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Sensory, Microbiological, Biochemical and Physico-chemical Assessment of Freshness and Quality of Fresh Lake Malawi Tilapia (*Chambo*) Stored in Ice

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Abstract Sensory, microbiological, biochemical and physico-chemical methods were used to assess freshness and quality of fresh Lake Malawi Tilapia (Local name: Chambo) to compare their effectiveness and reliability. Fresh Chambo samples were rejected by the sensory panel after 16 days from day of catch with a strong linear correlation ($P < 0.01$, $R^2 = 0.95$) between sensory quality scores and storage time in ice. Highest bacterial load of 1.6×10^7 cfu/g, cfu/cm^2 was observed on day 15 coinciding with sensory rejection time. TMA-N and TVB-N for freshly caught fish was 0.7 and 5.1 mg/100g, which increased to 3.4 and 26.4 mg/100g respectively at the time of sensory rejection also correlating with increased bacteria load in the fish. Initial pH of the fresh fish muscle was close to neutral (6.47), and reached its lowest point (5.84) on day 16 which was sensory rejection time. Findings suggest that sensory evaluation is reliable in the absence of the other methods. TMA-N is not a reliable method for assessing freshness quality of Lake Malawi Tilapia due to insignificant readings. pH showed to be a quick freshness indicator with an understanding that muscle pH for live fish is generally neutral and increases as deterioration of the quality of fish progresses in storage. Rejection of fish samples before reaching unacceptable microbial limits in this study, underpins the need for using more than one method for accurate freshness and quality assessments of fresh fish.

Keywords Sensory; Microbiological; Biochemical; Freshness; Quality; Lake Malawi tilapia

1 Introduction

Fish is a highly perishable commodity (Liu et al., 2010). After death, a series of complex biochemical processes set in culminating into rapid deterioration of fish freshness and quality over time (Huss, 1995). Wholesome freshness in fish can therefore be guaranteed only when it is still alive in water (Ghaly et al., 2010). Quality also involves safety aspects such as being free from harmful bacteria, parasites or chemicals (Gokoglu and Yerlikaya, 2015).

Due to the complexity in determining quality in fish, many indices have been used for the assessment of fish quality such as microbiological, chemical, sensory analysis and physical assessment (Huss, 1995). Freshness and quality assessment methods are broadly put into two categories namely, sensory and non sensory. While sensory methods are useful for identifying products of very good or poor quality, these must be performed scientifically under carefully controlled conditions to minimize effects of test environment, personal bias, etc. (Huss, 1995). On the non sensory methods, TVB-N is the widely used indicator of spoilage but cannot be used as a freshness indicator because it shows constant level during the first days of iced storage and does not reflect the mode of spoilage (bacterial or autolytic) (Baixas-Nogueras et al., 2002). TMA-N like TVB-N analysis also does not reflect the earlier stages of spoilage and are only reliable for certain fish species (Oehlenschläger, 1997). Microbiological analyses on the other hand, are laborious, time-consuming, costly, and require skill in execution and interpretation of the results (Huss, 1995). Accurate results in assessment of freshness and quality of fish can therefore be obtained by correlating indices from various methods as a way of validation. This study assessed freshness and quality of Lake Malawi Tilapia – a widely consumed commercial value fish in Malawi, using sensory, microbiological, biochemical and physico-chemical methods to establish the reliability of these methods.

2 Materials and Methods

Whole fresh Lake Malawi Tilapia (Chambo) fish (Figure 1) were bought from fish sellers early in the morning between 4 am and 5 am soon after landing their catch at the southeast arm of Lake Malawi in Mangochi district, southern Malawi. The fish were immediately packed in cool boxes preserved with block ice without washing with water to avoid influencing the sensory analysis process (Huidobro et al., 2001). To avoid bias in the shelf life estimation process, thus reducing variability factors (Azam et al., 2005); fish of approximately the same size (about 25 cm TL average) were deliberately selected during purchase. Size of fish is one of the intrinsic factors that affect the rate of spoilage in fresh fish stored in ice (Huss, 1995).

Figure 1 Collection and preservation in ice of fresh Lake Malawi Tilapia (*Chambo*)

2.1 Sensory evaluation of the whole fresh tilapia

A sensory evaluation panel of six students in the age ranges of 21 and 23 was pre-trained according to de Kock et al. (2002) and ISO (1993). The sensory panel then described daily changes in freshness and quality during storage of whole fresh Chambo in ice using the developed quality index method (QIM) Scheme (Mai et al., 2009) (Table 1).

Sensory quality of the fish was decided by scoring the attributes in Table 1 between 0 and 3 where 0 and 3 entailed highly and poorly liked freshness of the fish sample respectively. Lower scores (demerit points) were given to sensory attributes that are not very important as far as acceptability of raw fish is concerned and as such, appearance of skin and scales; stiffness of the belly and the backside of the fish were allocated lower scores while higher scores were given to commonly used parameters (Nielsen, 1997) mainly colour and mucus on the gills and colour of the eye cornea.

2.2 Microbiological analysis

A sample of fresh tilapia fish stored in ice was removed every two to three days for microbiological assessment for a period of 21 days. Samples for aerobic psychrotrophic bacteria counts were obtained on the external surfaces (skin), flesh (tissue/muscle) and gills; and enterobacteriaceae counts from samples on kidney and intestine. Microbiological analysis was done following a procedure described by Slaby et al. (1981).

Skin surface

A sterile cotton wool swab dipped in 0.10% sterile peptone water was rubbed over the surface of the fish on the area covered by the wire swab guide. The swab was then immediately placed in a sterile sample vial containing 100 mls of 0.10% (w/v) peptone water. The vial was vigorously shaken for 10 minutes and allowed to stand for 20 minutes. 6 fold decimal serial dilution of the bacterial suspension in peptone water was prepared in duplicate and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37 ℃ for 48 hrs as outlined by Okoro et al. (2010) and Slaby et al. (1981). All data on skin bacterial counts reported are based on this method. Results were reported in log cfu/cm^2 of skin surface.

Table 1 Quality Index Method (QIM) Scheme developed for the assessment of whole fresh Lake Malawi Tilapia (*Chambo*) stored in ice for 21 days

Quality parameter		Description	Scores
Appearance	Skin	Shiny grey	0
		Grey, not shiny	1
	Scale	Firm	0
		Loose	1
Eyes	Cornea	Very clear (glass-like)	0
		Cloudy	1
		Milky	2
		Opaque pupil	3
Gills	Colour	Bright red	0
		Pale red	1
		Dull red	2
		Brown	3
	Smell	Fresh, cut grass, aquatic weed	0
		Neutral	1
		Musty	2
	Mucus	Clear	0
		Cloudy	1
		Milky	2
		Brown-reddish	3
Texture	Backside	Firm and elastic (in-rigor)	0
		Soft	1
		Very soft/ depression when pressed	2
	Belly	Firm	0
		Soft	1
Quality Index (QI)			**0-16**

Intestines, gills, kidney and tissue (muscle/flesh)

One gram of the fish sample from a chosen part was removed, blended and mixed properly in a mortar then ascetically transferred to a sample vial containing 9 mls of 0.1% sterile peptone water. The vial was closed and shaken thoroughly for 10 minutes then allowed to standing for 20 minutes, after which a 6 fold serial decimal dilution was carried out in triplicates. Viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37 ℃ for 48 hours (Slaby et al., 1981). Results were reported in log cfu/g.

Identification of bacteria

Morphological characteristics of the various bacterial isolates were noted in the agar plates, and microscopy. After staining reactions and several biochemical tests, individual microbial species were identified as described by Slaby et al. (1981). Representative isolates were re-plated on various selective media to observe their habits and other specific colony attributes.

2.3 Biochemical analysis

A procedure earlier used by Okoro et al. (2010) modified from Conway (1968) was followed for the determination of Trimethlamine nitrogen (TMA-N) and Total volatile basic nitrogen (TVB-N).

Determination of Total volatile basic nitrogen (TVB-N)

25 g of the fish sample was removed, chopped and thoroughly mixed in 75 mls of distilled water in a 250 mls beaker. A few drops of 2N hydrochloric acid (HCl) were added to adjust the pH reading to 5.2 followed by heating at 70^{o}C then cooling to room temperature. When cooled, the sample was filtered into a conical flask using a Whatman No. 1 filter paper. 2 mls of 0.025N HCl was transferred to the central compartment of the micro diffusion dish using a pipette then adding 2 mls of the extract and 1 ml of saturated potassium carbonate (K_2CO_3)

solution into the outer part. The dish was then covered immediately with a glass plate and left at room temperature for 24 hrs. Later, the HCl in the inner part of the conical flask was titrated with 0.025N sodium hydroxide (NaOH) using 2-3 drops of methyl red indicator. Results were reported in mg, TVB-N/100g of fish (Conway, 1968).

Determination of Trimethylamine (TMA-N)

A slight modification of Conway (1968) Micro-diffusion Method (Okoro et al., 2010) was used by removing 25 g of the fish flesh, chop and mixing thoroughly with 75 mls of distilled water in a 250 ml beaker. A few drops of 2N HCl were added to obtain a pH of 5.2 then heating at 70^{o}C and cooling to room temperature. The sample was after cooling filtered into a conical flask using Whatman No. 1 filter paper. Later, 2 mls of 0.025N HCl were transferred into the micro diffusion dish (central area) using a pipette followed by 2 mls of the extract and 0.5 mls of 35% formaldehyde with 1 ml of saturated K_2CO_3 solution into the outer compartment. A glass plate was used to cover the dish and left at room temperature for 24 hrs. HCl in the inside chamber was then titrated with 0.025N NaOH using 2-3 drops of methyl red/methylene blue indicator. Results were reported in mg, TMA-N/100g of fish (Conway, 1968).

2.4 Physico-chemical analysis

Determination of pH

To determine pH of the fish, flesh (muscle) was removed and weighed out 10 g which was homogenized in 50 ml of distilled water and centrifuged using a Yamato Mag-Mixer Model MH 800 (Yamato Scientific Company Limited, Japan). The mixture was filtered using Whatman filter paper No.1. A pH meter (Wissenschaftlich-Technische Werkstätten, West Germany) electrode was then inserted into the homogenate to measure the pH at ambient temperature after calibration using standard buffers of pH 7 and 4 at 25^{o}C (Okoro et al., 2010).

Statistical analysis

Data were analysed using the statistical software SPSS 15.0 for Windows. A linear regression analysis of sensory changes against storage time of fish in ice was performed in Microsoft Excel 2010 (Sveinsdóttir et al., 2003). Means were compared using analysis of variance (ANOVA) and significantly different means were checked using Duncan's Multiple-Comparison Test. Means ±SD were reported and considered different at 95% level of significance. Pearson Correlations between sensory, biochemical and microbiological parameters were also performed. Principal Components Analysis (PCA) was used to reduce multivariate sensory attributes with full cross validation in The Unscrambler® X Version 10.1 Statistical Software Package ©2009-2011 (CAMO, Norway) (Mai et al., 2009).

3 Results

3.1 Sensory evaluation

Fresh Chambo samples were rejected by the sensory panel after 16 days (Figure 2) from day of catch when highest quality index (QI) demerit scores were recorded. QI for day 16 was 15.5 and 15.7 for day 19. A strong linear correlation ($P < 0.01$, $R^2 = 0.95$) between sensory quality index scores and days of storage in ice of the fish was observed (Figure 2). Results from the sensory evaluation apparently demonstrated that the appearance of the gills (colour), mucus; and appearance of eye cornea were the major attributes that the sensory panel observed noticeable changes in the freshness and quality of the fish during storage (Figure 3). Gill colour, mucus and eye cornea appearance are ironically, the commonest traditional freshness and quality parameters that consumers immediately look for before buying fresh fish on the market in Malawi. Changes in gill and eye cornea colour were more evident after 8 and 4 days respectively in ice storage. Gill colour changed from bright red in freshly caught fish to pale red then reddish brown at spoilage time. Eye cornea changed from glass clear on day 0 to grey and even opaque when fish was completely spoiled. Gaping in the prepared fillets was observed from day 12 of storage in ice and became more pronounced at day 16. Findings in Figure 3 were well supported by the principal components analysis (PCA) (Figure 4).

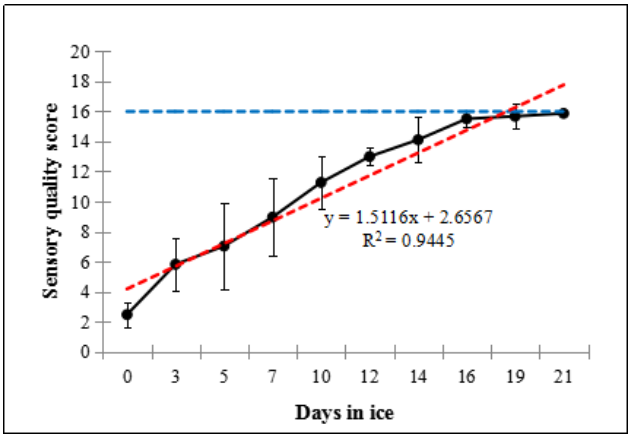


Figure 2 Quality Index (QI) of Lake Malawi Tilapia (*Chambo*) stored in ice for 21 days

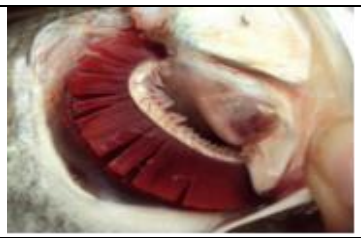
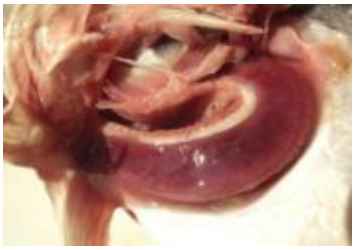
Day in ice	Gills	Eye cornea	Flesh
0			
4			
8			
12			
16			

Figure 3 Daily changes in appearance of gills, eye cornea and flesh of Lake Malawi Tilapia (*Chambo*) during storage in ice for 21 days

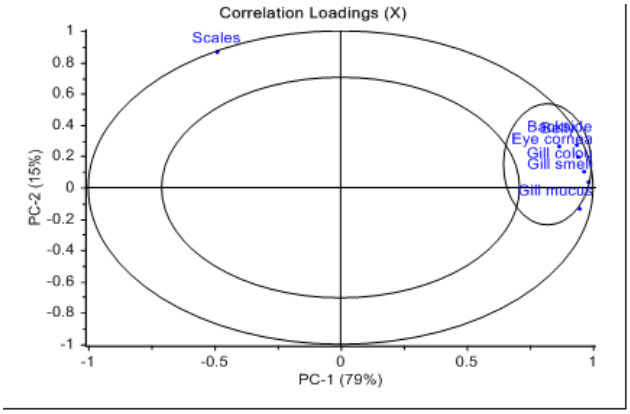


Figure 4 PCA correlation loadings of the various sensory parameters used for determining level of freshness and quality of Lake Malawi Tilapia (*Chambo*) stored in ice for 21 days

3.2 Microbiological analysis

Highest number of bacteria was observed on day 15 and bacterial load at the time of sensory rejection was 1.6×107 cfu/g, cfu/cm^2 (Table 2). Bacteria growth was slow at the beginning of storage but suddenly shot up after 12th day and stagnated after day 15 appearing to coincide with sensory quality scores. Highest quantities of bacteria were observed in the gills (4.0×105 cfu/g) on day 15 and intestines (9.6×107 cfu/g) on day 21.

Table 2 Mean population densities (TVC) of bacteria isolated from whole fresh Lake Malawi Tilapia (*Chambo*) fish samples during storage in ice for 21 days

Fish part	**Days in ice**						
	1	3	6	12	15	18	21
Skin surface (log cfu/cm^2)	4.4×10^3	8.6×10^3	1.2×10^4	1.7×10^4	2.8×10^4	3.9×10^4	5.6×10^4
Stdev.	$\pm2.2\times10^2$	$\pm7.7\times10^2$	$\pm1.0\times10^3$	$\pm1.5\times10^3$	$\pm3.3\times10^3$	$\pm1.1\times10^3$	$\pm3.0\times10^3$
Muscle (log cfu/g)	1.1×10^2	3.8×10^3	9.8×10^3	2.7×10^4	1.8×10^5	2.5×10^5	6.2×10^5
Stdev.	$\pm2.6\times10^1$	$\pm5.2\times10^2$	$\pm3.0\times10^2$	$\pm3.0\times10^3$	$\pm2.1\times10^5$	$\pm3.1\times10^4$	$\pm4.4\times10^4$
Gills (log cfu/g)	1.2×10^5	1.9×10^5	2.1×10^5	2.6×10^5	4.0×10^5	3.6×10^5	2.5×10^5
Stdev.	$\pm1.0\times10^5$	$\pm1.1\times10^4$	$\pm2.6\times10^3$	$\pm3.610^3$	$\pm5.0\times10^3$	$\pm5.4\times10^4$	$\pm7.6\times10^3$
Kidney (log cfu/g)	9.1×10^3	7.3×10^4	1.9×10^4	1.2×10^4	7.2×10^3	3.8×10^3	2.5×10^4
Stdev.	$\pm6.7\times10^2$	$\pm9.2\times10^4$	$\pm2.0\times10^3$	$\pm1.7\times10^3$	$\pm1.0\times10^3$	$\pm2.4\times10^3$	$\pm1.2\times10^4$
Intestines (log cfu/g)	4.0×10^3	1.4×10^6	1.7×10^6	7.7×10^6	8.0×10^7	8.4×10^7	9.6×10^7
Stdev.	$\pm6.4\times10^2$	$\pm1.2\times10^5$	$\pm1.5\times10^6$	$\pm8.5\times10^5$	$\pm5.2\times10^6$	$\pm1.1\times10^7$	$\pm1.5\times10^6$
TVC (cfu/g, cfu/cm^2)	2.9×10^4	3.5×10^5	3.9×10^5	1.6×10^6	1.6×10^7	1.6×10^7	1.9×10^7
Stdev.	$\pm6.4\times10^4$	$\pm5.8\times10^5$	$\pm9.0\times10^5$	$\pm3.1\times10^6$	$\pm3.3\times10^7$	$\pm3.4\times10^7$	$\pm3.9\times10^7$

3.3 Biochemical analysis

TMA-N and TVB-N for freshly caught fish was 0.7 and 5.1 mg/100g, which increased to 3.4 and 26.4 mg/100g respectively at the time of sensory rejection (Figure 5). TVB-N and TMA-N levels increased drastically after day 12 also coinciding with the significant increase in bacteria populations in the fish.

3.4 Physio-chemical analysis

Initial pH of the fish muscle at day 0 was close to neutral (6.47) but continued fluctuating around 6.40 before reaching the lowest point of 5.84 at day 16 which consequently was sensory rejection time, then rose sharply up to 6.48 and 6.86 on day 18 and 20 respectively (Figure 6). In general, the muscle/flesh pH of the fresh Chambo fish significantly decreased with storage time in ice after day 12 ($P < 0.05$).

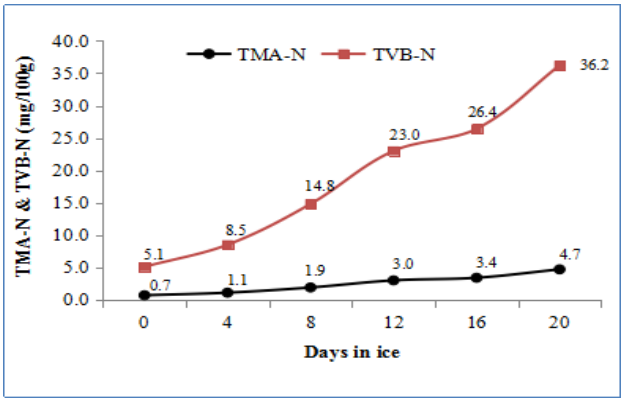


Figure 5 Total volatile bases-Nitrogen (TVB-N) and Trimethylamine levels in Lake Malawi Tilapia (*Chambo*) stored in ice for 21 days

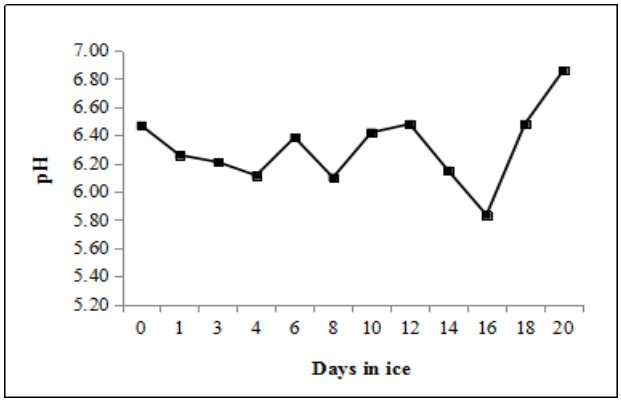


Figure 6 pH for Lake Malawi Tilapia (*Chambo*) muscle stored in ice for 21 days

4 Discussions

4.1 Sensory evaluation

Results suggest that whole fresh Chambo stored in ice can remain in acceptable condition for consumption until between day 16 and day 19. Also apart from explaining the significant relationship between fish freshness and quality deterioration with storage time in ice (Figure 2), the strong correlation also demonstrated the reliability of the sensory evaluation in determining freshness and quality attributes using the developed QIM scheme with minimal errors. The estimated shelf life of 16 days for Lake Malawi tilapia (Chambo) in this study appear to fall within the previously reported for tilapias (Surendran et al., 1989; Adoga et al., 2010). Results reported for this study also echoed earlier and most recent reports that tropical fish kept in ice exhibit a longer shelf life compared to temperate fish (Huss, 1995; Goliat et al., 2016).

4.2 Microbiological analysis

Bacterial populations in the study were within the recommended safe range of bacterial counts (5×105 cfu/g and 5×107 cfu/g) for good quality fish product stipulated by the International Commission on Microbiology Safety for Foods (ICMSF, 1986). Spoilage of fish under aerobic conditions becomes apparent when specific spoilage bacteria (SSO) reach the values of 108-109 cfu/g flesh or cm^2 (Gram and Huss, 1996). This suggests that despite the fish being sensorily unacceptable, microbial load had not yet exceeded levels that could be deemed dangerous to human consumption. While Hernandez et al. (2009) observed a correlation between bacterial viable counts and sensory parameter scores, findings in this study suggest the contrary supporting the observation by Ólafsson (1999) that bacterial total count and sensory evaluation do not always agree. This study observes a likelihood of condemning as unfit, products that are microbiologically safe for human consumption and hence, underscoring the importance of validating sensory results with other methods. Fish gills and intestines contain huge load of bacteria (Huss, 1995; Singh et al., 2011) underscoring the need for quick removal of these appendages to reduce microbial spoilage of fresh fish.

4.3 Biochemical analysis

Values for TBN-N and TMA-N reported in this study at sensory rejection were within the acceptable limits (Connell, 1975) and similar to those reported by Okeyo et al. (2009), but slightly lower while within range of those reported by Guo et al. (2011). The noticeable increase in TVB-N and TMA-N between day 8 and 12 may be attributed to the sharp increase in bacteria population (Table 2). TVB-N and TMA-N are volatile amines that are produced by spoilage bacteria hence their increase tends to be positively correlated with microbial population in fresh fish (Huss, 1995). In iced fish, TVB-N contents decrease over the first few days before rising again (Howgate, 2006; Okeyo et al., 2009). Although growth of bacteria starts immediately after resolution rigor, formation of TMA-N in stored fish only commences after several days (Connell, 1975). The relatively smaller observed values for TVB-N could be due to the washing effect (leaching) of ice as the melted ice in the cooler box

was replaced every one to two days, earlier reported by Howgate (2006). Lower levels of TVBN at the beginning could therefore be due to lower levels of endogenous ammonia as a result of reduced microbial activity during the first 12 days of storage of the fish in ice (Okeyo et al., 2009). This agrees with the assertion that volatile amines especially TMA-N does not present a reliable method for determining fresh fish spoilage because they are only detected when spoilage of fish has already progressed (Okoro et al., 2010). Mhongole (2009) also observed that sensory and microbiological methods were more reliable for quality assessment of fresh fish than chemical methods.

4.4 Physico-chemical analysis

pH is directly correlated to microbial growth (Huss, 1995), hence important in influencing post-mortem changes in the freshness of fish. Decreasing pH values of the fish with storage time in ice for this study have been previously reported (Obemeata et al., 2011). Generally, pH decreases during anaerobic formation of lactic acids during the first hours after death (Huss, 1995). Decrease in pH level may be due to the decomposition of carbohydrate of the fish by proteolytic microbes that produce acids thereby increasing the acid level of the medium (Eyo, 1993). This is well supported by the observed slow growth of bacteria until sensory rejection – an indication of the importance of ice in retarding microbial growth (Huss, 1995). Consequently, TVB-N and TMA-N started to increase sharply after day 12 which also coincided with increase in total viable bacteria counts on the fish indicating as associated between pH levels and microbial activity (Huss, 1995). Most gram negative spoilage bacteria cannot survive at low pH (acidic) hence lower pH eventually entails lower microbial activity (Huss, 1995). It has been suggested that tropical fish species reach a very low pH explaining why they have a longer shelf life (Huss, 1995). Increased pH values after sensory rejection could possibly be an indication of accumulation of alkaline compounds as well as volatile bases such as ammonia and trimethylamine produced by autolytic activities and metabolism of spoilage bacteria (Liu et al., 2010). Thus, increase in pH after day 16 could have been associated with rapid spoilage of the fish. Similarly, advanced gaping of the muscle was observed concurrent to the increase in pH around the time of sensory rejection (Figure 3) suggesting that temperature influences post-mortem pH (Huss, 1995). It is reported that even though changes in pH are generally rather small, they have great technological importance (Huss, 1995).

5 Conclusions

Results of different methods (sensory, microbiological, biochemical and physico-chemical) of assessing freshness and quality of Lake Malawi Tilapia have been discussed. Increasing bacteria numbers, increasing TVB-N and TMA-N, changes in pH against declining sensory quality suggest a strong correlation in fish spoilage. The general trend nevertheless, show that results from a well trained and experienced sensory evaluation panel can still be reliable in the absence of the other methods. pH can also be used as a quick freshness indicator with an understanding that muscle pH for live fish is generally neutral and increases as deterioration of the quality of the fish progresses in storage. Relatively low values of TMA-N suggest that this may not be a reliable method for assessing freshness quality of freshwater fishes such as the Lake Malawi Tilapia.

The strength of the study is that use of combined methods in freshness and quality assessment of fresh fish appears to be the first in Malawi and hence, could create more interest in similar studies in future. Probably, the main challenge or weakness was the complexity of analysing TVB-N and TMA-N using the modified Conway Diffusion Method. Further, analysis of TMA-N in freshwater fish such as tilapia appears to be a challenge.

6 Recommendations

Rejection of fish samples before attainment of unacceptable microbial limits underpins the need for using more than one method for accurate freshness and quality assessments of fresh fish. Such study should be conducted on fish at different storage conditions other than ice only.

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