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Modified icing system containing mint leaf and citrus peel extracts: effects on quality changes and shelf life of Indian mackerel

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ABSTRACT

The efficacy of ice containing mint (*Mentha arvensis*) leaf extract (700 mg l^{-1}) and citrus (*Citrus aurantium*) peel extract (1000 mg l^{-1}) in retarding the biochemical, microbiological and sensory changes in whole Indian mackerel was assessed. Presence of extracts in ice significantly ($p<0.05$) reduced the generation of total volatile base nitrogen (TVB-N), trimethyl amine nitrogen (TMA-N) and free fatty acids (FFA) during storage. Moreover, a marked inhibition of lipid oxidation was also observed in samples stored in ice with extracts, as indicated by its lower peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) compared to that of control. Additionally, icing with extract substantially reduced the count of total viable bacteria, as compared with the samples stored under conventional icing system. According to sensory evaluation, shelf life of Indian mackerel was determined to be 13 days for fishes stored under conventional icing system, 15 days for fishes stored in ice containing citrus peel extract and 17 days for the fishes kept in ice containing mint extract.

Keywords: Antioxidant activity, Icing system, Indian mackerel, Plant extracts, Shelf life

Introduction

Constituents or extracts from plant sources are considered as bio-preservatives and in recent years, researchers have been giving increased attention to the positive role of extracts obtained from plant sources for food applications. The antibacterial and antioxidant activities of extracts obtained from different plant sources against food borne pathogens and food spoilage bacteria are well demonstrated (Shan *et al.,* 2007; Babbar *et al.,* 2011). *Mentha* species, belonging to the family *Laminaceae* is a rich source of antioxidants and antibacterial compounds (Moreira *et al.,* 2005; Kannatt *et al.,* 2008). Citrus peel, a major contributor to fruit juice waste is rich in phenolic compounds such as phenolic acids and flavonoids with good antioxidant power (Wang *et al.,* 2008).

Icing and mechanical refrigeration are the most prevalent techniques to control microbial and biochemical spoilage of freshly caught fish during distribution and marketing in tropical countries. However, shelf life of ice stored fish is often limited due to post-mortem autolysis, microbial growth and lipid oxidation. Lipid deterioration is considered to be the major cause of off-flavour development and quality loss of fishes during iced storage, particularly in semi fatty/fatty fishes. Recent studies have shown significant inhibitory effects of ice incorporated with natural antimicrobials and antioxidants on the microbiological and biochemical mechanisms involved in fish spoilage, as compared to conventional icing (Oral *et al.,* 2008; Garcia-Soto *et al.,* 2013; Bensid *et al.,* 2014). These studies demonstrated that storage of fish in ice incorporated with extracts from plant sources retarded the biochemical, sensory and microbiological changes and extended the shelf life compared to control samples.

Indian mackerel (*Rastrelliger kanagurta)* contributes to a major quantity of pelagic fatty fishes harvested across the Indian coast. Being a semi-fatty fish and good source of marine lipids, the Indian mackerel attracts great attention owing to the positive role of marine lipids in human nutrition (Viji *et al.,* 2015). However, effective and eco-friendly methods for controlling spoilage and improving quality of fresh Indian mackerel are necessary. Shinde *et al.* (2015) reported positive effects of pomegranate peel and tea leaf extracts on the quality changes of mackerel during chilled storage. In our previous study (Viji *et al.,* 2015), dip treatment in mint

and citrus peel extract had shown shelf life extension of mackerel stored at 0 - 2^{O}C. The present study was aimed to investigate the efficacy of ice incorporated with extracts from mint leaf and citrus peel against that of conventional icing on improving the quality and shelf life of whole Indian mackerel during storage.

Materials and methods

Mint (*Mentha arvensis*) and citrus (*Citrus aurantium*) peels were procured from nearby vegetable market, Vashi, India. Fresh Indian mackerel (weight 180±20 g; length 15±2 cm) was procured from the fish landing centre, Vashi, Navi Mumbai, India and brought to the laboratory in iced condition within 15 min.

Preparation of extract and estimation of antioxidant activities

Extracts from mint leaf and citrus peel were prepared as described earlier by Viji *et al.* (2015). Total phenolic content in the extracts was estimated by the method of Singleton and Rossi (1965) and the results were expressed as mg gallic acid equivalent per g dry wt. of the extract. Total flavonoids of the extracts were determined by the method described by Ordonoz *et al.* (2006) based on the formation of a flavonoid aluminium complex. The total flavonoid content of the extracts was calculated from the equation derived from the standard curve of quercetin and the results are expressed as mg quercetin equivalent per g of extract. Ferric reducing antioxidant power (FRAP) assay was done according to the procedure of Benzie and Strain (1996). The standard curve was constructed using iron (II) sulfate solution (100–1500 µm) and the results were expressed as µmol Fe^{2+} per mg of the extract. All the measurements were taken in triplicate and the mean values were calculated.

Preparation of plant extract-ice and storage of fish

Mint extract solution (700 mg l^{-1}) and citrus extract solutions (1000 mg l^{-1}) were prepared in 2 ppm chlorinated water. Two litres of each solution were packed in a polyethylene bag and frozen to 0^{O}C in a commercial plate freezer. Conventional ice was prepared from 2 ppm chlorinated water. Concentrations of plant extracts were chosen based on the results of the preliminary trials (100 - 1000 mg plant extract l^{-1} distilled water), where the fishes stored were visually analysed and a concentration which gave the best appearance of fish with less presence of colour of plant extract was chosen. The ice blocks were crushed before use.

The whole mackerel was washed thoroughly in potable water and divided into 3 lots. First lot was stored in conventional ice (C ice), the second was stored in ice containing mint extract (ME ice) and the third lot in ice containing citrus extract (CE ice) in individual thermocole boxes in 1:1 fish to ice ratio. The boxes were further stored in a vertical chiller maintained at 0 - 2^{O}C. Re-icing was done every day to maintain the ratio of fish to ice as 1:1 in each group. Three fishes from each box were sampled at days 1, 4, 7, 10, 13, 15, 17 and 18 and analysed for biochemical, microbiological and sensory qualities. All the analyses were done in triplicate.

Quality assessment

Biochemical analysis

Proximate composition of the raw fish was determined by AOAC (1998) method. pH was determined using a glass electrode digital pH meter (Cyberscan 510, Eutech instruments, Singapore) after making a homogenate of fish muscle in distilled water (1:5 w/v). Non-protein nitrogenous (NPN) content of the samples was measured by estimating nitrogen in the TCA extract using Kjedahl distillation method (Alongo *et al.,* 1994). Total volatile base nitrogen (TVB-N) and trimethyl amine (TMA) was estimated by the microdiffusion method (Conway, 1950). Thiobarbituric acid reactive substances (TBARS) (Tarladgis *et al.,* 1960) as well as peroxide value (PV) (Yildiz *et al.,* 2003) of the muscle were measured to assess the oxidative rancidity. Free fatty acid (FFA) value was determined as per AOAC (1989) to assess the hydrolytic rancidity.

Microbiological analyses

Aerobic plate count (APC) of the samples was analysed according to the method of Ryser and Schuman (2013). Fifty gram of fish muscle was homogenised with 450 ml of phosphate buffer for 2 min. Decimal dilutions were made in phosphate buffer and 1 ml each of three consecutive dilutions were plated on plate count agar (Difco). Plates were incubated at 35 ± 2^{O}C for 48 ± 2 h for obtaining total viable count.

Sensory analysis

Raw and cooked pieces were analysed by a panel of 6 experienced members. A 9 point hedonic scale as described by Amerine *et al.* (1965) was used to score various characteristics like colour, odour, texture and flavour. The overall acceptability score was determined taking into account the total score obtained for raw and cooked samples. Samples were rejected when the overall acceptability score reached 4.

Statistical analysis

All the measurements were taken in triplicate and the data were subjected to analysis of variance (ANOVA) using SPSS software version 16. Difference between the mean values of various treatments and storage days was analysed by Duncan's multiple range test using SPSS and the significance was defined at $p<0.05$.

Results and discussion

Total phenolic and flavonoid content

Yield of mint leaf and citrus peel extracts were 14 -15% and 16 -18%, respectively. The analysis revealed significantly higher amount of phytochemicals (phenolics and flavonoids) in mint extract compared to citrus extract (Table 1). The total phenolic contents of citrus peel extracts were much lower than those reported by Ghasemia *et al.* (2009) (132.2 - 223.2 mg GAE per g dry matter) whereas the polyphenol content of mint extract in our study (127±8.2 mg GAE g^{-1}) was comparable to those observed in methanolic extract of *M. longifolia* (107.20±34.2 mg GAE g dry wt^{-1}) by Janifer Raj *et al.* (2010). Ghasemia *et al.* (2009) reported the flavonoid content in methanolic extracts of 6 citrus varieties which ranged from 0.3 - 31.7 mg quercetin equivalent g wt^{-1}, while Asjad *et al.* (2013) obtained flavonoid content varying from 0.2 - 25.7 mg quercetin equivalent g wt^{-1} in 6 common citrus varieties of Pakistan. The flavonoid obtained for citrus extract in the present work was much higher than that reported in literature, probably due to the difference in extraction procedure. Phenolic compounds of plants are present in different bound status depending on species and hence, effective processing steps for liberating phenolic compounds from various plants may be different (Jeong *et al.,* 2004). This reason might have accounted for the significant difference in total phenolic and flavonoid content between citrus peel and mint extracts.

Ferric reducing antioxidant power (FRAP) assay

In this assay, the antioxidant capacity is measured based on the ability to reduce ferric ion to ferrous ion by donating an electron and the results are expressed as µmol Fe^{2+} mg^{-1} of the sample. The FRAP of mint extract was significantly ($p<0.05$) higher than that of citrus extract (Table 1). This might be due to the higher concentration of phenolic and flavonoid compounds present in the same. It has been reported that the reducing power might be due to hydrogen-donating ability and is generally associated with the presence of reductones (Duh, 1998). Stanisavljevic *et al.* (2012) observed that the FRAP values of extracts of *M. longifolia* prepared using 70% ethanol varied from 1.22-2.76 mM $FeSo_4$ g dry wt^{-1}. In the present study, *M. arvensis* extract showed FRAP value of 1340±34.11 µm Fe^{2+} mg^{-1} which was comparable to that of butylated hydroxy toluene (BHT) (1712±42.76). The data available in literature on the FRAP assay of *Citrus* spp. and *Mentha* spp. are scattered and is often difficult to compare because of the differences in the methodologies and in the expression of results.

Proximate composition of mackerel

Fresh mackerel used in this study showed 74.32±0.85% moisture, 21.15±0.743% protein, 3.38±0.29% fat and 1.06±0.03% ash content. The results indicated higher protein content and hence, mackerel is considered to be one of the cheap sources of protein. Fat content indicated a semi fatty nature of mackerel muscle. Comparatively higher moisture content contributes to faster spoilage due to microbial growth (Viji *et al.,* 2015).

pH

A significant increase ($p<0.05$) in pH was noticed in all the samples over the storage period (Fig. 1). No significant difference between pH of different groups was observed till 7 days of storage in ice. However, after 7 days, the samples kept in conventional ice showed significantly higher ($p<0.05$) pH than its counterparts stored in ice containing plant extracts. On days 15 and 17, CE iced group presented significantly higher ($p<0.05$) pH than ME iced group. pH of the samples reached 7.12, 7.02 and 7.11 in samples stored under conventional ice, CE ice and ME ice, on their sensory rejection days *i.e.*, on 15th, 17th and 18th day, respectively.

Comparison among samples (Fig. 1) indicated that presence of extracts in ice significantly ($p<0.05$) reduced the pH value of whole mackerel during extended storage. Similar results have been reported by Quitral *et al.* (2009)

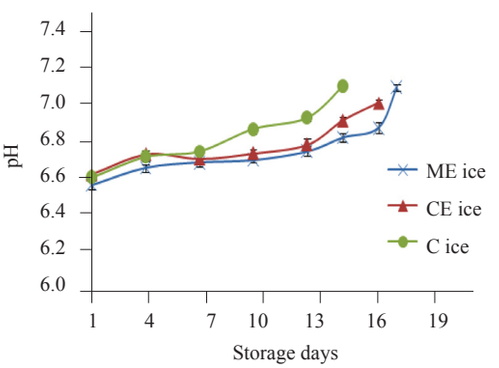

Fig. 1. Effect of different icing systems on the pH of indian mackerel during storage (Mean±SD, n=3)

Table 1. Antioxidant activities of the extracts

Parameter /Source	Mint leaf extract	Citrus peel extract	BHT
Total phenolic content (mg gallic acid eq. g wt^{-1})	108^{b}±6.7	74.8^{a}±5.3	NA
Total flavonoid content (mg quercetin eq. g wt^{-1})	91.14^{b}±6.32	66.94^{a}±5.43	NA
FRAP (µ mol Fe^{2+} mg^{-1})	1340^{b}±34.11	1076^{a}±20.31	1712^{b}±42.76

when Chilean jack mackerel was stored under oregano and rosemary extracts whereas Bensid *et al.* (2014) reported that the pH of anchovy was not significantly affected by icing with extracts from thyme, oregano and clove. The higher pH observed in fishes stored under conventional icing conditions during extended storage may be due to the generation of more bases associated with the degradation of tissue constituents.

TVB-N

The chemical spoilage of fish samples during storage is usually evaluated by measuring the changes in the content of TVB-N which mainly comprises ammonia and primary, secondary and tertiary amines produced as a result of microbial activity. The TVB-N of fresh fish was 13.2 mg%. TVB-N slightly increased till 7th and 10th day of storage, respectively for samples stored under conventional ice and extracts incorporated ice. Thereafter, a marked increase was observed in all samples with the progress of storage period in ice (Fig. 2a). For samples under conventional ice, the TVB-N formation reached up to a level of 27.1 mg% on the sensory rejection day (15th day). The development of TVB-N was significantly higher in conventional iced groups than that in CE and ME iced groups ($p<0.05$).

Concentration of TVB-N in freshly caught fish is typically between 5 and 20 mg N 100 g^{-1}, whereas levels of 30 - 35 mgN 100 g^{-1} fish are generally regarded as the limit of acceptability for ice-stored cold water fish (Connell, 1995). In our study, even though TVB-N showed an increasing trend, none of the samples crossed the established acceptable limit. Lower levels of TVB-N in extract iced samples can be attributed to its lower microbial counts compared to conventionally iced samples which further indicate the antibacterial effects of the extracts tested. An inhibitory effect by the presence of plant extracts in ice on TVB-N generation has been reported previously by Quitral *et al.* (2009) and Bensid *et al.* (2014).

TMA-N

TMA-N is derived from trimethylamineoxide (TMAO) during spoilage in marine fishes. At the beginning of the storage, TMA-N values were determined as 2.1 mg100 g^{-1} flesh which increased significantly ($p<0.05$) with time of storage in all groups (Fig. 2b). After 4th day of storage, samples stored in conventional ice indicated significantly higher ($p<0.05$) amounts of TMA-N than the samples stored under extract ices. Among the extract ices, mint extract ice significantly ($p<0.05$) reduced TMA-N formation on most of the sampling days. Presence of extract in ice might have reduced the activity of bacteria causing reduction of TMA-O to TMA-N, leading to significantly lower amounts of TMA-N in the treated samples.

Assessment of TMA-N in the present study did not provide a good indication of spoilage, as the evolution of TMA-N was quite low throughout the storage period. It has been reported that the level of TMA-N in numerous fatty fish never reached 5 mg% although the limit is 10 - 15 mg% (Sikorski *et al.,* 1990; Ozogul *et al.,* 2004). Although TMA-N is believed to be generated by the action of spoilage bacteria, the correlation with bacterial numbers is often not very good (Huss, 1995). Similarly, in this study, changes in the concentrations of TMA-N in mackerel during chilled storage did not correlate well with APC.

NPN content

Variations in the NPN content of all the samples are presented in Fig. 2c. A statistically significant reduction ($p<0.05$) in NPN content with storage period was observed in the samples stored under conventional as well as extract icing systems. The rate of reduction was lower in fishes under ME ice compared to all other samples. With slight fluctuations, NPN content reduced from an initial value of 512 mg% in fresh mackerel to a final value of 478.67, 436.0 and 474.18 mg% in samples stored under ME ice,

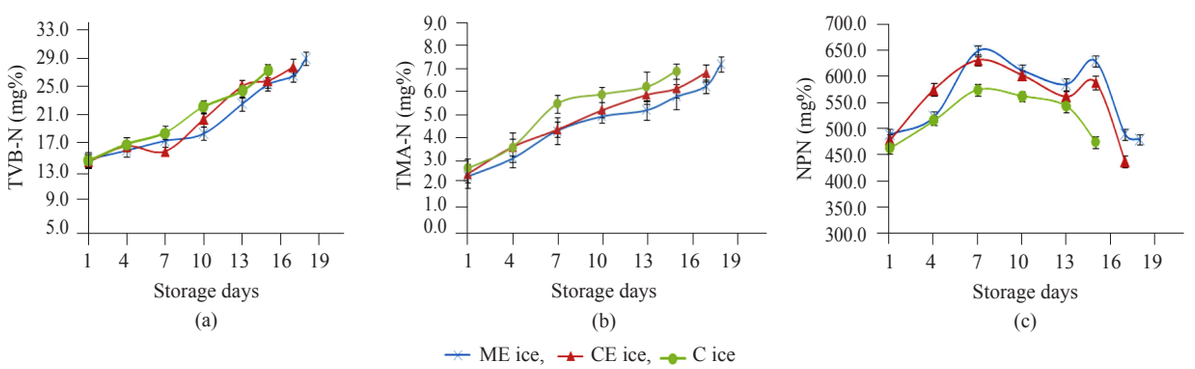

Fig. 2. Effect of different icing systems on (a) TVB-N, (b) TMA-N and (c) NPN of Indian mackerel during storage (n=3, mean±SD)

CE ice and conventional ice, respectively on their final day of storage. As most of the non-protein nitrogen in fish is water soluble sarcoplasmic fractions, leaching out of these fractions into the melting ice is responsible for reduction in NPN content during storage. Meanwhile, the degradation of proteinaceous matter and free aminoacids by bacteria can make a rise in NPN content. Lapa Guimaraeus *et al.* (2005) reported a reduction in NPN content when squid is stored under ice. Leaching out of NPN compounds is also observed by Vaz-pirez *et al.* (2008) during the iced storage of cuttlefish and shortfin squid. The reduction in NPN content during storage can also be associated with the degradation of the low molecular weight amino acids or nucleotides by microorganisms. Accordingly, the rate of reduction was lower in samples kept under mint extract and citrus extract ice compared to conventional icing system.

FFA

The FFA values of fish muscle gives an account of the degree of lipid hydrolysis during spoilage. Initial FFA values of fish samples were found to be 0.948 (oleic acid %). There was a marked increase in FFA content of all groups during storage (Fig. 3a). The FFA content increased from an initial value of 1.41, 1.48 and 1.74 to a final value of 7.15, 6.81 and 6.64 % oleic acid in mackerel stored under ME ice, CE ice and conventional ice, respectively. On and after 7 days of storage, the FFA content of samples in conventional ice was significantly higher ($p<0.05$) than that of samples stored under ME ice. Samples of CE iced group retained significantly ($p<0.05$) lower FFA content than that of C iced samples on 4th, 10th and 13th day of storage. The lower FFA values in extract iced samples could be attributed to the effect of phenolic compounds of plant extracts which may suppress lipolytic bacteria responsible for liberating free fatty acids. Mint extract was found superior to citrus peel extract for controlling FFA formation.

Peroxide value

Shelf life of oily fish species is limited due to the oxidation of lipid. The primary product of lipid oxidation is fatty acid hydroperoxide, measured as PV. In this study, PV was employed for determining the amount of primary oxidation products during the storage period under different icing systems. Initial PV of fresh mackerel used in this study was determined as 3.29 meq O_2 kg^{-1} of fat. The PV showed an increase in all groups ($p<0.05$) till a particular day of storage and thereafter, the values indicated a declining trend (Fig. 3b). This index exhibited a marked increase in the samples of conventional ice, in contrast to fishes kept under ME ice and CE ice conditions. The maximum PV recorded was 13.83 meq O_2 kg^{-1} of fat for samples under conventional ice on 13th day and 10.05 and 12.36 meq O_2 kg^{-1} of fat for samples stored in ME ice and CE ice, respectively on 15th day of storage. It is inferred from the results that the presence of both extracts in the chilling medium led to a significant inhibition of peroxide formation. Mint extract showed the highest antioxidant effect in agreement with its *in vitro* antioxidant activities in whole mackerel compared to citrus peel extract and yielded lower peroxide generation during storage. Since, the fishes were in direct contact with extract ice, the phenolics present in ice might have reduced the hydroperoxide formation through scavenging the reactive fatty acid free radicals, thereby breaking the chain reaction of lipid oxidation. Similar findings were obtained for icing with rosemary extracts during the storage of Chilean jack mackerel (Quitral *et al.,* 2009) and for icing with extracts from thyme, oregano and rosemary during storage of anchovy (Bensid *et al.,* 2014). The reduction in PV in all the samples towards the end of storage period may be related to the secondary reactions of the hydroperoxides and volatilisation (Vidya and Srikar, 1996).

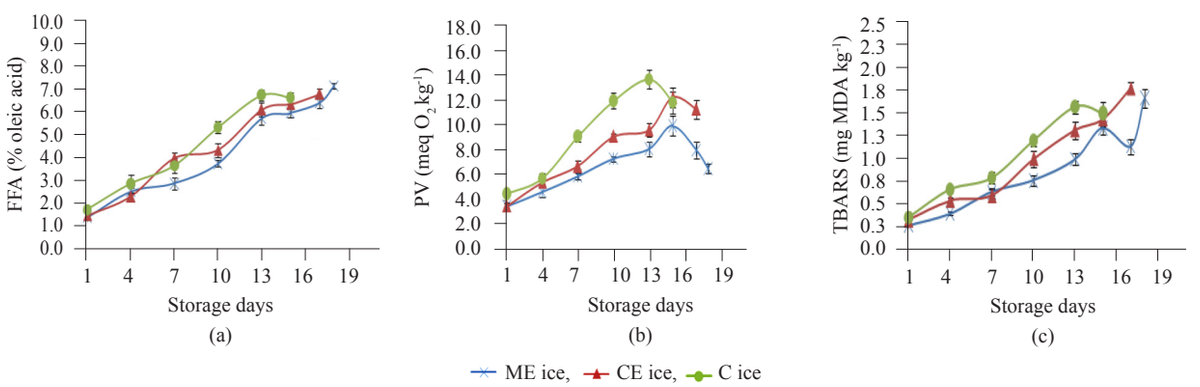

Fig. 3. Effect of different icing systems on (a) FFA, (b) PV and (c) TBARS of Indian mackerel during storage (n = 3, Mean ± SD)

TBARS

TBARS gives a measure of malonaldehyde (MDA), which is produced through secondary lipid oxidation from the degradation of hydroperoxides. As evident from Fig. 3c, a significant increase in TBARS was observed with storage time in ice, in all sample groups. On and after 3rd day, higher TBARs value ($p<0.05$) in the conventional iced samples was observed in comparison to mackerel stored in ice with mint extract. Except for the 1st, 4th and 15th day, the samples stored in ice with citrus peel extract also had significantly lower TBARS ($p<0.05$) than that stored in conventional ice. On the final sampling day, the TBARS reached up to 1.586, 1.683 and 1.43 mg MDA kg^{-1} fish, respectively for samples in ME ice, CE ice and C ice. Nunes *et al.* (1992) reported that the acceptability limits of TBARS value for fish stored in ice were 5 - 8 mg MDA kg^{-1} flesh. However, none of the samples crossed this limit during the entire storage period.

It was interesting to note that, although the samples showed comparatively higher PV, the TBARS values were comparatively lower. This could be probably due to a lower rate of degradation of peroxides formed during primary oxidation. In addition, the MDA can interact with nucleic acids, proteins, amino acids and phospholipids resulting in lower amounts of free MDA that can be detected by TBARS test (Pezeshk *et al.,* 2011). Hence, TBARS index may not always give the actual level of lipid oxidation occurred. However, as observed from the present results, addition of extracts, especially mint extract in ice significantly inhibited secondary lipid oxidation in mackerel. These results are in agreement with those of Quitral *et al.* (2009), who reported that icing system with oregano and rosemary extracts generally showed lower TBARS value for jack mackerel (*Trachurus murphyi*). Similarly, Ozyurt *et al.* (2012) also found that icing with rosemary extract was effective in controlling TBARS values in sardine. Among the two extracts, mint extract was more effective in controlling secondary lipid oxidation than citrus peel extract, which may be attributed to the higher antioxidant activities of the former.

APC

Aerobic plate count (APC) reflects the microbial quality of food and is useful for indicating potential spoilage of perishable products. The initial population of viable bacteria in fish used in this study was 4.94 log cfu g^{-1}. Bacteria grew significantly in all samples with progress of storage in ice (Fig. 4). APC of the samples under different conditions were comparable till 4 days of storage. On and after 7th day of storage, the fishes stored in conventional ice showed significantly ($p<0.05$) higher levels of viable counts than their counterparts

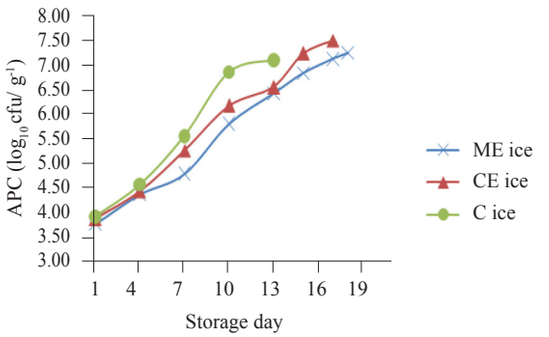

Fig. 4. Effect of different icing systems on APC of Indian mackerel during storage

stored in extract ices. In general, the counts of viable bacteria in conventional iced samples were ca. 0.5 - 1.0 log cycles higher than those for the extract iced fishes after 1 week storage. The upper acceptable limit (M) for marine and freshwater fish proposed by ICMSF (1998) is 7 $\log_{10}$ cfu g^{-1} and it has been considered as the standard for establishing microbial limits in fresh fish by many researchers (Pezeshk *et al.,* 2011; Houicher *et al*., 2013). Fishes stored in conventional ice, citrus peel extract ice and mint extract ice crossed this limit on 13th, 15th and 17th day of storage, respectively.

The results revealed that microbiological growth was significantly influenced by the addition of plant extract in ice. In ME and CE iced fishes, even though microbiological limit was crossed before the end of their storage life, it was not reflected on sensory analysis. This may probably be due to reduced spoilage activities of the bacteria in presence of extracts in the chilling medium. Accordingly, the fishes were not rejected by the sensory panellist on the day when the samples crossed the microbiological limit. Similar findings were earlier reported by Viji *et al.* (2015) in gutted sutchi catfish during ice storage.

The analysis indicated that mint extract with effective antimicrobial activities, could significantly delay microbial growth and extend the shelf life by 4 days. Work done by Sugandhi and Meera Bai (2011) showed that the ethanol extract of *M. arvensis* inhibited the growth of *Escherichia coli, Pseudomonas aeruginosa, Shigella flexineri, Klebsiella pneumoniae* and *Staphylococcus aureus in vitro*. Hence, the mint extract with effective antimicrobial and antioxidant activities, could significantly delay microbial growth and lipid oxidation and can act as a natural food preservative in ice. Similar to this finding, presence of thyme in ice extended the microbiological shelf life of anchovies by 3 days compared to conventional ice (Bensid *et al.,* 2014). However, so far no studies reported regarding the effect of icing with mint and citrus peel extracts on microbiological quality of fish.

Sensory evaluation

Changes in the overall acceptability scores over the storage period are presented in the Fig. 5. Sensory scores of fishes in conventional and extracts ice declined with the progress of ice storage. Sensory deterioration was faster in conventional iced groups followed by CE iced groups than their counterparts in ME group. Even though the samples of ME group had higher overall acceptability score, the colour and appearance of raw pieces scored lower towards the end of storage life. However, it was not observed in cooked pieces, which could be due to leaching of mint flavour during boiling. Progress of fish spoilage in the whole fish was evident by development of putrid odours, slimy and soft flesh. The samples were considered unfit for consumption when the overall score crossed 4. Accordingly, the panellists rejected the C, CE and ME group on 15th, 17th and 18th day of storage, respectively. Hence, taking into account microbiological and sensory analysis, the shelf life was determined to be 13 days for mackerel stored in conventional ice and 15 days and 17 days for fishes stored in citrus peel extract ice and mint extract ice, respectively. Ozyurt *et al,* (2012) studied shelf life of sardine (*Sardinella aurita*) stored in ice with rosemary and their results showed that the addition of natural extract improved the sensory quality of fish and extended the shelf life by 3 days compared with the control samples.

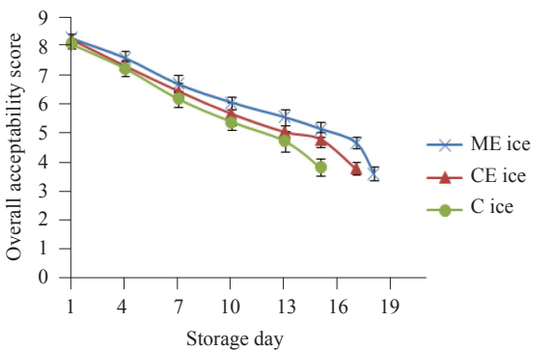

Fig. 5. Effect of different icing systems on the sensory score of Indian mackerel during storage (n=5, mean ± SD)

The preservative effects displayed by plant extracts incorporated ice could be attributed to the presence of bioactive phytochemicals in the extracts. The major phenolics reported in mint leaves are rosemarinic acid, caffeic acid, eriocitrin and luteolin (Padmini *et al.,* 2010; Kappa *et al.,* 2013) with potential antibacterial and antioxidant activities (Singh *et al.,* 2010). Manthey and Grohmann (2001) reported that polyphenol compounds such as *p*-coumaric, ferulic and sinapic acids and narirutin are present in citrus peel extract. Constituents such as gamma-terpinene, terpinolene, alpha-terpinene, hesperidin and neohesperidin are also found to be responsible for the preservative action in citrus species (Singh *et al.*, 2010).

The present study clearly indicates the potential use of a novel icing system incorporated with extracts from mint leaf and citrus peel in chilled storage of fish. Presence of extracts, especially mint extract significantly delayed the biochemical, microbiological and sensory quality changes and extended the shelf life of Indian mackerel by 5 days. The antioxidant effects of extracts were extremely prominent from the significantly lower values of lipid oxidation indices like PV and TBARS in the treated groups. Also, the presence of extracts in ice significantly ($p<0.05$) suppressed the growth of bacteria as determined by the counts of total viable bacteria. Findings of the present work are noteworthy as the shelf life of Indian mackerel is often limited due to lipid oxidation and rancidity. It can be concluded that modification of icing medium by incorporating natural plant extracts can be useful for the food industry to extent the shelf life during storage and distribution.

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