Quality Assessment of Fresh Lake Malawi Tilapia (*Chambo*) Collected from Selected Local and Super Markets in Malawi

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Abstract

The aim of this study was to assess quality of Lake Malawi Tilapia (local name: Chambo) from local and super markets in Malawi. Fish from local markets hadsignificantly higher numbers of viable bacteria counts(9.5×10^8CFU/g, cm^2) (P<0.01)which were above acceptable limits compared to fish from super markets(2.7×10^5 CFU/g, cm^2).Most isolated bacteria species were Corynebacterium, Micrococcus, Pseudomonas, Bacillus, Flavobacterium and Escherichia coli. Mean total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N) (g/100mg) and pH of fish from local and super markets were 15.40±0.00, 0.10±0.01, 6.11±0.12 and 14.90±0.58, 0.13±0.02, 6.20±0.07, respectively.Fish collected from local markets had significantly higher levels of TVB-N (P<0.05)compared to those from super markets. Fish from both local and super markets were contaminated generally due to poor handling by sellers but not necessarily at the selling points. The study nevertheless observed that despite being microbiologically contaminated, fish were not wholly spoiled. This suggests that product declared unfit for consumption through sensory evaluation may still be nutritionally good hence need for validating such results with other methods.

Key words:Tilapia, Lake Malawi, freshness, quality assessment, sensory evaluation.

Introduction

In Malawi, fish continue to be the most reliable and affordable source of dietary animal protein to nearly the whole population. Most of the fish comes from Lake Malawi which has about 1,000 species of fish - the highest species biodiversity in the world (EAD 2010). However, Tilapia (local: *Chambo*) is the most important commercial fish in Malawi,

widely consumed and even exported.After catch from Lake Malawi, the fresh fish isusually preserved in ice and transported to different selling points. While fish is kept in refrigerators and often sold frozen at most super market stores, these storage facilities are not available at local markets. Fish is therefore sold at open spaces and exposed to high ambient temperatures, which consequently increase its likelihood of spoilage.Also, marketing and handling chain involving fresh fish in Malawi is

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very long and complex due to the high consumer demand. After catch, the fish changes several hands starting from the fishers through the middle men (fish vendors), then wholesalers and eventually retailers before reaching the ultimate consumer. However, issues of safety and quality in fresh fish are ranking high due to its highly perishable nature (Huss 1995).Therefore, the need for establishing necessary pre-cautions to protect the consumer is indispensable.Unfortunately, in Malawi, fish quality inspection is not well organised probably due to lack of knowledge in fresh fish quality management. Institutional capacity and inadequate availability of resources further constrain fish quality checks in the country.

This study thus endeavoured to assess the quality of fresh Lake Malawi Tilapia fish sold at local and super markets in Malawi.

Materials and Methods

Research design and fish sample collection. Fresh Tilapiafish samples were collected from local and super markets from the three regions of Malawi - Mzuzu city in the north, Lilongwe city in the centre and Blantyre city in the southern region in March, 2012 (Fig. 1).
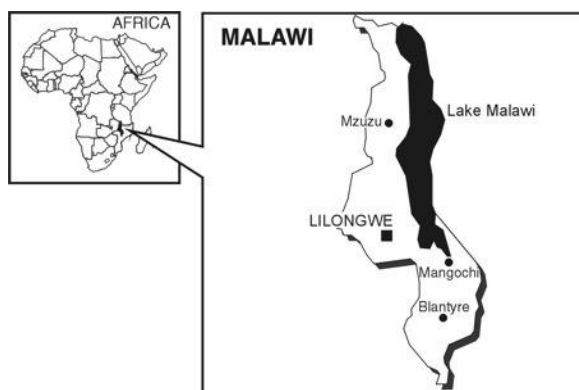
Figure 1. Map of Malawi showing the fish sample collection sites: Mzuzu (Northern Region), Lilongwe (Central Region) and Blantyre (Southern Region).
The fish were then carefully put in well-labeled plastic bags and kept in cooler boxes filled with clean fresh ice blocks and transported to the laboratory for analysis.While, most samples from super markets were collected in frozen form, fish from local markets were not frozen but preserved in ice.

Fish sample analysis. At the laboratory, sensory evaluation, microbiological andbiochemical analyses were carried out using appropriate methods.
Sensory evaluation.A pre-trained sensory evaluation panel consisting of six people described the freshness and quality of the fish samples using a generally agreed up-on and developed quality index method form (Table 1). Sensory quality of the fish was decided by scoring the attributes in Table 1 between 0 and 3 demerit scores where 0 and 3 entailedmost and poorly liked freshness of the fish sample, respectively.
Microbiological analyses. A procedure earlier used by Okoro et al. (2010) was followed for microbiological analysis of the fish samples. Samples for aerobic psychrotrophic bacterial counts were obtained from the external surface of the fish's skin, flesh and gills while samples for investigation of *enterobacteriaceae* counts were done on samples from kidneys and intestines.

Bacteria on the skin surface of the fish were determined by using two methods. The skin surface was laterally scraped using a sterilised rectangular wire swab guide measuring 5cm by 2cm. The swab was immediately placed in a sterile sample vial containing 100mls of 0.10% (w/v) peptone water. The vial was vigorously shaken for 10 minutes and allowed to stand for 20 minutes. A 6-fold serial decimal 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. All data on skin bacterial counts reported are based on this method. Results were reported in log CFU/cm^2 of skin surface.

Fish sample (1 g) from a selected part was dissected out, blended and mixed properly in a sterile 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 stand for 20 minutes, after which a 6 fold serial dilution was carried out in triplicates. Viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37 ℃ for 48 hours. Results were reported in log CFU/g.
Identification and enumeration of bacteria. Morphological characteristics of the various bacterial isolates *in vitro* were noted in the agar plates, and microscopy. After staining reactions and several biochemical tests, individual microbial species were identified. Representative isolates were re-plated on various selective media to observe their habits and other specific colony attributes.

Table 1. Quality Index Method (QIM) Scheme developed for the assessment of whole fresh Lake Malawi Tilapia collected from Local and Super Markets in Malawi.

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 & elastic (in-rigor)	0
		Soft	1
		Very soft/ depression when pressed	2
	Belly	Firm	0
		Soft	1
Quality Index			0-16

An automatic colony counter was used to count the number of colonies growing on the plates and the counts were expressed as colony forming units (CFU/g). Bacterial enumeration was done using the most probable number (MPN) procedure.

Biochemical analyses. A procedure used by Okoro et al. (2010) was was used in the biochemical analyses. pH was determined by removing and weighing 10g of muscle tissue of the fish and homogenized in 50 ml of distilled water. The mixture was centrifuged using a Yamato Mag-Mixer Model MH 800 (Yamato Scientific Company Limited, Japan). The mixture was then 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 $^{\circ}$C.

To determine Total volatile basic nitrogen (TVB-N) and Trimethylamine nitrogen (TMA-N), 25g of fish sample muscle tissuewas 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 $^{\circ}$C and then cooled to room temperature. When cooled, the sample was filtered into a conical flask using a Whatman No. 1 filter paper. The 2mls of 0.025N HCl was transferred to the central compartment of the micro diffusion dish using a pipette then adding 2mls 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 24hours. 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.The same procedure was used for TMA-N but the only difference was the addition of 0.5mls of 35% formaldehyde into the outer compartment of the micro-diffusion apparatus to block primary and secondary amines.

Statistical analysis. Data were analysed using the statistical software SPSS version 15.0 for Windows. Simple t-tests were performed to compare sensory scores (QI) and bacteria populations of the fish from super markets and those from local markets. To reduce the dimensionality of the sensory quality multivariate demerit attributes, principal components analysis (PCA) was performed using the Unscrambler® X Version 10.1 Statistical Software Package © 2009-2011 (CAMO, Norway).

Results and Discussion

High sensory quality index (QI) scoreswere recorded for fish collected from super markets than fish that were collected from local markets (Fig. 2).These values were nevertheless not significantly different (P>0.05) suggesting that sensory scoring of the quality attributes between fresh fish collected from super and local markets was not different.
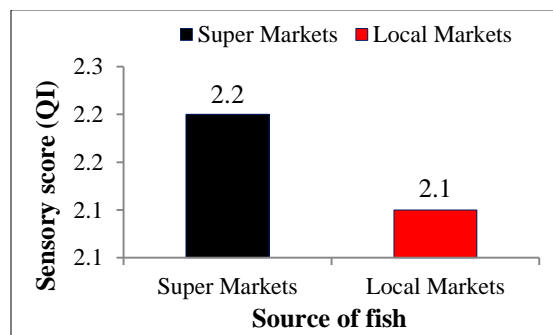
Figure 2. Sensory quality evaluation scores (QI) of Lake Malawi Tilapiacollected from Super and Local Markets in Malawi.

Except for scales, freshness scoring of the fish from both local and super marketsappeared to be influenced byappearance of the eyes, changes in the colour of the gills, mucus on the gills, texture of the belly and backside(Fig. 3). This observation was further confirmed by abi-plot of the principal components analysis (PCA) with full cross validationwhere PC1 and PC2 explained 45% and 28% of the observations respectively(Fig. 4).
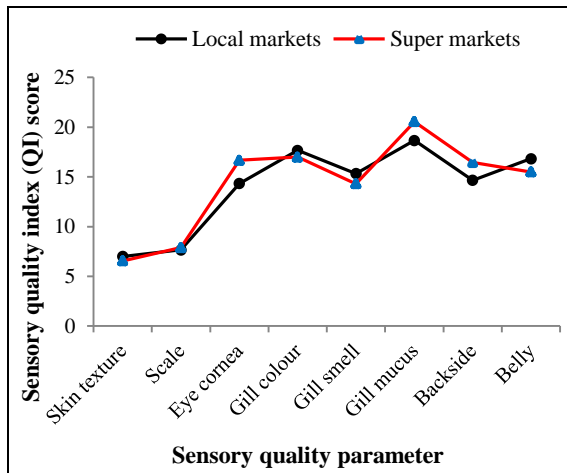

Figure 3. Sensory quality index (QI) scores for Lake Malawi Tilapia collected from Super and Local Markets in Malawi.

In reality, more sensory quality loss would be anticipated in fish from local markets due to unhygienic conditions and poor handling that necessitates increased bacterial loading (Diei-Ouadi and Mgawe, 2011). It was therefore interesting to note that sensory results between fish collected from super and local markets were not significantly different. However, fish from super markets were collected in frozen form and without prior history of storage time from catch because most fish sellers did not know. In Malawi, most fish sold in super markets are bought from fresh fish vendors who themselves buy the fish from the fishers or sometimes from other middle men. It is apparent therefore that the period that fresh fish take before storage is long and probably after changing many hands. Therefore, the likelihood that fish were frozen after rigormortis had already advanced cannot be ignored. This was apparent during thawing of the fish prior to sensory evaluation.

Though sensory evaluation lacks objectivity (Baygar et al. 2012), results show that attributes that are commonly and locally used for freshness evaluation in Malawi were significant. One possible explanation for disparities in sensory results could however be due to the fact that fish from super markets were frozen. It is reported that melting of ice through thawing affects sensory evaluation of fish (Huidoboro et al. 2001). Sensory scoring of smelling senses may therefore have been negatively influenced in this study. CAC-CL 31 (1999) showed that evaluating freshness of thawed whole fish based on appearance is problematic because freezing and thawing processes alter sensory characteristics such as eyes, skin and colour of gills and blood. These arguments could possibly explain why fish collected from super markets compared no better to fish from local markets using sensory evaluation.

Contrary to sensory results, fish collected from local markets had significantly (P<0.01) high counts of bacteria (10^7 CFU/g, cm^2)(Fig. 5 and Table 2) that were above the acceptable limits according to the International Commission on Microbiological Specifications for Foods (1986). On the other hand, bacterial load on fish from super markets was within the acceptable levels of 10^5 CFU/g, cm^2.
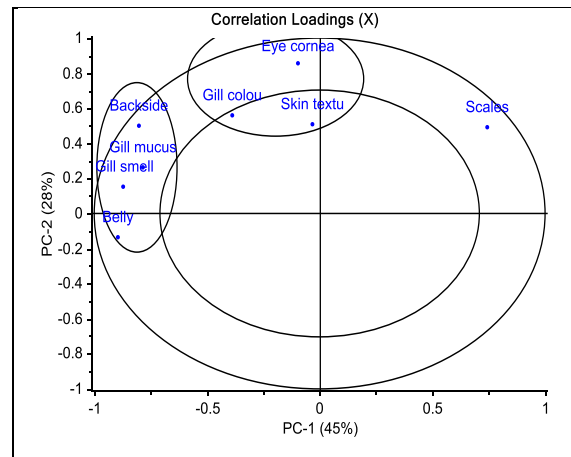
Figure 4. Bi-plot for principal components analysis (PCA) with full cross validation of all measured sensory quality parameters for fresh Lake Malawi Tilapia collected from Local and Super Markets in Malawi.

Findings for this study concurred with those earlier reported by Adebayo-Tayo et al. (2012) implicating poor handling and sanitary conditions for the high bacteria counts on fish from local markets. Conditions in most fresh fish markets in Malawi are unhygenic and this was also observed in this study where the fish were freely left on unsanitary places with a lot of dirt by fish vendors (Fig. 6).

Non correlating results between sensory evaluation scores and bacteria numbers demonstrated the challenge with the independent use of sensory

methods in quality evaluation of food products. Ólafsson (1999) observed that sensory evaluation and bacterial total count do not always agree.

Large bacteria colony countswere generally enumerated from gills and intestines in fish from both local and super markets though comparatively, the latter had fewer numbers of bacteria (Table 2).

Table 2. Comparison of bacteria populations isolated from Lake Malawi Tilapia collected from Super and Local Markets in Malawi.

Fish part	Super markets	Local markets
Skin surface (CFU/cm^2)	2.4×10^2	3.3×10^2
Muscle (CFU/g)	7.9×10^1	2.9×10^2
Gills (CFU/g)	8.8×10^3	5.2×10^6
Kidney (CFU/g)	4.5×10^4	4.2×10^5
Intestines (CFU/g)	2.2×10^5	9.5×10^8
TVC (CFU/g, cm^2)	2.7×10^5	9.5×10^8

Muscle tissue and skin surface had smaller populations of bacteria (Table 2). It was observed that in each case, not less than six bacteria species were isolated from all the parts of the fish except for the fish muscle tissue and kidney.
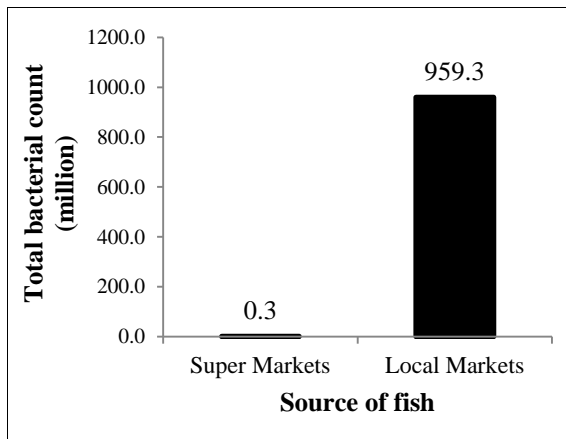
Figure 5. Bacteria population isolated from Lake Malawi Tilapia collected from Super and Local Markets in Malawi.

The commonest isolated bacteria species from fish from both super and local markets were *Corynebacterium* (20%), *Micrococcus* (16%), *Pseudomonas* (15%), *Bacillus* (15%), *Flavobacterium* (15%) and *E. coli* (10%) (Fig. 7 and Table 3).

Though pathogenic enteric bacteria species such as *E. coli*, *Vibrio* and *Staphylococcus* were not present on most fish samples, populations above acceptable

limits (10^8CFU/g,cm^2) (ICMSF, 1986) were found on fish from local markets (Table 3).

Table 3. Bacteria species and populations isolated from Lake Malawi Tilapia collected from Super and Local Markets in Malawi

| Source of fish	Super markets	Local markets
Bacteria species	Bacteria counts (CFU/g, cm^2)	
Corynebacterium	5.9×10^4	1.9×10^8
Micrococcus	5.9×10^4	1.5×10^8
Pseudomonas	5.0×10^4	1.4×10^8
Bacillus	3.5×10^4	1.4×10^8
Flavobacterium	3.4×10^4	1.4×10^8
Escherichia coli	2.2×10^4	9.5×10^7
Vibrio	8.8×10^3	4.7×10^7
Staphylococcus	4.4×10^3	9.7×10^7
Salmonella	2.4×10^3	9.6×10^6
Shigella	2.5×10^3	9.7×10^6

Presence of enteric pathogenic bacteria species in the fish from both super and local markets may be a clear indication of cross contamination due to poor sanitary standards and handling by the sellers, earlier observed by Mhango et al. (2010). This study echoed a report by Huss (1995) that proper handling of fresh fish between capture and delivery to the consumer is a crucial element in assuring final product quality. Similarily, Oramadike et al.(2010) concluded that the ultimate quality of frozen fish is decided by the sanitary and hygienic conditions prevalent at the selling point.This is a cause for concern because these organisms are potential enteric pathogens, which ideally must not be found on fresh fish. Doyle (2007) documented its public health threat in fishery products in fish from local markets.

As mesophilic pathogenic bacteria, the ambient temperature at which they are subjected to at local markets in Malawi, is a favourable condition for growth and multiplication.

Figure 6. Fresh Lake Malawi Tilapia fish being sold at Local Market (Note: flies on the fish and the garbbage far left) (Photo: F. Kapute).

Corynebacterium is a facultative anaerobic consistent with humans which Al-Harbi and Uddin (2003) isolated from Tilapia (*O. niloticus*) mainly in intestines. Facultative microbes are difficult to handle because they are found in both frozen and ambient environment stored fish (Doyle 2007) suggesting that regardless of the changes in the methods of handling, these bacteria may prove difficult to control. *Bacillus* is reported as the highest occurring bacterial isolate which causes toxin medicated disease rather than an infection (Adebayo-Tayo et al. 2012). *Staphylococcus* are mesophilic bacteria that has been isolated in tilapia fish from both super and local markets but high numbers were from super markets (Mhango et al. 2010). Pathogenic *Flavobacterium* species have been implicated for causing off-odors in fish (Omojowo and Sogbesan 2005). *Micrococcus* is a mesophilic bacterium and ranked among the common resident species of bacteria from freshwater fish (Doyle, 2007). In their study, Gelman et al. (2001) isolated *Micrococcus* species in 80% of the fish samples. The high occurence of *Micrococcus* species reported in this study could be indicative that this bacteria species may have contributed to the spoilage of the fresh fish. *Pseudomonas* species observed in this study have been reported as the dominant bacteria during the ice stored fish (Doyle, 2007).It is also known that*Pseudomonas* bacteria are only inhibited at pH values less than 5.4 (Doyle, 2007). However, pH values in this study were high (Table 4) suggesting why these bacteria flourished.
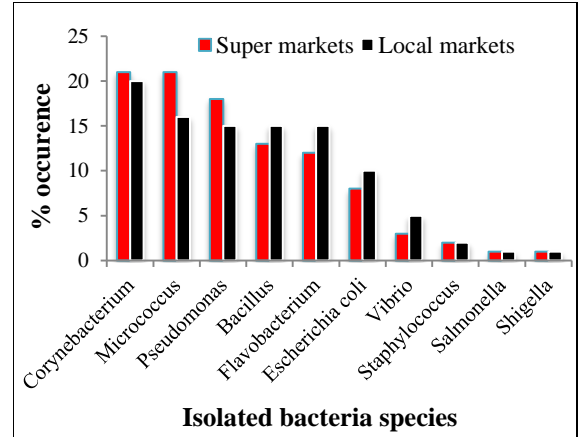
Figure 7. Frequency of occurrence of the bacteria in Lake Malawi Tilapia collected from Super and Local Markets in Malawi.

Since fish were preserved in ice during transportation to the laboratory to prevent further spoilage, it is not surprising that fish muscle tissue was not infested with a lot of bacteria species. Only two species of bacteria were isolated from fish muscle tissue compared to the other parts of the fish which registered diverse groups of bacteria comparatively. Also, in the case of frozen samples, the fish were thawed prior to analysis. Huidobro et al. (2001) observed that washing of the fish reduced number of microorganisms. It is suggested therefore that the bacteria found on the fish may be as a result of contamination due to handling from the source for both fish sourced from local and super markets. Few numbers of bacteria species isolated in the muscle could be an indication that the fish were not highly contaminated because bacteria would only get through to the tissues after some time (Huss, 1995).
Results for Total volatile basic nitrogen (TVB-N), Trimethylamine nitrogen (TMA-N) and pH for fish samples collected from local and super markets are presented in Table 4.

Table 4. Mean Total volatile basic nitrogen (TVB-N), Trimethylamine nitrogen (TMA-N) and pH of Lake Malawi Tilapia collected from Super and Local Markets in Malawi.

Area /Parameter	Local markets	Super markets	Sig.
TVB-N (mg/100g)	15.40±0.00	14.90±0.58	0.003
TMA-N (mg/100g)	0.10±0.01	0.13±0.02	0.192
pH (-log[H^+])	6.11±0.12	6.20±0.07	0.325

Note: Mean values are represented in standard deviation (±SD) and significant at 0.05%

These results were nevertheless not significantly different (P>0.05). This suggests that formation of volatile amines (TVB-N and TMA-N) in fish from local and super markets was not different hence same degree of spoilage.

Values for pH (Table 4) for fish from both local (6.11±0.12) and super markets (6.2±0.07) were close to neutral and were not significantly different (P>0.05).TVB-N and TMA-N levels reported in this study were far below the acceptable limits for human consumption of 10-15mg per 100g of fish flesh (Connell 1989) respectively. Also, these levels did not correlate with bacterial numbers and sensory quality scores agreeing with earlier studies that these are not reliable indices of spoilage in freshwater fish (Howgate 2010; Okoro et al. 2010). It is also known that freshwater fish such as tilapia contain very little or no trimethylamine oxide (TMA-O) at all which converts to TMA-N (Huss 1995). TVB-N and TMA-N values also depend to a great extent on the method of analysis (Huss, 1995). This suggests that varied results may be reported for same samples based on method used. The analysis method used in this study was the Conway micro-diffusion which was further modified to suit the available requirements during sample analysis.Thawing of the frozen fish samples collected from super stores prior to laboratory analyses may also have influenced results for TVB-N and TMA-N obtained in this study.Volatile compounds, in particular ammonia which is highly water soluble, can be lost when fish is in contact with ice due to its washing effect(Ruiz-Capillas et al. 2001). It could be with same notion that Magnússon and Martinsdóttir (1995) concluded that TMA-N is a poor spoilage indicator for thawed whole fish stored in ice.It is also evident from the study that frozen fish samples may pose even a greater danger by the fact that the fish were frozen after they were already subjected to contamination by the vendors. The need for proper sanitary measures throughout the production of fresh fish i.e. from catch to the ultimate consumer is therefore indispensable.

In summary, quality of whole fresh Lake Malawi Tilapia collected from local and super markets whereindifferent. However, bacteria loading was much higher and above acceptable limits in fish from local markets. Chemical and physical spoilage factors were not different between fish from local and super markets. The study echoed earlier reports that sensory evaluation and bacterial counts do not always agree. Further, the assertion that TVB-N and TMA-N are not reliable indicators of freshness in freshwater fish such as tilapia was observed. Effect of bacterial washing in fish kept in ice or frozen were also evident owing to significantly low numbers of bacteria on the skin surface of the fish.

Based on these findings, this study concluded that fresh fish obtained from both local and super markets had compromised sanitary and hygiene conditions suggesting that fresh fish consumers may not be better off buying fish from super markets than from local markets. It is concluded that spoilage of fresh Tilapia in super markets was iniated and more due to pre-handling activities by the fishers and vendors such that fish were frozen while in already adulterated form. At local markets, this problem was aggravated by unsanitary storage facilities and temperature abuse resulting into rapid bacterial multiplication in the fish.

The study also concluded that there is high probabillity of discarding rather good fish as unfit for consumption if sensory evaluation is used as a sole method for freshness and quality assessment.

Based on these conclusions, this study recommended promotion of sanitation and hygiene campaign in all fresh fish outlets whether local or super markets including fresh fish vendors through regular visitations and training by quality regulators.

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Appendix

Appendix 1:

Paired Two Sample t-Test for sensory scores (QI) of Lake Malawi Tilapia (*Chambo*) collected from Super and Local Markets.

	Super markets	*Local markets*
Mean	2.13333	2.06666
Variance	0.16333	0.30333
Observations	3	3
Pearson Correl.	0.23212	
t Stat	0.19156	
P(T<=t) one-tail	0.43288	
t Critical one-tail	2.91998	
P(T<=t) two-tail	0.86576	
t Critical two-tail	4.30265	

Appendix 2:

Paired Two Sample t-Test for Means of bacterial populations isolated from Lake Malawi Tilapia (*Chambo*) collected from Super and Local Markets in Malawi.

	Super Markets	Local Markets
Mean	23379.777	80032802
Variance	55804095	5.276E+15
Observations	12	12
Pearson Correl.	0.96606	
t Stat	-3.81684	
P(T<=t) one-tail	0.00143	
t Critical one-tail	1.79588	
P(T<=t) two-tail	0.00286	
t Critical two-tail	2.20099	

Pearson Correl.	0.99126
t Stat	0.01641
P(T<=t) one-tail	0.49385
t Critical one-tail	2.13185
P(T<=t) two-tail	0.98769
t Critical two-tail	2.77645

Appendix 3:

Paired Two Sample t-Test for Total volatile basic nitrogen (TVB-N), Trimethylamine nitrogen (TMA-N) and pH of Lake Malawi Tilapia (*Chambo*) collected from Super and Local Markets.

	Local markets	Super markets
Mean	7.20089	6.98012
Variance	59.4111	53.16138
Pearson Correl.	0.99951	
t Stat	0.80015	
P(T<=t) one-tail	0.25378	
t Critical one-tail	2.91999	
P(T<=t) two-tail	0.50756	
t Critical two-tail	4.30265	

Appendix 4:

Paired Two Sample t-Test for mean proximate composition values of Lake Malawi Tilapia (*Chambo*) collected from Local and Super Markets in Malawi.

	Local markets	Super markets
Mean	36.58	36.54
Variance	1140.317	1340.628
Observations	5	5