Biochemical, textural, microbiological and sensory attributes of gutted and ungutted sutchi catfish (*Pangasianodon hypophthalmus*) stored in ice

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Abstract *Pangasianodon hypophthalmus* (sutchi catfish) is a fresh water catfish extensively being cultured in the South East Asian countries in the recent years. The present study provides the first report on the effects of gutting on the quality characteristics of aquacultured sutchi catfish stored in ice. pH of whole ungutted and gutted catfish didn't show significant difference ($p>0.05$) during ice storage period. Total Volatile Base Nitrogen (TVB-N), Alpha Amino Nitrogen (AAN), Free Fatty Acids (FFA) and Thio Barbituric Acid Reactive Substance (TBARS) were lower in gutted fish compared to whole ungutted fish at any particular day during ice storage. However, gutted fish expressed higher rate of primary lipid oxidation than ungutted fish. Textural degradation of the fish muscle as indicated by hardness, cohesiveness, springiness and chewiness was lower in gutted fish. Results of sensory evaluation revealed that gutting has significantly improved the sensory quality of the fish. However, microbiological analysis revealed higher Total Plate Count (TPC) and Enterobacteriaceae count in gutted fish. The shelf life of gutted and whole ungutted sutchi cat fish as determined by microbiological analysis was 16–18 days and 18–20 days respectively while storage in ice.

Keywords Sutchi catfish · Gutting · Ice storage · Biochemical and microbial quality

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Introduction

Presently, catfish farming is gaining importance among Indian farmers as an alternative to carps in different parts of the country. The major species of catfishes recently adopted for culture is *Pangasianodon hypophthalmus* (sutchi catfish). The flesh of sutchi catfish has delicate flavour and characterised by the absence of intermuscular pin bones. As fish is a rapidly growing commodity of the modern diet, freshness and safety is becoming more and more important for the consumers. Compared to terrestrial meat, seafood deteriorates rapidly post mortem as a consequence of various biochemicals, autolytic and microbial breakdown mechanisms. Spoilage of fish occurs concurrently and independently, their relative importance varying with species of fish (size, lipid content, maturate stage, etc.), environmental conditions (feeding availability, temperature, microbial load, etc.), method of slaughter and post-mortem handling, storage procedures and processing conditions (Isabel et al. 2009).

Post harvest handling practices has a prominent role in determining the quality of the final fish product. Fish gut is known to be a reservoir of strong digestive enzymes and bacteria that can rapidly spoil the fish. Gutting or evisceration considerably reduces the microbial load in belly portion which otherwise fasten the process of autolysis that leads to strong off-flavour development and belly bursting. In fish, most of the blood volume is located on the venous side of the cardiovascular system. This means that by evisceration, most of the blood would be removed along with intestines thereby improves the color and appearance of fish flesh. However, many factors such as the age of the fish, species, amount of lipid, harvesting environment and method, etc., should be taken into consideration before deciding whether or not gutting is advantageous.

Various food preservation techniques have been utilized to improve the microbial safety and extend the shelf life of fish in

general, including icing, freezing, chemical preservation, salting and smoking. Presently, icing and mechanical refrigeration are the most prevalent techniques to control the microbial and biochemical spoilage in freshly caught fish during distribution and marketing in tropical countries. Furthermore, preservation in ice has an added advantage that it keeps the inherent flesh characteristics of the fish unchanged during storage unless spoiled. However, there is no regular or definite pattern for deterioration in ice stored fish as it may vary due to species, size and season etc.

Worldwide, sutchi catfish is having high demand as a fresh as well as a suitable candidate for developing value added products. However, very little information is available in literature regarding the preservation aspects of sutchi cat fish in ice. Information on biochemical, microbiological and sensory quality of the sutchi cat fish is of interest to retailers as well as consumers. In this context, the present study has been undertaken to evaluate quality characteristics as well as the effect of gutting on the quality indices of sutchi catfish during storage in ice.

Materials and methods

Materials

Recently harvested sutchi catfish having an average weight of 3–4 kg was procured from a local farm near to Cochin, Kerala. The fish was immediately iced (1:1 fish to ice ratio) in High Density Poly Ethylene (HDPE) boxes and brought to the institute laboratory within 2 h after harvesting. All the chemicals and glass wares used are of the analytical grade.

Methods

Preparation of samples

The raw fish was de iced and thoroughly washed with chilled potable water. One batch of the fish was immediately packed in insulated polypropylene box with ice in the ratio 1:1. Another batch of fish was beheaded and eviscerated. The cut surface and belly portion of gutted fish was cleaned and thoroughly washed under running water to remove the complete stain of blood. The gutted samples were packed in insulated box as described earlier. The melted ice was replaced daily to maintain the fish to ice ratio as 1:1 to maintain a temperature of 1–2 °C. Samples from both the batches were withdrawn at regular intervals to determine the extent of spoilage by biochemical, physical, microbiological and sensorial analyses. All the analyses were done in triplicate and the mean values were taken for deriving conclusion.

Chemical analyses

For chemical analyses, the fish muscle was taken and ground using a mixer grinder. Proximate composition of the raw fish was determined by AOAC (1998) method. The fish muscle sample was homogenised in distilled water (1: 5 w/v) and pH of the homogenate was determined by using a glass electrode digital pH meter (Cyberscan 510, Eutech instruments, Singapore). Total Volatile Base Nitrogen (TVB-N) content was estimated by the microdiffusion method after extracting fish muscle in 10 % Tri Chloroacetic Acid (TCA) (Conway, 1950). Alpha Amino Nitrogen (AAN) content of the sample was determined from the TCA extract of the samples according to the method of Pope and Stevens (1939). Oxidation stability of the sample was assessed from an acidified distillate of sample by measuring Thio Barbituric Acid Reactive Substances (TBARS) spectrophotometrically (Tarladgis et al. 1960). Peroxide Value (PV) was measured by iodometric titration (Yildiz et al. 2003) of the chloroform extract of fish samples. Free Fatty Acid (FFA) value was determined from the chloroform extract as per AOAC (1989) to assess the hydrolytic rancidity.

Texture analyses

Texture Profile Analysis (TPA) was measured with a universal testing machine (Llyod instruments LRX plus, UK), as described by Anderson et al. 1994, equipped with a load cell of 50 N. TPA was performed on uniform raw fish pieces of 2 cm³, compressed twice by a cylindrical probe having a diameter of 50 mm and a test speed of 12 mm/min. For obtaining a consistent result, pieces cut randomly from the fillet of fish was selected for analysis. The instrument generates a curve showing load resulting from deformation. Hardness, cohesiveness, springiness and chewiness of the fish muscle were calculated as defined in the texture analyser user manual.

Microbiological analyses

A 25 g portion of fish was aseptically weighed and transferred to a sterile stomacher bag and 225 ml of sterile physiological saline was added. This suspension was homogenised by a stomacher (Lab blender 400, Seward medical). For the enumeration of aerobic mesophilic count, 0.5 ml of the serial dilutions of homogenates was spread on the Plate Count Agar (PCA) followed by incubation at 37 °C for 2 days. For enumeration of Enterobacteriaceae, 1 ml of serial dilutions of the homogenates was inoculated to 10 ml of molten violet red bile glucose agar (VRBGA). After solidifying, a 10 ml overlay of molten agar was added and the plates were incubated at 30 °C for 24 h and the large colonies with purple haloes were counted.

The dominant aerobic microflora at the final sampling days was determined by isolating and identifying 20 % of the colonies from PCA plates. 20–25 numbers of colonies were randomly selected from PCA plates. To identify specific spoilage flora, 60 numbers of colonies were randomly selected from PCA plates and characterised morphologically and biochemically. They were then grouped according to the taxonomic schemes proposed by several authors for identification (Dainty et al. 1979; Molin and Ternstrom 1982; Krieg and Holt 1984; Sneath et al. 1986; Kirov 2001; Brenner et al. 2004). The isolated cultures were identified and confirmed using API 20NE and API 20NE system (Biomerieux, France).

Sensory analyses

Sensory evaluation of raw and cooked catfish pieces was carried out by a panel of 6 trained members. For taste panel scoring, uniform pieces from each fish sample were cooked in boiling water for 5 min. The members were pre-trained in distinguishing and discriminating between the various aspects of freshness quality assessment like changes in color, odour, appearance and taste of fish during spoilage by giving them the representative samples. Various sensory characteristics of the fish viz. color, appearance, odour and taste at each sampling date were evaluated by the panellists. Scoring was based on a 9 point hedonic scale as described by Amerine et al. (1965). The overall impression of the product by the assessor was estimated as overall acceptability, by adding the scores for all the attributes and dividing by the total number of attributes. A score of below 4 was considered as 'rejected'.

Statistical analyses

All the analysis was done in triplicate. The data was subjected to ANOVA by statistical software, SPSS version 16. Duncan's multiple range tests was carried out to find out the significant difference between mean values of experimental data of the treatments at 5 % level of significance.

Results and discussion

Proximate composition

Proximate composition of the fresh catfish meat showed 17.63 ± 0.33 % protein, indicating that the fish can be considered as a good table fish. Fresh water fish in general contain 17–22 % protein and fat content in a broad range of less than 2 % to as high as 20 % depending on type of feed given, age, size etc. (Natarajan and Sreenivasan 1961). Fat content of sutchi cat fish meat was found to be 2.12 ± 0.26 % which contributes to the delicious taste of the fish. Moisture and

ash content of the fish were found to be 77.43 ± 1.46 % and 1.07 ± 0.12 %, respectively.

pH

Mean pH values of the samples are presented in the Fig. 1a. pH of fresh fish was 6.33 indicating the freshness of the fish. The initial reduction in the pH on 5th day in both the samples might be due to the accumulation of lactic acid by anaerobic glycolysis and the liberation of inorganic phosphates by the degradation of ATP. This is in agreement with the result reported by Ayala et al. (2010) for sea bream during 22 days

Fig. 1 Changes in **a** pH, **b** TVB-N and **c** AAN of whole ungutted (WUG) and gutted **g** sutchi catfish stored in ice ($n=3$, mean $\pm$ standard deviation)

ice storage. The reduction in pH after 3rd day indicates the stress which the fish encountered during harvesting. The more the fish struggled during harvesting, the more the lactic acid production during post mortem and the lesser the pH. After 5th day of storage in ice, pH increased marginally and reached 6.68 and 6.66 on 24th and 26th day of storage, when the samples were rejected based on sensory evaluation, respectively for whole ungutted and gutted sutchi cat fish. Increase in pH during storage may be due to the production of amines and other volatile bases by the autolytic and microbial action on protein and other compounds (Binsi et al. 2007). The post-mortem pH limit of acceptability is usually 6.8–7.0 (Zang and Deng 2012). In the present study, whole as well as gutted fish never crossed this limit at any time during the entire storage period in ice. However, no significant difference in pH was observed between the samples under study. Similar results were also reported by Lokuruka et al. (2012) for gutted and whole ungutted tilapia and Nile perch during storage in ice. The results of our study indicate that pH is a poor indicator of quality in sutchi catfish during ice storage.

Total volatile base-nitrogen content

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. Variations in the mean TVB-N values of the sample are depicted in the Fig. 1b. Fresh fish has shown a TVB-N value of 5.6 mg%. An initial lag phase of 5 days with no marked increase in TVB-N was observed in whole ungutted as well as gutted catfish. Subsequently, a significant increase in TVB-N ($p<0.05$) content was noticed in both the samples with the storage period. The increase in TVB-N during storage is a consequence of liberation of basic compounds by microbial activity on protein and non protein nitrogenous compounds. Over the entire storage period, whole ungutted cat fish presented significantly higher TVB-N ($p<0.05$) values compared to gutted fish. This observation is in agreement with the findings reported by Lokuruka et al. (2012) for ungutted tilapia during ice storage. The low levels of TVB-N in gutted fish samples may be attributed to the reduced bacterial decomposition of nitrogenous compounds in the fish flesh.

The concentration of TVB-N in freshly caught fish is typically between 5 and 20 mg N/100 g, whereas levels of 30–35 mg N/100 g fish are generally regarded as the limit of acceptability for ice-stored cold water fish (Connel 1995). However, various authors have reported different acceptability levels for TVB-N value depending on fish species, specific treatments, and processing conditions: 35–40 mg/100 g (Lakshmanan 2000); 25–30 mg/100 g for oyster (Lopez-Caballero et al. 2000); 25–35 mg/100 g for sardine (Ababouch et al. 1996) etc. In our study, the maximum

TVB-N value registered for whole and gutted samples were 28.96 and 30.57 mg N/100 g on their sensory rejection day, respectively on 22nd and 26th day of storage. Hence, the results of the present study suggest a maximum acceptable limit of 25–30 mg/100 g for TVB-N in ice stored sutchi cat fish.

Alpha amino nitrogen content

AAN is a major component of non-protein nitrogenous compounds in fishes and in teleost fishes it ranges from 17–81 mg/100 g of meat. In the present study, the AAN content in fresh cat fish was found to be 8.75 mg%. Changes in the mean AAN content of the samples are depicted in the Fig. 1c. During storage in ice, whole and gutted sample showed a significant increase ($p<0.05$) in AAN content up to a certain period during ice storage and thereafter shown a progressive reduction until the end of storage. From 5th to 18th day of ice storage, whole ungutted samples presented significantly higher ($p<0.05$) values for AAN than that of gutted sample. In gutted fish, the rise in AAN content was comparatively slower, registered a peak on day 20th, and then maintained a decreasing trend till the end of storage period. The rise in AAN content is attributed to the release of free amino acid through the proteolytic action of endogenous as well as microbial enzymes on muscle protein. During the later period of storage, the free amino acid produced undergoes further degradation into volatile bases and other low molecular weight compounds, causing a reduction in total AAN content. Moreover, leaching of free amino acid to the surrounding media can also contribute to the final reduction in AAN content in both the samples during ice storage. The higher AAN content observed in whole ungutted fish up to 18th day of storage may be associated with a higher proteolytic activity in the same compared to gutted fish.

Free fatty acid content

Degree of lipid hydrolysis can be evaluated by measuring the amount of free fatty acids. Triglyceride in the depot fat is cleaved by triglyceride lipase originating from the digestive tract or excreted by certain microorganisms. Variations in the mean FFA values of the samples are given in Fig. 2a. Both the samples showed a marginal increase till 16th day of storage followed by a significant progressive increase up to the end of storage period. Difference in the mean FFA values between the whole ungutted and gutted samples has become significant ($p<0.05$) after 20 days of storage. Gutting followed by washing might have considerably reduced the microbial load in the belly area and hence the hydrolysis of lipid by microbial enzyme got reduced in gutted fish than that in whole ungutted fish towards the end of storage period. There are some previous reports that gutting causes lower degree of hydrolytic lipid

Fig. 2 Changes in **a** FFA, **b** PV and **c** TBARS of whole ungutted (WUG) and gutted **g** sutchi catfish stored in ice ($n=3$, mean $\pm$ standard deviation)

spoilage in fish (Lehmann and Aubourg 2008; Erkan and Ozden, 2008). However, in the present study, the difference in FFA value has become significant only towards the end of storage period, indicating that the effect of gutting on lipid hydrolysis was insignificant in the early stages of ice storage period. Presence of FFA content in fresh water fish is not regulated from the safety point of view in legislation (Jezek and Buchtová 2007).

Peroxide value

Lipid in the fish is susceptible to oxidation and PV measures the amount of hydroperoxides formed i.e., the primary lipid

oxidation products in fish muscle. The variations in mean PV values are presented in the Fig. 2b. PV of fresh fish was 3.3 MEq O₂/Kg sample. Whole and gutted samples showed a gradual increase in PV till 16th day of storage, followed by a sharp increase to a maximum value on 20th and 22nd day respectively and thereafter showed a reduction towards the end of storage period in ice. Whole ungutted sample showed significantly lower ($p<0.05$) values for PV than gutted catfish which indicates that hydroperoxides formed in whole ungutted fish might have undergone subsequent oxidation to tertiary compounds at a faster rate than that in gutted catfish sample. It has been reported that, in fatty fishes like mackerel and sardine, gutting increased the peroxide development (Lehmann and Aubourg 2008; Erkan and Ozden, 2008) during storage. In our study also, comparison of PV among the samples revealed a greater degree of primary oxidation in gutted sample. This could be attributed to the fact that gutting exposes the belly area and cut surfaces to the air thereby rendering the lipid more susceptible to oxidation. This accounts for the higher PV values in gutted sutchi catfish.

Thio barbituric acid reactive substance

TBARS is a measure of one of the secondary lipid oxidation products, the malonaldehyde. Changes in TBARS value of the samples over the storage period is depicted in the Fig. 2c. Fresh fish showed a TBARS value of 0.038 mg malonaldehyde/kg sample. There was a significant difference ($p<0.05$) between the TBARS of whole ungutted and gutted samples. In ungutted fish, TBARS increased markedly and reached 1.091 mg malonaldehyde/kg sample on 18th day and thereafter showed a reduction towards the end of storage period. Whereas, gutted fish showed a gradual increase in TBARS and the value never crossed 1 mg malonaldehyde/kg sample at any time during the entire storage period. The limit of acceptability for TBARS is 2 mg malonaldehyde/kg sample, beyond which the fish generally develops an objectionable odour and taste (Connell 1990). In the present study, TBARS was within the acceptable limit for both the samples throughout the entire storage period. Tejada and Huidobro (2002) reported that gutting of ice stored sea bream seemed to have no significant effect on rancidity. However, in this study, whole catfish sample showed significantly higher TBARS than gutted sample till 20th day of storage. The higher TBARS values in whole catfish sample can be attributed to the higher rate of secondary lipid oxidation compared to that in gutted catfish sample.

According to Aubourg (1993) TBARS records may not reveal the actual rate of lipid oxidation since malonaldehyde can interact with other components of fish muscle. Such components may be amines, nucleosides and nucleic acid, proteins, amino acids of phospholipids, and other aldehydes that are end products of lipid oxidation and this interaction may vary greatly with fish species. As many authors reported

(Maqsood and Benjakul 2010; Goulas and Kontominas 2007), declining trend in TBARS of whole ungutted catfish toward the end of storage period is attributed to the interaction of these low molecular unstable compounds as well as its break down to organic acid, alcohols etc. which are not determined by TBARS test.

Texture profile analyses

Hardness

In texture profile analysis, the response of a sample to a compressive or tensile force is measured by means of time. Basic mechanical variables that characterise texture of food are hardness, springiness, adhesion and cohesion (Casas et al. 2006). Hardness represents the peak force required to compress the fish flesh and it gives an indication of resistance of the muscle to deform by external pressure. Hardness 1 refers to the peak force during first compression and Hardness 2 refers to the peak force during second compression. Changes in hardness 1 and hardness 2 values of the samples over storage period are shown in Fig. 3a and b respectively. In the present study, hardness 1 of whole and gutted cat fish increased from an initial value of 11.22 Kgf to 12.38 and 12.04 Kgf respectively on 1 day of storage in ice. Thereafter, both the samples have shown a significant decrease in values over the storage period. A similar trend was also observed for hardness 2 of whole ungutted and gutted fish during storage. Since the captured fish was immediately packed in ice after death, the fish entered into the stage of rigor mortis during the first day of storage in ice. This accounts for the increase in hardness 1 and hardness 2 values observed on the first day in both the samples. Decreases in hardness 1 and hardness 2 values after first day might be attributed to the weakening of connective tissue of fish muscle during storage. Similar observations were also made by Hatae et al. (1985) who reported a softening of the texture in several fish species stored at 4 °C for up to 14 days, in a study using the General Foods (GF) texturometer.

During storage in ice, some myofibrillar protein degrades and fish muscle generally becomes softer (Verrez-Bagnis 1997). It has also been reported that the reduction in textural properties is attributed to the weakening of connective tissue and the Z-lines completely extinguishes after storage (Laksmanan and Piggott 2003). These undesirable changes are due to the activity of autolytic enzymes (e.g. collagenase, ATPase) degrading different proteins and breaking down the connective tissue in muscle meanwhile the spoilage microorganisms multiply rapidly and promote the progress of spoilage. The hardness values were significantly lower ($p < 0.05$) in whole ungutted catfish than that in gutted fish, which further indicates the softness of the muscle in the earlier.

Cohesiveness, springiness and chewiness

Cohesiveness indicates the property of being cohesive and sticky of the fish. Cohesiveness gives an indication of how well the samples withstand the deformation during compression. A value of 1 indicates total elasticity and a value of 0 indicates that the sample did not recover at all (Manju et al. 2007). The change in cohesiveness of whole ungutted and gutted fish is shown in the Fig. 3c. Even though not significant, cohesiveness slightly decreased from an initial value of 0.289 to 0.223 and 0.221 in whole and gutted fish on 22nd and 26th days of storage, respectively. This indicates that there was not much change in the internal bonding of fish muscle during storage. This is in agreement with the result of Manju et al. (2007) who observed a slight reduction in cohesiveness value of pearl spot during ice storage.

Springiness indicates the elasticity of muscle that can be stretched and returns to its original length. Springiness is the elastic or recovering property of the fish muscle during compression. Variations in the springiness values are presented in the Fig. 3d. In general, a decreasing trend was observed for springiness values in whole as well as gutted fish over the storage period. The values indicate that the fish muscle is losing its elasticity during storage. The mean values of springiness in whole ungutted fish became significantly lower ($p < 0.05$) to that of gutted fish after 5 day of storage.

Chewiness is the mouth feel sensation of laboured mastication due to sustained, elastic resistance from the fish, defined as the product of hardness $\times$ cohesiveness $\times$ springiness. Changes in the mean chewiness values of whole and gutted fish sample over the storage period is depicted in the Fig. 3e. The fresh fish showed a value of 2.064 Kgf/mm for chewiness. A significant reduction in chewiness values was observed for both the sample during the ice storage period which reached 1.363 and 1.423 Kgf/mm respectively, for whole and gutted fish on their respective rejection days. Decrease in chewiness indicates that the fish muscle becomes soft during storage. In general, whole ungutted fish presented significantly lower values of chewiness as well as springiness than that of gutted fish. In addition, the significant reduction in springiness and chewiness was coincidental with the muscle softening, degradation and deterioration. The results revealed that gutting delayed the progress of muscle tissue degradation. It has been reported that strong digestive proteases from digestive tract can invade the muscle tissue which can cause muscle tissue degradation (Pedrosa-Menabrit and Regenstein 1988). Hence, in the present study, by removing the viscera, the digestive enzyme load might have reduced to a greater extent in gutted fish compared to whole ungutted fish, leading to lower tissue degradation. Ghaly et al. (2010) stated that autolytic degradation can limit the product quality and shelf life even with relatively low levels of spoilage organisms, which was found to be true in the case of whole ungutted catfish.

Fig. 3 Changes in **a** Hardness 1, **b** Hardness 2, **c** Cohesiveness, **d** Springiness and **e** Chewiness of whole ungutted (WUG) and gutted **g** sutchi catfish stored in ice ($n=3$, mean $\pm$ standard deviation)

Changes in total mesophilic and enterobacteriaceae counts

Changes in mesophilic and Enterobacteriaceae counts of whole ungutted and gutted samples are presented in the Fig. 4. The initial aerobic, mesophilic count was $4.241 \log_{10} \text{cfu g}^{-1}$. Icing of fish samples caused a reduction in the bacterial counts and thereafter, the counts gradually increased. In this study, initial mesophilic counts of whole ungutted and gutted sutchi catfish is indicative of superior flesh quality considering the proposed lower limit for aerobic plate count of $5 \log_{10} \text{cfu g}^{-1}$ for fresh fish (ICMSF International Commission on Microbiological Specifications for Foods 1998). The upper limit (M) for fresh fish proposed by ICMSF (International Commission on Microbiological Specifications for Foods) (1998) for human consumption is $7 \log_{10} \text{cfu g}^{-1}$. In the present study, mesophilic counts for whole ungutted catfish

and gutted cat fish exceeded this limit on day 20 and day 18, respectively at $0-2^\circ \text{C}$ during ice storage.

Fig. 4 Changes in total aerobic and Enterobacteriaceae counts of whole ungutted (WUG) and gutted **g** sutchi catfish stored in ice

The initial count of Enterobacteriaceae was $2.3 \log_{10} \text{ cfu g}^{-1}$ which increased to a final levels of 5.4 and $5.6 \log_{10} \text{ cfu g}^{-1}$ in whole ungutted and gutted catfish respectively while stored in ice. However, the initial level of Enterobacteriaceae noticed in the present study is lower than the earlier observations made on farmed freshwater trout (Ninan et al. 2011) and farmed brackish water fish such as pearl spot (Ravishankar et al. 2008) and milkfish (Sneha 2011). Assessments of the levels of Enterobacteriaceae in foods allow an estimation of their general bacteriological condition ('indicator' function) and to a certain extent the risk of presence of pathogenic enteric organisms ('index' function) (Mossel 1982). Adopting a reference value of 10^3 g^{-1} Enterobacteriaceae with the usual tolerance (Mossel and Tamminga 1980), sutchi catfish sample used in this study has a lower value. In this study, their proliferation was slow, possibly because their growth rate is lower than that of other Gram-negative aerobic bacteria, reaching final levels of $5.4\text{--}5.6 \log_{10} \text{ cfu g}^{-1}$. Gram et al. (1999) reported that *N*-Acyl Homoserine-Lactone (AHL) mediated gene regulation in many strains of Enterobacteriaceae isolated from foods boost production of AHL at cell densities of 10^5 to 10^7 cfu/g , thereby contributing to quality changes at relatively low cell numbers. The contribution of Enterobacteriaceae in the microflora of fish and its spoilage potential must be taken into consideration especially in the case of harvesting from polluted waters and delay in chilling after catch.

In general, the counts of aerobic mesophilic bacteria and Enterobacteriaceae of gutted catfish were ca. 0.3–1.0 log cycles higher ($p < 0.05$) than those for the whole ungutted samples throughout the entire storage period. The higher counts noticed on gutted fish throughout the entire period of storage in ice can be attributed either to cross-contamination of fish during gutting procedures or to the exposure of larger fish flesh surface area to environmental microbial contamination during icing. This is in agreement with the observations made on gutted and whole ungutted aqua-cultured sea bass (*Dicentrarchus labrax*) stored in ice (Papadopoulos et al. 2003). Little information is available on mesophilic counts and Enterobacteriaceae counts on whole and gutted sutchi catfish. Maqsood and Benjakul (2010) reported a shelf life of 3 days for sutchi cat fish fillets stored at 4°C under air. Shelf life of frozen sutchi catfish stored at $4.0 \pm 0.7^\circ\text{C}$ was found to be 7 days (Noseda et al. 2012). In this study, based on the microbiological data, a shelf life of 18–20 days was noticed for whole ungutted sutchi catfish and for gutted samples shelf life was only 16–18 days.

On the basis of the microbiological data obtained in this study, this is the first report on microbiological changes associated with whole ungutted and gutted sutchi catfish stored in ice. The initial microflora on fresh catfish was dominated by *Pseudomonas*, *Aeromonas*, *Burkholderia*, *Enterobacteriaceae*, *Staphylococcus* and *Bacillus*. At the end of chilled storage, the dominant genera belonged to *Aeromonas*, *Pseudomonas*,

Burkholderia and *Stenotrophomonas* (data not shown). It is well documented that bacterial spoilage in refrigerated fish and fish products under aerobic storage conditions is caused by Gram-negative psychrotrophic organisms such as *Pseudomonas*, *Alteromonas*, *Shewanella* and *Flavobacterium* spp. (Hubbs, 1991). In this study, in addition to *Pseudomonas* and *Aeromonas*, a significant number of isolates from catfish at the end of storage were partially identified as members of *Stenotrophomonas* and *Burkholderia*. They are psychrotrophic organisms and these genera comprise species considered as emerging pathogens and species that exhibit multiresistant traits (Alonso et al. 2000; Levy 2002).

Sensory evaluation

Changes in the overall acceptability scores over the storage period are presented in the Fig. 5. Sensory scores showed a significant reduction in both whole and gutted catfish with the progress of ice storage. 6th day onwards, gutted sample presented significantly higher scores compared to that of whole ungutted cat fish sample. Moreover, gutting has appreciably improved the colour and appearance of the fish flesh compared to whole fish. Progress of fish spoilage was evident by development of putrid odours, slimy and soft flesh. Accordingly, a sensory acceptability of 20 and 24 days under ice storage is estimated for whole and gutted sutchi cat fish, respectively.

It was interesting to note that the sensory data was not in agreement with microbiological data. Ninan et al. (2011) reported that spoilage of fresh trout stored aerobically in ice was due to the activity of more than one specific spoilage organism and genera *Moraxella*, *Acinetobacter*, *Pseudomonas*, *Shewanella*, *Aeromonas* and *Flavobacterium* dominated in the microflora. In contrary, in the present study, towards the end of storage, the specific spoilage organisms were limited to *Pseudomonas* and *Aeromonas* alone, whereas significant number of members of *Stenotrophomonas* and *Burkholderia* was observed among the microflora. The members of *Stenotrophomonas* and *Burkholderia* are considered as

Fig. 5 Changes in the sensory score of whole ungutted (WUG) and gutted g sutchi catfish stored in ice ($n = 5$, mean $\pm$ standard deviation)

emerging pathogens which need not necessarily cause spoilage reactions in fish. Thus, even though the Total Plate Count was more in gutted fish, it was not reflected on sensory and biochemical analysis. Huss et al. (1974) also reported that not all the bacteria species isolated from fish cause spoilage. However, considering the microbiological safety of fish, the shelf life of gutted and whole ungutted catfish is determined as 16–18 days and 18–20 days in ice, respectively.

There are variations among the reports on effect of gutting on the shelf-life of other fishes in the literature. Chang et al. (1998) reported 19 days shelf-life for gutted and headed sea bass whereas Papadopoulos et al. (2003) has reported a shelf-life of 13 and 8 days respectively for whole ungutted and gutted sea bass samples during ice storage. The study of Tejada and Huidobro (2002) indicated that both the slaughter method and gutting of the fish seabream had no influence on the quality during chill storage. All these results indicate that there is a variation among species with regard to the efficacy of gutting in extending shelf life.

Conclusions

In the present study, whole ungutted as well as gutted sutchi catfish sample never crossed the prescribed acceptance limit of any of the biochemical quality parameters studied. pH of both whole ungutted and gutted fish has shown no significant changes during storage. Gutting and evisceration significantly reduced volatile base generation and free amino nitrogen content, whereas it enhanced the peroxide development during storage at 4 ± 2 °C. Even though the sensory data indicated an extended acceptability up to four days for gutted sutchi catfish (24 days) than whole ungutted pangasius (20 days) during iced storage, the microbiological shelf life was determined as 16–18 days and 18–20 days, respectively for gutted and whole ungutted fish. The present study revealed that although gutting improves the sensory qualities, it render the fish more prone to microbial cross contamination, thereby limiting its shelf life during prolonged ice storage.

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