

RESEARCH PAPER

Post-harvest Quality of Fresh Pama fish (*Pama pama*) From Three Fish Market in Patuakhali District

Biplob Dey Mithun¹, Md. Sazedul Hoque^{1*}, S.M. Oasiqul Azad¹, Md. Shahin Alom¹, Papri Biswas¹, Suprakash Chakma¹, Mst. Niloy Jaman¹, Md. Jahangir Alam² and Nusrat Shahnewaz³

¹Department of Fisheries Technology, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh.

²Department of Fisheries Management, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh.

³Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

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*Corresponding author
E-mail: sazedul.fst@pstu.ac.bd

ABSTRACT

Post-harvest quality losses of fish are the great concern for the fisheries sectors of Bangladesh especially in aspects of food security and public health issues. Therefore, the present study was conducted aimed for evaluation of organoleptic, biochemical and bacteriological quality of Pama (*Pama pama*) fish in local fish market of Patuakhali district. The fish sample was collected from three different markets namely Pirtola, Rajakhali and New market of Patuakhali district and the study was conducted from July to November 2016. Organoleptic method was used for sensory quality assessment of fish sample by ten trained pannelist. Proximate composition of fish sample was determined following AOAC method and bacteriological study was carried out according to the Standard Plate Count method. From the results, organoleptically the fish from the respective three different fish market were excellent and highly acceptable. Furthermore, biochemical study revealed that there were no significant different in nutrient content of fish sample collected from three different markets ($p > 0.05$). Among the compositions, higher moisture content (79.87 to 84.67%) and lower ash content (0.99 to 1.99 %) was observed for *Pama pama*, irrespective of fish market. In general, similar sensory and biochemical quality was observed for fish from three different market referred acceptable post-harvest handling and preservation of fish. However, significant different and cooperatively higher bacterial load was observed 4.82×10^9 , 4.43×10^9 and 1.63×10^9 CFU/g for Pirtola fish market, Rajakhali fish and New market fish market, respectively ($p < 0.05$). Therefore, the post-harvest quality evaluation through organoleptic and biochemical aspects of Pama fish (*Pama pama*) from three different fish market in Patuakhali district suggested acceptable fish quality which leads to supply of higher nutrient content without any public health concern. Besides, bacteriological study indicated that further improvement in post-harvest handling, preservation and hygiene practice are required.

Key words: Pama fish, sensory analysis, proximate composition, microbial load

Introduction

Bangladesh is in the Asia continent and the latitude and longitude for the country are 23.8511° N, 89.9250° E. The country is located in South Asia, bordered by India in the north, the Bay of Bengal in the east and west, while Myanmar surrounds it to the south. Bangladesh is endowed with a vast expanse of inland open waters such as rivers, canals, natural and man-made lakes, freshwater marshes, estuaries, brackish water impoundments

and floodplains. Bangladesh has the third largest aquatic fish biodiversity in Asia with about 800 species in fresh, brackish and marine water (Hussain and Mazid, 2001). The country has a total of 260 freshwater species, 475 marine species, 12 exotic species, shrimp 36 and prawn 24 species (DoF, 2016). About 80% of animal protein in our diet comes from fish alone. (Rubbi *et al.*, 1978).

The fisheries sector contributes 3.69% to the national GDP and 23.12% to the agricultural GDP in Bangladesh (DoF, 2016). Southern region of Bangladesh especially in Patuakhali district under Barisal division are full of fisheries resources. In this region fisheries production are based on capture fisheries due to large number of river named Paira, laukhati, karkhana, Bishkhali, Lohalia, Andar manik but culture fisheries are not so developed. These fisheries resources are vital sources of different freshwater and estuarine fish of which Pama fish is one of the economic and commercial important fish species in southern region of Bangladesh. Pama fish are locally available in fish market of Patuakhali district. This is the very popular fish in Patuakhali district. It contains 18.6% crude protein, 1.7% fat and 75% water (Basu and Gupta, 1939).

Pirtola fish market is stand beside the Paira River. Fish are brought to the market from nearest river and some are transported from fish landing center in kalapara, mahipur and other landing center in Patuakhali. The local fish market are not well developed and the facilities are not satisfied to the consumers. Despite the lack of infrastructure and fish preservation facilities, the fish play a major role to supply the nutrient for all people lived in Patuakhali district. Dumki and Rajakhali fish market is a local market in Patuakhali district near the Karkhana River. The market is a great source of fish for total sadar upazilla and others upazilla in Patuakhali district.

The quality of fish and fishery products has become a major concern in all over the world (Huss et al., 2003). Freshness is one of the most important attributes of fish quality and it can be measured by different analytical methods. Sensory analysis is used to ensure that fishery products completely with food quality and safety select and test some simple, cheap and rapid methods suitable for freshness evaluation of fish in different fish market.

Proximate composition generally comprises the estimation of moisture, protein, fat and ash contents of the fresh fish body. The percentage composition of these constituents accounts for about 96-98% of the total tissue constituents in fish (Nowsad, 2007). The assessment of the proximate composition of the fish is not only important to know its nutritive value, but also for its better processing and preservation (Mridha, 2005). On the other hand, the microbial load affects the composition, freshness, spoilage and nutritional quality of the fish. Considering different unfavorable factors, investigations on microbiology of fishes have been worked globally in both freshwater and marine environment. However, there were no sensory, proximate composition and microbial report has been carried out especially in pama fish from different fish market in the Patuakhali district. Considering the quality fish, spoilage, food safety and public health aspects; the objective of the present study was to analyze the proximate composition, sensory and microbiological quality of fresh pama fish (*Pama pama*) from different local fish market in Patuakhali district.

Materials and Methods

Study Location

For this study Patuakhali district under Barisal division of Bangladesh was selected. Different fish market in Patuakhali district named Pirtola fish market, Rajakhali fish market and New market fish market were the source for sample for the study.

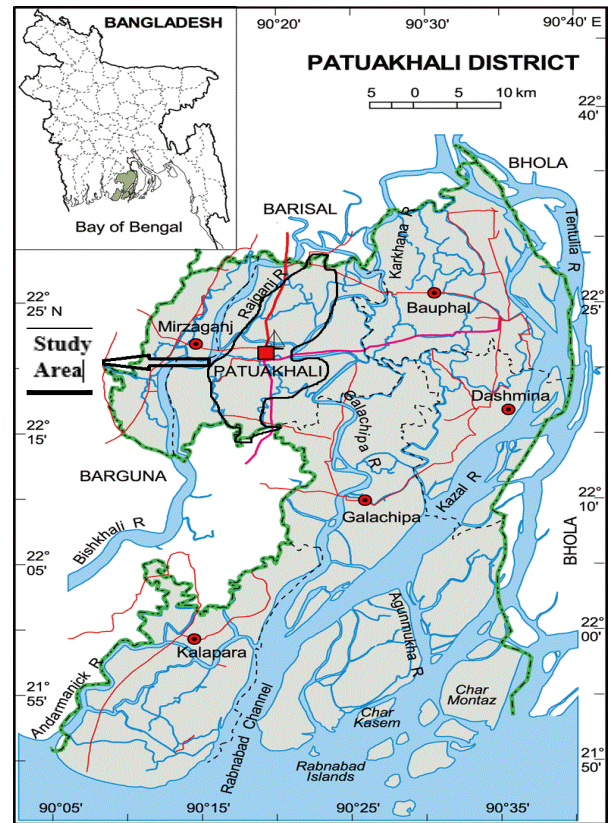


Figure 1. Location of the study area.

Duration of the study

The present study was conducted during the months of July to November, 2016.



Figure 2. Collection of fresh Pama fish (*Pama pama*) from fish market in Patuakhali district

Collection and Preparation of Sample

The study methodology was followed to achieve the objectives of the study and explains the choice for selecting study tools and methods for data collection.

Pama fish (*Pama pama*) was collected from three different local fish market namely Dumki (Pirtola) fish market, Rajakhali fish market and New market in Patuakhali district town (Figure 1). Those areas were designated as Site No. 1(P), Site No. 2(R), and Site No. 3(N). Collected Pama fish (Figure 2) sample was transported to the laboratory of Faculty of Fisheries, Patuakhali Science and Technology University for sensory, proximate composition and bacteriological analysis using appropriate analytical methods.

Sensory or organoleptic assessment of Pama fish

Sensory methods was carried out on whole Pama fish in the laboratory by using the Quality Index method to access the degree of freshness based on organoleptic characteristics such as gill odor, color and slime, general

appearance, eyes, body slime general appearance and consistency of flesh. A sensory method such as organoleptic method is one of which subjective judgments was assess by ten trained panelists. Numerical scoring system was developed to evaluate the judgments or results, simply in the form of excellent/highly acceptable, acceptable/good, deteriorating/not acceptable and spoiled/rejected etc. Numerical scores of the each samples were averaged to give the quality index score and recorded in the quality index scheme for fresh Pama fish. The guidelines and methods, given here are based on the organoleptic characteristics of fish as described by European Commission freshness grade for fish quality (Howgate *et al.*, 1992) as in Table 1 and 2.

Table 1. Determination of defect points

Characteristics of fish	Defect characteristics	Defect point
Odor of gill	a. Nature odor	1
	b. Faint sour odor	2
	c. Slight moderate sour odor	3
	d. Moderate to strong sour odor	5
Color of gill	a. Slight pinkish red	1
	b. Pinkish red to brownish	2
	c. Brown to grey	3
	d. Bleached color	5
Slime of gill	a. Thin Colorless	1
	b. Sticky greenish slime, filaments separate	3
	c. Yellowish slime, filaments attached	5
Body slime	a. Clear transparent, uniformly spread	1
	b. Turbid, opaque	3
	c. Thick, sticky, yellowish or greenish	5
Eye	a. Bulging with protruding lens, transparent eye cap	1
	b. Slight cloudy lens, sunken	2
	c. Dull, sunken, cloudy, blood line, reddish cornea	3
	d. Sunken eyes covered with yellow slime	5
Consistency of flesh	a. Firm elastic	1
	b. Moderate soft & some loss of elasticity	2
	c. Some softening of muscle	3
	d. Limp or floppy	5
General appearance	a. Full bloom, bright, shining, iridescent	1
	b. Slight dullness, loss of bloom	2
	c. Definite dullness and loss of bloom	3
	d. Reddish lateral line & caudal region dull, no bloom	5
Total Defect Point (DP)		
Average Defect Point (DP)		

Organoleptic assessment was calculated by using following formula-

$$\text{Average grade points} = \frac{\text{Total Grade point}}{\text{Number of Characteristics}}$$

Table 2. Grading of fresh Pama fish (*Pama pama*)

Grade	Point	Degree of freshness
A	<2	Excellent, Highly acceptable
B	2 to <5	Good/ acceptable
C	5	Rejected

Determination of bacterial load

The following procedure for Standard Plate Count (SPC) was adopted for bacteriological study (Cappuccino and Sherman, 1992).

Preparation of Agar media

For bacteriological analysis, agar media was prepared by the following composition and procedure. For preparation of media, the estimated quantities (14g) of nutrient plate count agar media was weighed and suspended into 500 ml of distilled water in a conical flask for each sample. Then the mixture was boiled on electric heater to dissolve completely and sterilized.

Sterilization

Sterilization was accomplished by placing the media in an autoclave for 20 minutes at a temperature of 121°C under 15 lbs/sq. inch pressure. Tips, distilled water, and cotton were sterilized as the same procedure same as media. Different glassware such as petridish, test-tube and glass rod were also sterilized by placing in a hot air oven at a temperature of 150°C for 6 hours. Then media was cooled down to around 50°C and was poured into previously sterilized petridish. The process was repeated for each sample.

Preparation of sample

Five gram of Pama fish samples was homogenized and mixed in 200 ml sterile distilled water for each sample preparation. Then shaking was done properly. After that we prepared stock solution for dilution for each sample.

Dilution and culture for bacteria

One ml sample (fish stock solution) was transferred with a micropipette to test tube containing 9 ml of sterile distilled water. The test tube was shaken thoroughly on a vortex mixture in order to get 10^{-1} dilution of original sample solution. Using the similar process several dilutions of 10^{-2} to 10^{-6} was made for Pama fish. One milliliter of each homogenate solution was serially diluted (10^{-1} to 10^{-6}) with sterile distilled water. Aliquots of 0.1 ml of the serial dilutions was inoculated onto nutrient media in triplicate using the spread plate method as this medium recovered most of the bacteria. The samples pipetted was spread homogenously and carefully by sterile flamed L-shaped glass rod. The plates were incubated at 36°C in an inverted position in the microbiological lab of PSTU for 24 hours. Similar process was done for each sample.

Aerobic plate count (APC)

After 48 hours of incubation colonies developed was counted. Plates containing 30-300 colonies was used to calculate bacterial load results, recorded as *cfu* per unit of fish sample by using following formula:

$$\text{Cfu/g} = (\text{No. of colonies on Petridis} \times 10 \times \text{dilution factor} \times \text{volume of total stock solution}) / \text{Wt. of fish sample}$$

Determination of proximate compositions

Moisture, protein, ash and lipid composition of fish was determined by the following Association of Official Analytical Chemicals (AOAC, 2000) method.

Moisture

Moisture was determined by placing an accurately weighed known amount of ground sample in a pre-weighed porcelain crucible in an electric oven at 105°C for about 24 hours until constant weight was obtained. The loss of moisture was calculated as percent moisture.

$$\text{Moisture content (\%)} = \frac{\text{Weight of wet material} - \text{Wt. of dry material}}{\text{Weight of wet material}} \times 100$$

Ash

About 3-5g prepared sample was taken in pre-weighed porcelain crucible and was placed in muffle furnace at 550°C for 6 hours. Then the crucibles were cooled in desiccators. The average in percentage of each sample of the remaining materials was taken as ash.

The lipid content was calculated by using the following formula-

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Protein

Kjeldhal method was used to determine protein content of the Pama fish samples.

Reagents for protein estimation

Different analytical grade reagents like Digestion mixture (100g $\text{Na}_2\text{SO}_4/\text{K}_2\text{SO}_4$, 10g CuSO_4 and 1g selenium powder), $\text{Na}_2\text{S}_2\text{O}_3$ (8%), Concentrated H_2SO_4 , H_3BO_3 (2%), NaOH (40%), Standard HCl (0.1N) and Mixed indicator (0.2g methyl red and 0.1g methyl blue in 100ml ethanol) were used.

Procedure for estimation of protein

For the determination of crude protein by kjeldahl method first of all, the fish sample was taken and chopped into small pieces and was grinded by grinder. Approximately 1.0g of sample was taken in a clean kjeldahl flask and 4g of digestion mixture was added along with 25ml of conc. H_2SO_4 by swirling the flask. Then the kjeldahl flask were placed in inclined position on heating device of kjeldahl apparatus and were heated at 70°C for about 1-1.5 hours. The end point of digestion was indicated by a completely clear and of light blue color solution. The content of the flask was cooled at room temperature and 100ml of distilled water and 25ml of $\text{Na}_2\text{S}_2\text{O}_3$ were continuously added in each flask and were mixed and cooled. A few glass beads were added in each flask to prevent bumping. Then 100-120ml of 40% NaOH was added in each flask to make the solution

sufficiently alkaline. The flask was immediately connected to distilling bulb on condenser. A conical flask containing 50ml of 2% H₃BO₃ with 2 drops of mixed indicator was placed under the condenser against kjeldahl flask to collect the distillate. After completion of distillation (about 100ml distillate) the collected distillates were titrated with standard HCl. The end point was indicated by light pinkish color.

Total nitrogen was calculated by using the following formula-

$$\text{Nitrogen (\%)} = \frac{\text{ml. Acid titrate} \times \text{normality of acid titrated} \times \text{mili equiv. of N (0.014)}}{\text{Weight of sample}} \times 100$$

$$\% \text{ Crude Protein} = \text{Nitrogen (\%)} \times 6.25$$

Lipid

Lipid content was determined by soxhlet apparatus using acetone as solvent. Accurately weigh samples (2-3g) were taken in thimbles and were dipped in pre-weighed aluminum cups with acetone. At first boiling was done for 15 minutes, and then rising for 25 minutes and finally extraction was done for 10 minutes. After extraction, the aluminum cups were taken out from chamber and acetone was placed in an oven at 1000⁰c for 30 minutes. The cups with lipid was cooled in desiccators and weighed again. The calculated value for lipid content was obtained as percent sample.

The lipid content was calculated by using the following formula-

$$\text{Lipid content (\%)} = \frac{\text{Weight of lipid}}{\text{Weight of sample}} \times 100$$

Result and discussion

Organoleptic Evaluation of fresh Pama fish

The results of the organoleptic quality assessment of fresh Pama (*Pama pama*) are presented in Table 3. The qualities of the fishes were graded using the score from 1 to 5 according to grading scoring method (table 1 and 2). The grades were defined in terms of the total number of defects or demerit points. The score points less than 2 was considered as excellent, 2 to 3 were judged as good or acceptable conditions, above 3 to less 4 showed deteriorating or not acceptable while 4 to 5 considered as bad or rejected. In general, similar organoleptic characteristics were observed for Pama fish irrespective of fish market resulted with excellent and highly acceptable. Natural odor and slight pinkish red color of gill was observed for each fish from three different markets. No gill or body slime and transparent eye was observed for from any market. The general appearance of fresh Pama fish was from full bloom, bright silver, shinning, iridescent which refer to fresh and good quality of fish. Texture was firm and elastic for all fish samples from three different markets. The flavor and color which are important factors influencing the overall consumer acceptability. The lowest label of defect point present in Rajakhali fish market (1.27) then Pirtola fish market (1.31) and the highest score was for fish from New market in Patuakhali town but the quality of fish in all market was excellent and acceptable. Finally organoleptic characteristics referred the excellent and highly acceptable Pama fish from the respective three markets in Patuakhali district.

Table 3. Organoleptic characteristics of fresh Pama fish (*Pama pama*) from different fish market in Patuakhali district

Source of fish	Organoleptic Quality							Defect Point	Grade	Grade characteristics
	Odor of Gill	Color of Gill	Slime of Gill	Body Slime	Eye	Consistency of flesh	General appearance			
Pirtola fish market	Natural odor	Slight pinkish red	Thin colorless slime	Clear & transparent	Transparent eye	Firm, elastic flesh	Full bloom, bright, shinning	1.31	A	Excellent, highly acceptable
Rajakhali fish market	Natural odor	Slight pinkish red	Thin colorless slime	Clear & transparent	Transparent eye	Firm, elastic flesh	Full bloom, bright, shinning	1.27	A	Excellent, highly acceptable
New market fish market	Natural odor	Slight pinkish red	Thin colorless slime	Clear & transparent	Transparent eye	Firm, elastic flesh	Full bloom, bright, shinning	1.54	A	Excellent, highly acceptable

Determination of bacterial load of fresh Pama fish

The total aerobic plate count expressed as colony forming unit in one gram of sample (CFU/g) of the representative samples of fresh Pama collected from different fish market in Patuakhali district was determined by standard plate count method. The bacterial load of fresh Pama in different fish market were shown in table 4.

Table 4: Total Aerobic Plate Count of fresh Pama fish (*Pama pama*) from different fish market in Patuakhali District

Sources of fish sample	APC (CFU/g)
Pirtola fish market	4.82×10 ⁹
Rajakhali fish market	4.43×10 ⁹
New market fish market	1.63×10 ⁹

Among the three different markets, similar bacterial load was observed in fish sample from Pirtola and Rajakhali fish market. On the other hand, Pama fish obtained from New market fish market had the lowest value microbial (1.63×10^9 cfu/g) and the highest value (4.82×10^9 cfu/g) was in fish from Pirtola fish market. Use of ice for preservation of fish might leads to lower microbial content in fish from Patuakhali New market. The maximum bacterial counts for fresh and frozen fish samples recommended as 5×10^5 cfu/g (ICMSF, 1986). The bacterial load was found $1.22 \pm 0.12 \times 10^6$ cfu/g in fresh hilsa from Karwan Bazar (Rohomania *et al.* 2015). The result also exceeds the limit of bacterial load of hilsa. In the present study, Pama fish samples exceed acceptable limit of bacterial content recommend further improvement of personnel sanitary and hygiene condition of the respective fish market. Bacterial growth is the main cause of fish spoilage therefore it is logical to use bacterial content as an index of fish quality.

Biochemical characteristics evaluation of fresh Pama fish

A part from sensory and microbial quality, proximate composition of fish was also analyzed of the collected fish from the respective fish market. The results of proximate analysis of fresh Pama are shown in figure 3.

Moisture

Moisture content of fresh Pama (*Pama pama*) that collected from different fish market in Patuakhali district were shown in figure 3. The range of moisture content of fresh Pama fish from different fish market were varied from 79.87% to 84.67%. The lowest moisture value (79.87%) was observed in fresh Pama fish from New market fish market and the highest value (84.67%) was in fish from Pirtola fish market. The high moisture content of the fish sample would increase the deterioration level of fish when kept for a long time. High moisture favors the growth and multiplication of bacteria which leads to higher microbial decomposition of fish. Basu and Gupta (1939) reported that Pama fish contains 75% water. This also supports with the findings for *M. aculeatus* (Nabob and Hossain, 1989), *P. gonionotus* (Salam *et al.*, 1995), *A. mola*, *P. chola*, *G. chapra* and *P. Atherinoides* (Mazumder *et al.*, 2008). These findings also agreed with observation of several freshwater fish species (Marias and Erasmus, 1977). Moisture content was 73 to 80% for *Lates niloticus*, *Bagrus bayad*, *Oreochromis niloticus*, *Synodontis schall* and *Tetraodon lineatus* (Mohamed *et al.*, 2010). Sutharshiny and Sivashanthini (2011) also estimated the moisture content in *S. lysan*, *S. tol* and *S. commersonianus* from 72.57% to 75.67 %.

Ash

Ash content of fresh Pama (*Pama pama*) that collected from different fish market in Patuakhali district were shown in figure 3. The range of ash content of fresh Pama fish from different fish market vary from 0.99% to 1.99 %. The lowest value (0.99%) obtained from fresh Pama fish in Rajakhali fish market. Mazumder *et al.*, (2008) reported the ash percentage varied within 1.6% to 3.2 % for *A. coila* and *A. mola*. Lower amount of ash

content in six freshwater fishes *L. rohita* (1.31%), *Catla catla* (0.93%), *Cirrihinus cirrhosus* (1.40%), *L. calabasu* (1.02%), *Mystus seeghala* (0.91%) and *Wallagu attu* (0.72%) was observed by Devadsan *et al.*, (1978). The ash content estimated in *S. lysan*, *S. tol* and *S. commersonianus* were 1.42, 1.49 and 1.6%, respectively (Sutharshiny and Sivashanthini, 2011).

Protein

Protein content of fresh Pama (*Pama pama*) that collected from different fish market in Patuakhali district were shown in figure 3. The range of protein content of fresh Pama fish from different fish market were varied from 14.26% to 15.4%. The lowest value and highest value of protein obtained from fresh Pama fish in Patuakhali new market and Pirtola fish market, respectively. In another study, Basu and Gupta, (1939) found Pama fish contains crude protein 18.6%. Protein content was estimated as $19.47 \pm 0.16\%$, $18.99 \pm 0.51\%$ and $21.68 \pm 0.65\%$ in *S. lysan*, *S. tol* and *S. commersonianus*, respectively (Sutharshiny and Sivashanthini, 2011).

Lipid

Lipid content of fresh Pama (*Pama pama*) that collected from different fish market in Patuakhali district were shown in figure 3. The range of lipid content of fresh Pama fish from different fish market vary from 3.00% to 5.00%. The lowest value (3.00%) obtained from fresh Pama fish in new market fish market and the highest value (5.00%) from Rajakhali fish market. Basu and Gupta (1939) reported Pama fish contains 1.7% fat. The deep sea fish species rough head grenadier (*Macrourus berglax*), mora/deep-sea cod (*Mora moro*), Portuguese dogfish (*Centroscymnus coelolepis*), black dogfish (*Centroscyllium fabricii*), leafscale gulper shark (*Centrophorus squamosus*), greater lantern shark (*Etmopterus princeps*), small-eyed rabbitfish/ghostshark (*Hydrolagus affinis*), birdbeak dogfish (*Deania calcea*) and two species of smooth head (*Alepocephalus bairdii* and *Alepocephalus agassizii*), the first eight species contained less than 1% fat in the muscle, while the last two contained 3.0% and 3.6% fat, respectively (Økland, 2005). Lipid content was 1.8 to 17.3% in *Lates niloticus*, *Bagrus bayad*, *Oreochromis niloticus*, *Synodontis schall* and *Tetraodon lineatus* (Mohamed, *et al.*, 2010). Lipid content for *S. lysan*, *S. tol* and *S. commersonianus* was recorded as $0.89 \pm 0.005\%$, $0.594 \pm 0.113\%$ and $1.00 \pm 0.12\%$, respectively (Sutharshiny and Sivashanthini, 2011). Based on the fat content, Pama fish could be considered as low to medium fatty fish but not fatty fish.

Conclusion

In the present investigation, the post-harvest quality aspects of Pama fish (*Pama pama*) from different fish market in Patuakhali district were evaluated based on the sensory quality, proximate composition and total bacterial load. Based on sensory analysis the lowest defect point of fresh pama indicate the fish sample from Rajakhali fish market were excellent and highly acceptable among the three markets. Based on bacterial load New market fish market was lower microbial load as compared with other two market. Higher moisture and protein content and lower

to medium fat content was observed in Pama fish irrespective of fish market. The higher total bacteria count in different fish market samples indicated the unhygienic, rough handling and lack of personnel hygiene. The retailers and fish handlers should take proper training on the proper practice of post-harvest

handling thus to avoid the cross contamination and associated health risks. Finally, it could be concluded that quality of Pama fish in different fish market of Patuakhali district is acceptable and safe for consumer; however, further improvement for post-harvest handling, preservation and personnel hygiene are required.

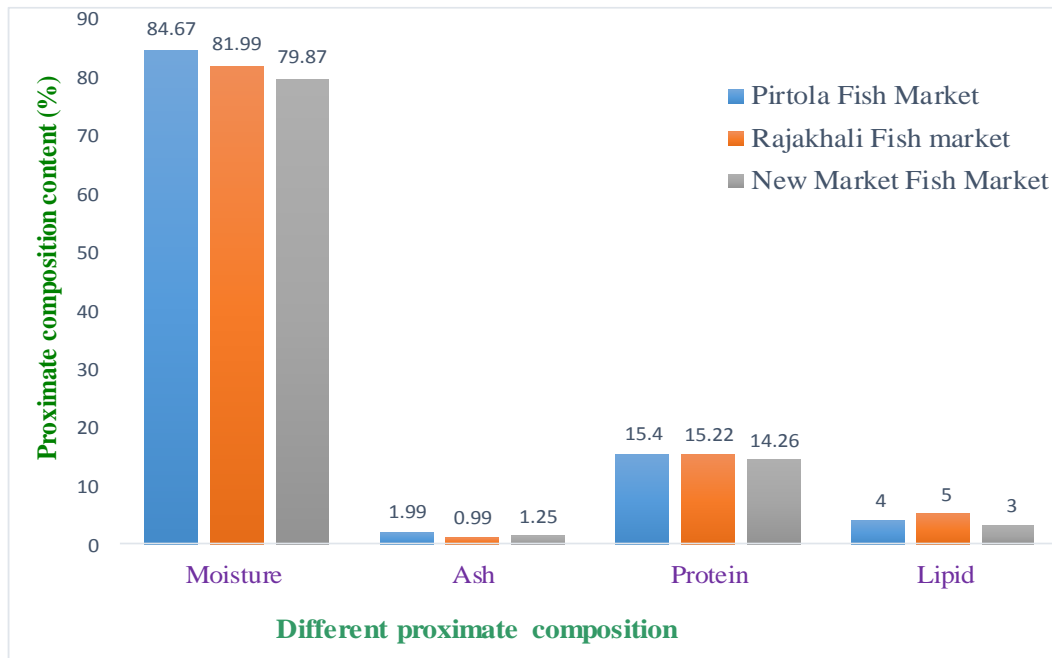


Figure 3. Moisture, ash, protein and lipid content of fresh Pama fish (*Pama pama*) from different fish market in Patuakhali district

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