS 9.2.

Results and Discussion

The spoilage caused by microorganisms, often detected as a fishy odour, is due to the decomposition of trimethylamine oxide (TMAO) by the enzyme TMAO reductase. TMA can be used as a spoilage indicator since it appears after 3 or 4 days of storage.

The concentration of TMA-N increased with storage in both methods of icing (Fig.1-A). The average



Means with different letters differ significantly at 5% level of significance

Fig. 1. Change in biochemical parameters under different icing methods on chilled storage

increase in TMA-N in the fish muscle was less during initial storage in gel iced condition (up to 8th day) and later it was found to increase at a faster rate. Based on the fitted regression model, the rate of change of TMA-N was increasing for both types of icing methods. The rate of increase was maximum for GI (0.556) and minimum for FI (0.486). Though TMA-N increased during both icing condition, the rate of increase is significantly less for gel icing compared to flake icing (5% level of significance) initially and there was significant increase in TMA-

N for GI stored fillet towards the end of storage. The fish is considered to be spoiled when levels between 10-15 mg of TMA-N 100 g⁻¹ (Connell, 1995). In this study, the TMA-N content increased from 0.41 mg N g⁻¹ to 8.2 mg N 100 g⁻¹ and 8.8 mg N 100g⁻¹ in flake iced (FI) and gel iced (GI) fish respectively during the storage period of 16 days, and the value was much lower than the limit of acceptance. These results are in accordance with others obtained for farmed sole, farmed turbot, in which significant TMA-N formation was not observed during accept-

able sensory period (Rodríguez et al., 2003). During iced storage of farmed turbot (*Psetia maxima*), Rodríguez et al., 2003 observed a shelf life of 19 days on Sensory analyses, though the production of total volatile base nitrogen (TVB-N) and trimethylamine-nitrogen (TMA-N) was low o the extent of 40 mg TVB-N 100 g⁻¹ muscle and 3.5 mg/TMA-N 100 g⁻¹ muscle, even after 40 d of refrigerated storage. Specific bacterial composition will influence TMA production. Although *S. putrefaciens* is a TMA producer, other spoilage organisms such as *Pseudomonas* spp do not produce TMA. In addition, the potential presence of *Pseudomonas* spp can inhibit the growth of *S. putrefaciens* causing a reduction below the densities required for TMA production (Gram & Melchiorson, 1996).

The formation of TVB-N, an index of spoilage during chilled condition (Cobb & Venderzont, 1975), during storage in different icing methods is shown in Fig. 1. During the storage TVB-N increased from the initial concentration of 0.86 mg N 100 g⁻¹ to 13.44 0.86 mg N 100 g⁻¹ and 14-22 0.86 mg N 100 g⁻¹ respectively for flake icing and gel icing methods. Initially upto 8 days of storage the TVB-N content was less in fish stored in gel ice whereas after 10 days condition reversed. The average increase in TMA-N in the fish muscle was significantly less during initial storage in gel iced condition (up to 8th day) and there was no significant difference in the TVB-N in the fish muscle during 10th, 12th and 14th days of storage for both types of icing system but it was significantly different during 16th day. This could be due to the increase in temperature during the later stage of storage as there was no replacement of ice in gel icing unlike in flake icing. Based on the fitted regression model, the rate of change of TVB-N was increasing for both types of

icing methods. The rate of increase was maximum for gel icing (0.89) and minimum for flake icing (-0.027). But even after 16 days of chilled storage, the TVB-N values were within the limits of, 30 mgN 100 g⁻¹ as proposed by Connel (1995). According to European Council directive the limits of acceptability for fish species based on TVB-N were 25-35 mgN 100 g⁻¹ muscle (EC. 1995).

The pH of the meat at the start of the study was 6.7 (Fig. 1), which increased slowly up to 6th day after which the pH of the meat stored in gel icing increased marginally, but after 10th day there was significant difference in the pH of the meat (7.3 against 7.8 for gel ice). The increase in pH is due to the production of various amines and basic substances from proteolytic products derived from microbial activity. A good correlation between change in pH and organoleptic qualities of the fish samples was observed where the organoleptic qualities deteriorated with the increase in pH. Based on the fitted regression model, the rate of change of pH was increasing for both types of icing methods. The rate of increase was maximum for GI (0.074) and minimum for FI (0.038).

The peroxide value gives a measure of the first stages of oxidative rancidity. *L. lentjan* is a lean fish with a fat content of 0.45%. Peroxide formation in ice stored fish fillets was found to be very slow during chilled condition (Fig. 1). From the initial value of 2.09 meq kg⁻¹, the value increased to only up to 5.16 meq kg⁻¹ in gel iced storage and 5.30 meq kg⁻¹ in flake iced storage at the end of 16 day storage. Based on the fitted regression model, the rate of change of PV was increasing for both types of icing methods. The rate of increase was more for GI (0.200) compared to FI (0.171).

Table 1. Regression equation for change in different biochemical parameters on storage for Gel-icing and Flake icing
Y = a+bx, where x = days

Parameters	Gel icing	Flake icing
pH	Y = 6.493 + 0.074 x, R ² = 0.89	Y = 6.621 + 0.038 x, R ² = 0.87
TMA	Y = - 1.288 + 0.556 x, R ² = 0.86	Y = - 0.975 + 0.486 x, R ² = 0.84
TVBN	Y = - 1.903 + 0.890 x, R ² = 0.86	Y = 1.037 - 0.027 x, R ² = 0.86
TBA	Y = - 1.903 + 0.890 x, R ² = 0.86	Y = - 1.037 - 0.026 x, R ² = 0.98
FFA	Y = 0.252 + 0.195 x, R ² = 0.88	Y = 0.253 + 0.058 x, R ² = 0.68
PV	Y = 1.672 + 0.200 x, R ² = 0.88	Y = 1.741 + 0.171 x, R ² = 0.82

Changes in FFA values in fish fillets during chilled storage under two different icing methods (Fig. 1) showed an increase from an initial value of 0.46 to 1.56 in flake icing and 2.14 in gel iced storage. The FFA formation in fish meat is not significant up to a period of 6 days of storage and there after FFA concentration increased considerably. The increase in FFA was more significant in the case of fish stored in gel ice. This could be due to activation of lipase due to the altered pH environment shown during the storage in gel ice. Based on the fitted regression model, the rate of change of pH was increasing for both types of icing methods. The rate of increase was maximum for GI (0.195) and minimum for FI (0.058). The increase in FFA was associated with loss of freshness as is seen from sensory studies. Similar results were reported by Barassi et al., 1987; Ozogul et al., 2005.

The TBA index is widely used as an indicator of degree of lipid oxidation. It was reported that TBA values may not give actual rate of lipid oxidation

since malonaldehyde can interact with other components of fish such as nucleosides, nucleic acid, proteins, amino acids, phospholipids and aldehydes which are the end products of lipid oxidation. The level of tissue aldehydes, the secondary degradation products of lipid oxidation as a result of peroxide breakdown into smaller molecules, is often assessed in biological systems (Khayat & Schwall, 1983). The initial TBA value of 0.81 mg MA kg⁻¹, in which, significantly increased to 3.85 and 3.55 mg MA kg⁻¹ for Gel iced and Flake iced fillets ($p < 0.05$) at the end of storage period.

The concentration of TBA increased with storage in both methods of icing. The average increase in TBA in the fish muscle was small during initial storage in gel iced condition (up to 8th day) and later it was found to increase at a faster rate. Based on the fitted regression model, the rate of change of TBA was increasing for both types of icing methods. The rate of increase was maximum for GI (0.89) and minimum for FI (-0.026). Though TBA increased

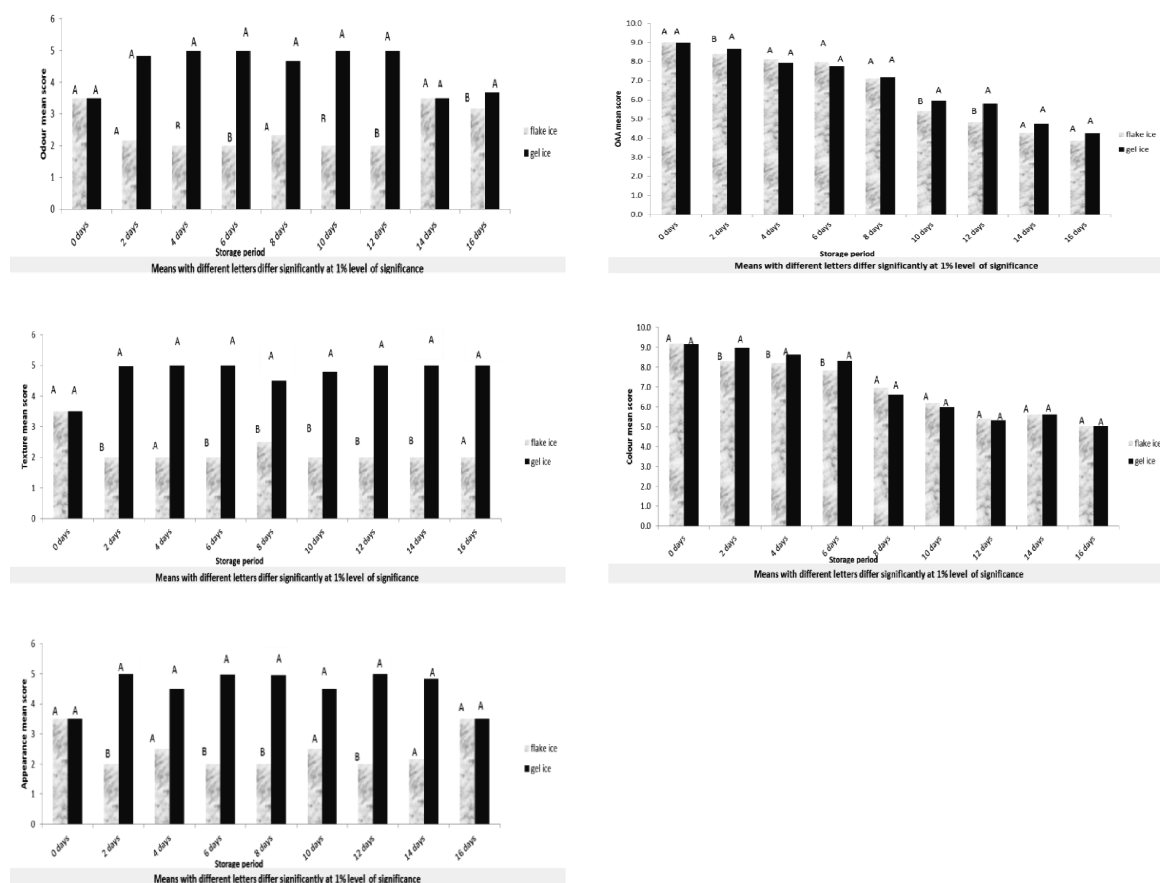


Fig. 2. Change in organoleptic parameters under different icing methods on chilled storage

during both icing condition, the rate of increase is significantly less for gel icing compared to flake icing (5% level of significance) initially and there was significant increase in TBA for GI stored fillet upto 10th day and there was no significant difference in TBA was noticed between the two methods of icing on 12th day. Nishimoto et al. (1985) reported for mackerel 4 and 27 mg malonaldehyde (MA) kg⁻¹ muscle for good and low quality fish, respectively. The peroxyradicals are highly reactive and further abstracts the hydrogen from another hydrocarbon chain. The reaction yields hydro peroxides and form a new free radical. Lipid peroxides formed during the primary phase of oxidation are very unstable. They undergo cleavage and yield free alkoxy radicals, which are further broken to aldehydes, ketones and hydrocarbons. The increase in TBARS value during the iced storage may be attributed to the increased oxidation of unsaturated fatty acids. TBARS is produced due to the second stage of auto oxidation during which peroxides are oxidized to aldehydes and ketones (Gram & Melchiorson, 1996).

Simple linear regression model was fitted with high R² value to quantify the rate of change in quality parameters in different methods of icing under chilled storage. Table 1 depicts the regression equation for different biochemical parameters for Gel-icing and Flake icing. When the entire storage period is considered as a whole, the rate of increase in TMA-N in gel icing was found to be 0.556 and for flake icing it was 0.486. The increase in TVBN content was 0.890 for gel icing and + 0.027 for flake icing. The rate of increase in TBA, FFA and PV was found to be 0.890, 0.195 and 0.2 respectively in gel icing and -0.026, 0.058 and 0.171 for flake icing.

A significant decrease in sensory scores ($p < 0.01$) was noticed throughout the period of storage in both flake iced and gel iced fish. Sensory evaluation data (Table 2) showed highly negative correlation

between mean panel scores for all sensory attributes and storage period. From the table it is seen that the rate of decrease in quality with respect to appearance and overall acceptability is more for gel icing, the value being -0.251 and -0.350 respectively. The corresponding value for flake icing being -0.248 and -0.305. Whereas in the case of colour, odour and texture the rate of quality decrease for gel icing being -0.265, -0.328 and -0.313. On the other hand for flake icing the corresponding values are -0.296, -0.337 and -0.310.

Mean sensory evaluation score for the attribute, appearance have shown significant difference up to 12 days after that there is no significant difference. Up to 12 days this attribute is superior for Gel ice stored fish. From Fig. 2 it is clear that the quality attribute colour is significantly differing for gel iced and flake iced fishes the storage days advances up to 6 days and after that there is no significant difference between the two, though there is reduction in scores. Short duration in storage gel ice was found to be superior in this case. The attribute score for odour is depicted in Fig. 2 shows that for storage up to 12 days the values are significantly better for gel ice fish fillets. The statistical evaluation of sensory attribute texture (Fig. 2) indicated a significantly better quality for gel iced fillet for 14 days.

When the sensory scores for different attributes were analyzed to find variation between the days, it was seen that in gel iced fish fillets there was significant decrease in all attributes ($p < 0.01$). The sensory evaluation scores, which indicate consumer preference depicts that for short term chilled storage, Gel icing is the best option.

In consistent with the present study, Goncalves et al. (2007) reported a shelf life of 15 days for farmed whole Senegale sole (*Solea senegalensis*) stored in ice.

Table 2. Regression equation for change in organoleptic parameters on storage for Gel-icing and Flake icing

$Y = a + bx$, where x = days

Parameters	Gel icing	Flake icing
APPEARANCE	$Y = 9.017 - 0.251 x, R^2 = 0.94$	$Y = 9.369 - 0.248 x, R^2 = 0.94$
COLOUR	$Y = 9.091 - 0.265 x, R^2 = 0.94$	$Y = 9.445 - 0.296 x, R^2 = 0.91$
ODOUR	$Y = 9.177 - 0.328 x, R^2 = 0.94$	$Y = 9.568 - 0.337 x, R^2 = 0.95$
TEXTURE	$Y = 8.955 - 0.013 x, R^2 = 0.95$	$Y = 9.347 - 0.310 x, R^2 = 0.95$
OAA	$Y = 9.359 - 0.350 x, R^2 = 0.94$	$Y = 9.261 - 0.305 x, R^2 = 0.94$

According to Losada et al. (2005) horse mackerel stored in slurry ice maintained good quality up to 8 days. They also reported that on 19th day this lot was not acceptable. The same authors also reported that fish stored in flake ice was maintained in good quality only for two days. In the current work, the fish fillets stored under both icing system were in acceptable condition even after 16 days. This is because; the samples were kept under chilled condition after icing. In addition in flake iced fish, re-icing was done every day, after removing last day's ice.

Microbial counts represented as aerobic plate count of fish fillets preserved under gel icing and flake icing is shown in Fig. 3. Initial total viable counts of fish fillet was 3.75 log cfu g⁻¹ (day 0) and population of microorganisms significantly ($p < 0.01$) increased to 6.58 log cfu g⁻¹ and 6.61 log cfu g⁻¹ for flake icing and gel icing respectively on 16th day of storage.

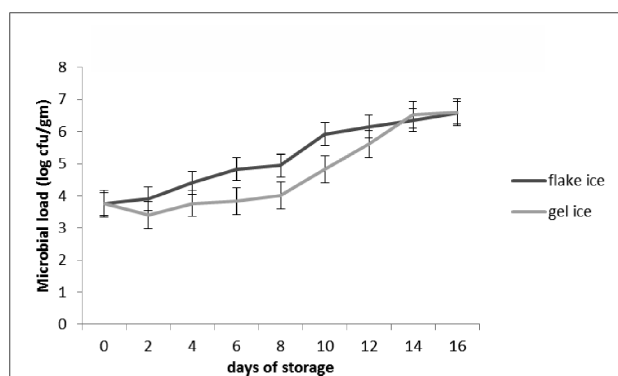


Fig. 3. Microbial load (Mean $\pm$ SD) under different icing methods

marginally higher (10^7 CFU g⁻¹) microbial safety criterion is normally applied for determining storage life of fresh seafood (IFST, 1999). The shelf life of common sole was approximately ~16-18 days, indicating that sensory analysis of common sole correlated well with microbiological analysis. Similar results were obtained for others species (Rodríguez et al., 2006; Ozogul et al., 2006).

The gel ice is equally good as a medium for the preservation of fish, particularly in first few days of storage. But if the sachets can be replaced once in two days the effectiveness of gel ice can be improved several folds and can be considered as an effective medium for fish preservation. Gel ice however has the advantage of reuse and therefore the recurring cost on ice can be reduced considerably.

In the present study it was observed that for short duration transport upto 8 days gel icing was found to be superior to flake icing.

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