

QUALITY OF FRESH FROZEN TILAPIA FROM SELECTED SUPERMARKETS IN MALAWI

**Bwanamudogo I^{1,2,3*}, Kapute F¹, Lina A^{2,3},
Mbalassa M^{2,3} Zaabwe T^{1,4} and W Singini¹**



Irengé Bwanamudogo

*Corresponding author email: irengalicet2017@gmail.com

¹Department of Fisheries and Aquatic Science, Mzuzu University, Private Bag 201, Luwinga, Mzuzu, Malawi

²Department of Biology, Official University of Bukavu, P.O. Box 570, Bukavu, DR Congo

³Laboratoire d'Hydrobiologie Aquaculture et Gestion des Ressources Naturelles « LHAGREN » Université Officielle de Bukavu, P.O. Box 570, Bukavu, DR Congo

⁴National Agricultural Research Organisation, Bulindi Zonal Agricultural Research and Development Institute, P.O. Box 101, Hoima, Uganda



ABSTRACT

Fish provides a major source of dietary animal protein to Malawi's population. Majority of tilapia in supermarkets are of different origins and bought from different suppliers. Fish is highly perishable commodity and its quality degrades even in frozen form due to microbial activity. The quality of frozen tilapia (the most commonly traded and consumed fish in Malawi) sold in some reputable supermarkets in Malawi was determined. Fish were collected from nine (9) reputable supermarkets in three (3) regions of the country (north, central and south) and analysed in the laboratory for sensory quality, microbiological, chemical and proximate analyses. Sensory quality evaluation was performed following guidelines earlier developed for fresh tilapia (*Oreochromis spp.*) in Malawi. Differences and changes in the fish sensory quality were attributed to the effect of storage duration and conditions within the freezer compartment. Two types of bacteria namely, *Salmonella spp.* and *Escherichia coli* were identified on the frozen tilapia, suggesting poor and unhygienic pre-handling. Despite the presence of bacteria on the fish and differences in sensory quality, the frozen tilapia were within the acceptable range for human consumption. Nutrient composition of frozen tilapia was high despite differences ($p < 0.05$) in moisture, ash and crude fat. Fish from different origin were sold mixed in all supermarkets, poor handling along the fish market chain was identified as the major source of fish contamination. Mechanical damages were reminiscent of the effects of frozen storage. There is a need to establish optimum storage time for frozen tilapia in supermarkets to provide products with good quality in terms of sensory properties, nutrient content and safe microbial loads.

Key words: fish quality, fresh fish, frozen fish, tilapia, supermarkets, Malawi, *Oreochromis spp.*



INTRODUCTION

Fish make an important contribution to food and nutritional security in low and middle-income countries [1]. In fresh fish, "quality" usually refers to the aesthetic, look and freshness or negatively to the degree of spoilage which the fish has undergone. The changes in texture, smell, taste, or appearance cause them to be rejected by consumers [2]. Some supermarkets do not have improved equipment and little or no instruction of vendors who are involved in keeping and managing fish. Thus, this should be obligatory because fish vendors have a role to accomplish. Habitually, characteristics such as gill colour, gill mucus and the appearance of the eye cornea are the most reliable visual attributes for defining quality and freshness of fresh fish in Malawi [3]. However, tilapia is sold frozen in most supermarkets making it difficult to determine its quality before buying.

Fresh fish spoil due to microbial, chemical and enzymatic activities that ensue immediately after capture making it a highly perishable commodity [4]. Preservation of fresh fish immediately after catch is also important due to the long distance and slow transportation from the source of fish capture (Lake Malawi) to the main markets [3]. For the preservation of fish, time (duration) and temperature are two factors which require attention to minimize spoilage or degradation [5]. Temperature accelerates activities of bacteria, enzymes and chemical oxidation of fat in fresh fish [5], and spoilage also increases with storage time. On the other hand, super-chilling is not recommended because it leads to denaturation of the muscle proteins as well as structural damage of membranes, which can result in increased drip loss and textural change [6].

Microbial contamination in fish is usually a challenge mainly due to poor handling, presenting consumer safety challenges, and most of the pathogenic bacteria isolated from fresh fish are due to poor sanitary and hygienic conditions at the markets [7]. Earlier reports showed that fresh fish from local as well as supermarkets contained potentially pathogenic microorganisms [8]. It is estimated that >200 known diseases are transmitted through food and fish is one of the common carriers [9].

Proximate composition of fish flesh offers a good indicator for fish quality because generally nutrients such as moisture, protein, and fat decline with spoilage [10]. Declining levels of nutrients are usually consistent with spoilage and loss of freshness qualities [11].

A study regarding quality assessment of fresh Lake Malawi tilapia collected from selected local and supermarkets in Malawi is accessible [12]. However, tilapia sold in supermarkets are from different provenance such as Lake Malawi, Maldeco Fisheries Company (cage cultured) and Lake Kariba in Zimbabwe (cage cultured) and are sold mixed. Most Lake Malawi tilapia sold in supermarkets are also bought from different suppliers or vendors [12]. Thus, ensuring that frozen tilapia from supermarkets in Malawi is in line with respecting the hygienic standards' preservation investigation to check their quality, is a need.



MATERIALS AND METHODS

Fish sample collection

Frozen tilapia were collected randomly in 9 reputable supermarkets in three regions (north, central and south). Freezing temperatures were indicated on each freezer compartment and oscillated between -20 and -21°C in all supermarkets but duration of tilapia within the freezer compartment were unknowable by all vendors. Fish samples were collected within same freezer and each fish was wrapped in well-labelled plastic bag while wearing gloves to avoid contact with hands and water from the melted ice during transportation. The samples were immediately packed in cool boxes containing block ice. Fish samples were transported to the laboratory for analyses. Fresh block ice was added periodically into the cool boxes and the water from the melted ice was drained during the entire period of fish sample collection [3]. Fish samples were later kept at the same temperature as within supermarkets (between -20 & -23°C) in a freezer throughout the entire period of laboratory work.

Sensory evaluation

Quality attributes evaluated included appearance of the skin, eye cornea, scales, gills (colour, smell, and mucus), the backside, and belly texture, using a previously developed protocol for the sensory evaluation of fresh tilapia from Lake Malawi (Table 1) [3]. The sensory quality parameters were assigned scores between 0 and 3, where 0 means very fresh and 3 for spoiled fish. Lower demerit scores (0 to 1 and 0 to 2) were assigned to sensory attributes that are not very important as far as acceptability of fresh fish is concerned mainly appearance of skin, scales, elasticity of the belly and the backside. The higher scores (0 to 3) were given to the commonly used parameters, mainly the gills (colour, presence of mucus and smell) and also the appearance of the eye cornea (shape and colour). An overall sensory score value with a maximum value of 16 was obtained when highest scores for each descriptor are grouped [3]. The same was done for scoring all quality attributes, quality which lead to maximum of overall sensory score value for frozen tilapia from each region of Malawi followed by the overall sensory score value for frozen tilapia from each region.

Microbiological analysis

While there are many spoilage and pathogenic bacteria, this study focused on the determination of two species namely *Salmonella spp.* and *Escherichia coli* because these are consistent with poor handling of fresh fish [13]. The microbiological analysis was done on selected fish parts including skin, gills, muscle and intestine [3].

Determination of bacteria from skin surface and gills

To determine the presence or absence of *E. coli* on the skin surface and gills, a sterilized rectangular wire swab guide measuring 5 x 2 cm was used to measure a surface on the fish skin. Later, a sterile cotton wool swab dipped in 0.10% sterile peptone water was rubbed over the gills and the surface of the fish skin area covered by the wire swab guide. The swab was then, immediately placed in a sterile sample vial containing 100 ml of 0.10% (w/v) peptone water [3]. The vial was shaken for 10 min and allowed to stand for 20 min. A 6-fold serial dilution of the bacterial suspension in peptone water was prepared in duplicate and viable *E. coli* were determined using



standard plate count violet Red Bile agar [3] after incubation at 37°C for 48 hr. For *Salmonella spp.*, a sterile cotton swab was rubbed over a different portion of the surface of the fish skin using the guide and the gills. The swab was then, immediately placed in a sampling bag containing 10 ml of *Salmonella* enrichment base with a *Salmonella* enrichment supplement [14]. The mixture was mixed and incubated at 41.5°C for 18 to 24 hr. The samples within the enrichment medium were removed from the incubator and agitated by hand. With a swab, the sample was then streaked onto the hydrate surface of the 3M Petrifilm SALX Plate to gel [14]. Using a single streak to obtain isolated colonies. The presence of *Salmonella spp.* was determined as yellow colonies on the Petrifilm Plate after incubation at 41.5°C for 24 hr [14].

Determination of bacteria from intestines and tissue (muscle/flesh)

One gram of fish tissue was removed, blended with a sterilized stainless steel laboratory knife and mixed in a mortar then aseptically transferred to a sample vial containing 9 ml of 0.1% sterile peptone water. The vials were closed and shaken thoroughly for 10 min and allowed to stand for 20 min, after which a 6-fold serial dilution was carried out in triplicate. The presence of *E. coli* was determined in standard plate count violet Red Bile agar after incubation at 37°C for 48 hr. The results were reported as the presence or absence of *E. coli* for the fish part selected. For *Salmonella spp.*, 1 g of the fish flesh and intestines were handled as previously but put into 10 ml of *Salmonella* enrichment base with the *Salmonella spp.* enrichment supplement and incubated at 41.5°C for 18 to 24 hr. The rest of the procedure continued as previously described in this study for *Salmonella spp.* on the skin and gills.

Identification of bacteria

Representative colonies isolated from the violet Red Bile agar were reported as the presence (+) of *E. coli*. For *Salmonella spp.*, the colony characteristics were the presence of red to brown colonies with a yellow zone or associated bubbles, or both, on the Petrifilm plate.

Microbiological analyses by using the Hygiena, SystemSURE Plus

Microbiological samples were also analysed using an ATP testing device following the Hygiena-monitoring guide-rvb-04 2013 to validate results from the other methods. Samples were collected from the gills using a swab, which was placed back into the swab tube. The ATP testing device was activated by breaking the plastic valve at the top of the device by bending the bulb backward and forward. The bulb was squeezed twice to expel the liquid (enzyme) in the bulb to the bottom of the tube. The swab bud was bathed in the liquid by shaking gently in a side-to-side motion for 5-10 sec. The entire test device was placed into the luminometer following the Hygiena-monitoring guide-rvb-04 2013. The results were reported in relative light units (RLU).

Determination of pH

A muscle sample weighing 10 g was homogenized in 50 ml of distilled water. The mixture was then centrifuged using the Yamato Mag-Mixer Model MH 800 (Yamato Scientific Company Limited, Japan) and the mixture filtered using Whatman filter paper No.1 [3]. The pH of the homogenate solution was determined at ambient temperature after calibration using standard buffers at pH 7 and 4 at 25°C [3]



Proximate analysis

Moisture, ash, crude protein, crude fat and crude fibre were determined following the AOAC methods [15]. Moisture content was determined by oven drying of 5 g of fish at 105°C until a constant weight was reached. Ash content was determined after incinerating in a muffle furnace at 550°C for 16 hr. The crude protein content was determined using the Kjeldahl Method, crude protein was determined using a 6.25 conversion factor. The crude fat was determined using a Soxhlet extraction with XX as the solvent. The crude fibre was done to determine separately the sample moisture by heating in an oven at 105°C to constant weight. Percentage crude fibre was then, calculated as follows: % Crude fibre (wet) = (wet wt of residue – wet wt of original sample) / wet wt of original sample x 100.

Statistical data analysis

Data was analyzed using Microsoft Excel (2016) (Microsoft, Redmond, WA, USA) and the Statistical Package for the Social Sciences, SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA or Armonk, NY, USA). One-way Analysis of Variance (ANOVA) was used to compare treatment means at the 5% level of significance. Statistically significant treatment means were separated using Tukey's multiple comparison.

RESULTS AND DISCUSSION

Sensory evaluation

The overall sensory score value (Table 1) showed significant differences ($p < 0.05$) between the fish samples. The frozen fish from North region showed the highest quality score of 10 from North region is indicative of fish collected with good condition, followed by fish from Central and South regions with quality scores of 9 and 7, respectively; which are indicative of very good freshness condition (Figure 1). North and Central Regions border on Lake Malawi whereas South doesn't.

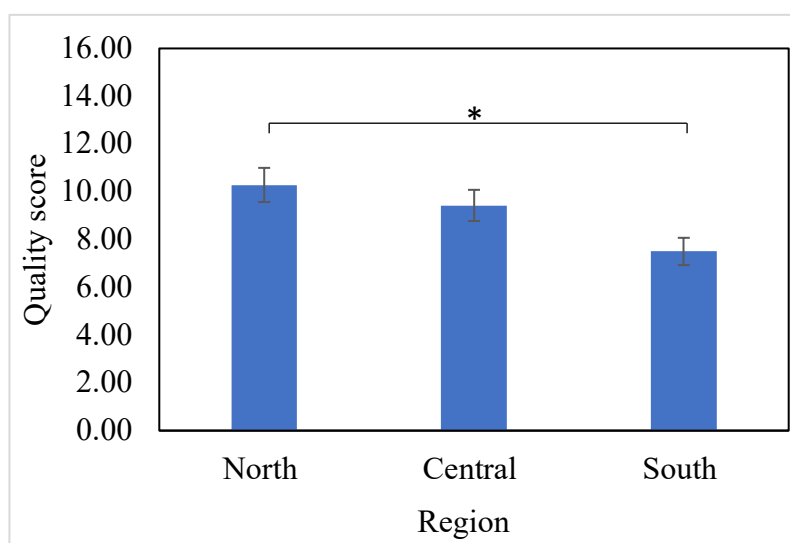
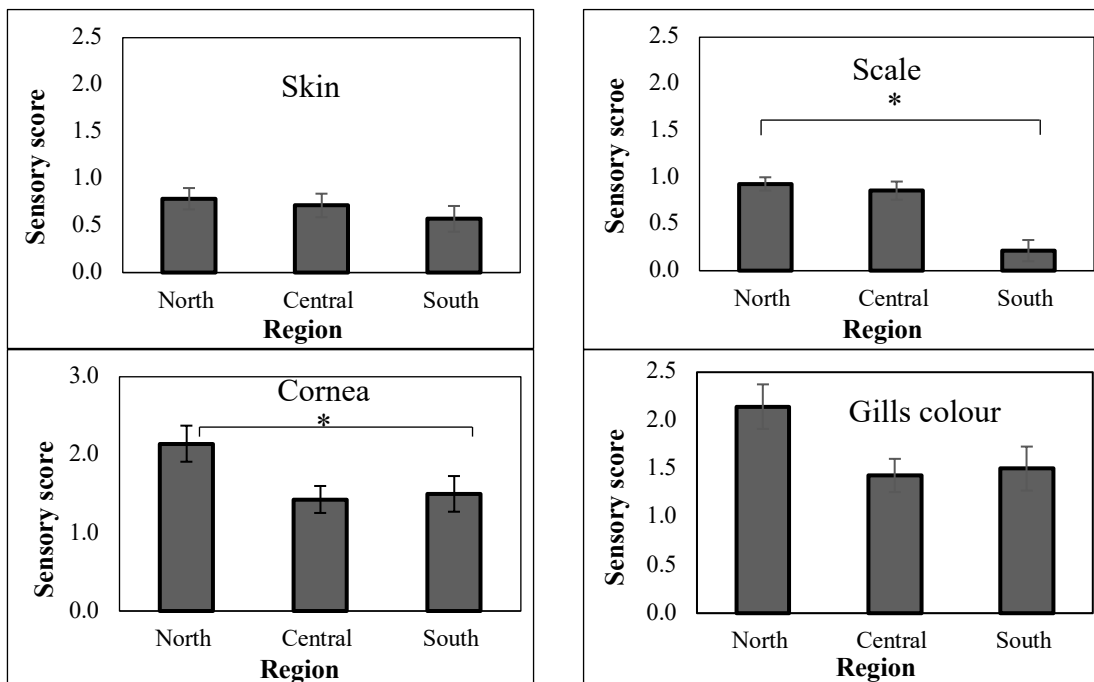


Figure 1: Maximum of overall sensory score value for frozen tilapia from each region of Malawi

The above results reveal that, frozen tilapia's freshness quality were acceptable despite the differences in the sensory attributes. The differences may be due to the fact that the frozen fish were mixed with some relatively low quality fish from different suppliers and origins then stored for long time within the same freezer compartment. Tilapia were mixed in supermarkets without looking at the degree of spoilage which fish has undergone. It has been reported that spoilage of fresh fish increases with storage duration [3, 11, 16]. In this study, some sensory attributes namely skin, gills and belly did not show any difference ($p \geq 0.05$) between frozen tilapia; but significant differences ($p < 0.05$) were observed for scales, cornea and backside (Figure 2). The eye cornea of fish from Central and South regions changed from clear to cloudy or milky and the shape of the eyes changed to concave while the backside texture of fish from North and South regions changed from firm to soft (Figure 3). The results indicate that these observed changes could be due to the low storage temperatures because freezing denatures fish flesh due to ice crystals that form causing mechanical damage to fish flesh by breaking down the structure and texture [17]. Gill colour of fish from Central and South regions changed from bright to pale or dull red (Figure 3). Freezing is a common practice and preserves fish quality for an extended time but can also deteriorate some factors such as colour [11, 18]. Fresh odour of the fish's gills was neutral suggesting that the fish were not spoiled.



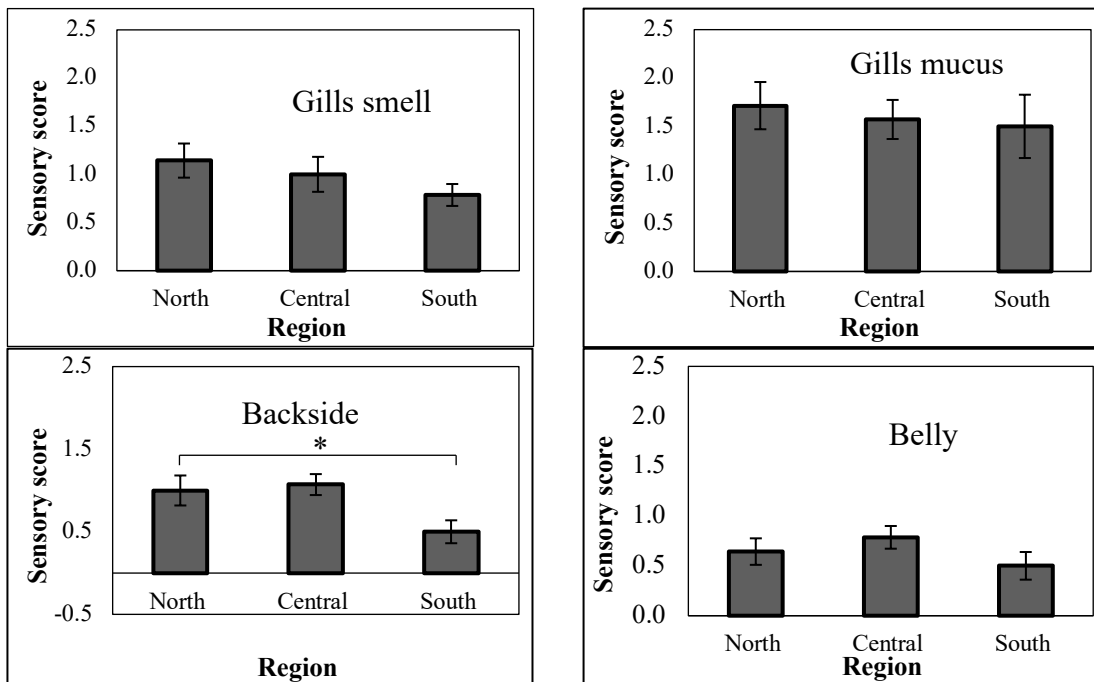


Figure 2: Average sensory scores for frozen tilapia from supermarkets in Malawi

Spoilage odour is a result of bacteria activities that produce enzymes responsible for breakdown of amino acids in the fish producing volatile compounds [19]. Spoilage odours are one of the significant characteristics of volatile compounds, which can be used to assess the freshness of fish [20].

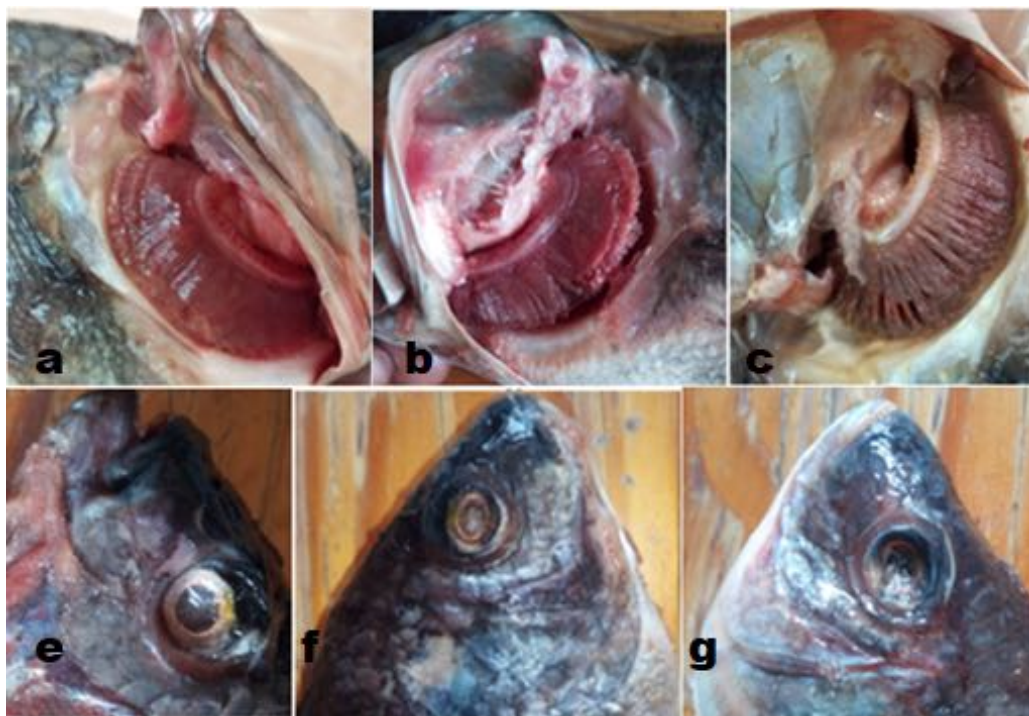


Figure 3: Gill colour (a,b,c) and eye cornea changes (e,f,g) for frozen tilapia from supermarkets in Malawi (Photo: I. Bwanamudogo)

The freshness of fresh fish can be assessed using techniques such as organoleptic evaluation of factors such as odour [21]. The eye cornea, gill colour and gill mucus were the significant and important sensory attributes used to determine the sensory quality of frozen tilapia in this study. The most important things that consumers look for in the freshness of fish are the general appearance of the fish including the eyes, gills, odour of the gills and texture of the belly cavity [22]. In Malawi, these parts of the fish are traditionally the most reliable way of judging freshness quality of fresh fish [3].

Microbiological analysis

Two types of pathogenic bacteria were investigated and were more present on frozen tilapia from North region (Table 2) with significant differences ($p < 0.05$) between fish from North and South region ($p < 0.05$). The highest RLU was 10 (Figure 4) suggesting that the fish were acceptable for human consumption (RLU < 30). The typical RLU limits for the fish product are less than thirty (< 30) as outlined in Hygiene-monitoring-guide-rvb-04 2013 (Business Centre Colne Way, Watford, Hertfordshire WD24 7 ND, UK). The contamination on the fish and consequently the pathogenic bacteria isolated, point to poor pre-handling at or from the point of capture along the market chain and within the supermarkets at different stages before freezing and during freezing [7]. Excessive handling of fish may lead to their harbouring significant numbers of bacteria, which can cause spoilage or be pathogenic [9].

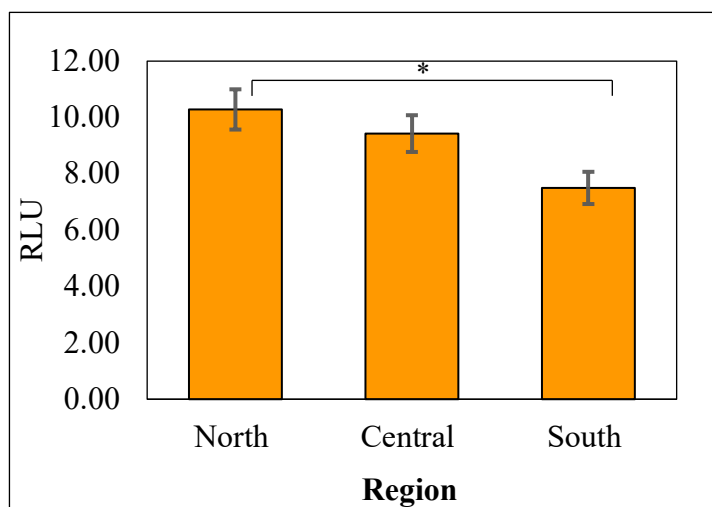


Figure 4: Microbiological results for frozen tilapia from supermarkets in Malawi measured in relative light units (RLU)

Microbiological results showed that, the highest and lowest presence of bacteria were observed on the gills and fish flesh, respectively. The presence of bacteria on fish skin and gills may be due to the fact that the fish were not well washed with clean water before freezing. Although fish can be contaminated from their habitats acting as a source of some pathogenic bacteria [8], results in the present study suggested poor handling. The highest presence of bacteria on the tilapia gills has also been reported previously [23]. The smaller number of bacteria on the fish flesh could be due to freezing preventing bacteria from invading the flesh. Temperature is a factor that

requires attention to minimize fish spoilage and temperatures may increase bacteria in the fresh fish [5]. Some collected fish were of low quality probably because they could have been frozen while already undergoing spoilage process and contaminated. The highest risk of fish contamination by *E. coli* in Malawi was reported to be within the transition from transport to vendor along the fish market chain [13]. Fish changes hands many times before reaching the ultimate consumer. Fishers spend long hours on boats with no provision of sanitary facilities and the landing environment is also without good sanitary facilities [13]. More than 200 known diseases are transmitted through food [10], and >2000 *Salmonella* serotypes can cause food poisoning with the major serotypes vary between countries [24]. The results indicate that despite different origins of tilapia sold in supermarkets, frozen tilapia were not highly contaminated but rather had got some compromised hygiene and sanitary conditions.

Chemical analysis of frozen tilapia from different supermarkets in Malawi

Measurement of pH is a quick method of determining freshness quality in fish since fresh fish usually have a neutral pH and any deviation suggests loss in quality [3, 27]. No significant differences ($p \geq 0.05$) in pH were observed between frozen tilapia. The highest pH value (7.44) was recorded from fish of Central region and the lowest pH value (7.30) was recorded from fish of South region (Figure 5). The results show insignificant variations of pH Value in frozen fish between the regions. The observed variations in pH value could be attributed to the fact that fish with both best and low quality were coming from diverse provenances (suppliers) and were mixed in all supermarkets.

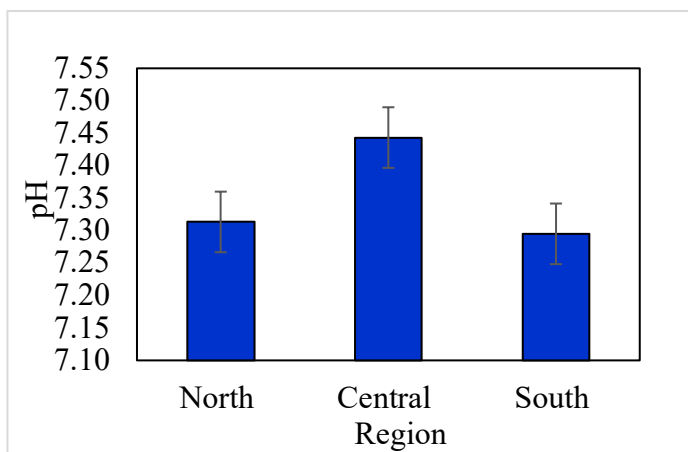


Figure 5: Mean pH for frozen tilapia from supermarkets in Malawi (Mean \pm S.E)

Natural pH for fresh fish muscles usually oscillates and depends on different factors such as fish species, season, and regimen, level of activity or stress during capture and storage conditions, with the typical pH of live fish muscle being ≈ 7.0 [26, 27]. The low pH value could be indicative of fish that were fresh and frozen shortly after capture. Similar findings were observed on frozen tilapia subjected to different freezing periods [11], and kept in ice [3]. The increase of fish muscle tissue pH correlated with storage time and bacterial activities [3, 11]. Accumulation of alkaline compounds within flesh muscle produced by bacteria could be the reason for the high pH values measured [28].

Alkaline compounds as well as volatile bases produced by autolytic activities and metabolism of spoilage bacteria increase the pH value after the initial pH decrease due to the build-up of lactic acid as the end product of anaerobic glycolysis [28].

Proximate composition

Results for moisture, crude protein, ash, crude fat and crude fibre of the frozen tilapia collected from supermarkets in Malawi are shown in Table 3. Significant differences were observed for moisture, ash and crude fat ($p < 0.05$) but not for crude fibre and crude protein ($p \geq 0.05$). The moisture content of the fish muscle is generally between 70-80% [29]. A moisture content of 81.4% was observed for frozen tilapia (*Oreochromis niloticus*) [30] and between 78.5 and 79.6% for Nile perch (*Lates niloticus*) [16]. The high moisture in this study may also be related to water being the principal component of fish with a value as high as 90% [31]. Ash content varied between fish could be due the different fish sizes. The nutritional value of freshwater fish can vary with size [32]. Ash content was found to be influenced by fish size. It has been reported that in that small sized fish species have higher ash content due to a high bone to flesh ratio [33]. Crude protein content of frozen tilapia was high, similar to an earlier study on frozen tilapia [11]. Furthermore, the high value of the protein content may suggest less bacteria contamination. Fish with less bacteria and declared unacceptable by sensory evaluation may still be nutritionally good for consumption [12]. Crude protein content decreases with increased storage time and increased numbers of bacteria in fresh fish [33]. Decreases in protein associated with freezing duration were observed on frozen tilapia [11]. Crude fat varied between 15.2 and 17.3% and $>13\%$ has been reported for *O. niloticus* [34] and 9.72% for tilapia (*Sarotherodon galiaenus*) [11]. The differences observed in this study, could be due to the different sizes of the fish samples [32]. Variations could also be because some frozen fish sold within supermarkets were from aquaculture (cage culture) such as Maldeco Fisheries Company in Mangochi, South region of Malawi and Lake Harvest (imported from Lake Kariba in Zimbabwe) while other were wild catch. Aquaculture raised fish tend to be fattier due to the high protein feed [31]. Crude fibre was insignificant probably due to the fact that fish contain low levels of fibre hence this is not important and may help explain the high digestibility of fish [31, 35].

CONCLUSION

The quality of the majority of frozen tilapia in most supermarkets in Malawi were within acceptable freshness and quality levels. However, some frozen tilapia showed relatively low sensory score value that was attributed to the fact that fish were frozen unsorted together regardless their degree of decreasing quality while coming from different origins (suppliers) and sold mixed within the same freezing compartment. Mechanical damages were reminiscent of the effects of frozen storage that could have changed the appearance and texture of fish attributes, thereby may deter consumers from purchasing the product. Fresh tilapia should, therefore, be well sorted based on their sensory quality and origins and not mixed before the storage. Fish should not be kept for long in freezers in order to minimize loss of its sensory quality and so that consumers avoid purchasing mixed fish of both low and good quality. Poor post-harvest handling of fresh fish by fishers and sellers along the market chain before



freezing could likely be the cause of the contamination. Thus, fresh tilapia should be checked and washed with clean water before the freezing. Majority of fish collected had high nutritional composition despite the presence of bacteria. Based on the results within one freezer compartment, there is more of good quality frozen tilapia and less of low quality suggesting that frozen fish consumers may buy mixtures of both good and low quality frozen tilapia. It is necessary to determine an optimum duration for tilapia freezing storage to maintain the sensory, nutritional and microbial quality of fresh fish.

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Table 1: Sensory quality scheme used for the sensory evaluation of frozen tilapia collected from supermarkets in Malawi

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

*(Adapted from Kapute et al., 2013)

Table 2: Determination of *Escherichia coli* and *Salmonella* bacteria species from selected parts of frozen tilapia collected from supermarkets in Malawi (+ = present, - = absent)

Fish part	Bacteria isolate type	Northern Region	Central Region	Southern Region
Skin	<i>E. coli</i>	-	+	+
	<i>Salmonella spp.</i>	-	-	-
Muscle	<i>E. coli</i>	+	+	-
	<i>Salmonella spp.</i>	-	-	-
Gills	<i>E. coli</i>	+	+	+
	<i>Salmonella spp.</i>	+	-	-
Intestine	<i>E. coli</i>	+	+	-
	<i>Salmonella spp.</i>	-	+	-

Table 3: Proximate composition of frozen tilapia collected from supermarkets in Malawi

Nutrient (%)	Northern Region	Central Region	Southern Region
Moisture	93.7 ± 0.05 ^a	92.6 ± 0.02 ^b	93.1 ± 0.2 ^b
Ash	14.9 ± 0.1 ^a	14.0 ± 0.1 ^b	15.4 ± 0.1 ^a
Crude fat	16.9 ± 0.02 ^a	17.3 ± 0.1 ^a	15.2 ± 0.2 ^c
Crude fibre	0.33 ± 0.00 ^a	0.35 ± 0.01 ^a	0.35 ± 0.00 ^a
Crude protein	63.1 ± 0.2 ^a	63 ± 1 ^a	62.8 ± 0.1 ^a

Means with a different superscript in a row are statistically different (p<0.05), (Mean ±SE)

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