



Effect of frozen storage on the biochemical, microbial and sensory attributes of Skipjack Tuna (*Katsuwonus pelamis*) fish loins

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ABSTRACT

Skipjack tuna (*Katsuwonus pelamis*) belongs to the Scombridae family to determine the consistency characteristics of tuna fish loins for 28th days at frozen ($-18 \pm 2^\circ\text{C}$) storage. The biochemical, microbial, and sensory quality were analyzed by association of official analytical chemists method, plate count, and Quality Index method (QIM), respectively. Protein, lipid, and moisture content were found to decrease significantly ($p < 0.05$) over the study period. Nonetheless, the values of ash, total volatile base nitrogen, tri-methyl amine nitrogen, pH, and peroxide increased significantly ($p < 0.05$). After 28 days of frozen storage, the total bacterial load in tuna fish loins was reduced from 3.8×10^5 to 3.3×10^4 colony forming unit/g. Total coliforms reduced from 113 to 5 Most Probable Number (MPN)/g, fecal coliforms declined to undetectable level from 13 MPN/g, and total *Salmonella* sp. also decreased from 1 MPN/g to unnoticeable, respectively. Organoleptically tuna loins were found in excellent condition up to 14th days although remained acceptable up to 28th days of the study period. The present findings should that the skipjack tuna loins can be retained under an acceptable limit for human consumption at frozen storage conditions up to 28th days.

1. INTRODUCTION

Nature makes fish one of the most perishable food items. The freshness of this highly perishable food item greatly affects the market acceptability [1–4]. Fish spoilage is triggered by the action of microorganisms and enzymes embedded in fish [5]. Ammonia and tri-methylamine (TMA) are developed due to these action presents in fishes, which led to the firmness and contamination in the seafood. The degree of reduction in quality relies on several factors, such as the freezing and thawing rate, storage temperature, and storage temperature fluidity [6]. Tuna is a popular fish species with much economic value relative to its increasing global market demand and is typically served fresh, canned, and frozen. There

were more than 50 species of which 5 species of tuna fishes plays an important role in European Union market [7]. *Katsuwonus pelamis* (skipjack tuna) is one of important pelagic fish distributed in torrid zone and warm temperate water area and also very valuable fishery species [8].

Loin from tuna fish is very delicious and can use in many kinds of seafood dishes that anybody can want but sadly, it was only available during a certain season. With the advancement of technology now fish loin product is available in any season. The buyer, however, needs the tuna loin products in fresh condition. But, the consistency measures impaired by freezing include changes in the composition of color, texture, water-retaining capacity, etc. Quicker freezing levels preserved the structural consistency and reduced the chemical reaction of several fish product forms [9–11]. That is why shelf-life studies, as well as a biochemical composition, must be needed or analyzed to

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sustaining the product or available in any season. Nevertheless, work is done on the shelf life; biochemical composition and microbiology of skipjack tuna fish loins are comparatively inadequate, especially from tropical waters. The current study was therefore undertaken for assessing the biochemical, microbiological, and sensory aspects of tuna fish loins in frozen storage condition. These values would be useful references for consumers, nutritionists, and food processors to choose the tuna fish loins, based on their appropriate uses.

2. MATERIALS AND METHODS

2.1. Sample Collection and Preparation

Freshly skipjack tuna fishes was purchased from Mohipur Fish Landing Station (Fig. 1) at Kalapara Upazila of Patuakhali District and were carried out by an insulated ice box and transported to the Fish Processing Laboratory at the Fisheries Technology Department, Patuakhali Science and Technology University, Bangladesh. The samples were washed with rinse cooled water and later by beheaded, gutted, washed, skinned, again washed, then filleted and skinless fillets were cut in the center into two pieces. Deeply dark meat is trimmed off and the tuna loins were made for analysis and samples were sealed in vacuum polyethylene bags and kept frozen at $(-18 \pm 2^\circ\text{C})$ until further analyses.

2.2. Determination of Biochemical Composition

Proximate compositions (crude protein, fat, ash, and moisture) of fish loin were analyzed in triplicate for each of sample according to the methods of Association of the Official Analytical Chemists [12]. The total volatile base nitrogen (TVB-N) and tri-methyl amine nitrogen (TMA-N) were determined according to the given methods in AMC [13]. P^{H} and peroxide values (PV) were determined according to Eagan [14] and Wood and Aurand [15], respectively.

2.3. Microbial Quality of Tuna Fish Loins

About 5 g of samples were taken aseptically from the zipper polyethylene bag and homogenized in 195 ml with 0.9% NaCl solution. Serial dilutions of homogenates were made up to 6 or 7 dilution with triplicates and aerobic plate count (APC), *Escherichia coli* count (TEC), *Salmonella* count (TSC) and vibrio counts (TViC) were analyzed by pour plate method, using Plate Count Agar and Anumbra 100 mm model plate. Nutrient Agar media for APC, Eosin Methylene Blue (EMB) Agar media for TEC; Xylose Lysine Deoxyo holate (XLD) Agar media for TSC and TCBS Agar media for TViC were used, respectively, for culture. The plates were incubated at 37°C for 24–72 hours on the basis of the selective media used for the specific bacterial culture. Bacterial population were counted under ≥ 30 –300 colonies per

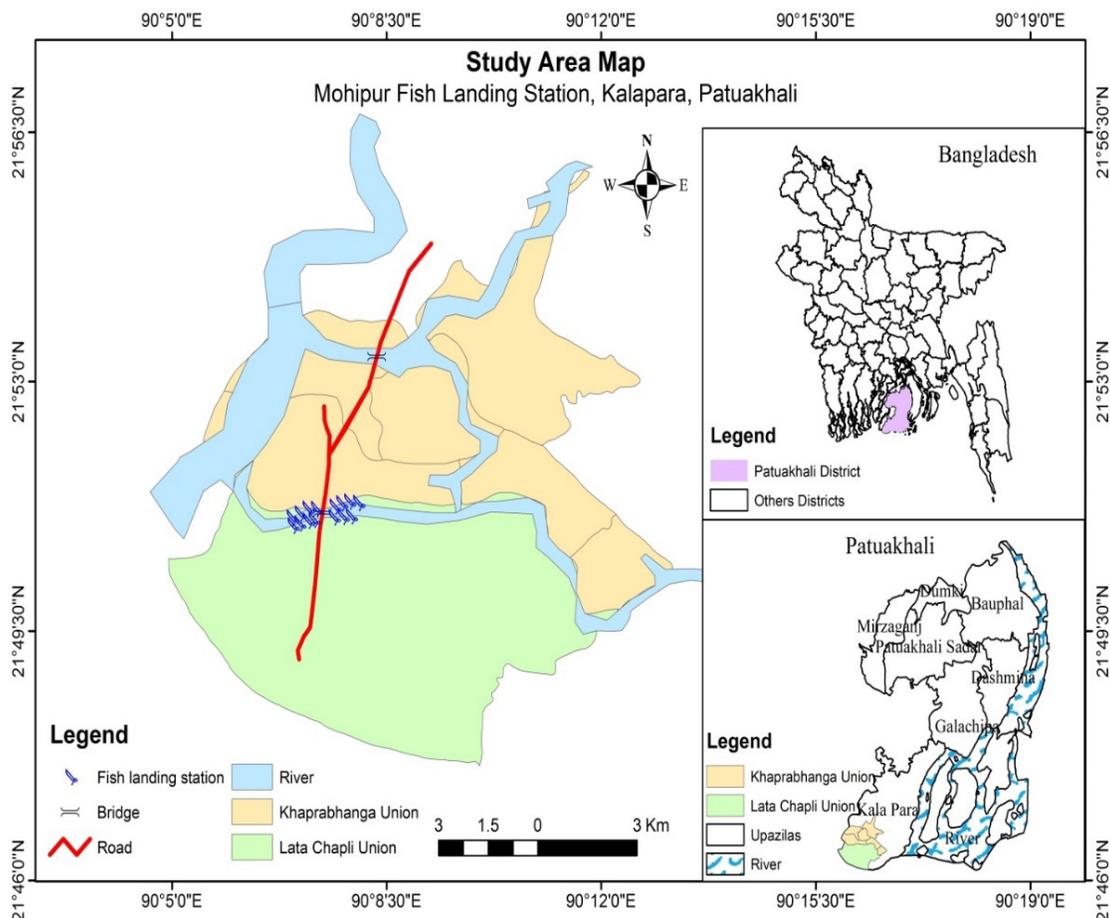


Figure 1: Map showing the collection point of the skipjack tuna fish (*K. pelamis*).

plate and were indicated as colony forming unit (cfu)/g per unit (weight in g) of samples.

The APC was determined by using the formula:

$$\text{APC or TPC} = \frac{\text{No. of colony} \times 10 \times \text{dilution factor} \times \text{volume of sample}}{\text{Weight of used sample}} \times 100$$

2.4. Organoleptic Study

A testing panel evaluated the developed skipjack tuna fish loin's for the organoleptic assessment. A prepared test-scoring sheet with hedonic scales was given to the each of the individual panelist for evaluation of the products. Ten experienced panelists were selected and evaluated the organoleptic test of tuna fish loin's samples using the narrative value listed in Tables 1 and 2. All panelists were checked based on their knowledge and sensory performances on tuna loins. This performance was conducted in a room designed for sensory analysis.

Table 1. Sensory defects and defects points (DP) for assessment of quality loss of tuna fish loins.

Characteristics	Defects	DP	Tuna fish loins	
			Fresh loins	Frozen loins
Odor	a. Natural odor	1		
	b. Faint sour odor	2		
	c. Slight moderate sour odor	3		
	d. Moderate to strong sour odor	5		
Color	a. Slight pinkish red	1		
	b. Pinkish red to brownish	2		
	c. Brown to grey	3		
	d. Bleached color	5		
Texture	a. Firm, elastic	1		
	b. Moderately 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 and caudal region dull, no bloom	5		
Average Defect Point (DP)				
Grand Average Defect Point (DP)				

Table 2. Grading of skipjack tuna fish loins acceptance.

Grade	Defects points	Degree of acceptance
A	Less than 2	Excellent, Highly acceptance
B	2 to less than 5	Good/acceptable
C	5	Rejected

Sensory analysis is calculated by using following formula:

$$\text{Average grade point} = \frac{\text{Number of characteristics}}{\text{Total grade point}}$$

2.5. Statistical Analyses

All analysis was performed through SPSS software version 23.0 and ANOVA test was used to compare treatments. Difference between means was assessed using the Duncan at a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Biochemical Composition of Tuna Fish Loins

Analysis of biochemical composition of tuna fish loins indicated that tuna fish loin was rich in protein (21.54%) while lipids/fat content of tuna fish loin was 3.66% (Table 3). Tuna red meat is of great nutritionally comparable with white meat [16]. There were differences in protein, lipid, ash and moisture value were found in frozen storage condition ($p < 0.05$).

3.1.1. Protein

The protein content of frozen tuna loins was decreased ($p < 0.05$) significantly from 0 days to 28th days although no significance difference between 0th days and 7th days. The highest protein content was recorded as 21.54 ± 0.25 g/100 g in the initial time and the lowest protein content was also recorded as 19.68 ± 0.08 g/100 g during 28th days of storage period (Table 3). Present finding was agreement with the finding of Matsumoto *et al.* [17] but the study of [16,18,19] revealed that skipjack tuna fish muscle protein was more than the present findings. This is an agreement with the findings of Mazonra-Manzano *et al.* [20] recorded that protein content of bluefin tuna fish muscle had high level of protein. The muscle protein of Bluefin tuna was more with the present findings [21]. Protein denaturation occurs due to the changes of chemical proportion and breakdown of protein. Protein denature in frozen muscle has been reviewed by several authors [22,23].

3.1.2. Lipids/fat

Oxidation of lipid is the serious reason of deterioration in most fatty fishes. Polyunsaturated fatty acid contains in large quantity of marine fishes which is more susceptible to lipid oxidation by an autocatalytic process [24]. Gradually, oxidation of lipids creating off-flavor and reduce nutrition as well as generate other problems, such as toughness and texture [25]. The current result showed that content of fat in tuna loin was decreased significantly ($p < 0.05$) due to entire study period and ranged from $3.66 \pm 0.03\%$ to $2.53 \pm 0.02\%$ (Table 3). This reason indicates that lipid was directly inverse relationship with the moisture [25,26] revealed lipid content drastically provoked ($p < 0.05$) significantly in increasing with storage time which is contradictory with the present study. The lipid content of rainbow trout (*Oncorhynchus mykiss*) is increased during frozen ($-18 \pm 2^\circ\text{C}$) storage [27]. Lipid changes occurs due to differences in period, geographical area, age, and size [28], the lipid content in tilapia fish fillets changes under

frozen storage was consistent with descriptions presented by other authors [26].

3.1.3. Moisture and ash content

The content of moisture and ash of tuna loins were significantly differences ($p < 0.05$) during 28th days ($-18 \pm 2^\circ\text{C}$) of storage period (Table 3). The values of moisture was decreased significantly ($p < 0.05$) from $73.79 \pm 0.25\%$ to $72.11 \pm 0.03\%$ during the study period, although there is no significance ($p > 0.05$) difference between 7th days and 14th days (Table 3). A decreasing trend in the moisture was also found by Younis *et al.* [29]. Ash content in sea bass fillets is decrease during 60th days of frozen storage condition [26]. Thaw rigor and drip loss could be the

possible reasons for decreasing in the content of moisture and ash. Moreover, moisture loss could also happen due to the desiccation and temperature fluidity in freezing process.

3.1.4. TVB-N and TMA-N

The level of TVB-N and TMA-N value expressed that low temperature has significant ($p < 0.05$) effect on the decreasing the quality of the frozen tuna loins Figure 2a and b. In the present study, the concentration of these two compounds were upward ($p < 0.05$) significantly in frozen condition where ranged from 7.89 ± 0.05 and 3.30 ± 0.03 to 13.29 ± 0.38 and 8.23 ± 0.02 mg N/100 g, respectively. This compound in fish is generally between 5 and 20 mg N/100 g muscle and even more this value

Table 3. Biochemical composition changes at different storage time of tuna fish loins.

Parameters	Storage time (days)				
	0	7	14	21	28
Protein	21.54 ± 0.25^a	21.38 ± 0.07^a	20.89 ± 0.10^b	20.03 ± 0.08^c	19.68 ± 0.08^d
Lipid	3.66 ± 0.03^a	3.32 ± 0.01^b	3.08 ± 0.01^c	2.80 ± 0.02^d	2.53 ± 0.02^e
Ash	2.19 ± 0.02^a	2.89 ± 0.02^b	3.09 ± 0.03^c	3.81 ± 0.03^d	4.92 ± 0.02^e
Moisture	73.79 ± 0.25^a	73.20 ± 0.07^b	73.20 ± 0.05^b	72.80 ± 0.03^c	72.11 ± 0.03^d

Values are presented as mean \pm SD; values of a same row that do not share a same superscript are significantly different ($p < 0.05$).

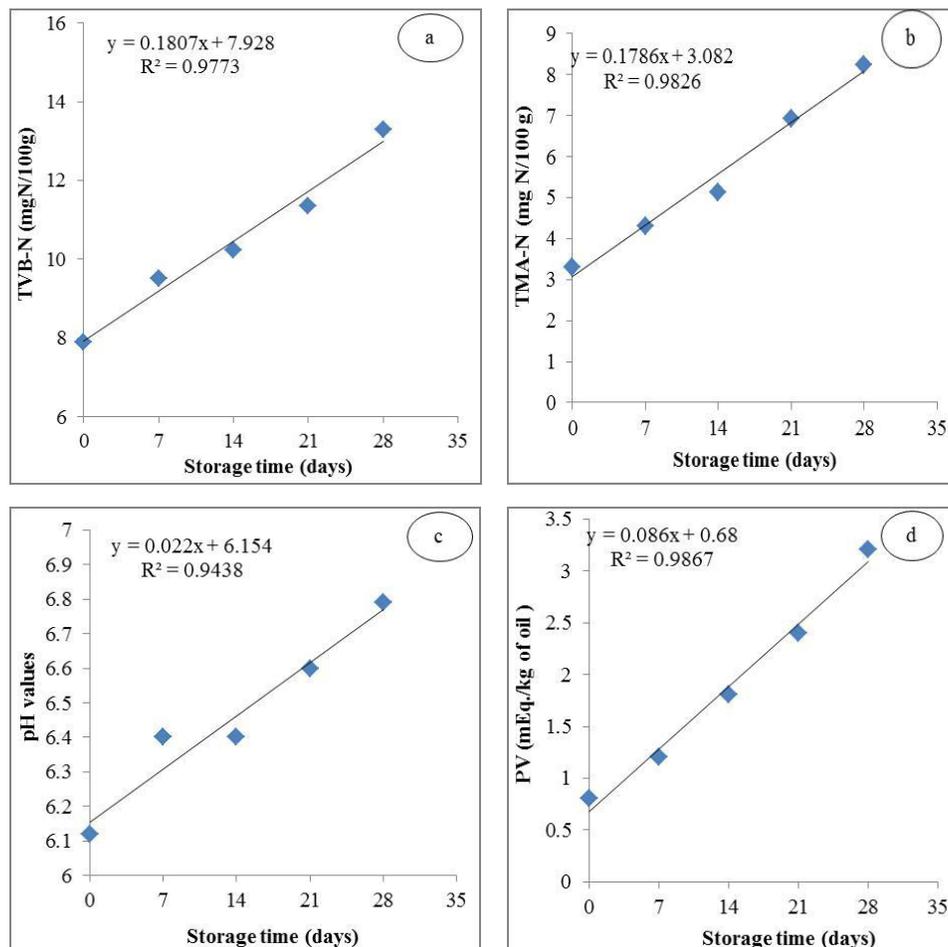


Figure 2: Correlation of TVB-N (a), TMA-N(b), pH^{H} (c) and PV (d) with frozen storage of tuna fish loins.

is also happened as spoilage [14]. These two compounds in tuna loins increased progressively under frozen period but did not go across over the acceptable level for human consumption [14] until the 28th days at frozen storage. In case of most fishes, tri-methyl amine comprises mostly the volatile compounds until spoilage [30]. However, the present findings showed dissimilarities with the finding of Mazorra-Manzano *et al.* [20], Salah and Salouca [31], and Ben-Gigirey *et al.* [32], but Similar findings were reported by Xue *et al.* [33] for yellowtail, [34] for croaker, and [35] in iced cuttlefish. Increasing of TVB-N concentration may be ascribed to the formation of TMA, ammonia and other nitrogenous bases resulted from the deposition of TMA-N by internal enzymes [14].

3.1.5. Peroxide and pH value

The PV showed a gradual significant increase over the whole storage time at $-18 \pm 2^\circ\text{C}$ for skipjack tuna loins (Fig. 2c and d). However, values obtained at the end of storage period remained relatively low with the finding of Sagar *et al.* [36] for pink perch mince; [37] for European hake; [25] for cod and haddock. pH value for the frozen stored skipjack tuna increase progressively throughout the experiment and is generally extend up to 6.79 during 28th days of frozen state. The concentration of pH in fishes is usually neutral position for human consumption [38] and the level of pH did not cross the acceptable limit in the present finding. Increasing of pH is correlated with the accumulation of basic substances like ammonia and TMA [39]. Although changes of muscle pH in fishes under storage period are differs depend on the basis of fish species and other biological factors. Besides, the concentration of pH not only important parameter but can be used as a quality indicator of fishes [38,40].

3.2. Microbial Quality of Frozen Fish Loins

Seafood product is fully depends on the sanitation condition of the water quality and the different stages of seafood processing [40]. For the determination of freshness quality of skipjack tuna loins at frozen storage, Aerobic Plate Count (APC), total coliforms, total fecal coliforms, and total *Salmonella* sp. were analyzed. In the current study, the original microbial load of tuna loins were observed at 3.8×10^5 cfu/g, while total coliforms, fecal coliforms and *Salmonella* sp. represented to be 170, 25, and 1 Most Probable Number (MPN)/g, respectively. Higher bacterial load in original loins might be regarded due to the ignorance of public awareness prevailing during harvesting and used contaminated knives, etc. during preparing tuna fish loins. The changes in total coliforms, fecal coliforms, and *Salmonella* sp. of skipjack tuna loins stored for 28th days in frozen ($-18 \pm 2^\circ\text{C}$) condition (Fig. 3). Total bacterial load in tuna loins was decreased from 0 days to 21th days but again started to increase on 28th day of frozen storage. Total *E. coli* drastically declined up to 21th days but again increased on the 28th days of frozen period. Both the coliform bacteria gradually laid down to 1 MPN/g during 28th day of frozen state. In case of *Salmonella* sp. was not detected during whole present study except the initial sample of tuna fish loins. Total bacterial load decreased which might cause due to sudden cold shock under frozen state. In general, the bacterial load is increased which is

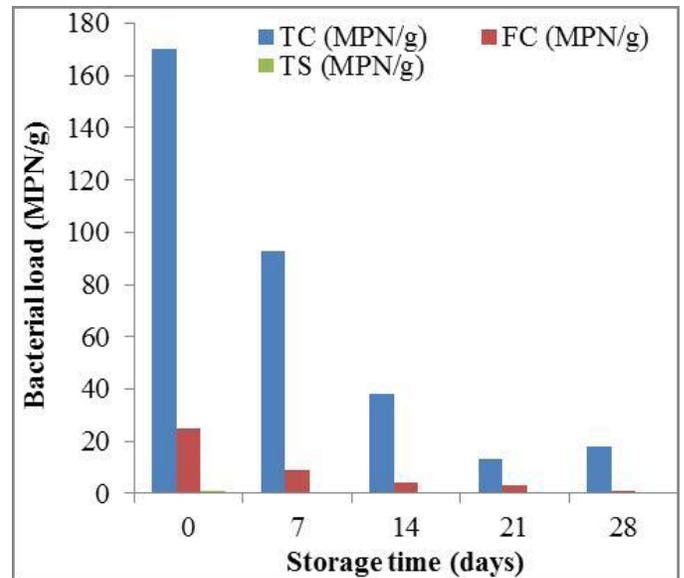


Figure 3: Microbiological quality of skipjack tuna fish loins at frozen storage ($-18 \pm 2^\circ\text{C}$) condition.

highly related with the increasing of time and temperature. The present finding is highly related with the study of [41]. Similar results were also reported by Thailambal [35], Sagar *et al.* [42], and export inspection council of India [43]. A considerable study was found by Wu *et al.* [44] reported that loins of skipjack tuna fish were lower than the present study. The bacterial load was similar but the total *E. coli* was more than the present results [45]. The frozen Nile tilapia (*Oreochromis niloticus*) fish fillet which contain more microbes than the present findings [46]. The total plate count raised to 7.2×10^5 cfu/g after 1th day during 25°C and other dominant Enterobacteriaceae bacteria was dominant throughout the storage period [47] which is not related with the findings of the present study. In the present study, skipjack tuna loin remained considerable microbial community, which might be related to inadequate human operations during fishing and processing in the laboratory. It is recommended that microbial quality of fishes may not be surpassed 10^6 cfu/g in fresh state which were agreement with the results of this study [48].

3.3. Sensory Attributes of Tuna Fish Loins at Frozen Storage Condition

The study revealed a general decline in the physical attributes, such as color, the grade reduced from A to B in the $-18 \pm 2^\circ\text{C}$ stored samples. The grade for texture of loins varied from 1.03 ± 0.063 to 2.58 ± 0.24 in the sample. While the grade for odor of the fish during ice storage showed decline from 2.48 ± 0.20 to 1.03 ± 0.06 in the sample. There was also changed in the general appearance of the loins that varied from 1.1 ± 0.21 to 3.21 ± 0.43 . Statistical analysis of the four parameters assessed for organoleptic characteristics: color, texture, odor, and general appearance revealed significant differences in the organoleptic characters of the tuna loins (Table 4). The fish remained in good condition about 30 days of storage [20,49] which are similar to the present study but Reza *et al.* [50] stated that, all fishes were being accepted not

Table 4. Organoleptic assessment of frozen stored tuna fish loins.

Organoleptic characters	Storage time				
	0 days	7 days	14 days	21 days	28 days
Color	1.065 ± 0.16 ^a	1.98 ± 0.18 ^b	2.15 ± 0.20 ^c	2.80 ± 0.20 ^d	2.97 ± 0.04 ^e
Texture	1.03 ± 0.06 ^a	1.09 ± 0.16 ^a	2.02 ± 0.20 ^b	2.25 ± 0.23 ^c	2.58 ± 0.24 ^d
Odor	1.03 ± 0.06 ^a	1.08 ± 0.08 ^a	1.84 ± 0.36 ^b	2.22 ± 0.25 ^c	2.48 ± 0.20 ^d
General appearance	1.10 ± 0.21 ^a	2.17 ± 0.33 ^b	2.40 ± 0.39 ^b	3.05 ± 0.44 ^c	3.21 ± 0.43 ^d
Grade	A	A	A	B	B

Values are presented as mean ± SD; values of a same row that do not share a same superscript are significantly different ($p < 0.05$).

more than 2 weeks under ice condition but in this study the fish loins remained edible until 28th days of storage condition.

4. CONCLUSION

Increasing of frozen period is directly correlated with the biochemical and microbial quality changes, which considerably accomplish to the sensory attributes of tuna loins. But the high bacterial population in primary level can be taken into consideration due to lack of personal hygiene and poor handling conditions prevailing during fishing and processing in the laboratory. However, microbial community which is linked with quality deterioration and spoilage and are associated with the increasing of proximate composition level during freezing process, representing that the stored skipjack tuna loins would be better for human consumption for up to 14 days.

CONFLICT OF INTEREST

The authors declared that they do not have any conflicts of interest.

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