

EFFECT OF STORAGE TEMPERATURE ON THE BACTERIAL FLORA AND SENSORY QUALITY OF VACUUM PACKAGED SMOKED CATFISH (*Clarias anguillaris*)

¹Obolu, N., ^{2*}Amakoromo, E.R., and ³Oku, I.

¹Department of Microbiology, University of Port Harcourt, Port Harcourt, Rivers State

²Department of Food, Nutrition and Home Science, University of Port Harcourt, Port Harcourt, Rivers State

³Department of Microbiology, Niger Delta University, Amasoma, Bayelsa State

*Corresponding author: ebi.amakoromo@uniport.edu.ng

obolunelson@gmail.com, ikpebieoku@yahoo.com

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ABSTRACT

This study evaluated the effect of two storage temperatures on the bacterial flora and sensory quality of vacuum packaged smoked catfish (Clarias anguillaris). The catfish was hot smoked using a traditional kiln, with mangrove wood as the source of heat. Total bacterial count (TBC) and sensory parameters (colour, smell, dryness and general appearance) were determined during storage at ambient (28 ± 2°C) and refrigeration (4°C) temperatures for 0-8 weeks. Bacteria were enumerated, isolated and identified using standard methods. Vacuum packaged smoked catfish stored at 4°C (VPCR) had the lowest TBC which increased from 3.23 – 3.58 log₁₀ cfu/g within 2 weeks of storage and stabilized at 3.44 log₁₀ cfu/g for 4 – 8 weeks. The TBC of the vacuum packaged smoked catfish stored at ambient temperature (28 ± 2°C) ranged from 3.32 – 6.88 log₁₀ cfu/g within 6 weeks of storage. The bacteria isolated from fresh catfish belonged to nine genera, namely, Bacillus, Chryseobacterium, Escherichia, Lysinibacillus, Proteus, Pseudomonas, Staphylococcus, Streptococcus and Vibrio. Only Bacillus and Proteus species were isolated from freshly smoked catfish. Isolates from vacuum packaged samples were Bacillus, Lactobacillus, Proteus and Staphylococcus. Molecular characterization of three isolates from fresh catfish revealed that the species were Chryseobacterium aquifridigense, Lysinibacillus mycoides and Vibrio parahaemolyticus. The sensory scores for sample VPCR stored for 8 weeks did not differ significantly (P ≥ 0.05) from freshly smoked catfish, except for the colour. Non-packaged and vacuum packaged smoked catfish samples were of good quality during storage at ambient temperature for 4 weeks, and 4 – 6 weeks, respectively.

Keywords: Catfish, *Clarias anguillaris*, vacuum packaging, hot smoking, bacterial flora

INTRODUCTION

The African catfish is one of the types of fish that is widely consumed in Nigeria and many other African countries. *Clarias anguillaris*, the species used in this study, is often

harvested in the wild and also from fish farms. FAO (1997) reported that fish is an important source of protein, supplying over one-third of animal protein in some countries. On the other hand, fish is highly perishable and need to be preserved in order to prevent

or minimize postharvest losses. Various methods are available for the preservation of fish which include smoking, drying, salting, freezing and fermentation. Hot smoking is a traditional method of fish preservation used in Southern Nigeria. Smoking is also used globally to preserve fish, especially in the third world countries; about 70% of harvested fish is smoked in Africa (Ward, 1995; Cardinal et al., 2001). The process involves drying, cooking and deposition of bactericidal chemicals that are present in the wood used for smoking (Garrow & James, 2000).

Although smoking preserves fish, the smoked product can spoil during storage. Smoked fish is often distributed and sold without packaging, making it prone to postprocessing contamination. Oku and Amakoromo (2023) reported that unsalted hot smoked catfish had good quality when stored at ambient temperature ($28 \pm 2^\circ\text{C}$) up to 4 weeks. The quality of the smoked product deteriorates as a result of the breakdown of components by microorganisms, enzymes and fat oxidation (Brainherd and Junianto, 2001). Vacuum packaging can be used to extend the shelf-life of food. It involves expelling air from the package before sealing, to protect the product from air and moisture. Vacuum packaging has been reported to extend the shelf-life of products 3-5 times longer than without vacuum (Jay, 1996). This method has been reported to extend the shelf-life of garfish (Koral et al., 2009), rainbow trout (Khanipour and Mirzakhani, 2013) and tilapia (Masniyom et al., 2013). Ahaotu et al. (2021) reported that vacuum packaged smoked periwinkle was more resistant to spoilage than the sample packaged without vacuum, during storage at ambient temperature. Most of the studies on vacuum packaging of fish have been on raw or cold-smoked fish. Cold smoking involves the use of lower temperatures than those used in hot smoking, and imparts a desirable flavor without cooking the fish. The objective of this study was to evaluate the effect of two different storage temperatures on the bacterial flora

and sensory quality of vacuum packaged hot smoked catfish (*Clarias anguillaris*).

MATERIALS AND METHODS

Sample Preparation

Catfish (*Clarias anguillaris*) of $100 \pm 10\text{g}$ weight was purchased from a pond in Port Harcourt, Rivers State. The fish was transported in ice pack to the laboratory within 2 hours. The fish was eviscerated, washed, rinsed in tap water and allowed to drain. The drained fish was placed on an iron rack on a traditional drum kiln, and smoked for 8 hours at a temperature range of $60 - 70^\circ\text{C}$. The source of heat was mangrove wood. The smoked catfish was then left to cool.

The smoked catfish was vacuum packaged in polyethylene bags using a sealing machine (Vacuum Food Sealer, model V5230-I UK). Each bag contained 1 fish, and the machine expelled air before sealing the bags. Half of the vacuum packaged fish was stored in a refrigerator at 4°C for 0 – 8 weeks. The other half, and a non-packaged sample were stored at ambient temperature ($28 \pm 2^\circ\text{C}$) for 0 - 6weeks.

Bacteriological Analyses

The total bacterial count (TBC) of samples was determined using nutrient agar (NA). The TBC was determined at intervals of 2 weeks during the storage period. Catfish flesh (25g) was homogenized with 225ml of sterile peptone water in a stomacher (Stomacher 400, Lab. Blender, London, UK). Ten-fold serial dilutions were prepared using peptone water and pour plated in NA. The NA plates were incubated at 37°C for 24 hours and the colonies were counted. The results were expressed as the logarithm of colony forming units per gramme (cfu/g). Discrete bacterial colonies were subcultured on NA. Slants of the purified isolates were prepared and stored at 4°C . Bacterial isolates were identified based on morphological and biochemical tests (Holt et al., 1994).

Molecular characterization of 3 bacterial isolates was done by sequencing the 16S rRNA of the isolates followed by construction of phylogenetic tree. DNA was extracted using the boiling method. The isolates were identified by 16S rRNA sequencing employing universal primers 27F (5'-AGAGTTTGATCMTGGCTGAG-3') and 1492R (5'-GGTTACCTTACGACTT-3') used on ABI 9700 Applied Biosystems thermal cycler. The PCR mix used was X2 dream taq master mix supplied by Inqaba, South Africa (taq polymerase, dNTPs, MgCl₂). The temperature of the reaction for initial denaturation was 95°C for 5min; denaturation, 95°C for 30s, annealing at 52°C for 30s, and extension at 72°C for 30s for 35 cycles, with a final extension step at 72°C for 5min. The PCR product was proved by agarose gel electrophoresis and the gene segment was sequenced using the BigDye Terminator Kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria, South Africa. The sequence obtained was compared with the 16S rRNA GenBank database by using BLASTN of NCBI. Phylogenetic tree was constructed by the neighbor – joining method in MEGA 6.0 (Saitou and Nei, 1987).

Sensory Analysis

Ten panelists who were familiar with smoked fish evaluated the smoked catfish samples for colour, smell, dryness and general appearance. Sensory analysis was performed using a modification of the 5-point hedonic described by Huss (1988) (where 5 = excellent, 4 = good, 3 = fair, 2 = poor and 1 = unacceptable).

Statistical Analysis

The data are presented as means and standard deviations. Differences in mean values were analyzed using one-way ANOVA implemented in statistical package for social sciences (SPSS) version 23.

RESULTS

Total Bacterial Count

The total bacterial counts (TBC) of vacuum packaged smoked catfish samples during storage are shown in Fig. 1. The counts for the refrigerated sample (VPCR) increased slightly within the first 2 weeks and reduced while the counts for the sample (VPCA) stored at ambient temperature increased rapidly within the first 2 weeks and continued to increase till the 6th week. The TBC for sample VPCR ranged from 3.32 – 3.58 log₁₀ cfu/g while the counts for sample VPCA ranged from 3.32 – 6.88 log₁₀ cfu/g.

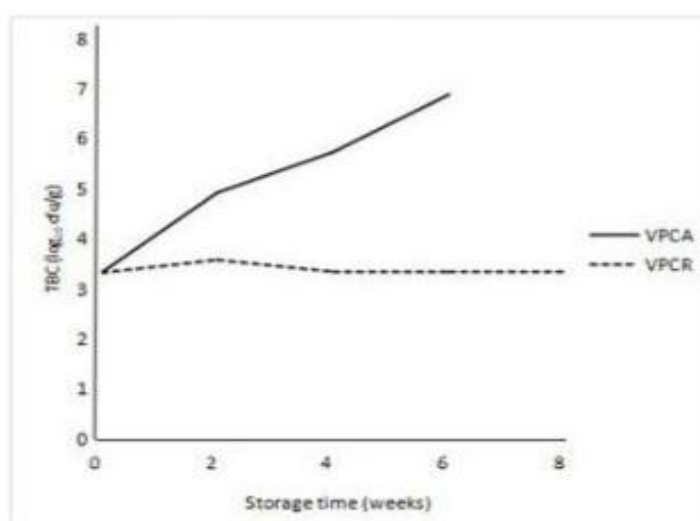


Fig. 1: Changes in the total bacterial count (TBC) of vacuum packaged smoked catfish (*Clarias anguillaris*) during storage. VPCA – catfish stored at 28 ± 2°C; VPCR – catfish stored at 4°C

The bacteria isolated from fresh and smoked catfish samples are presented in Table 1. The bacteria belong to 10 genera, namely, *Bacillus*, *Chryseobacterium*, *Escherichia*, *Lactobacillus*, *Lysinibacillus*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, and *Vibrio*. Bacteria belonging to 9 of these genera were found in fresh catfish while freshly smoked and vacuum packaged catfish samples had bacteria from 2 and 4 genera, respectively. The result of

agarose gel electrophoresis for 3 bacterial isolates obtained from fresh catfish is shown in Fig. 2. The isolates produced the 16S rRNA gene band at 1500bp. The phylogenetic tree for the isolates is shown in Fig. 3. The isolates had 100% similarity index with *Chryseobacterium aquifrigidense*, *Lysinibacillus macroides* and *Vibrio parahaemolyticus*. The accession numbers of the isolates are presented in Table 2.

Table 1: Bacteria isolated from fresh and hot smoked catfish (*Clarias anguillaris*)

Sample	Storage time (weeks)	Bacteria
Fresh catfish	0	<i>Bacillus</i> sp. <i>Escherichia coli</i> <i>Chryseobacterium</i> sp. <i>Lysinibacillus</i> sp. <i>Proteus</i> sp. <i>Pseudomonas</i> sp. <i>Staphylococcus</i> sp. <i>Streptococcus</i> sp. <i>Vibrio</i> spp.
Freshly smoked catfish	0	<i>Bacillus</i> sp. <i>Proteus</i> sp.
Non-packaged smoked catfish (stored at 28 ± 2°C)	6	<i>Bacillus</i> sp. <i>Lactobacillus</i> sp. <i>Staphylococcus</i> sp.
Vacuum packaged smoked catfish (stored at 28 ± 2°C)	6	<i>Bacillus</i> sp. <i>Proteus</i> sp. <i>Staphylococcus</i> sp.
Vacuum packaged smoked catfish (stored at 4°C)	6	<i>Bacillus</i> sp. <i>Lactobacillus</i> sp. <i>Proteus</i> sp.

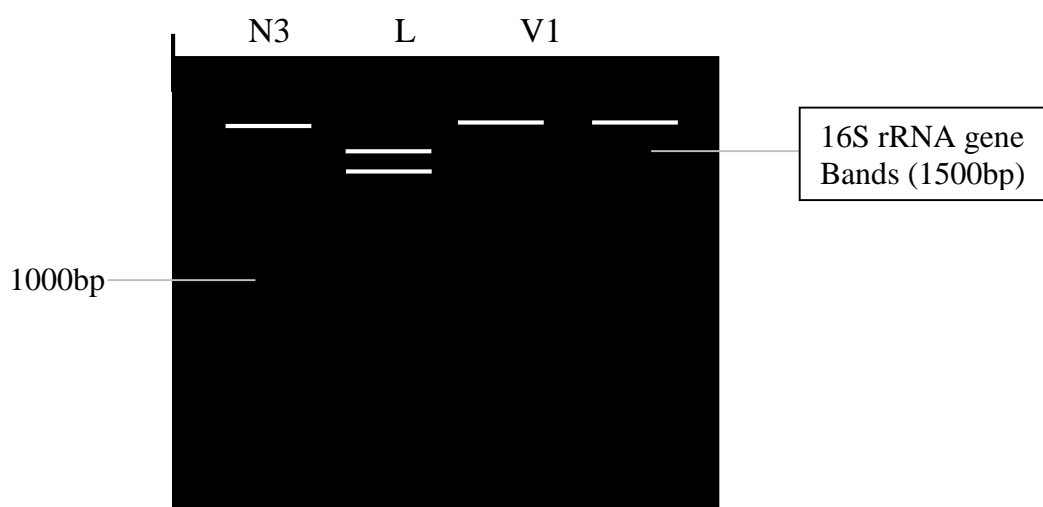


Fig. 2: Agarose gel electrophoresis of the amplified 16S rRNA gene. Lanes N3, V1 and B1 represent the amplified 16S rRNA gene bands at 1500bp, lane L represents the 1000bp molecular ladder.

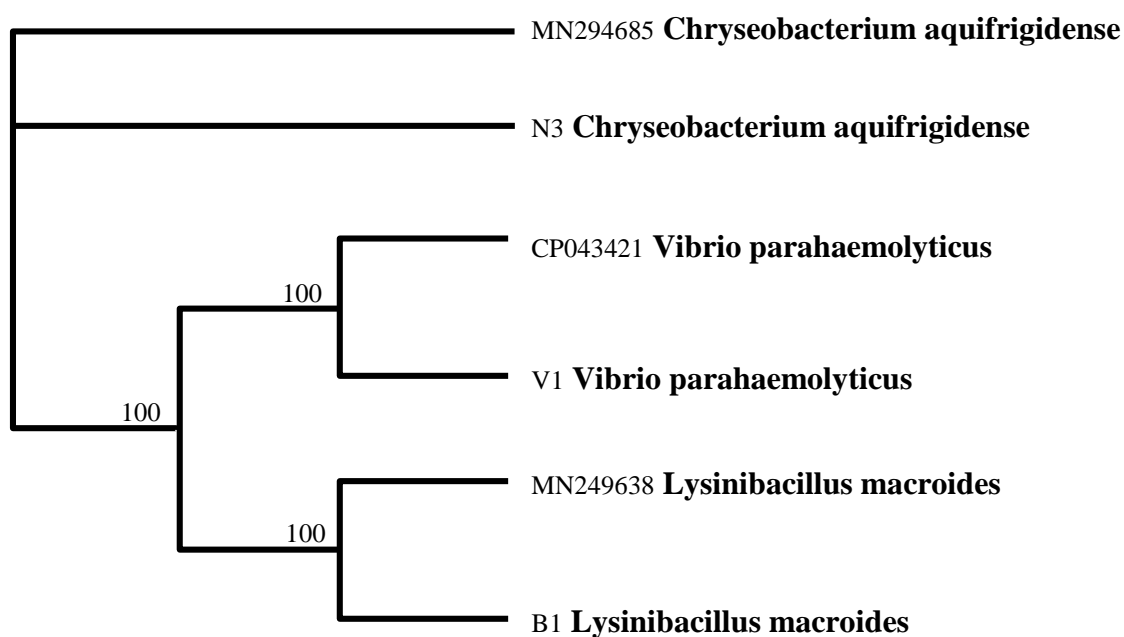


Fig. 3: Phylogenetic tree of bacteria isolated from fresh catfish (*Clarias anguillaris*)

Table 2: Accession numbers of bacterial isolates from catfish (*Clarias anguillaris*)

Isolate code	Accession number	Similarity Index (%)	Bacterial species
N3	MN508786	100	<i>Chryseobacteria aquifrigidense</i>
V1	MN508787	100	<i>Vibrio parahaemolyticus</i>
B1	MN508788	100	<i>Lysinibacillus macroides</i>

Sensory Quality

The changes in sensory quality of non-packaged and vacuum packaged smoked catfish samples during storage are shown in Table 3. Freshly smoked catfish generally had the highest scores for colour, smell, dryness and general appearance. The scores declined during storage at ambient and refrigeration temperatures. However, the scores for sample VPCR which was vacuum packaged and refrigerated were not significantly different ($P \geq 0.05$) from scores for freshly smoked catfish throughout the 8 weeks of storage,

with the exception of colour. On the other hand, scores for colour and general appearance of the vacuum packaged sample stored at ambient temperature (VPCA) were significantly ($P \leq 0.05$) lower than scores for freshly smoked catfish by 4 weeks, and scores for smell and dryness by 6 weeks of storage. The scores for the colour and general appearance of the non-packaged sample stored at ambient temperature were significantly ($P \leq 0.05$) lower than scores for freshly smoked sample by the 2nd week, and scores for smell and dryness by 4 weeks of storage.

Table 3: Sensory characteristics of non-packaged and vacuum packaged smoked catfish (*Clarias anguillaris*) during storage

Sample	Storage time (weeks)	Colour	Smell	Dryness	General Appearance
Non-packaged smoked catfish (at $28 \pm 2^\circ\text{C}$)	0	4.5±0.3 ^a	4.3±0.3 ^a	4.0±0.5 ^a	4.5±0.3 ^a
	2	3.4±0.4 ^b	3.8±0.7 ^{ab}	3.8±0.6 ^{ab}	3.9±0.5 ^b
	4	3.0±0.6 ^b	3.7±0.5 ^b	3.3±0.5 ^b	3.2±0.4 ^c
	6	2.8±0.3 ^{bc}	2.3±0.3 ^c	2.1±0.7 ^c	2.3±0.5 ^d
Vacuum packaged smoked catfish (at $28 \pm 2^\circ\text{C}$)	0	4.5±0.3 ^a	4.3±0.3 ^a	4.0±0.2 ^a	4.5±0.6 ^a
	2	4.3±0.2 ^a	4.3±0.4 ^a	4.1±0.3 ^a	3.9±0.5 ^{ab}
	4	4.0±0.5 ^b	3.9±0.2 ^{ab}	3.7±0.5 ^{ab}	3.6±0.2 ^{bc}
	6	3.6±0.5 ^{bc}	3.5±0.6 ^b	3.5±0.6 ^{bc}	3.3±0.4 ^c
Vacuum packaged smoked catfish (at 4°C)	0	4.5±0.3 ^a	4.3±0.2 ^a	4.0±0.5 ^a	4.5±0.3 ^a
	2	4.3±0.4 ^a	4.0±0.5 ^a	4.1±0.4 ^a	4.5±0.5 ^a
	4	4.2±0.3 ^{ab}	4.0±0.3 ^a	4.2±0.4 ^a	4.3±0.2 ^a
	6	4.0±0.2 ^b	4.0±0.4 ^a	4.1±0.2 ^a	4.0±0.6 ^a
	8	3.8±0.3 ^b	4.0±0.4 ^a	3.8±0.2 ^a	3.8±0.5 ^a

Each value is the mean \pm SD of the scores of ten judges. Means with different superscripts within a column are significantly different ($P \leq 0.05$).

DISCUSSION

The bacterial counts for the vacuum packaged catfish samples increased slightly at refrigeration temperature, and rapidly at ambient temperature, during the first 2 weeks of storage. This can be attributed to incomplete expelling of air from the packages. Masniyon et al. (2013) observed the growth of psychrotrophic and mesophilic bacteria in vacuum packaged tilapia fillets stored at 4°C . According to these authors,

modified atmosphere packaging was more effective than vacuum packaging for the preservation of the fish. The total bacterial count (TBC) of the vacuum packaged smoked catfish sample (VPCR) stored for 8 weeks at refrigeration temperature was $3.43 \log_{10}$ cfu/g while the count for the sample at ambient temperature (VPCA) for 4 weeks was $5.73 \log_{10}$ cfu/g. The count for sample VPCR at 8 weeks was below the maximum acceptable limit of 10^5 (5 log) cfu/g for smoked fish

(EOS, 2005) but the count for sample VPCA exceeded the limit at 4 weeks of storage. The lower storage temperature could be responsible for the lower counts of the refrigerated sample. Apart from the lower counts obtained for the refrigerated sample, this sample would be considered to be safe for human consumption as there are concerns about the possible growth and production of toxins by the mesophile, *Clostridium botulinum* in vacuum packaged fish products. The Finnish Food Authority (2018) reported that food poisoning from this microorganism is due to the use of too high storage temperature and too long storage time. According to OSU (2002), a refrigeration temperature of 4.4°C is appropriate for the storage of reduced oxygen packaged (e.g. vacuum and modified atmosphere) fish products as it will limit the growth of *C. botulinum* and other pathogens.

The bacteria isolated from fresh catfish were more diverse than those from non-packaged and vacuum packaged smoked samples. This could be attributed to the effect of heat and antimicrobial substances produced during the smoking process. The bacteria isolated from fresh and smoked catfish samples belong to 10 genera; *Bacillus*, *Chryseobacterium*, *Escherichia*, *Lactobacillus*, *Lysinibacillus*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Streptococcus* and *Vibrio*. Bacteria from these genera have been associated with raw and smoked seafood in various studies (Oku & Amakoromo, 2013; Efiuwewewere & Amadi, 2015; Emoghene et al., 2021; Miwore et al., 2021; Oghenemawho & Ahaotu, 2021). *Bacillus* species were isolated from fresh and smoked catfish; bacteria from this genus have been reported as predominant bacteria in smoke-dried seafood sold in Port Harcourt, Rivers State (Aminigo & Okoro, 2002). Psychrotrophic bacteria such as *Pseudomonas* and *Vibrio* species which cause microbial spoilage of seafood (Khan et al., 2005) were found in fresh catfish but not in the smoked samples.

Vibrio parahaemolyticus which was isolated from fresh catfish is known to have strains that are pathogenic to humans. Other bacterial species identified by molecular characterization were *Chryseobacterium aquifridigense* and *Lysinibacillus macrolides*. In a recent study, Oghenemawho and Ahaotu (2021) isolated *C. aquifridigense* from freshly harvested periwinkle. This species of *Chryseobacterium* was isolated from a water-cooling system in Gwangyang, Republic of Korea and reported as a novel species in 2008 (Park et al., 2008). The genus, *Lysinibacillus* is a novel genus into which some *Bacillus* species have been reclassified (Ahmed et al., 2007). Some strains of *L. macrolides* have been reported to possess probiotic potentials. Chen et al. (2022) found that 2 strains of *L. macrolides* (38327 and 38328) selected from 506 *Bacillus* isolates obtained from traditional fermented food and soil had ability to protect against *Salmonella* infection in animal trials, and could be used as novel probiotics for intestinal health. Reports on other species of this genus include *L. endophyticus* being common in dried and smoked fish sold in Cameroon (Miwore et al., 2021), *L. sphaericus* and *L. fusiformis* as potential agents that control several plant diseases (Ahsan and Shimizu, 2021).

It was observed that the sensory scores for smoked catfish decreased during storage, and this was more pronounced for the non-packaged and vacuum packaged samples stored at ambient temperature. The sensory scores for the non-packaged sample stored at ambient temperature were the lowest and were below 3.0 (fair) by the 6th week of storage. This is in agreement with the findings of Akise et al. (2013) which reported that smoked fish stored at ambient temperature for 6 weeks was marginally acceptable. The scores for the vacuum packaged sample stored at ambient temperature were between 3.3 and 3.6 by the 6th week of storage, indicating better quality than the non-packaged sample. Ahaotu et al. (2021) also found that the sensory quality of vacuum packaged smoked periwinkle during

storage at ambient temperature was better than that of the product packed in polythene bags without vacuum sealing. The sensory scores for the vacuum packaged sample stored at refrigeration temperature were not significantly different ($P \geq 0.05$) from scores for the freshly smoked sample throughout the 8 weeks of storage, except for colour which was significantly different ($P \leq 0.05$) by the 6th week. The reduction in sensory scores during storage could be partly due to microbial activity since the TBC increased during storage, and was higher for the sample stored at ambient temperature. This is in agreement with Masniyom et al. (2013) who reported a decline in the sensory scores for vacuum packaged tilapia fillets during storage at 4°C for 18 days.

CONCLUSION

The changes in the total bacterial counts of vacuum packaged smoked catfish stored at ambient temperature were more pronounced than those in the refrigerated sample. The bacteria isolated from fresh catfish (*Clarias anguillaris*) were more diverse than those isolated from smoked samples and included *Chryseobacterium aquifridigense*, *Lysinibacillus mycoides* and *Vibrio parahaemolyticus*. The sensory quality of freshly smoked catfish and the vacuum packaged smoked catfish refrigerated for 8 weeks were comparable except for colour. Non-packaged and vacuum packaged smoked catfish were of good quality during storage at ambient temperature for 4 weeks, and 4 – 6 weeks, respectively. It is recommended that hot smoked vacuum packaged catfish be stored at 4°C.

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