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Nutritional profiling and shelf life assessment of monosex tilapia steaks during ice storage

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ABSTRACT

In freshwater aquaculture, monosex tilapia is emerging as a potential alternative to common tilapia which is a prolific breeder. The nutritional profile as well as the shelf-life stability of monosex tilapia stored under iced condition was assessed. The fatty acid profile pointed out that the major class of fatty acids present in tilapia was monounsaturated fatty acids (42.36%). Amino acid compositional analysis indicated higher levels of glutamic acid and aspartic acid. Tilapia steaks were divided into two lots for ice storage *viz.,* control (AP: air packed) and vacuum packed (VP). AP samples crossed the total volatile base nitrogen (TVB-N) acceptability limit of 35 mg% towards 16th day of storage whereas VP samples were acceptable throughout the storage period of 19 days. Increase in thiobarbituric acid (TBA) value was more prominent in air packed lots during storage period. The decrease in hardness and lightness values were more prominent in vacuum packed samples. Results of the biochemical and microbiological analyses indicated that air packed samples were acceptable upto 16th day while it was extended to 19th day in vacuum packed samples.

Keywords: Air packaging, Ice storage, Monosex tilapia, Nutritional profiling, Shelf life, Vacuum packaging

Introduction

Fish, nutritionally loaded on account of its unique composition of all essential amino acids as well as polyunsaturated fatty acids, has gained importance and demand as a health food in human diet (Tidwell and Allan, 2001). Due to the stagnating growth in capture fisheries sector, more focus has been given to inland aquaculture to meet the consumer demand for fish. Freshwater aquaculture sector has been found to have a remarkable growth in the past few years with more and more emerging freshwater species gaining commercial importance in the domestic as well as export market. Among the freshwater candidate species, tilapia has emerged from mere obscurity to one of the most productive and internationally traded food fish in the world, to such an extent that they have been called the fish of the future. Monosex tilapia, is produced by hormonal sex reversal of male tilapia, *Oreochromis niloticus*. The growth rates of the monosex tilapia are twice faster than female tilapia and hence its commercial production is expanding globally (Laly *et al*., 2016).

Fish protein is the cheapest among animal proteins and growth rate of fishes are faster with growth coefficient often >1, compared to any other animal protein sources, including poultry (Venugopal, 2006). These biological advantages offer considerable benefits to fish as a tool to achieve nutritional and social security. Moreover, the contribution of fisheries to GDP is increasing in most countries in spite of the decreasing contribution of agriculture to GDP. However, though highly nutritious, it is one of the most perishable food items which has been marked as a major disadvantage. Hence processing and preservation of fish plays a major role for maintaining its quality and researchers have been constantly focusing on improved methods to preserve or extend the shelf life and safety of these unstable aquatic food products (Chang *et al.,* 1998). Different processing and preservation methods like chilling, freezing, salting, drying, smoking or intelligent combination of two or more of these methods commonly referred to as hurdle technology (Leistner, 2000), are used for the preservation of fish. The role of packaging in food processing is equally significant in maintaining the safety and quality of the food product. Vacuum packaging is a type of modified atmospheric packaging in which air is completely removed from the pack and sealed (Church, 1998). The fresh and healthy appearance of vacuum packed (vp) products has gained a huge consumer preference in many countries.

Quality and shelf life studies on freshwater fishes are very few compared to the abundance of data available on marine species. Rong *et al*. (2009), Emire and Gebremariam (2010), Arekemase *et al*. (2012) and Kulawik *et al*. (2013) have reported on the quality and shelf life of tilapia under low temperature storage. However, so far only Laly *et al*. (2016) have reported on the quality changes of monosex tilapia during storage. Hence a study was carried out to determine the nutritional status and shelf-life of ice stored monosex tilapia under normal and vacuum packaged conditions.

Materials and methods

Raw material

Fresh monosex tilapia samples were procured from a fish farm at Kochi, Kerala. The fish samples were immediately layered with flake ice (1:1 (w/w) in high-density polyethylene (HDPE) insulated boxes with polyurethane foam (PUF) and transported to the laboratory. Further, they were dressed and cut into steaks of 1.8-2 cm thickness and divided into two lots; the first lot kept as control (AP: air packed) and the second lot was vacuum packed (VP). Bags made of 12 µ polyester laminated with 300 gauge low density polyethylene were used for packing the samples. After packing, all the samples were iced immediately with flake ice (1:1 (w/w) in an HDPE box insulated with PUF and were kept at room temperature. Re-icing was done every day to compensate the melted ice. Triplicate samples were drawn randomly from each lot at regular intervals and were subjected to physical, chemical, microbiological and sensory analyses. Fatty acid and amino acid profiling were done in duplicate.

Nutritional profiling

Fresh tilapia was evaluated for its nutritional aspects in terms of proximate composition as per AOAC (2012). Total nitrogen and crude protein contents were estimated by Micro Kjeldahl method. Fat from the samples was extracted as per Folch *et al*. (1957). Fatty acids present in the sample were converted to fatty acid methyl esters (FAMEs) using BF3 methanol and fatty acid composition analysis was performed using gas chromatograph (Varian, Model CP-3800) with a chrompack capillary column (25 m × 0.32 mm ID; 0.30 µm film thickness) and a flame ionisation detector. Amino acid analysis was accomplished by HPLC (high-performance liquid chromatography) following Ishida *et al.* (1981). HPLC was equipped with cation exchange column packed with a strongly acidic cation exchange resin *i.e.,* styrene di vinyl benzene co polymer with sulphinic group. The instrument was equipped with Shimadzu FL 6A fluorescence detector and the oven temperature was maintained at 60°C.

Biochemical analysis

The biochemical parameters *viz*., moisture (AOAC, 2012), pH (pH meter - Cyberscan 510, Eutech Instruments, Singapore), total volatile base nitrogen (TVB-N) (Conway, 1962), thiobarbituric acid (TBA) (Tarladgis, 1960) and water-holding capacity (WHC) (Mohan *et al*., 2011) were analysed during ice storage.

Instrumental texture analysis

Instrumental texture (Anderson *et al.,* 1994) was analysed using Universal Testing Machine (LRX plus, Lloyd Instruments Ltd, Hampshire, UK) wherein the load cell used was a cylindrical probe of 50 mm dia equipped with a sensor of 50 N. The central part of the fish steak was uniformly cut and used for analysing the texture. Sample was analysed to produce numeric results indicative of the colour of the sample by measuring the degree of lightness (L^*), degree of redness/greenness (a^*) and the degree of yellowness or blueness (b^*) (Hunter Lab colorimeter, Miniscan XE Plus Hunter Associates Lab inc., USA).

Microbial analysis

A known quantity of the sample (25 g) was aseptically removed from each lot and macerated well for one minute with 225 ml of a suitable diluent (0.85% sterile physiological saline) in a stomacher (Lab Blender 400; Seward Medical, London, UK). The samples were serially diluted and plated for enumeration of total aerobic plate count by the spread plate method. The inoculated plates were incubated at 30ºC for 48 h. Microbiological counts were performed in triplicate and expressed as cfu g^{-1} (AOAC, 2002).

Sensory evaluation

Six trained panelists assessed different attributes like appearance, odour, flavour, colour and texture using a 9-point hedonic scale as prescribed by Meilgaard *et al.* (2006) of both raw and cooked samples (cooked in 1.5% brine for 10 min), 4 being the acceptability limit. The overall impression of the product on the assessor was estimated as overall acceptability, by adding the scores for all the attributes and dividing by the total number of attributes.

Statistical analysis

Data obtained were analysed by analysis of variance (ANOVA) using the statistical software SPSS 16 (SPSS Inc. Chicago).

Results and discussion

Nutritional profile

In the present study, tilapia meat had 73.44±0.24% moisture content, 17.11±0.16% crude protein, 6.75±0.04%

fat and 1.18±0.01% ash content. According to Arekemase *et al*. (2012), protein content of fresh samples of tilapia ranged from 15.56 to 15.72% and fat content ranged from 5.12 to 5.44%. Emire and Gebremariam (2010) reported 79.87% moisture content, 18.52% protein, 0.37% fat and 0.98% ash in Nile tilapia fish fillets. In the present study, the fat content of tilapia samples was higher which could be attributed to an influence of diet. El-Zaeem *et al.* (2012) pointed out that increase in parameters like feeding rate and fish size brings about enhanced adipose deposition and decrease water content in the fish body. Study on fatty acid profile analysis indicated that the major class of fatty acids in tilapia was monounsaturated fatty acids (MUFA) (42.36%), followed by saturated fatty acids (SFA) (35.49%) and polyunsaturated fatty acids (PUFA) (22.16%). According to Chukwuemeka (2008), in some species of Cichlidae and Claridae family, the SFA component ranged from 37 to 74%, MUFA varied from 21 to 47% and PUFA were present in low levels of 18%. Among the fatty acids (Table 1), oleic (33.28%) and palmitic acids (24.70%) were found in higher levels whereas caprylic acid was the limiting one. Zuraini *et al*. (2006) observed that the most abundant fatty acid in freshwater fishes like *Channa* spp. was palmitic acid ranging from 25.6 to 30.4%. Similar findings were reported by Osibona *et al.* (2009) in *Tilapia zillii*. Observations made by Kulawik *et al.* (2013) on the fatty acid composition of farmed Nile tilapia indicated that the major class of fatty acids in tilapia was the MUFA (39%), which was mostly due to the high content of oleic acid. The fatty acid compositions of freshwater fishes *viz.,* North African catfish, common carp, wels catfish, tench, kutum and zander consisted of 28.0-34.6% of SFA, 10.7-22.7% of MUFA and 23.2-43.8% of PUFA (Yesim *et al.*, 2007). The ratio of omega-3 to omega-6 fatty acids was observed to be 0.795 in the present study. Al-Souti and Claereboudt (2014) reported that the ratio of total omega-3 to omega-6 fatty acids is much lower for freshwater fish than for marine fish typically in the range 0.5-3.8% due to the relatively higher proportions of omega-6 PUFA; especially linoleic acid.

Table 1. Fatty acid composition of monosex tilapia meat (Mean ± SD)

Fatty acids	% of total fat
C8:0	0.028 ± 0.006
C12:0	0.058 ± 0.01
C14:0	2.075 ± 0.237
C14:1	0.122 ± 0.014
C15:0	0.261 ± 0.023
C16:0	24.697 ± 1.051
C16:1	5.792 ± 0.336
C17:0	0.174 ± 0.003
C17:1	0.233 ± 0.004
C18:0	6.123 ± 0.125
C18:1(c+t)	33.283 ± 0.469
C18:2n6c	0.047 ± 0.004
C18:2n6t	7.839 ± 0.047
C18:3n6	0.322 ± 0.004
C18:3n3	0.486 ± 0.023
C20:0	0.206 ± 0.020
C20:1	1.546 ± 0.111
C20:2	1.136 ± 0.084
C20:3n6	0.649 ± 0.005
C21:0	0.118 ± 0.001
C20:3n3	1.112 ± 0.027
C20:4n6	0.144 ± 0.005
C20:5n3	0.309 ± 0.036
C22:0	0.227 ± 0.045
C22:1n9	0.166 ± 0.119
C22:2	4.867 ± 0.625
C23:0	1.523 ± 0.199
C24:1	1.212 ± 0.412
C22:6n3	5.245 ± 0.798
SFA	35.5
MUFA	42.4
PUFA	22.2

The amino acid analysis indicated higher levels of glutamic acid, aspartic acid, alanine and lysine whereas tryptophan was the limiting aminoacid (Table 2). Proline, tyrosine, methionine and histidine were also found in low levels. Chukwuemeka (2008) reported that glutamic acid (17.81-18.16%) and aspartic acid (11.17-11.35%) were the dominant amino acids in both Cichlidae and Claridae species and glutamic acid content in all the species were higher compared to aspartic acid. Osibona *et al.* (2009) observed that the major aminoacids present

Table 2. Amino acid composition of monosex tilapia (Mean ± SD)

Aminoacid composition	% of total amino acids
Essential amino acids	
Arginine	5.04 ± 0.14
Histidine	2.09 ± 0.14
Isoleucine	4.06 ± 0.03
Leucine	7.87 ± 0.08
Phenyl alanine	4.18 ± 0.72
Threonine	3.93 ± 0.13
Valine	4.45 ± 0.77
Methionine	1.86 ± 0.33
Lysine	9.26 ± 0.53
Tryptophan	0.03 ± 0.002
Non-essential aminoacids	
Alanine	8.09 ± 0.46
Aspartic acid	16.15 ± 0.56
Glycine	5.04 ± 0.15
Glutamic acid	22.52 ± 1.84
Proline	1.05 ± 0.12
Serine	3.86 ± 0.25
Tyrosine	1.69 ± 0.14
Cysteine	ND

in *T. zillii* were glutamic acid, aspartic acid, arginine, lysine and leucine ranging between 9.49 to 18.16%. According to Zuraini *et al*. (2006), the major amino acids in *Channa* spp were glutamic acid, aspartic acid and lysine ranging from 9.7 to 21.7% of total amino acids.

Biochemical analyses

Variations in moisture content indicated a slight increasing trend initially followed by a decline which was not significant ($p>0.05$) throughout the storage period in both the lots (Fig. 1a). Similar observations were made by Binsi *et al*. (2015) in chill stored eviscerated and vacuum packed freshwater catfish and reported it to be associated with the poor barrier property of packaging material and the seal which might have promoted the entry of moisture from storage environment. Indira *et al*. (2010) observed a slight increase in moisture content from an initial value of 79.35 to 82.60% in common carp during 3 weeks of storage at refrigeration temperature. Loss of waterholding capacity of myofibrillar proteins during extended chill storage might have resulted in increased drip loss and thereby decrease of moisture content in samples. VP samples exhibited lower moisture content compared to AP samples which may be on account of more exudates released during vacuum packing. Similar observations were made by Manju *et al*. (2008) in pearlspot where drip loss was found to be significantly ($p<0.05$) more in the case of vacuum-packaged samples than that of air packaged samples. According to the study by Arekemase *et al*. (2012), the moisture content ranged from 75.15 to 76.71% in preservative treated tilapia during refrigerated storage which is in accordance with the results observed in the present study where the moisture content ranged from 73.4 to 76.7% in all the samples.

pH is an important factor that affects microbial growth and spoilage of foods (Salaudeen *et al*. 2010). pH value of tilapia samples were found to increase gradually but significantly ($p<0.05$) throughout the storage period in VP samples (6.0 to 6.2) whereas an increasing trend followed by slight fluctuations of decrease and increase was observed in AP samples. No significant difference ($p>0.05$) in pH was observed between the AP and VP lots (Fig. 1b). Similar results in pH were reported by Laly *et al.* (2016) in both whole and gutted monosex tilapia samples which showed an increasing trend initially followed by slight fluctuations of increase and decrease of pH during later stages of storage. Ruiz-Capillas and Moral (2001) opined that carbon dioxide diffusion to muscle tissue and the parallel formation of carbonic acid play a role in pH reduction. An increase of pH was

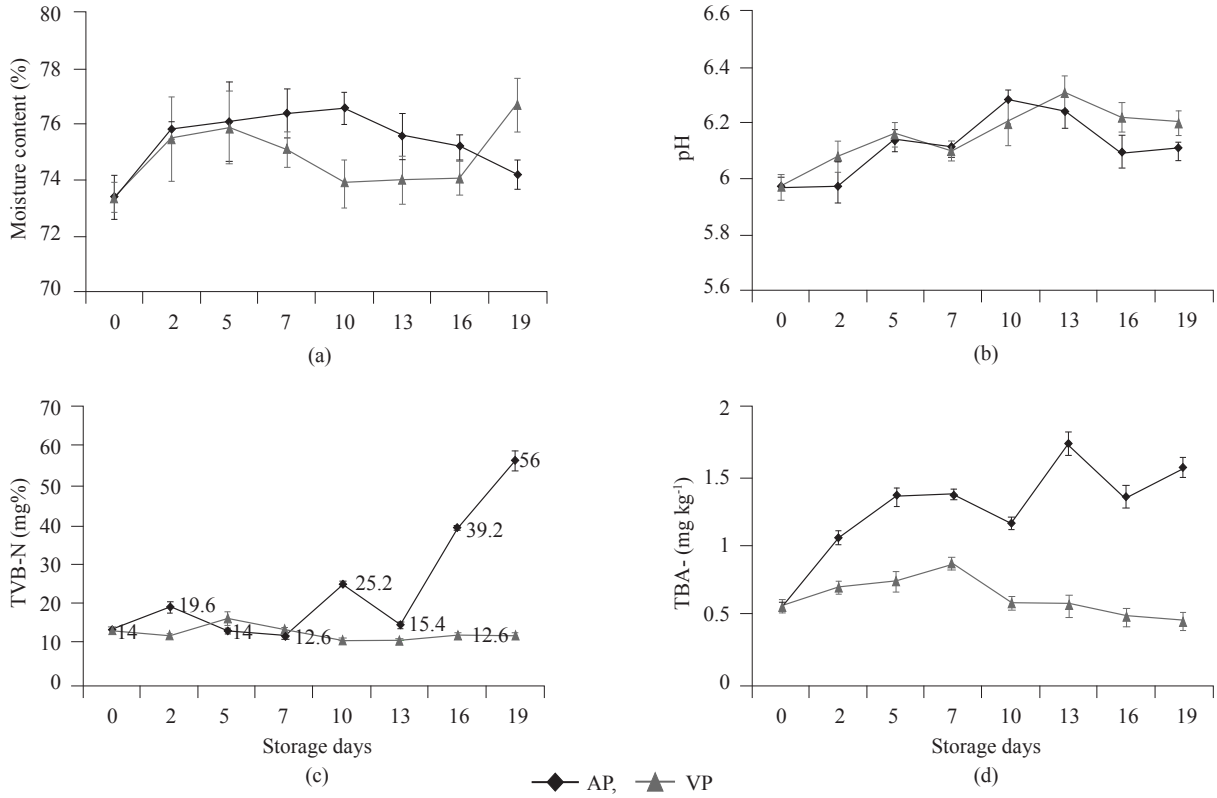

Fig. 1. Variations in biochemical composition of air packed and vacuum packed monosex tilapia during ice storage. (a): Moisture content; (b): pH; (c): TVB-N; (d): TBA

postulated to be due to the rapid spoilage of the product and the formation of alkaline compounds of autolysis and bacterial metabolites (Ruiz-Capillas and Moral, 2001; Benjakul *et al*., 2002).

Total volatile base nitrogen (TVB-N) has been reported as spoilage compound and proposed as a good fish spoilage indicator (Koutsoumanis and Nychas, 2000). A significant increase ($p<0.05$) in TVB-N was found in AP sample lots. However it remained more or less constant with slight fluctuations in the VP lots (Fig. 1c). The increase in TVB-N may be attributed to the bacterial decomposition taking place during storage. According to the study of Adoga *et al*. (2010), TVB-N contents of Nile tilapia showed slow increase during the early stages of storage and the level increased more rapidly towards the later stage during ambient storage. A level of 30-35 mg% is usually regarded as the limit of acceptability (Lakshmanan, 2000) and in the present study, AP samples crossed the acceptability limit reaching 39.2 mg% towards 16th day whereas VP samples were within the acceptability limit throughout the storage period. In the present study, poor correlation was observed between pH and volatile bases in AP samples as changes in the concentrations of TVB-N did not vary in accordance with pH fluctuations.

Thiobarbituric acid (TBA) value, a major index of fat oxidation was found to vary significantly between samples from an initial value of 0.52 mg malonaldehyde kg^{-1} of fish to 1.41 and 0.42 mg malonaldehyde kg^{-1} of fish in AP and VP lots respectively on 19th day of ice storage which may be on account of the exclusion of air from the latter thereby reducing fat oxidation in the samples (Fig. 1d). A level of 2 mg malonaldehyde kg^{-1} is regarded as the limit beyond which fish will normally develop an objectionable odour/taste (Connel, 1990; Goulas and Kontominas, 2007) and the present study revealed that TBA was within the acceptability limit for both the samples throughout the storage period. An observation by Yesudhason *et al*. (2010) in seerfish steaks revealed that TBA values in control air packed, control modified atmosphere packed and potassium sorbate modified atmosphere packed steaks were within the TBA limit value on day 12, 18 and 28 respectively. Simeonidou *et al*. (1998) reported significant increase in TBA values during ice storage of fish and suggested that it could be used as a biochemical index for monitoring quality changes of fishes.

Water holding capacity (WHC) of the samples decreased from an initial value of 27.71 to 24.57% and 17.25% for AP and VP lots respectively towards 19th day of ice storage which indicated that air packed samples had better WHC than vacuum packed ones. Manju *et al*. (2008) revealed an inverse relationship between drip loss and WHC in pearlspot samples. They observed that the drip loss was found to be significantly ($p<0.05$) more in the case of vacuum packaged and potassium sorbate drip treated samples than that of air packaged samples.

Instrumental texture analysis

Texture of raw fish which is an important index for consumer acceptability, can be measured by different methods using mechanical food testing equipment. When the texture of fish is measured, hardness and springiness are often the major variables (Botta, 1991). Hardness values (I and II) were found to decrease significantly in both lots during ice storage and the decrease was more for vacuum packed samples compared to air packed ones (Fig. 2a and b). Similar findings were made by Manju *et al*. (2008) in pearlspot samples where hardness I and II showed a significant difference ($p<0.05$) between the control air packed, control vacuum packed and the treated samples.

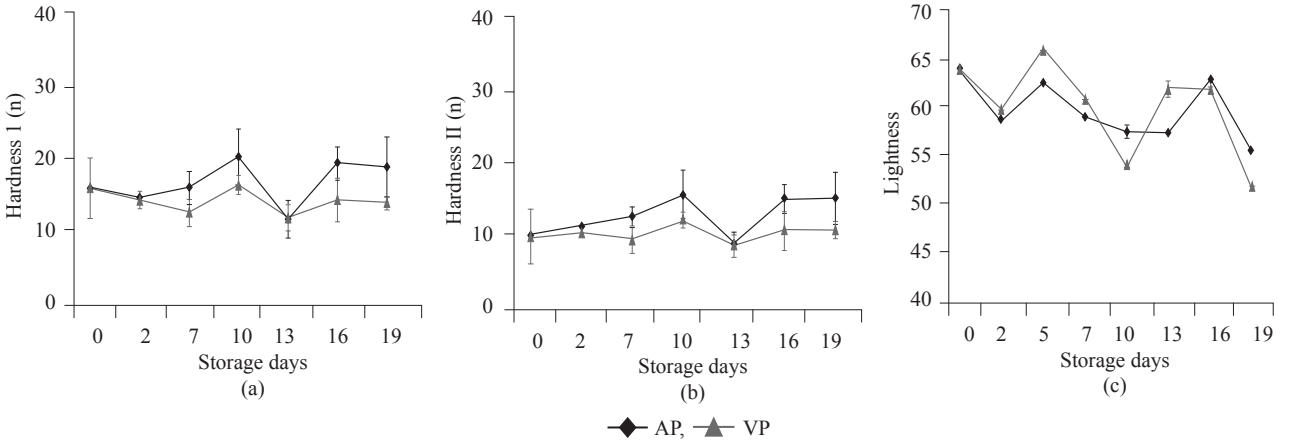

Fig. 2. Variations in (a): Hardness I, (b): Hardness II and (c): Lightness (L*) of air packed and vacuum packed monosex tilapia during ice storage

The lightness value (L) of tilapia samples significantly decreased ($p<0.05$) from an initial value of 63.7 to a final value of 55.31 and 51.88 for AP and VP respectively on 19th day of ice storage (Fig. 2c.) whereas a^* value showed a slight increase (towards redness) and b^* showed a slight decreasing trend (towards blueness) with no significant difference ($p>0.05$) during the study. Similarly Mohan *et al*. (2011) observed that in double filleted oil sardine, the lightness value of all the samples decreased with the storage period with significantly ($p<0.05$) higher decrease for untreated samples compared to chitosan treated ones, whereas both a^* and b^* did not show much variations after initial treatment.

Microbial analysis

Microbiological analyses indicated that AP samples were acceptable up to 16th day with total plate counts (TPC) of 6.81 log cfu g^{-1} whereas VP samples were acceptable upto 19th day with TPC of 6.59 log cfu g^{-1}. The microbiological limit proposed by International commission of microbiological specification for foods (ICMSF, 1998) for fresh fish is 10^7cfu g^{-1}. A significant increase ($p<0.05$) in TPC was observed between the sample lots throughout the storage period (Fig. 3). According to Manju *et al*. (2008), the total plate counts in chill stored pearlspot were found to increase significantly ($p<0.05$) in air packaged samples compared with treated vacuum packaged samples. Laly *et al*. (2016) observed an increase in TPC in gutted monosex tilapia samples reaching 6.49 log cfu g^{-1} after 24 days of ice storage.

Sensory evaluation

Sensory analysis indicated that both AP and VP samples were acceptable throughout the storage period of 19 days. However AP samples exhibited lower score (5.0) compared to VP samples on 19th day. Appearance of vacuum packed samples were inferior compared to air packed ones due to the presence of blood stained exudate in the pack. Adoga *et al*. (2010) reported changes in organoleptic quality in tilapia during ice storage and found that the samples were acceptable upto 15 days in iced condition and 12 h at ambient temperature. Mohan *et al*. (2010), reported a declining trend with storage period, for all the sensory attributes in seerfish steaks.

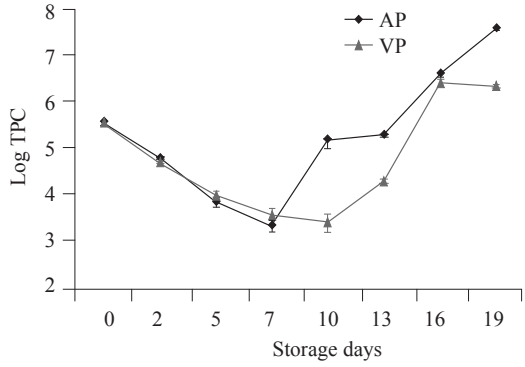

Fig. 3. Variations in TPC of air packed and vacuum packed monosex tilapia during ice storage

Freshwater fishes have comparatively extended shelf life than marine fishes on account of their body composition. Results of the present study, revealed that under iced condition, vacuum packed steaks of monosex tilapia had extended shelf life of 19 days as compared to 16 days observed for air packed steaks. Further studies are needed to explore advanced preservation and processing techniques applicable for this promising freshwater fish.

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