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Assessment of Quality and Safety Parameters of the Market Tilapia (*Oreochromis niloticus*) Fish

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Abstract:- People are searching for wholesome and nourishing cuisine these days. Polyunsaturated fatty acids and nutrients are abundant in tilapia. The current study was carried out for evaluating the market tilapia's safety and quality aspects. In order to evaluate and assess the quality and safety criteria (proximate analysis, microbiological assessment, heavy metal determination, storage research), market tilapia was bought from the local market (Kewatkhali Bazar) for this study. The tilapia's relative moisture, ash, fat and protein contents were 65.083%, 0.64%, 17% and 14.52% respectively. The carcass traits (head, fillet, skin, carcass) of tilapia were 23.90±0.056%, 44.13±0.118%, 4.37±0.153% and 45.72±0.031% in that order. The tilapia's respective rigor mortis indices were 33.33%, 66.67%, 70% and 83.34% respectively up to 12 hours. The muscle pH decreased from 7.75 to 6.18 with a standard deviation of 0.02; this indicated increased acidity and the weight loss of tilapia was increased from 17.5 to 25.82 with standard deviation of 0.01. The peroxide value of the tilapia rose from 7 to 9 both before and after cooking. Four hours after thawing, the drip loss or protein loss of tilapia was 105.12 mg/3 ml respectively. Both raw and cooked fish received satisfactory ratings for color, flavor, texture and overall acceptability. Both *Salmonella sp.* and *Escherichia coli* isolates were medium-shaped, gram-negative rods.

In the flesh with skin, gills and intestine of the tilapia, the total bacterial viable counts were 13.34×10^{6} CFU/ml, 18.74×10^{8} CFU/ml, 20.10×10^{12} CFU/ml respectively and total mold viable counts of the flesh with skin, gills and intestine of tilapia were 5.20×10^{16} CFU/ml, 7.72×10^{16} CFU/ml, 8.56×10^{16} CFU/ml respectively. Both ranges exceeded the intended value by a small amount. The heavy metal accumulation in muscles of tilapia was Cu (49.2656)>Mn (23.67)>Ni (15.165)>Cr (8.939)>Pb (6.488)>Se (5.67)>As (1.182)>Cd (0.374)>Co (0.3268) as ppm. The identification results of formalin came back negative. The study concluded that tilapia fish was better for nutrition and consumption despite the little variations.

Keywords:- *Tilapia, Physicochemical Properties, Storage Stability, Microbiological Analysis, Sensory Attributes.*

I. INTRODUCTION

The fishery sector in Bangladesh is directly responsible for a significant portion of the country's efforts to ensure food security together with the national economy (GDP). One of the healthiest food options in Bangladesh is tilapia (*Oreochromis niloticus*), the freshwater fish species which has a great demand with significant amount of high-quality protein and omega-3 fatty acids all around the world (Hernandez et al., 2020). The Nile tilapia (*O. niloticus*) which has excellent growth rates, a high potential for intensive fish farming, the ability to adapt to various climates and environmental conditions, a wide range of cultivation methods and meat of exceptional quality, makes it one of the most popular species raised commercially. Additionally, the white color, lack of intramuscular bones, excellent texture and flavor of tilapia meat make it popular on the market. The fish processing industry's primary method of marketing the finished product is through the fillet (Sahu et al., 2017). Fish's quality and nutritional content mostly depend on how recently it was caught because after fish mortality, enzymatic digestion, lipid oxidation and bacterial breakdown, lastly fish rotting occur that adversely affect the quality and safety of fish (Dutta et al., 2018). Sensory and non-sensory approaches are the two major ways to evaluate fish quality in order to determine its freshness and shelf life. While non-sensory approaches include physical, chemical, biochemical and microbiological means, sensory methods predominantly rely on the look, smell, texture and taste of fish (Okoro et al., 2010). Fish and fishery products are extremely perishable foods and their rapid perishability has been the principal barrier to their preservation (Dewi et al., 2011; Goja, 2013). Proteins, minerals, vitamins and other nutrients, among others are all found in large quantities in fish meat. But fish meat spoils more quickly than other muscle meals, especially when handled carelessly and this type of spoiling is predominantly bacterial in origin (Ghaly et al., 2010; Goja, 2013). Fish have microorganisms in their guts, stomach and on their exterior surfaces including slime, skin that continue to thrive and reproduce after death, swiftly invading the body. Initial chemicals include those with tart, fruity or acidic notes; then bitterness, sulfide or rubberizes; and lastly, the rotten state. The quality of fish and fisheries products is significantly influenced by the bacterial population in fish (Shikha et al., 2019). Several elements, including morphological, physiological, environmental, food habitat, etc. affect the body composition of fish (Salam et al., 1994).

Consumers are paying more attention to carcass and flesh quality because it is directly tied to nutrition and human health (Sahu et al., 2017). To quantify overall changes under storage conditions, a variety of physical techniques are used including the rigor mortis index, muscle pH value, drip-loss and weight-loss. Drip loss during thawing, which can also convey nutritious components like proteins and lipids along with the lost water, might result in changes in the texture of the meat (Viegas et al., 2013). When a fish dies, a condition known as rigor mortis occurs. The kind of fish, the temperature and treatment of the fish before slaughter, the stress of the slaughter, the fish's biological condition and the temperature of pre-rigor storage all affect rigor mortis (Le et al., 2020). Other factors that affect fish quality while being stored include organoleptic properties, peroxide value. The results of a fish's freshness test reveal the fish's quality in terms of odor, color and look. With time, peroxide value also rises due to storage (Shikha et al., 2019). As the typical diet of Bangladeshis consists of various fishes with a high carbohydrate content, the author had attempted to determine all the fundamental safety and quality parameters of the tilapia fish from farm to fork to ensure that the marketed fish was safe and of good quality for the public health, whilst other publications had highlighted some specialized metrics.

In light of the explanation above, the primary goals of the current study are to evaluate the sensory, quality and safety characteristics of market tilapia as well as the stability of raw fish during storage with regards to selected physiological indicators.

II. METHODOLOGY

A. Materials and Methods

This research work was carried out in the laboratory of the department of Food Technology and Rural Industries, Aquaculture, Microbiology & Hygiene, and Central Laboratory, Bangladesh Agricultural University, Mymensingh.

➢ *Collection of Raw Fish, Chemicals and Equipment*

Fresh and live tilapia was purchased from the neighborhood market (Kewatkhali bazar), Mymensingh. The average weight and size of the Fifteen tilapias were 0.366±0.454 kg and 16.002±2.54 cm, respectively. The price of tilapia in market was 40-45 tk. per kg. The fish were first organoleptically inspected. The eyes were clear, convex with a black and distinct pupil. The skin of fish was shiny, gills were red in color and fish meat had a translucent, intact belly. Hot air oven, Muffle furnace, Kjeldahl apparatus, Soxhlet apparatus, pH meter (HI 2211 & ORP Benchtop meter), Shimadzu 2300 ICP-MS, UV-Visible spectrophotometer (Photolab® 7600, UV-Vis, EU) etc. were used. The laboratory of the Department of Food Technology and Rural Industries, Department of Microbiology & Hygiene, Department of Aquaculture, Department of Soil Science at BAU provided all of the necessary chemicals, including 99.85% pure food grade Glacial acetic acid, ACS grade Chloroform, Laboratory grade Standard KI solution, 38%concentrated HCl, Secondary standard reagent $Na_2S_2O_3$, Starch indicator, Phenyl Hydrazine, Biuret reagent, Reagent grade Acetone, Conc. H_2SO_4 (N-free) and for microbial analysis, plate count agar, potato dextrose agar, PBS solution and other tools.

➢ *Experimental Design*

The experimental design for this investigation is shown in Table-1. The purpose of the experiment was to precisely assess the physicochemical, biochemical, sensory and microbiological quality characteristics of tilapia fish. The feasible experiment is carried out in a short amount of time. The remaining samples were then placed in a zippered bag and preserved for the remainder of the experiment at -18±1^{0}C.

B. Physicochemical Analysis

According to the guidelines set forth by the AOAC (Association of Official Analytical Chemists), the proximate characteristics of tilapia were carried out. The moisture content was determined using the methodology outlined in standard 930.15 (AOAC, 2023), by drying the sample at 105 °C for 8 hours. The crude protein was determined by the Kjeldahl technique in standard 954.010 (AOAC, 2023) through completing the digestion, distillation and titration. The ashes were heated in a muffle furnace until light gray ash (or constant weight) was produced, in accordance with norm 942.05 (AOAC, 2023). Crude fat analysis was conducted according to standard 920.39 (AOAC, 2023), by ether extraction method. One hundred percent of the previously listed components were subtracted from the sample in order to determine the amount of carbohydrates (Spanopoulos-Hernandez et al., 2010).

$$\text{Carbohydrate} = 100 - (\%\text{ of moisture} + \%\text{ of protein} + \%\text{ of ash} + \%\text{ of fat}) \quad (1)$$

Total energy value was calculated by the accumulation of the protein, lipids and carbohydrates (Jahan et al., 2021).

$$\text{Energy} = (\%\text{ of carbohydrate} \times 4.1) + (\%\text{ of protein} \times 4.1) + (\%\text{ of fat} \times 9.1) \quad (2)$$

Table 1: Experimental Design of the Current Study

Sample no.	Observation time	Parameters observed	
Tilapia	15 days with every 3 days interval	**Physicochemical:** moisture, crude protein, fat, ash, muscle pH, heavy metal determination, formalin test, carcass yield, rigor mortis index, drip loss, weight loss	
		Biochemical: peroxide test	
		Microbial analysis: Total Viable Count for bacteria, Total Viable Count for mold, cultural, morphological characters of ***Salmonella sp.*** and ***E. coli***	
		Sensory attributes (before and after cooking)	**Treatment**
			S_1 (cooked by steaming)
			S_2 (cooked by electric oven)
			S_3 (cooked by gas oven)

Formalin was identified by adopting Schryver S.B., 1909, using phenyl-hydrazine method (Tankard et al., 1926). Muscle pH of tilapia was determined with digital pH meter (Perez et al., 1987). With minor adjustments, the weight loss during storage, carcass yield, drip loss and rigor mortis index were calculated using the methodology described in Viegas et al., (2013). The soluble protein content of the drip loss was determined by the Biuret test (Chutipongtanate et al., 2012).

$$\text{Weight loss with respect to the fresh and thawed fillets (\%)} = \frac{W1-W2}{W1} \times 100 \quad (3)$$

Where, W_1= Weight of fresh fillet, g and

W_2= Weight of thawed fillet, g

Carcass yield was calculated as follows:

$$\text{\% Carcass yield} = \frac{\text{Weight of body Component}}{\text{Total weight of each specimen}} \times 100$$

The soluble protein content of the drip loss = (mg protein × mL drip)

The rigor mortis index was calculated using the following formula:

$$\text{Rigor Mortis Index (\%)} = \frac{D0-D}{D0} \times 100 \quad (4)$$

Where,

- D_0 = The distance on the table's horizontal line from the caudal fin's base (shortly after death)
- D = The caudal fin's base distance following each chosen time period

Heavy metal of tilapia was ascertained by using ICP-MS (Shimadzu 2300) as per procedure outlined by İslamoğlu et al., (2021) after finishing the powder preparation, digestion and analysis stages. Fish samples weighing roughly 103 g were prepared, placed on petri dishes (cleaned beforehand on acid bath at 40^0C for two hours) and dried at 65^0C for 72 hours until the weight remained constant.

Fig 1: The Determination Process of Rigor Mortis Index on a Horizontal Table Conducted by the Laboratory of the Department of Food Technology and Rural Industries

In order to complete the digestion steps (5:2, 69% HNO_3: 30% H_2O_2), the oven-dried fish samples were ground into powder in a ball mill and about 200 mg of each powdered sample was then weighed out using Shimadzu digital weighing scales and placed in the digestion block (VELP digestion system) at 140^0C for five hours. Following digestion, a dull white solution was allowed to cool before being used for analysis. Forward RF power of 1550 W and a nebulizer sample flow rate of 0.35 ml/min were the operational parameters for the ICP-MS. At a flow rate of 5 ml/min, helium was employed as the collision gas. Samples were examined by contrasting them with the previously stated criteria.

C. Biochemical Analysis

The peroxide value (meq/kg) was evaluated in accordance with the sallam (2004) method.

D. Microbiological Analysis

For microbial assessment, Total viable count (TVC) of bacteria & mold was enumerated (Ikhlas et al., 2012) and isolation & identification of *Salmonella sp.* and *Escherichia coli* were performed by adopting Gram's staining method (Merchant & Packer, 1967).

E. Organoleptic Evaluation

Three various types of the cooked tilapia were assessed by 10 semi-trained panelists for flavor, color, overall acceptability and texture. A nine-point hedonic rating scale, with 1 denoting extremely dislike and 9 denoting extremely like, was used for the statistical analysis of the degree of acceptability of the cooked tilapia (Mazumder et al., 2020). The panelists were chosen from the teachers, students and employers with different age of the Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh. The results were evaluated by Analysis of variance (ANOVA) and Least Significant Difference (LSD) procedures of the statistical analysis system by using the IMB SPSS 26.0 software.

Many sensory aspects of raw fish have been assessed using the eyes. The recommendations and techniques presented here (Table 2 and Table 3) described by Howgate et al., (1992) to assess fisheries goods for freshness based on organoleptic qualities of fish.

F. Statistical Analysis

Every experiment was carried out three times and the average value was reported. Software called SPSS 26.0 (IBM SPSS Inc., Chicago, IL, USA) was used to conduct the statistical analysis. Excel software was used to draw each graph. At $p<0.05$, the levels were deemed substantially different using LSD test.

III. RESULTS

A. Physicochemical Properties of Market Tilapia

The physicochemical properties of tilapia are the crucial factors which impact the growth, reproduction, nutritional profile and other biological features of fish. The physicochemical characteristics of tilapia, such as proximate composition, muscle pH, carcass yield, rigor mortis index, drip loss, weight loss, formalin and heavy metal content are substantially ($P<0.05$) different from the control during the entire experimental period. The result of proximate composition of cooked tilapia is displayed in the Figure 2 that demonstrates the moisture, ash, protein and fat content of market cooked tilapia found to be 65.083%, 0.64%, 14.52% and 17% respectively. The calculated value of carbohydrate and energy is 2.757% and 225.55 Kcal respectively. From exploring research paper, the proximate composition of tilapia is 81.39% moisture, 13.66% protein, 0.54% fat and 1.36% ash respectively (Olopade et al., 2016). From the datasets, it reveals that the percent of moisture content of tilapia is reduced from the research value, consequent with the significant increase in protein, fat and ash which implies that higher solid content gives higher amounts of nutrients as well as higher food value. This variation between research value and market value may occur due to different feeding habits, habitants, species variation, environmental condition, location and metabolism systems. According to Stansby (1962) and Salam et al. (1995), Fish species, season, age, and eating habits can all affect the proximate composition of their flesh. Fish fillets often have inversely correlated moisture and fat concentrations, summing up to about 80% (FAO, 1999). The summation of fat and moisture content of market fish and research value coincide with approximately 80%. The ash content of market tilapia is less than the research value. That's because the inorganic compounds are less due to high value of organic compounds (protein, fat, carbohydrate). Consequently, the high fat content of market tilapia contributes more to its energy level. This is because the fish used in the tests are moderately large (1.0±0.39 kg), less than a year old and may have a higher range of fat content.

Table 2: Grading of fresh fish

Grade	Point	Degree of freshness
A	<2	Excellent/Acceptable
B	2 to <5	Good/ Acceptable
C	5	Bad/Rejected

Table 3: Determination of Defect Points

Parameters		Attributes	Grading point
Appearance	Skin	Very bright	
		Bright	2
		Dull	
	Slime	Clear-transparent	
		Slightly cloudy/milky	
		Cloudy	5
Eyes	Clarity	Clear-translucent	1
		Slightly opaque	
		Opaque	
	Shape	Convex	
		Flat	2

		Concave	
Texture	Elasticity	Elastic (finger mark disappears immediately)	1
		Slightly marked by pressure	
		Clearly marked by pressure	
	Belly	Intact	
		Slightly intact	2
		Soft	
		Very soft	
Gills	Color	Bright/dark red	
		Brownish red	2
		Discolored	
	Odor	Fresh, sea weedy	1
		Neutral	
		Fishy / sour	
		Off-odor / rotten	

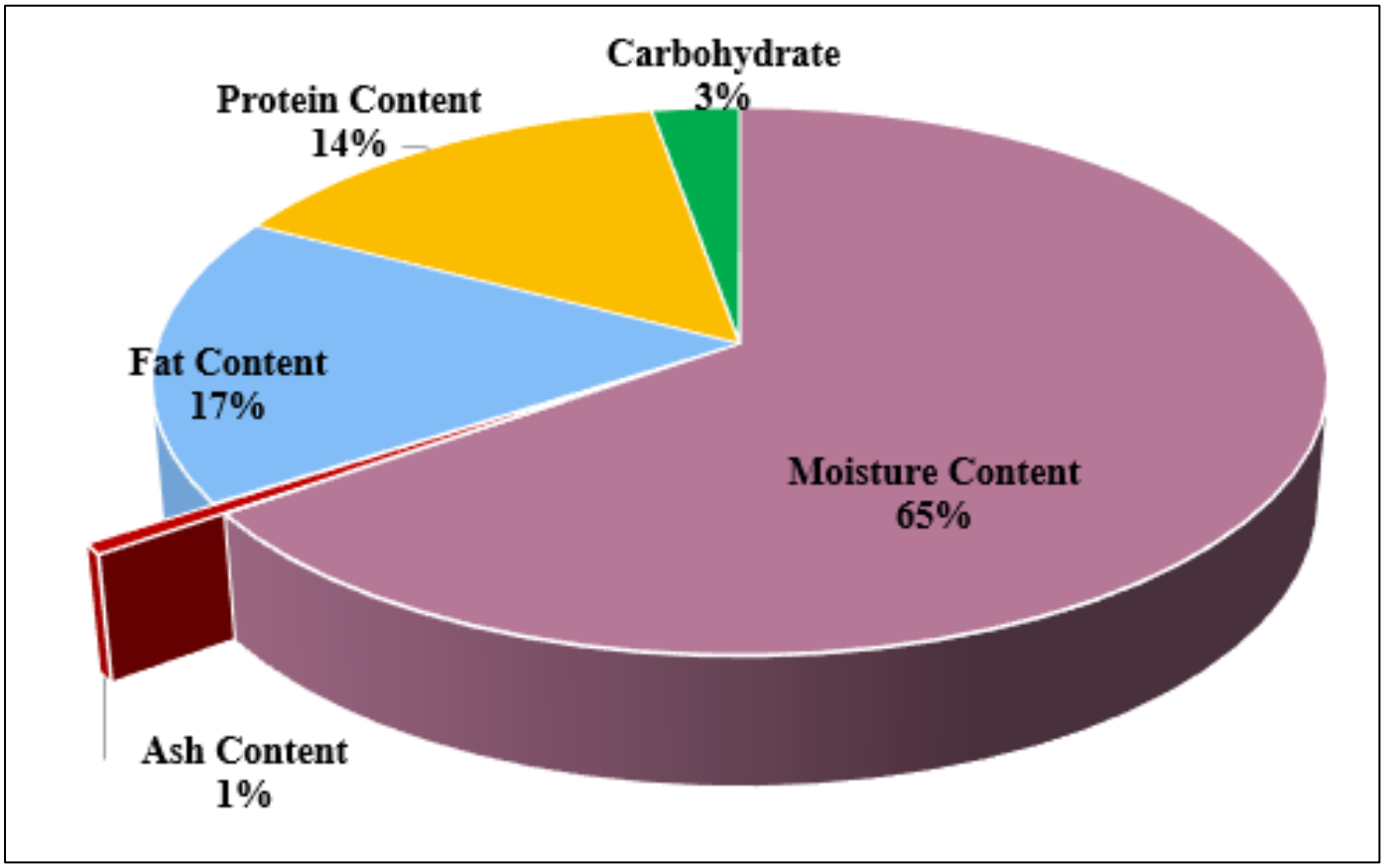

Fig 2: Graphical Presentation of Proximate Analysis of Tilapia

Figure 3 displays the muscle pH value of tilapia, which starts at 7.75 with a standard deviation of 0.02. Since tilapia must be kept cold for 15 days, its pH value steadily drops; as a result, after 9 days, it rises and then falls once more. Tilapia has pH values of 7.75, 6.35, 6.18, 6.86, 6.70 and 6.62 respectively three days apart. Due to cold storage, the pH value of tilapia falls in a down-up-down pattern and increases acidity. According to Viegas et al., (2013), When fish are alive, their pH tends to stay near neutral 7.4 and when they die, their muscles' pH drops because of anaerobic glycolysis, which turns glycogen into lactic acid. A slight but consistent decrease in muscle pH for storage is noted in tandem with the progression of rigor mortis and at the conclusion of the time, there was a slight increase in pH readings.

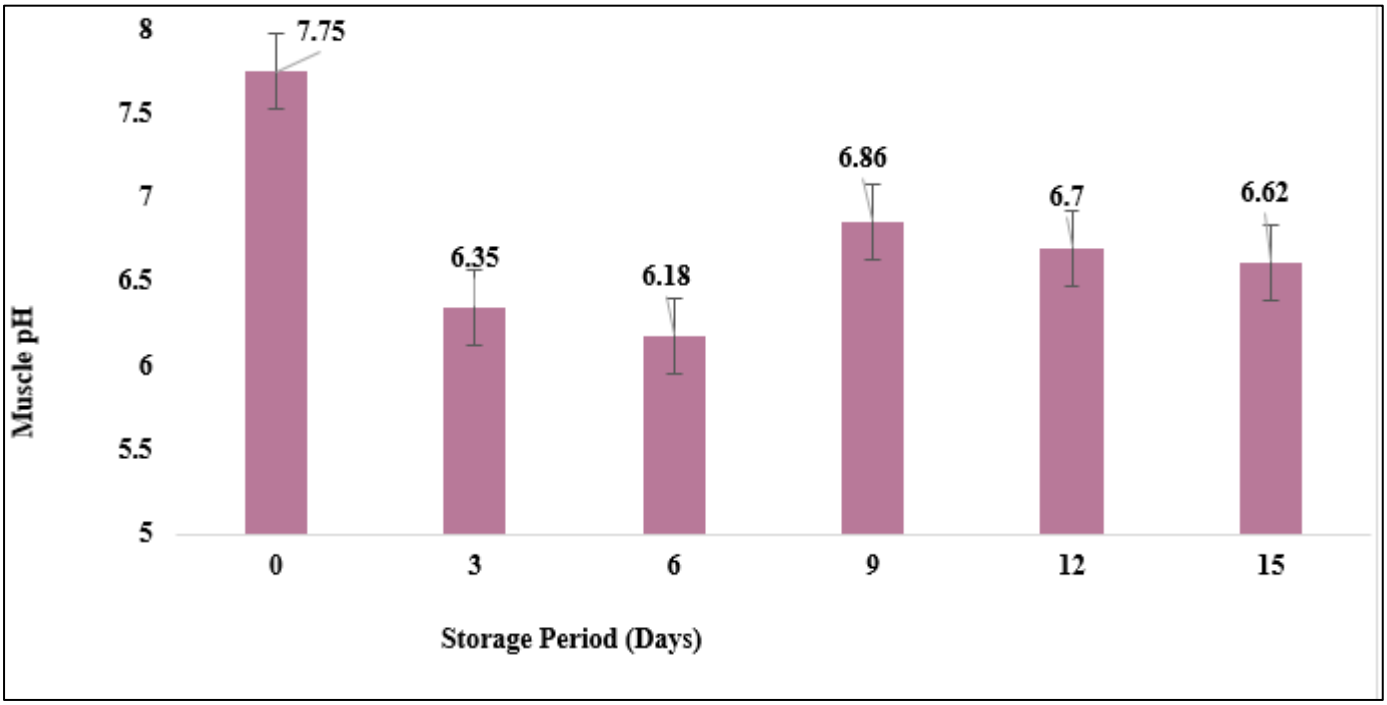

Fig 3: Graphical Presentation of Muscle pH of Tilapia

Rodrigues et al., (2008) observed that the pH levels gradually elevated. This phenomenon was linked by Viegas et al., (2013) to the existence of catabolites, including ammonia, in the last stages of the storage period, which are caused by bacterial activity on the meat's amino acids. It is shown that the muscle pH of tilapia is initially 7.75 with a standard deviation of 0.02 that's consistence with neutral value. Due to cold storage for 15 days, the value of pH of tilapia decreases gradually, therefore, about 9th days, the pH value also increases then again decreases. The pH decreases for producing the lactic acid in fish meat and increases for producing alkaline compounds such as: ammonia, trimethylamine derived from microbial action during fish muscle spoilage. This falling and upping pH influences the quality of fish particularly fish muscles and texture. For reducing the pH value, fish's metabolism badly affects and for increasing pH value, fish's muscle tissue is weak that deteriorates the quality of fish.

Table 4: Drip loss of tilapia fish samples

Drip loss (mg/3 ml) of tilapia at slow freezing after 24 hours storage time	**105.12**

According to Table 4, the drip loss of tilapia is 105.12 mg/ml for 3.30 hours after thawing during a 24-hour period of storage in a 3 ml solution. With regard to calculations, the drip loss of tilapia reveals that the vital nutrients are progressively being taken away from the fish. Kristoffersen et al., (2007) mentioned that the denaturation of muscle proteins and the breakage of membranes, cytoskeleton and extracellular matrix cause the drip loss, which results in the loss of intracellular chemicals in addition to proteins. Therefore, the creation of a considerable volume of drip loss during thawing not only reduces the nutritional quality due to the leaching of the proteins, but it also makes the fillets less appealing to the consumer in terms of look, succulence, texture and flavor. Viegas et al., (2013) showed that there is an increase in the soluble protein of tilapia fillet contained in the drip loss, for 3 hours storage period remaining 96.70±14.99 mg/mL protein. Due to 24 hours of storage period and 3.30 hours of freezing and thawing process, the drip loss of tilapia is more about 105.12 mg/ml from research value. Due to drip loss, the fish quality is spoiled because the protein content is decreased gradually from fish fillet through thawing that happens the gap of nutritional demand. Basically, drip loss is happened due to lowering pH or temperature differences that promotes the protein denaturation. Figure-4 shows that when tilapia is stored, the percentage of weight loss gradually increases. The percentage of weight loss for tilapia stored for 15 days at a distance of 3 days is 17.5%, 20.62%, 21.56%, 24.68% and 25.82% with a standard deviation of 0.01. The percentage weight loss of tilapia is medially higher from 0 to 15 days at 3 days interval which suggests less moisture is present. The weight loss of fish fillet is increased keeping on cold storage conditions. The fresh and thawed fillets' weight loss differed from 3.03% for 1 hour to 8.09% for 92 hours (Viegas et al., 2013). Emire et al., (2010) showed decreasing the moisture content results in the weight loss in fish fillets stored frozen. It indicates, due to reduce moisture content and consequently increase percent weight loss, the fish quality is rapidly spoiled through rupturing the fish skin.

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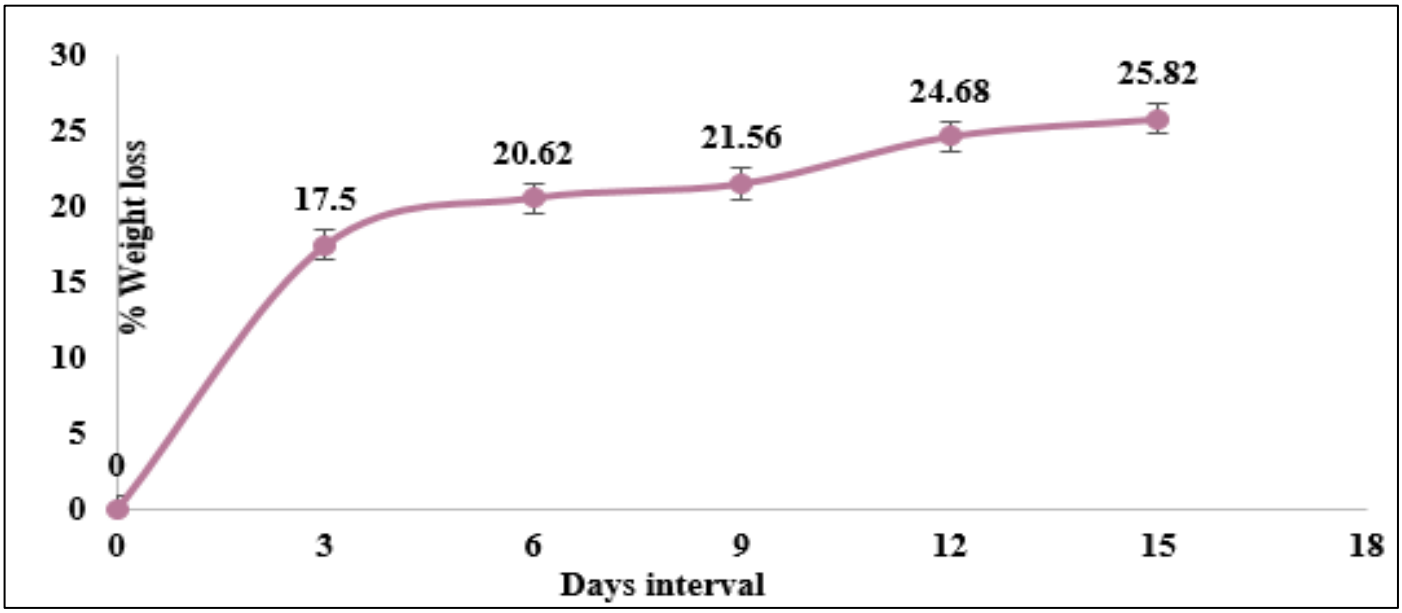

Fig 4: Graphical Presentation of Percent Weight Loss of Tilapia

Figure 5 demonstrates that the rigor mortis index of tilapia is 33.33%, 66.67%, 70%, 83.34%, 83.34% and 83.34% for up to 12 hours.

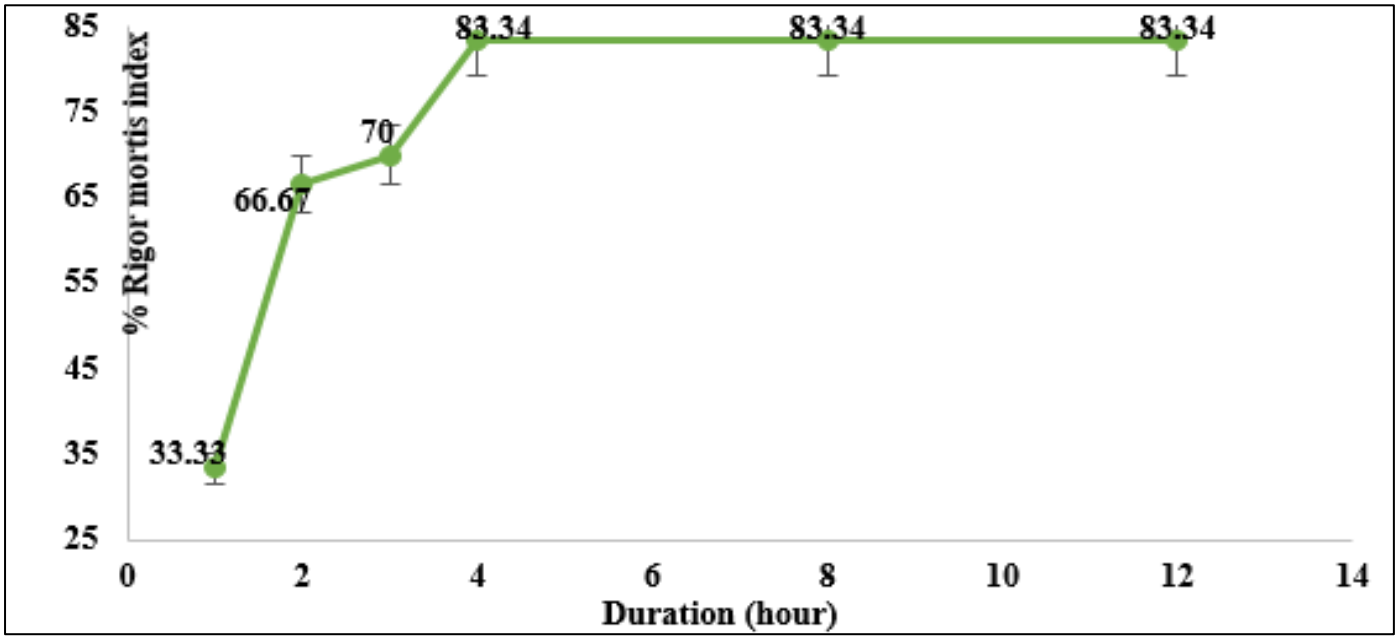

Fig 5: Graphical Presentation of Rigor Mortis Index of Tilapia

Table 5: Carcass Yield Traits of Tilapia

Parameters	Tilapia
Traits parameters (%)	
Body weight	363±1.0
Fillet	44.13±0.118
Carcass	45.72±0.031
Offal yield traits (%)	
Head	23.90±0.056
Skin	4.37±0.153
Fin	4.53±0.208
Viscera	9.39±0.031
Spine	1.62±0.035
Scales	1.91±0.056

After 30 minutes or even up to 3 hours of storage at room temperature, tilapia fish begins to rigor fast. After 4 hours, the tilapia's rigor mortis index stays at 83.34% for up to 12 hours of storage. Due to lack of ATP, tilapia exhibits rapid rigor (33.33%–83.34%) during the course of a 12-hour experiment. Viegas et al., (2013) stated that after just four hours of storage, all of the fish were in a 100% rigor mortis state and the rigor mortis index of tilapia also developed rapidly. Batista et al., (2004) observed the rigor mortis evolved more quickly, reaching full rigidity condition after about 1 hour and 15 minutes. At room temperature, the tilapia fish started to rigor quickly remaining 0 after 30 minutes and even up to 3 hours. After 4 hours, the highest rigor mortis is

gotten 83.34% for tilapia and remains uniform up to 12 hours. According to Contreras-Guzman (2002), in the pre-rigor phase, the muscle is typically supple and pliable; nevertheless, during the rigor phase, the animal's ATP and glycogen reserves biochemically decline at the time of death. Muscle glycogen and ATP levels can also be impacted by a number of other variables, including stress, the time of year, and physiological status. A lack of ATP causes rigor mortis, which makes the fish's body rigid and degrades its quality.

Table 5 indicates, the total body weight, fillet, carcass of tilapia is 363±1.0%, 44.13±0.118%, 45.72±0.031% respectively. Among offal yield, head, skin, fin, viscera, spine, scales of tilapia are 23.90±0.056%, 4.37±0.153%, 4.53±0.208%, 9.39±0.031%, 1.62±0.035%, 1.91±0.056% in that order. The carcass traits of tilapia are yielded approximately with proper way. According to Simoes et al., (2007); Vieira et al., (2009) and Viegas et al., (2013), The yield percentage of tilapia is observed and the percentage for the carcass without head (58.22 to 76.10%), head (14.29 to 29.14%), skin (3.68 to 8.0%) and residues (9.76 to 26.71%), the filleting yield of 32.42 ± 3.05% for fishes weighing 373.71 ± 61.91 g. The carcass yield of the tilapia fish is lower amount from research value. Other yield traits coincide approximately the research value. The carcass and fillet percentage indicate the better flesh quality. Certainly, the size of the head always influences the overall outcome. The increased fat content in body typically results in a lower slaughter yield, exclusive fat deposit reduces the quality of the fish. The offal yield along with carcass yield produces, if the offal is higher than carcass, the flesh quality isn't good. The variation in filleting and carcass yield of fish between experimental and research value can be impacted principally by factors such as: the degree of filleting automation, filleting capabilities, species attributes (size, sexual behavior, physical makeup and anatomical structure) and filleting techniques. Moreover, the filleting and carcass yields of fishes found in the present experiment are close to research value.

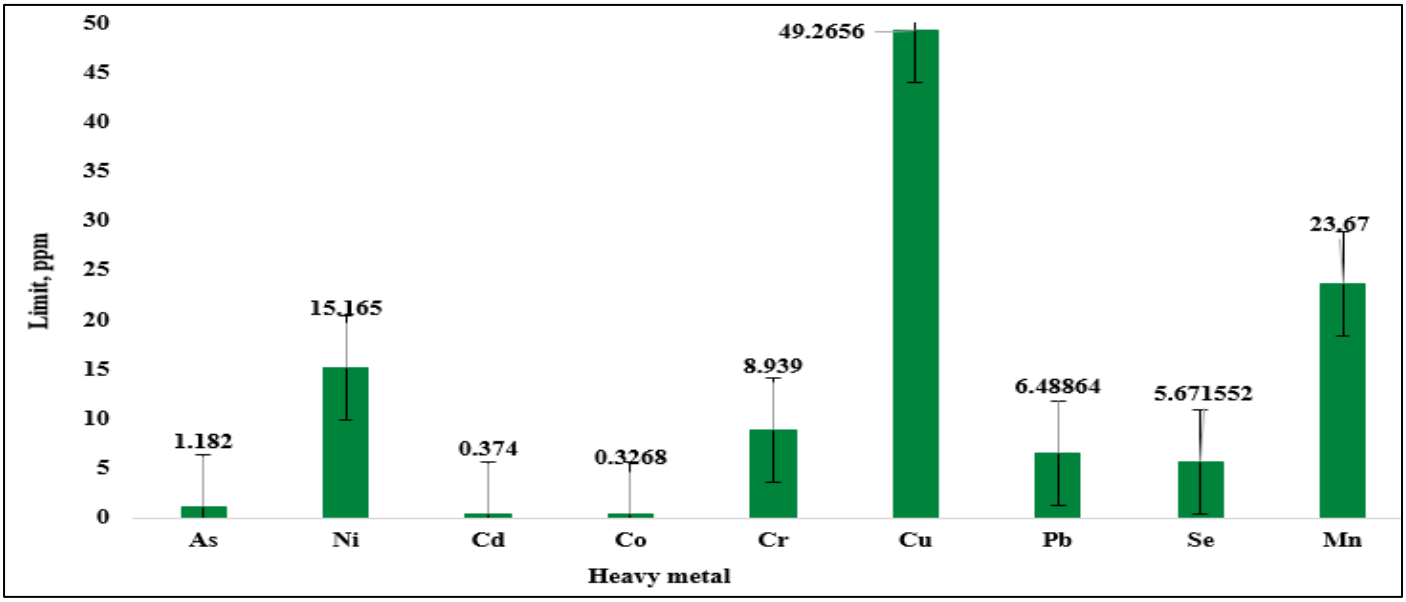

Fig 6: Graphical Presentation of Heavy Metal of Tilapia

B. Formalin Test

The outcome is negative.

The ICP-MS identifies several micronutrients including Ca, Fe, Mg and Zn in addition to analyzing the heavy metal level. Ca (896.40 ppm), Fe (51.188 ppm), Mg (5503.328 ppm) and Zn (49.5059 ppm) are the micronutrients found in tilapia. The graphic representation of tilapia's heavy metal spectrum is shown in Figure 6. The concentration of heavy metals in tilapia is extremely high, especially Cu (49.2656 ppm) and Cr (8.939 ppm). In all of the tilapia's tissues, Cu is found in the highest amounts, followed by Ni, Cd, Mn, Cr, Se, As, Co and Pb. Cu>Mn>Ni>Cr>Pb>Se>As>Cd>Co are the descending orders of metal accumulation in tilapia muscles. This finding demonstrates that the levels of heavy metals in tilapia tissues are variable. Standard permissible limit of heavy metal for fish is 0.5 ppm for Cd, Pb and 30 ppm for Cu according to FAO/WHO, (1989), 80 ppm for Ni according to USFDA, (1993) and 0.05 ppm for Cr, 0.05 ppm for As of organic form, 100 ppm for Ca, 150 ppm for Mg (WHO, 2011), 0.03 mg of inorganic As/kg of fish (EFSA, 2009) and 100 mg/kg for Zn (MFA, 1983). Concentration of Cu in tilapia fish is recorded at 3.40±0.74 ppm (Abdulali et al., 2011). The value of Cu for tilapia from this study is slightly higher than the reported value. Cu plays a vital role in enzymatic processes and are essential for the synthesis of haemoglobin but very high intake will cause health problems (Demirezen et al., 2006). The concentration of Cd of this study is within the limit for tilapia. The concentration of As and Pb measured in this study for tilapia is higher than reported value. The increased As creates toxicity and incidence of lung, skin and bladder cancer. Ni concentrations in fish sample is lower than reported value but Cr concentration of fishes in this study is higher than the reported value. Small doses of manganese must be consumed daily for children to grow and be healthy. However, serious skeletal and reproductive defects can result from Mn deficits (Baharom et al., 2015). Overconsumption of zinc is harmful to human health and can result in fever, diarrhea and poisoning (Chi et al., 2007). The study found that tilapia had

the greatest zinc concentration (49.5059 mg/kg), which is within the recommended range. The concentration of Ca, Mg, Fe for tilapia is higher than reported value. The limits of heavy metal for tilapia exceed the reported value. It's because due to food, habitant, environmental variation, ecosystem, health problem of fishes, the average quality fish feed in the farm, discharging of the industrial pollutants and other anthropogenic ingredients directly into the natural waterways and the improper purification of the source water.

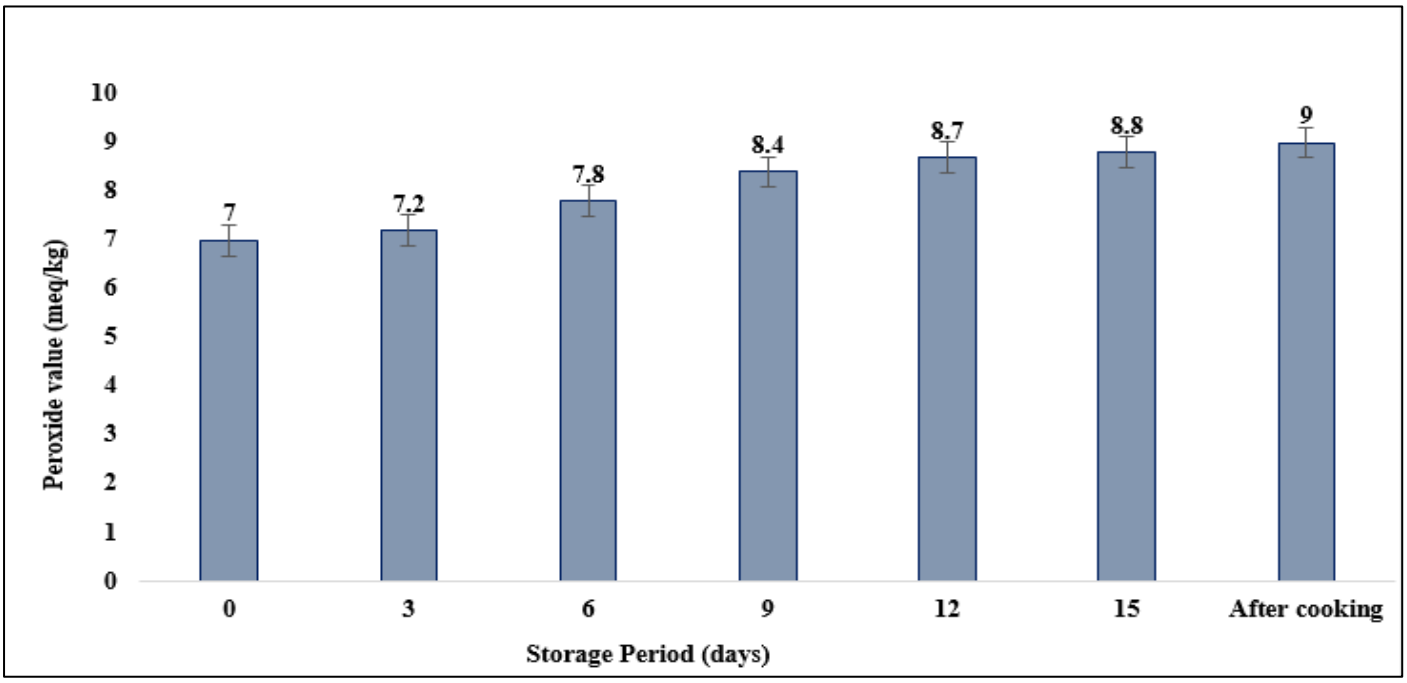

Fig 7: Peroxide Values of Tilapia on Storage before to Cooking and Following Cooking

C. Biochemical Test

The peroxide test, out of all the biochemical tests, is crucial for keeping up the safety and quality of tilapia fish since it truly measures the amount of rancid fat in the fish.

Figure 7 shows the elevated peroxide value pattern of tilapia throughout storage before to cooking and following cooking. During 15 days of storage at 3-day intervals in -18±1^{0}C, the peroxide value of tilapia is gradually upgraded through the pattern of 7, 7.2, 7.8, 8.4, 8.7, 8.8 and 9 (after cooking) meq/kg, respectively. The peroxide value of tilapia (7 meq/kg) is almost identical to or meets the required value after a day of storage. During storage, the peroxide value is progressively raised ($p<0.01$) at intervals of three days, reaching 8.8 meq/kg of tilapia at the fifteen-day mark. It has been demonstrated that frying increases the tilapia's peroxide value (9 meq/kg). Due to cooking, fat rancidity is slightly increased. Because of this, the peroxide value rises as well. Peroxide value in O. niloticus in ice was 3.24 meq/kg of oil for day 0, 7.21 meq/kg of oil for day 9 and 9.74 meq/kg of oil at the end of the 21 days storage period (Adoga et al., 2010). Results suggest that the fish samples are in good condition throughout the storage period based on values of 10-20 meq/kg of oil as recommended by Connell (1995). The peroxide value of the tilapia fish is increased during storage that indicates the possibility of deterioration of food quality. Due to increase peroxide value it indicates the fat oxidation is happened swiftly. For tilapia fish, the peroxide value is within the standard limits (10-20 meq/kg of oil). According to Hossain et al., (2005), lipid oxidation limits the shelf life of oily fish. Fluctuations in peroxide value indicates the formation of oxidative products and binds or couples with others through removing of moisture. Peroxide value may not be considered a good indicator of freshness in this study as values are within the range of acceptability throughout the storage period. Though the peroxide value of fishes increases gradually for 15 days of storage, for meeting up to research value the fish quality is considered as good.

Table 6: Sensory Attributes of Cooked Tilapia

Samples		**Sensory Attributes**			
		Color	**Flavor**	**Texture**	**Overall Acceptability**
Tilapia	*S_1	7.5^{ab}	6.70^{b}	7.10^{b}	7^{b}
	S_2	7.3^{ab}	7.30^{b}	7.80^{ab}	7.70^{b}
	S_3	8^{b}	8^{b}	8^{ab}	8^{b}
LSD (P = 0.05)		**0.291**	**0.182**	**0.269**	**0.197**

*S_1 = Cooked by Steaming; S_2 = Cooked by Electric Oven; S_3 = Cooked by Gas Oven; ^{b}The Mean Difference was Statistically Significant at 0.05 Level

D. Sensory Evaluation of fish samples

To promote public acceptability and trust, external fish monitoring is crucial for maintaining fish safety and quality. Because of this, numerous sensory characteristics of raw and cooked tilapia

have been assessed organoleptically. The mean scores for tilapia at three different cooking ways in terms of flavor, texture, color and overall acceptability are shown in Table 6. After doing a one-way analysis of variance, the findings showed that there is a substantial (P=0.05) variation that tilapia finds acceptable. The LSD test is used to rate sensory (flavor, texture, color and overall acceptability) variability. A one-way analysis of variance for tilapia revealed that the samples have a significant impact on overall acceptability color, texture and flavor at the 0.05 level in the case of texture, overall acceptability, color and flavor preference among samples. Of the three samples, the texture, color, flavor and overall acceptability of the tilapia in S_3 sample is noticeably superior to that of the other two.

The organoleptic characteristics of raw tilapia are shown in Figure 8. The instructions of Table-2, as stated in table-3, provide the sensory evaluation approach employed in this study to evaluate the raw tilapia. With the exception of one characteristic (slime), the degree of freshness for tilapia is essentially satisfactory. These exception parameters have a grade point of 5, which denotes poor quality. The real grade point, which denotes an exceptional degree of freshness, is satisfied by the grade points of additional parameters such as eyes (clarity-1, shape-2), texture (elasticity-1, belly-2), gills (color-2, odor-1) and appearance (skin-2).

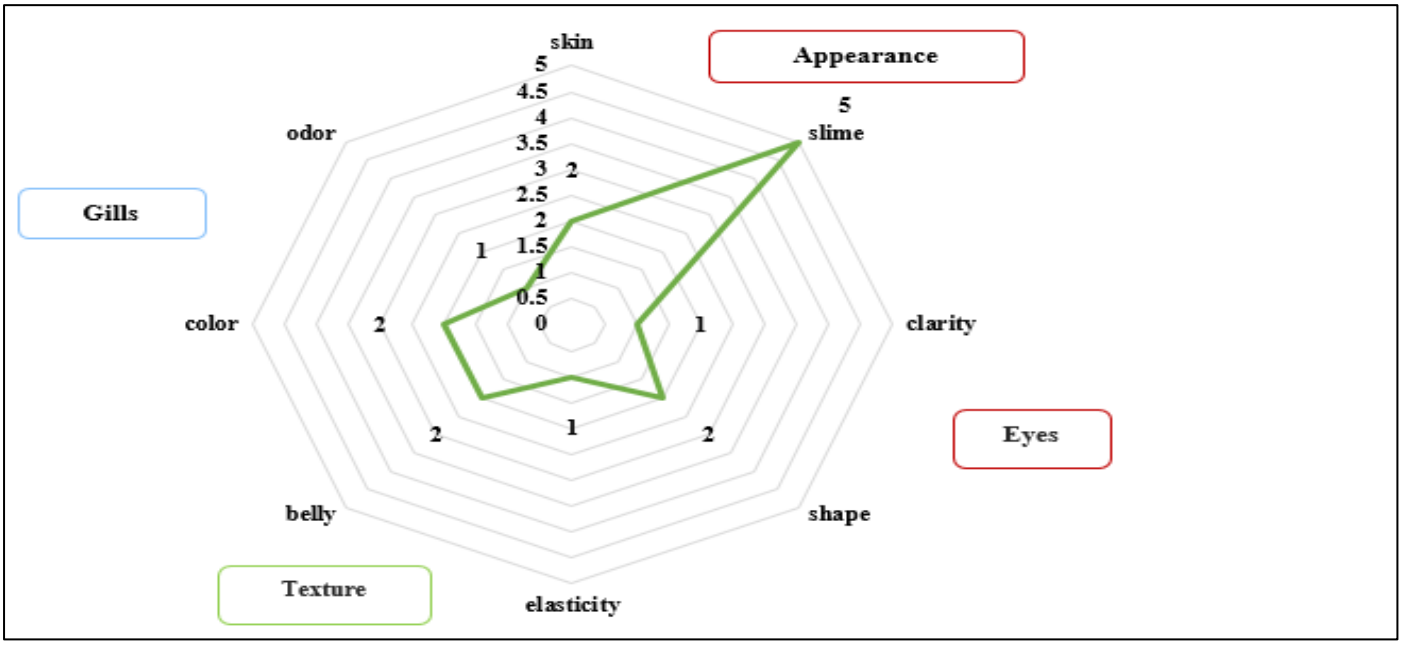

Fig 8: Graphical Presentation of Sensory Attributes of Raw Tilapia

Figure 9 indicates the total viable count for mold and bacteria of tilapia. The total viable count for bacteria of tilapia at expected parts is $13.34\times10^{6}\pm0.651$ CFU/ml for flesh with skin, $18.74\times10^{8}\pm0.508$ CFU/ml for gills, $20.10\times10^{12}\pm0.953$ CFU/ml for intestine respectively. For mold count, tilapia has $5.20\times10^{16}\pm0.367$ CFU/ml for flesh with skin, $7.72\times10^{16}\pm1.166$ CFU/ml for gills, $8.56\times10^{16}\pm0.629$ CFU/ml for intestine respectively. Figure 9 shows that for all predicted parts, the total viable mold count of tilapia is higher than the total viable bacterial count. The intestine has a higher total viable mold and bacterial count than the skin and gills and the predicted sections are intestine>gills>skin in descending order. While the TVC of tilapia for bacteria occur in a separate dilution, the TVC of tilapia for mold appears in the sixteenth dilution. Viable bacterial counts were significantly higher in fish from local markets (9.5×10^{8} CFU/g, cm^2) ($P<0.01$), above permissible limits (Kapute et al., 2012). Fish products used in TVC analysis typically have an acceptable limit of 10^{6}–10^{7} CFU/g (Fan et al., 2009; Piotr et al., 2016). The total microbial count of fish skin contains 5×10^{4} CFU/cm^2 for bacteria and 1.2×10^{4} CFU/cm^2 for mold (Ibrahim et al., 2020). Other research showed that the bacterial count for gills is 8.7×10^{6} CFU/gm and 5.8×10^{7} CFU/gm for intestine (Ahmed et al., 2003). Because the gills come into direct touch with the water, particularly in plankton feeders, they have a more diverse microbiota both qualitatively and statistically (Pao et al., 2008). The total viable count of bacteria and mold for tilapia fish is visibly more because of higher moisture content. Contamination of fish by bacteria is occurred due to higher moisture content, deterioration of fish and contamination of fish by mold is due to presence of microorganisms on the skin surface, gills and intestine of fish. The limit of total viable count for bacteria and mold of tilapia fishes is slightly upper than the research value and only flesh with skin is within the acceptable limit. The bacterial load varies in the expected parts of the fishes. This may be occurred due to the detached procedure slightly during distribution, processing and freezing at right time. The bacterial load in all samples is reasonably high and one of the reasons may be that the ambient temperature, working environment place. The presence of a high bacterial load in gills and intestine of fish may be due to moisture content and

high metabolic activity of fish associated with increased feeding rates at temperatures. The morphological features of the bacteria isolated from tilapia fish are displayed in Table 7. The process of isolating bacteria involves growing it on primary medium (nutrient agar) for E. coli and Salmonella sp., then growing it on specific media (EMB agar for E. coli and XLD agar for Salmonella sp.), and lastly using staining to study the cultural patterns and morphological characteristics. The two bacteria have opposite (-) staining characteristics. Both bacteria have a short rod shape when stained, with Salmonella sp. exhibiting a distinct Enterobacter characteristic. E. coli is arranged in pairs and a short chain, while Salmonella sp. is arranged in pairs and singles. E. coli's smooth, round, colorless colonies on nutritional agar and moist, round colonies with black centers and a metallic sheen on EMB agar are indicative of its cultural characteristics. On nutritional agar, Salmonella sp. exhibits a smooth, round, opaque colony appearance, but on XLD agar, the colony appearance is red with black centers. Normally, Gram-negative bacteria caused the food spoilage particularly fish and chilled fish. For ensuring safety and assessing the better quality, Gram-negative bacteria is identified and counted the total number.

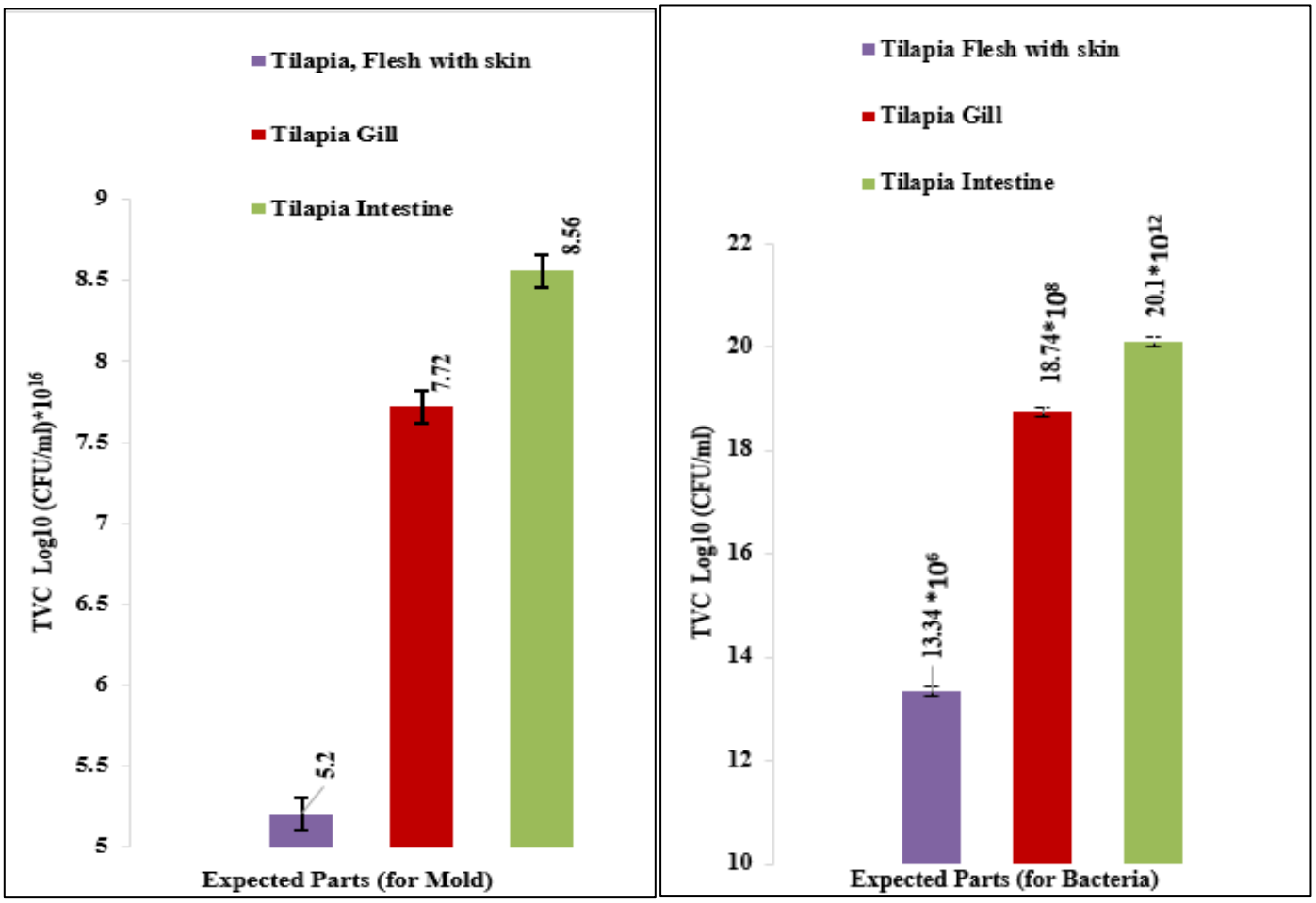

Fig 9: Graphical Presentation of the TVC for Bacteria and Mold of Tilapia

Table 7: Identification of Isolates from Tilapia

Cultural Characters			Morphological & Staining Characters			Identified Organisms
Nutrient agar	EMB agar	XLD agar	Shape	Arrangement	Gram's staining reaction (+/-)	
Smooth circular, colorless colony	Moist circular colonies with dark centers yellow green metallic sheen	-------	Short rods	Paired and short chain	-	*E. coli*
Smooth circular, opaque colony	-------	Red colonies with black centers	Short rod Enterobacter	Singles and paired	-	*Salmonella sp.*

IV. CONCLUSION

Around the world, fish is a staple diet and a vital source of protein. The fishing industry in Bangladesh plays a significant role in both generating foreign currency and supplying the country's local demand for animal protein. For high palatability, low cholesterol, nutritious, mild taste of fish, the popularity and consuming ability are getting increased. Tilapia (*O. niloticus*) is one of the healthiest food choices in Bangladesh which contains a rich amount of high protein and omega-3 fatty acids. For this contrast tested study, market tilapia was purchased from local market for evaluating and assessing their quality and safety parameters from farm to fork. The key intention of this research is to lead safety parameters and improve quality for human consumption. Through performing different experiment, it is demonstrated that the acceptability of tilapia is admirable for both of safe and nutritious aspects despite being market fish. This research work is handled properly from physical hazards and safety parameters met approximately with standard values. From this study, Tilapia is nutritionally safe and quality food by choosing fresh fish. The storage stability for the fish is satisfactory but obviously the quality of frozen fish is lower acceptable than the fresh fish. The sensory attributes of cooked tilapia by using gas oven are more preferable and the organoleptic properties of raw fishes, tilapia is satisfactory and acceptable. The microbial study of the fishes slightly is high than reasonable value. The total viable bacteria and mold of the fish, intestine organ contains more microbes than other organ such as flesh with skin and gills. The heavy metal concentration of tilapia, Cu ion concentration is the highest for tilapia as 49 ppm whereas the permissible limit is 30 ppm. Other metal accumulation of fishes is slightly variated. From this research study, it is indicated that there is a dazzling expectation where nutritious, safe and quality based food is gotten along with fulfilling the basic needs. Therefore, proficient hygienic handling, rapid cooling of fish, using of clean water during fish wash can reduce the quality loss of fish. To evaluate better quality and economic features for commercial exploitation, more research is required to determine alternative sources and samples, decreasing heavy metal buildup and increasing storage stability.

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[4]. AOAC Crude protein AOAC 954.01. Association of Official Analytical Chemists (2023).

[5]. AOAC Determination of ash in animal feed AOAC 942.05. Association of Official Analytical Chemists (2023). ch 4 p 8.

[6]. AOAC Ether extract in animal feed AOAC 920.35. Association of Official Analytical Chemists (2023). ch 4 pp 39-40.

[7]. AOAC Moisture content AOAC 930.15. Association of Official Analytical Chemists (2023). ch 4 pp 1-2, 4-8.

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APPENDICES

The data and attachments needed to generate the results are provided herewith.

➢ *Drip Loss of Tilapia*

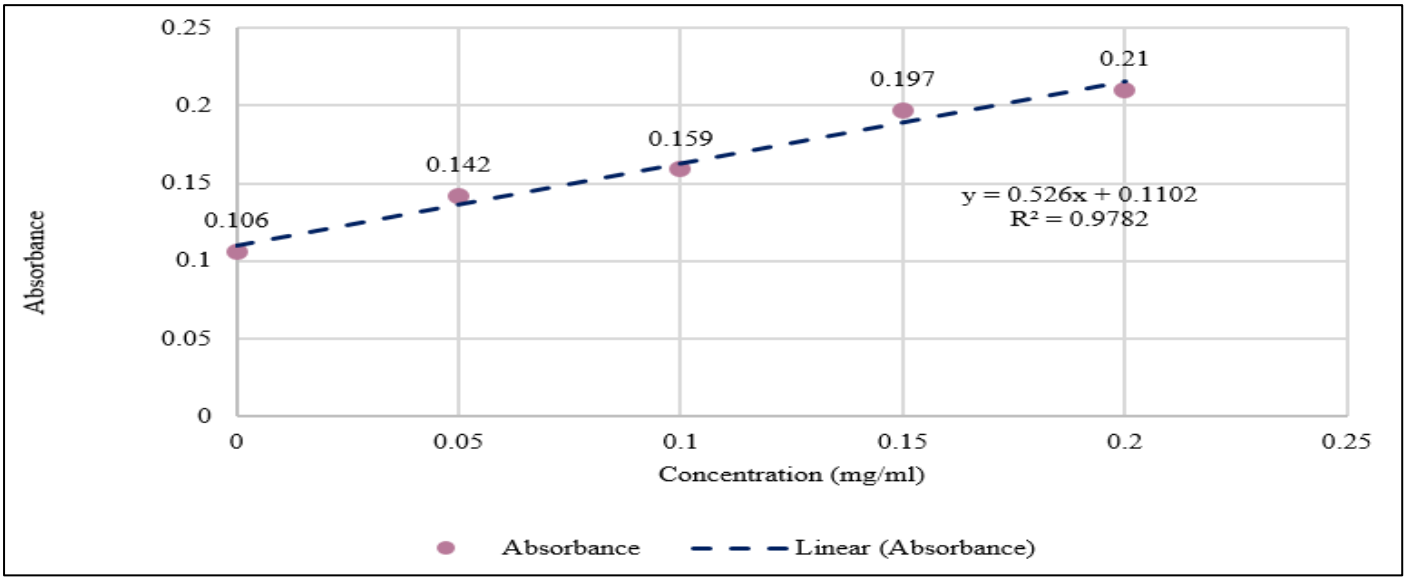

Fig 1: Graphical Presentation for Absorbance Reading of Standard Solution

From this graph and calculation, Average absorbance value for tilapia was 1.65±0.015. For 1.65 absorbance, Concentration of protein for tilapia = 2.92 mg/ml

For determining protein concentration of tilapia, absorbance reading is recorded and by using y=0.526x+0.1102 equation from the graph, protein concentration is calculated. The average absorbance reading for tilapia is 1.65±0.015 that indicates the light blue in color. The protein concentration of tilapia is 2.92 mg/ml. Due to linear relationship between absorbance and concentration, for higher absorbance reading of tilapia, the protein concentration is also more using the volume (0.25ml) of tilapia.

For tilapia,

Total Protein loss in 3 ml = 2.92×3 mg
= 8.76/0.25 mg/ml
= 35.04×3 mg
= 105.12 mg

➢ *Heavy Metal Residues in Frozen Fillets of Tilapia*

Table 1: Heavy Metal Residues Presented in Tilapia

Heavy metal residues	**Tilapia**	
	ppb	**ppm**
Ca	3730	896.40
Cd	1.56	0.374
Co	1.36	0.3268
Cr	37.2	8.939
Cu	205	49.2656
Fe	213	51.188
Mg	22900	5503.328
Mn	98.5	23.67
Ni	63.1	15.165
Pb	27	6.48864
Se	23.6	5.671552
Zn	206	49.5059
As	4.92	1.182

Table 2: Total Viable Count for Bacteria and Mold of Tilapia

Market fish	Expected parts of fish	Sample size (mean value), g	TVC for bacteria (CFU/ml)	TVC for mold (CFU/ml) $\times 10^{16}$
	Flesh with skin	1.74	14.32×10^{6}	5.7
Tilapia 1	Gill	1.32	18.36×10^{8}	8.76
	Intestine	1.18	19.07×10^{12}	7.66
	Flesh with skin	1.34	13.67×10^{6}	5.2
Tilapia 2	Gill	1.09	18.25×10^{8}	6.80
	Intestine	1.43	19.45×10^{12}	9.15
	Flesh with skin	1.34	13.10×10^{6}	5.40
Tilapia 3	Gill	1.45	19.46×10^{8}	9.15
	Intestine	1.35	20.32×10^{12}	8.54
	Flesh with skin	1.32	12.75×10^{6}	4.90
Tilapia 4	Gill	1.36	19.05×10^{8}	7.34
	Intestine	1.32	20.10×10^{12}	8.31
	Flesh with skin	1.32	12.87×10^{6}	4.80
Tilapia 5	Gill	1.40	18.56×10^{8}	6.57
	Intestine	1.47	21.55×10^{12}	9.16

Table 3: Rating Score for Sensory Evaluation of Tilapia

Panelists	Color			Flavor			Texture			Overall acceptability		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
1	7	9	8	7	8	9	8	9	9	6	8	9
2	6	8	9	6	7	8	7	8	7	6	8	8
3	8	6	8	7	7	8	7	6	8	7	8	8
4	8	7	7	7	7	7	7	8	8	8	8	7
5	6	6	9	7	8	7	6	8	9	8	7	8
6	7	7	9	6	7	9	7	8	9	7	7	7
7	8	7	7	7	8	8	8	7	7	7	8	8
8	9	8	8	6	7	8	9	7	8	7	9	9
9	9	8	7	7	7	7	6	8	7	7	7	8
10	7	7	8	7	7	9	6	9	8	7	7	8