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RESEARCH ARTICLE

Evaluation of the Biochemical and Functional Characteristic of Stinging catfish, *Heteropneustes fossilis* Muscle Tissue with Emphasize on Myofibrillar Protein and Collagen

Treesa Varghese[1,2]*, **Saleena Mathew**[2]

1 School of Biosciences, Mar Athanasios College for Advanced Studies, Tiruvalla, Kerala, India
2 School of Industrial Fisheries, Cochin University of Science and Technology, Kochi (Retd), Kerala, India

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* **Corresponding author**.

treesav@gmail.com

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Abstract

Objectives: To evaluate the physico-chemical and functional characteristics of the stinging catfish muscle tissue during the storage time of 18 hours following the death of the fish. **Methods:** In the present study, stinging catfish were collected from the paddy fields and streams near Tiruvalla Kerala. Fish were slaughtered immediately and analyzed for changes in pH value, cook loss, expressible water content, water holding capacity, sensory demerit score values, and textural profile and were compared with progress of rigor mortis. Study also evaluated the changes in protein fractions present in the muscle tissue and correlated them with autolytic enzyme activities. **Findings:** In stinging catfish, the rigor mortis occurred within four hours of storage (78.37 %) at ambient temperature. The post rigor stage was attained after the 6^{th} hour and the K value reached above 60% within the 8^{th} hour which indicates the unacceptable range for consumption. The present study also proved that the stinging catfish is an inosine producer. The initial concentration of sarcoplasmic protein in fish muscle (13.71%) decreased to 7.62% at the end of 18 hours of storage. The muscle tissue myofibrillar protein also showed a decrease from an initial concentration of 44.87% to a final concentration of 18.02%. Protein denaturation was evident with an increase from 39.65% to 73.20% at the end of the storage period. Collagen concentration was found to be reduced (31.85% reduction) after the 18^{th} hour of storage. Pepsin soluble collagen was less susceptible to the activity of collagenase enzyme in the pre-rigor stage, confirming its role in post-rigor muscle softening when compared to acid soluble and insoluble collagen. **Novelty:** The present work authenticated that post-rigor softening of stinging catfish muscle is typically due to the degradation of pepsin soluble collagen and not reported elsewhere. Additionally, myofibrillar proteins have more influence on total hardness and stiffness than collagen, while cohesiveness is a better function of collagen than myofibrillar protein. These findings make it useful to perceive the process that

induces softening in catfish and to create effective strategies to prevent it.

Keywords: Collagen; Myofibrillar Protein; Adenosine Nucleotides; Autolytic Enzymes; Textural Profile

1 Introduction

Stinging catfish, *Heteropneustes fossilis*, is a member of Heteropneustidae, mainly found in tropical, subtropical, and temperature waters, lakes, swamps, marshes, muddy rivers, and ditches. Even though the stinging catfish are freshwater fishes, they can tolerate slightly brackish water. They are bottom feeders omnivorous in nature and possess air sacs and four pairs of barbells [(1)]. Soft, boneless white flesh with a pleasant flavor makes them one of the most delicate fish as food organoleptically accepted and is often sold at high prices in many parts of India.

Immediately after the fish die several physical and biochemical changes are initiated which is of utmost concern and can affect fish quality and market value. One of the foremost indications of the freshness of fish is its firmness. But during death and postmortem storage, firmness is lost and there will be a decline in fish quality due to bacterial spoilage, autolytic degradation, and lipid oxidation [(2,3)]. Total firmness of muscle tissue in fish is linked to its total collagen content [(4)] and degradation of type I and type V collagen resulted in gapping and total softening in muscle food [(5)]. Weakening of pericellular connective tissue by degradation of thin collagen fibrils has also been reported but the collagen located in the interstitial connective tissue was irresponsive in such conditions [(6)].

Endogenous proteinases such as connective tissue collagenases and elastases, lysosomal cathepsins and cytosolic calpains proteases and collagenases involved in the deterioration of muscle tissue collagen and myofibrillar proteins leading to the loss of firmness [(7)]. Studies of Suarez et al reported that the softening of fish muscle during postmortem storage is a result of collagenase activity rather than proteolysis of myofibrils [(4)].

Even though there is great number of works relating to collagen and myofibrillar proteins along with collagen fractions together with the autolytic enzyme activity in fish muscle softening, similar studies have not included species such as stinging catfish, which is of great economic as well as nutritional impact in fish farming. In this concern, the aim of the present work is to contribute to a better understanding regarding the influence of collagenase and lysosomal proteolytic enzyme activity on post mortem quality changes in the myofibrillar protein and collagen content over storage times, in order to get a better understanding on what degree these variations induce the loss of freshness in catfish.

2 Methodology

2.1 Fish sample collection

Stinging catfish with a uniform size (weight of 250 $\pm$ 20g and length of 20 $\pm$ 5cm) were collected from the paddy fields and streams near Tiruvalla, Kerala, India in alive condition and slaughtered without delay by a blow to the head. Muscle tissues for the analysis were collected from the dorsal side of the fish between the gills and the dorsal fins. During the storage, the fish were randomly taken at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 15 and 18 hours for analysis in triplicates.

2.2 Analysis

2.2.1 Determination of rigor index

Rigor index of the whole fish was determined according to the method of Bito et al. [8]

2.2.2 Measurement of pH

One gram of fish muscle was weighed out and was homogenized in 10 mL of distilled water using a tissue homogenizer in cold condition, and the pH values were determined with a pH meter (ELICO pH Meter LI 127).

2.2.3 Nucleotide Analysis

Determination of adenosine nucleotides and its degradation compounds were done using HPLC according to the procedure of Ryder [9]. HPLC system(Waters 2487 model) and the separations were performed on a Symmetry C 18 column. The eluate was monitored by UV absorption at 256 nm. The chromatographic peaks of ATP and its breakdown products were determined by comparing the retention time of HPLC peak between samples with authentic external standard calibration using known concentrations of the following standards: ATP, ADP, AMP, IMP, inosine and hypoxanthine procured from Sigma Aldrich. Data analysis was performed using EMPOWER Chromatography software. K-values were calculated according to Saito et al [10] commended as the freshness limit and 60% as the rejection point.

2.2.4 Sensory evaluation

Sensory evaluation was carried out using a quality index method (QIM) developed by Bremner [11].

2.2.5 Moisture content

Moisture content of the homogenized sample was determined by drying the sample in an oven at 105°C till the weight became constant. The water content was determined by weight loss after drying.

2.2.6 Water holding capacity

Water holding capacity (WHC) was determined according to the method described by Borresen [12] with slight modifications. 2 g of filleted sample was centrifuged at 4500 rpm for 15 min by keeping a Whatman filter paper No 1 at the bottom of the centrifuge tube to remove the water content from the fillet. After centrifugation, the fillet was weighed again, and the difference between before and after centrifugation was calculated. The moisture content determined is used to calculate the water holding capacity. The WHC was calculated as the ratio of water remaining compared to the water content in the sample before centrifugation.

2.2.7 Expressible water content

Expressible water content (EWC) was determined according to the method of Benjakul [13] and was calculated as a percentage of sample weight.

2.2.8 Cook loss

Cook loss was determined according to a modified version of the method described by Borresen [12] and the cook loss was presented as % lost sample.

2.2.9 Protein fractionation

Muscle tissue proteins were extracted from the fish muscle according to the method of Devadasan and Nair [14]. Protein fraction in the extract was determined in terms of nitrogen content using micro-kjeldhal method.

2.2.10 Collagen fractionation

Extraction of acid soluble, pepsin soluble and insoluble collagen was carried out according to the method adopted by Makiand the nitrogen content was determined [15].

2.2.11 Instrumental textural profile analysis

Instrumental textural profile analysis was done by using Texture Analyzer (Lloyd Instruments, UK, model LRX PLUS) and Nexygen software. Flat-faced cylindrical probe of 50 mm diameter equipped with a load cell of 50 N and a test speed of 12

mm/min with a trigger force of 0.5 Kgf compressed the bite size piece (2 cm^3) twice in a reciprocating motion, imitating the mouth action. From the force-time plot, hardness 1- after first compression (Kgf), hardness 2- after second compression (Kgf), cohesiveness, springiness (mm) and stiffness (Kgf/ mm) were evaluated.

2.2.12 Determination of lysosomal acid phosphatase activity

The lysosomal enzyme activity was determined by the method of Warrier et al.[16]. Free, total and membrane bound lysosomal enzyme activity was determined in terms of acid phosphatase using p- nitrophenyl phosphate as substrate. The lysosomal activity was expressed as μmole of p-nitro phenol liberated/min/g tissue by the activity of acid phosphatase enzyme.

2.2.13 Determination of collagenase enzyme activity

2.2.13.1 Extraction of collagenase enzyme. Catfish muscle tissue was homogenized (1:10 w/v) with 50 mM Tris-HCl buffer containing 5 mM $CaCl_2$ (pH 7.5), was centrifuged at 12,000 rpm for 30 minutes at 4 °C. Repeated the procedure twice, pooled the supernatant and was used as the enzyme source.

2.2.13.2 Determination of collagenase enzyme activity. Collagenolytic activity was measured according to the method of Moore and Stein[17] with slight modifications. The reaction mixture of 1 ml of 50 mM Tris- HCl buffer (pH- 7.5) containing 5 mg collagen and 5 mM $CaCl_2$, and 0.1 ml of enzyme solution was incubated at 37 °C for 1 hour. The reaction was stopped by adding 0.2 ml of 50% Trichloro acetic acid. After 10 minutes of incubation at room temperature, the solution was centrifuged at 3000 rpm for 10 minutes. 0.2 ml of the supernatant was mixed with 3.8 ml of distilled water and 1 ml of ninhydrin reagent, and incubated for 15 minutes in a boiling water bath, then cooled to room temperature. The mixture was diluted with 1 ml 50% 1- propanol. The absorbance was read at 540 nm. 50 mM Tris-HCl buffer (pH- 7.5) that contained 5 mM $CaCl_2$ was used as reference. A standard curve was prepared using a solution of L- leucine amino acid. Specific activity of enzymes is calculated as the micromoles of leucine/minute/g tissue.

2.3 Statistical analysis

All statistical calculations was performed in IBM SPSS Statistics 20.0 Software. Data analysis was performed using one-way analysis of variance (ANOVA) ($p < 0.05$) with post-hoc with multiple comparison analysis performed using the Duncan test. Correlation analysis between parameters analyzed was done using Pearson Correlation.

3 Results and discussion

3.1 Change in rigor mortis and pH

Fish is regarded as one of the most sensitive foods, and as a result, storage conditions, mainly time and temperature, are the critical determinant factors of the food's quality. Stability of quality is influenced by the various physico-chemical parameters of the fish muscle tissue. Due to this, evaluation of physico-chemical changes in the fish muscle during post-mortem storage conditions is crucial. The results obtained for the progression of rigor mortis and variation in the pH level of stinging catfish muscle tissue stored at ambient temperature is given in Table 1 . The present studies exemplify that within four hours of storage, the whole stinging catfish entered the full rigor stage (78.37 %), which persisted for six hours of storage. Thereafter, a gradual decrease was noticed during the period of storage. Data obtained from the results of one-way ANOVA revealed that there is a significant level difference in rigor index ($p < 0.05$). Fresh fish muscle does have a pH value that is almost neutral (7.07). The value of pH significantly decreased ($p <0.05$) during storage, reaching a low level (6.96) in the third hour and being significantly associated with the commencement of the full rigor stage. During this stage the fish muscles were in fully stiffened condition. Diminution of ATP content is also related to the muscular pH value at in-rigor condition. It is reported that hydrolysis of ATP followed by accretion of phosphoric acid along with release of protons is strongly related to drop in pH in the muscle tissue[18]. An increase in pH value was noted when the fish proceeded through the post-rigor stage. This result is in good agreement with Tingting[19] , who stated that increase in post-mortem fish muscle tissue pH value is due to the accumulation of basic compound like amines and ammoniacal compounds arising from autolytic and microbial breakdown of complex organic compounds which serve as substrates for spoilage inducing bacteria.

3.2 Changes in adenosine nucleotide analysis

Data obtained from the analysis of adenosine nucleotide and its catabolites in muscle tissue of stinging catfish shows that there is a significant level of changes in the concentration of all nucleotides of fish muscle as storage period increases ($p < 0.05$)

Table 1. Change in rigor index and muscle pH value

Storage time (hours)	Rigor index (%)	pH
0	0.00±0.00[a]	7.07±0.03[b]
1	11.95±1.34 [b]	7.04±0.03[b]
2	52.80±1.68 [e]	6.97±0.00[a]
3	68.37±0.89 [g]	6.96±0.01[a]
4	78.37±0.88 [i]	6.97±0.02[a]
5	78.37±0.88 [i]	7.04±0.02[b]
6	78.37±0.88 [i]	7.05±0.03[b]
7	73.66±0.93 [h]	7.17±0.05[c]
8	64.49±2.11 [f]	7.24±0.00 [d]
10	53.41±2.00 [e]	7.26±0.01[d, e]
12	31.13±1.59[d]	7.26±0.01[d, e]
15	15.06±1.50 [c]	7.3±0.01[e, f]
18	10.45±2.05 [b]	7.32±0.02[f]

All values are given as mean (n=3) ± standard deviation from triplicate analysis
Different superscripts in the same column indicate significant difference ($p < 0.05$)

(Figure 1). Initial concentration of ATP in fish muscle was 2.03 μmoles/g. A steep decrease in concentration was noticed, and it decreased below 1.0 μmoles/g below after 4th hour of storage. The concentration of ADP and AMP was noticed as 2.67 and 2.29 μmoles/g, respectively and were gradually decreased throughout the storage period. Reduction in muscular ATP content typically induces the development and progress of rigor-mortis in fish muscle. Present result shows that fish species reached the full-rigor condition when ATP level in muscle tissue falls down to1μmole/g tissue. The present study suggested that postmortem unavailability of ATP for actomyosin cross-bridge breakage during muscle contraction could be the typical reason for post-mortem toughness. Initial concentration of IMP was found to be 6.27 μmoles/g and decreased to 0.95 μmoles/g by 18th hours of storage. Concentrations of inosine and hypoxanthine remain below 0.01μmoles/g till the 3rd hour of storage, and were increased to 6.96 μmoles/g and 5.62μmoles/g, respectively, showing that the rate of formation of inosine (HxR) was higher than hypoxanthine (Hx) content. Accumulation of IMP develops a pleasant flavor, while hypoxanthine brings about an off-odour in spoiled fish. High value for IMP content was found in fresh condition. Buildup of hypoxanthine concentration pointed out bacterial spoilage as well as autolytic deterioration [20]. Upon storage, accumulation of inosine was found to be higher than hypoxanthine, proving that stinging catfish is an inosine producer.

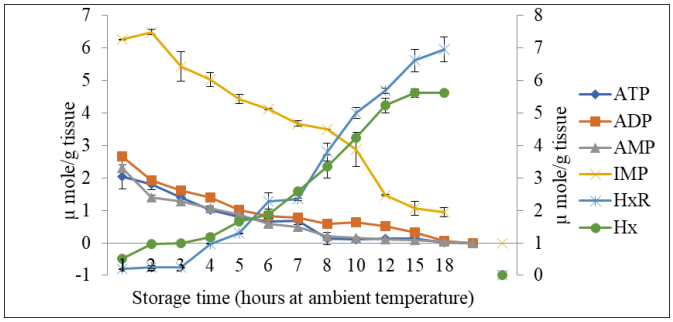

Fig 1. Changes in the concentration of adenosine triphosphate and its catabolism products. All values are expressed as mean ± standard error, n=3. On primary y-axis –ATP, ADP, AMP & IMP. On secondary y-axis –HxR & Hx

3.3 Determination of K value and sensory demerit score value

K value gives an information regarding the buildup of hypoxanthine and inosine content in the tissue, an indication of quality degradation and were increasing in a significant manner ($p < 0.01$) during storage (Table 2). The K value reached above 20% within 4th hours of storage and at that time muscular ATP level fell below 1.00 μmol/g tissue, meanwhile, the fish became

rigid at this stage (Table 2). Additionally, when K value reached above 40%, fish entered in to the post-rigor stage. The K value reached above 60% within an 8th hour in stinging catfish, which is considered as unacceptable for consumption. Results for sensory demerit score evaluation shows quality demerit score reached above five within 4 hours of storage, indicating that its quality would be excellent only for the first three hours at ambient temperature, as good for five hours and unacceptable after 7 hours of storage. Data fromTable 2 shows that there was a significant level of change in K value and sensory demerit score value during the storage period. Ihuahi et al. reported that Tilapia (Oreochromis niloticus) organoleptic acceptability was about 12 hours at room temperature [21].

Table 2. Changes in the K value and sensory demerit score value

Hours	K Value	Sensory demerit score
0	1.56±0.11 ^{a}	0.00±00^{a}
1	5.05±0.35 ^{b}	0.00±00 ^{a}
2	9.56±0.38 ^{c}	0.33±0.58^{a}
3	11.27±0.26 ^{c}	2.67±0.58^{b}
4	20.20 ±0.21^{d}	5.67±0.58^{c}
5	29.36±0.24 ^{e}	7.67±0.58^{d}
6	40.22±0.57 ^{f}	11.33±0.58^{e}
7	46.82±0.14 ^{g}	20±1.00^{f}
8	61.72±1.08^{h}	24.33±0.58^{g}
10	70.88 ±0.34^{i}	28.33±1.15^{h}
12	83.07±0.41 ^{j}	31.67±0.58^{i}
15	88.53±0.44 ^{k}	32±0.00^{i}
18	92.34±0.22 ^{l}	32±0.00^{i}

All values are expressed as mean, n=3. Different superscripts in the same column indicate significant difference ($p < 0.01$)

3.4 Determination of expressible water content, water holding capacity and cook loss

In order to study the quality changes in stinging catfish stowed at ambient temperature, changes in expressible water content, water holding capacity and cook loss during storage period was also conducted and is given in Table 3. The Expressible water content in the fresh condition was noticed as 9.32% and increased gradually throughout the storage period ($p < 0.05$). 63.35 percent of increases in cook loss were noticed during the storage period in stinging catfish muscle during the storage period ($p < 0.05$). Small increase in water holding capacity was noticed at the pre-rigor stage and thereafter a steep decrease in water holding capacity was observed when the whole fish was entered into the post-rigor condition ($p < 0.05$) and it is inversely related to the change in cook loss and expressible water content.

Another causative factor for the water loss from tissue is the change in pH value during post-mortem storage. According to a survey, the breakdown of ATP molecules and a drop in pH could each account for one third of the decline in the WHC of fish muscle tissue [22]. Results obtained from the correlation analysis shows that there is a significant level of correlation between muscle pH with WHC, EWC and CL ($r < 0.05$). More precisely, change in muscle pH inversely linked to the WHC and directly proportional to the EWC and CL, confirmed that pH variation in the post-mortem fish muscle tissue significantly influenced its water holding capacity and hence expressible water content. This result is strongly supported by Lin [23], who testified that the water holding capacity and pH are interrelated.

3.5 Protein fractions

Functional properties of the proteins will govern the structure–stability relationships in the muscle tissue [24]. Post-mortem storage causes the denaturation of fish muscle protein by altering the structural level organization. Protein fractions from the muscle tissue of catfish also shown some variation ($p < 0.01$) (Figure 2). The initial concentration of sarcoplasmic protein in fish muscle was 13.71%, which decreased to 7.62% by the 18th hour of storage. Compared to other fractions, only a slight decrease of sarcoplasmic protein was noticed in muscle tissue fractions, proving that the sarcoplasmic protein content displayed a certain degree of stability during the post-mortem storage. Peculiarity of sarcoplasmic protein is the presence of highly active autolytic enzymes. Additionally, leaching reduces the total sarcoplasmic protein in the muscle tissue extract considerably during the later period. Concentration of myofibrillar protein and denatured protein at the initial stage of storage were 44.87% and

Table 3. Postmortem changes in expressible water content, water holding capacityand cook loss

Storage time (Hours)	EWC (%)	WHC (%)	CL (%)
0	9.32±1.02 ^{a}	70.06±0.91^{e,f}	10.48±1.20^{a}
1	10.02±0.89^{a}	69.44±0.93^{e,f}	9.82±2.02 ^{a}
2	9.03±1.02^{a}	70.25±0.48^{e,f}	9.73±1.92 ^{a}
3	9.02±2.04^{a}	70.91±2.22^{f}	10.20 ±1.11^{a}
4	9.98±1.01^{a}	69.14±4.73^{e,f}	11.90±3.93^{a,b}
5	10.02±2.72^{a}	70.50±0.27^{f}	13.44±2.44^{a,b}
6	10.74±3.98^{a}	66.67±2.70^{e,f}	16.44±4.80^{b,c}
7	13.55±2.48^{a,b}	65.57±1.39^{d,e}	19.97±0.16^{c}
8	15.55±4.50^{b,c}	61.42±1.50^{c,d}	21.38±4.55^{c}
10	17.48±2.94^{b,c}	58.33±0.33^{c}	27.42±4.19^{d}
12	19.31±2.99^{c}	54.04±0.80^{b}	32.02±1. 46^{d,e}
15	20.00±2.13^{c}	52.16±5.32^{b}	32.97±3.61^{e}
18	25.92±2.80^{d}	47.15±3.21^{a}	35.59±2.78^{d,e}

EWC-Expressible water content, WHC- Water holding capacity, CL- Cook loss. Values are given as mean ± standard error from triplicate determinations. Different superscripts in the same column indicate significant differences ($p < 0.05$).

39.65%, respectively. A gradual decline in the myofibrillar protein along with an upsurge in denatured protein was observed with the progress of post-mortem storage time. Feng et al also reported that in Pseudosciaena crocea, the myofibrillar protein was decreases with increase in storage time in slurry ice[25]. Total collagen in the fish muscle declined from 1.12% to 0.36% during the storage period. It was reported that reduction in collagen content was noticed over storage time in farmed sea breammuscle and was significantly related to the total firmness but inversely related to the water-holding capacity[4].

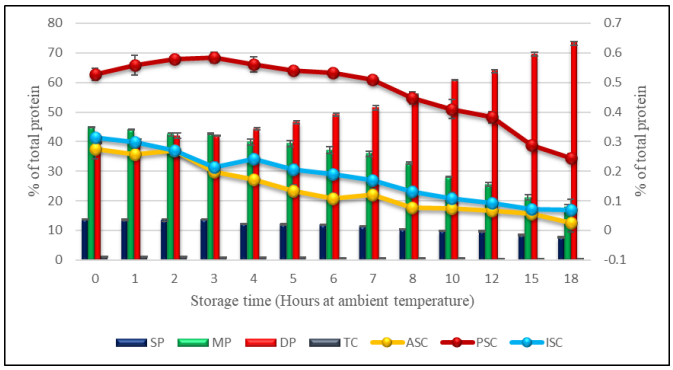

Fig 2. Protein fractions in the muscle tissue. SP- Sarcoplasmic protein, MP- Myofibrillar protein, ASP-Alkali soluble protein, TC- Total collagen, ASC-Acid soluble collagen, PSC- Pepsin soluble collagen, ISC-Insoluble collagen. All values are expressed as mean ± standard deviation, n=3. On primary y-axis – SP, MP, DP, TC. On secondary y-axis –ASC, PSC, ISC

3.6 Collagen fractionation

By considering the collagen fractions, the pepsin soluble collagen constitutes the highest concentration in the fresh catfish muscle tissue whereas acid soluble collagen constitutes the lowest concentration (0.52% and 0.27%, respectively of the total protein concentration). The insoluble collagen concentration in the fresh muscle tissue of catfish was 0.31% of total protein content (Figure 2). The pepsin soluble collagen content remained nearly stable for the first four hours, followed by a subsequent 52% reduction in its concentration at the end of storage period. A gradual reduction in acid soluble and insoluble collagen were noticed during the study. The results also show that there was a 91% depletion in acid soluble collagen noticed in muscle tissue on the last hours of storage, whereas insoluble collagen showed only 77% of reduction during the same storage period. The results obtained from statistical analysis shows that all the collagen fractions were significantly varied as the post-mortem storage period advanced ($p < 0.01$). Additionally, compared to acid soluble and insoluble collagen, the pepsin soluble collagen

was less susceptible to post-mortem proteolysis confirming that the pepsin soluble collagen is crucially responsible for post-rigor softening than two other fractions. This high concentration of pepsin soluble collagen content points out a high initial proportion of collagen with cross-links and hence of great firmness. Studies of Monika et al[26] also supported the present work, who reported a remarkable level of elevation in total collagen and acid soluble collagen content along with a depletion in insoluble collagen in bovine M. infraspinatus during aging between 5th to 10th day in vacuum at 3 °C.

3.7 Textural profile analysis

Results of catfish muscle tissue textural profile analysis in-terms of hardness 1 and hardness 2, stiffness, springiness, and cohesiveness is depicted in Table 4. A minor level increase in the values for hardness, stiffness and cohesiveness noticed in the early post-mortem storage period. Formation of a three-dimensional protein network could be a reason for the increase in hardness and stiffness together with actomyosin cross bridge formation during pre-rigor storage conditions at ambient temperature. Detachment of myofibers from sarcolemma- endomysium and myocommata along with degradation of collagen and myofibrillar protein could be the reason for reduction in post-rigor muscle tissue hardness. Studies have been reported that reduction in toughness and successive drop in connective tissue total strength is typically due to the denaturation of collagen, resulted in shrinkage followed by gelation of collagen. It was previously demonstrated that structural and textural changes in fish products during traditional and sous1 vide cooking were due to denaturation and gelation of collagen[27].

Table 4. Textural profile analysis in the stinging catfish muscle tissue

Storage time (hours)	Hardness 1 (kgf)	Hardness 2 (kgf)	Springiness(mm)	Stiffness (kgf/mm)	Cohesiveness
0	0.43±0.00 ^{e,f}	0.18±0.01 ^{g}	0.89 ±0.01^{d}	0.59 ±0.15^{b,c}	0.08±0.00 ^{e}
1	0.37±0.03 ^{c,d,e}	0.14±0.01 ^{f}	0.68±0.01 ^{c}	0.64±0.05 ^{c,d}	0.07±0.00 ^{e}
2	0.46±0.06 ^{f}	0.11±0.00 ^{e,f}	0.64 ±0.01^{c}	0.79 ±0.00^{d,e}	0.14±0.02 ^{g}
3	0.45±0.03^{e,f}	0.14±0.00 ^{f}	0.61±0.02 ^{c}	0.83 ±0.00^{e}	0.12 ±0.01^{f}
4	0.48±0.06 ^{e,f}	0.07±0.04 ^{c,d}	0.40 ±0.10^{a,b}	0.87 ±0.05^{g}	0.13±0.02 ^{f,g}
5	0.48±0.02 ^{f}	0.11±0.01^{e,f}	0.58 ±0.03^{a,b}	0.94 ±0.05 ^{h}	0.04 ±0.00^{a}
6	0.41 ±0.01^{d,e,f}	0.09±0.01 ^{d,e}	0.54±0.03 ^{b}	0.85±0.00 ^{f}	0.06±0.01^{d,e}
7	0.34±0.00 ^{c,d}	0.09 ±0.00^{d,e}	0.45±0.04 ^{b}	0.84±0.17 ^{e}	0.06 ±0.01^{d,e}
8	0.30 ±0.00^{c}	0.09±0.01 ^{d,e}	0.46 ±0.07^{b}	0.77±0.03 ^{d,e}	0.04±0.01 ^{c,d}
10	0.21±0.06 ^{b}	0.04 ±0.01^{b,c}	0.34 ±0.02^{a,b}	0.65 ±0.15^{c,d}	0.04±0.00 ^{b,c}
12	0.20 ±0.00^{a,b}	0.04±0.00 ^{a,b}	0.34±0.01 ^{a,b}	0.65±0.01 ^{c,d}	0.04 ±0.00^{a,b,c}
15	0.13±0.01 ^{a}	0.02 ±0.00^{a}	0.29 ±0.02^{a}	0.49±0.12 ^{a,b}	0.02±0.00 ^{a,b}
18	0.10±0.04 ^{a,b}	0.01±0.00 ^{a}	0.25 ±0.01^{a,b}	0.59±0.15 ^{a}	0.01±0.00^{a,b}

All values are expressed as mean ± standard error, n=3. Different, superscripts in the same column indicates significant difference ($p < 0.01$).

A decreasing trend is also noticed in springiness over the storage period ($p < 0.01$), indicating the loss of elastics recovering property of the muscle tissue. The result obtained for correlational analysis also shows that the total hardness and stiffness of the catfish muscle tissue is more dependent on the concentration of myofibrillar protein than collagen (Table 5). Cohesiveness is a functionality of collagen, whereas springiness is equally influenced by both collagen and myofibrillar protein.

Table 5. Correlation between muscle tissue protein and textural profile parameters

		Hardness1	Hardness2	Cohesiveness	Springiness	Stiffness
MP	Pearson Correlation	.938**	.913**	.715**	.930**	.633*
	Sig. (2-tailed)	.00	.00	.006	.000	.020
TC	Pearson Correlation	.907**	.886**	.769**	.937**	.546
	Sig. (2-tailed)	.00	.00	.006	.000	.053

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). MP: Myofibrillar protein TC: Total collagen.

3.8 Determination of autolytic enzyme activity

Evaluation of total, bound and free lysosomal enzyme activity were evaluated in terms of acid phosphatase enzyme activity were evaluated (Table 6) in the stinging catfish muscle during postmortem storage period stored at ambient temperature and gives relevant information regarding their role in post-mortem softening. It was reported that 40-60% of acid phosphatase enzymes were bound to the membrane of lysosomes [28]. The results obtained for lysosomal acid phosphatase activity and collagenase enzyme activity is given in Table 6. The data shows that the free and bound lysosomal enzyme activity in the fresh catfish muscle tissue were 1.2742 mM p-nitrophenol/g tissue/min and 2.6194 mM p-nitrophenol/g tissue/min respectively. There was a 49% reduction in the bound enzyme activity noticed at the end of the storage period. While free enzyme activity increased with increase in storage period. Additionally, a slight reduction in their activity was also noticed by the end of the storage period. Results obtained thus reveal the instability of the lysosomal membrane and leakage of stored lytic enzymes into the cytoplasm. Besides this, leaching of water-soluble proteins from the muscle tissue resulted in a reduction in free and total enzyme activity during the later stage of post-mortem storage.

Table 6. Autolytic enzyme activity inthe muscle tissue of stinging catfish

Hours	Total lysosomal enzyme activity	Bound lysosomal enzyme activity	Free lysosomal enzyme activity	Collagenase activity
0	$3.8936\pm0.0615^{b,c}$	2.6194 ± 0.0929^{f}	1.2742 ± 0.0315^{a}	0.0128 ± 0.0185^{a}
1	$3.9503\pm0.1635^{b,c,d}$	2.5602 ± 0.1631^{f}	$1.3901\pm0.0004^{a,b}$	0.0667 ± 0.0052^{a}
2	$3.9927\pm0.0867^{b,c,d,e}$	$2.4794\pm0.0833^{e,f}$	1.5133 ± 0.0034^{b}	0.2497 ± 0.0329^{b}
3	$4.1184\pm0.2978^{d,e}$	$2.2451\pm0.0371^{d,e}$	1.8733 ± 0.0241^{c}	0.3485 ± 0.0209^{c}
4	$4.1243\pm0.0106^{d,e}$	$2.0174\pm0.0285^{c,d}$	$2.1069\pm0.0391^{d,e}$	0.7705 ± 0.0161^{d}
5	$4.1138\pm0.1293^{d,e}$	1.9506 ± 0.0628^{c}	1.9506 ± 0.0665^{e}	0.8876 ± 0.0079^{e}
6	$4.1225\pm0.0748^{d,e}$	1.8716 ± 0.1749^{c}	2.2509 ± 0.0059^{e}	0.9842 ± 0.0147^{f}
7	4.1796 ± 0.199^{e}	$1.7851\pm0.1364^{c,d}$	$2.1618\pm0.0189^{d,e}$	1.1062 ± 0.0095^{g}
8	$4.0361\pm0.1065^{c,d,e}$	$1.6869\pm0.0638^{c,d}$	$2.0062\pm0.0427^{c,d}$	1.2651 ± 0.0216^{h}
10	$3.9098\pm0.0858^{b,c}$	1.5359 ± 0.113^{c}	$1.9944\pm0.0577^{c,d}$	1.3752 ± 0.0095^{i}
12	3.9112 ± 0.1015^{b}	$1.4975\pm0.0854^{b,c}$	$2.0442\pm0.0856^{c,d}$	1.5437 ± 0.0069^{j}
15	3.5759 ± 0.1883^{a}	$1.4689\pm0.0748^{a,b}$	$2.0193\pm0.0709^{c,d}$	1.6889 ± 0.0242^{k}
18	3.4824 ± 0.2263^{a}	1.3315 ± 0.0633^{a}	$2.1509\pm0.0077^{d,e}$	1.7155 ± 0.0079^{k}

Lysosomal enzyme activity was expressed as mM p-nitro phenol/g tissue/min.Collagenase enzyme activity was expressed as μmole of leucine/g tissue/min.TA-Total activity, FA- Free activity, BA- Bound activity.All values are expressed as mean $\pm$ standard deviation, n=3.Different, superscripts in the same column indicates significant difference ($p < 0.01$)

Degradation of collagen molecules in the muscle tissue results in 'gaping' followed by unusual softening. Studies of Manuela reported that action of collagenase enzyme activity is more responsible than proteolytic cleavage of myofibrillar protein in postmortem softening of ice stored cod muscle [29]. Data obtained in the present study says that the activity of collagenase enzyme activity increases with the progress of storage time, establishing the role of collagenase enzyme in the post-mortem fish muscle softening. The results obtained are strongly supported by the studies of Suarez et al [4], who says that post-mortem softening of muscle tissue is mainly a by-product of specific collagenase activity. It was also reported that, the initial degradation of collagen fibril is specifically by collagenase enzymes, once the primary cleavage has been introduced, further breakdown could be catalyzed by other non-specific proteases enzymes [30].

Collagenase enzyme activity was low till the 3rd hours of storage, thereafter a steep increase with storage period was noticed. Proteolytic activity of collagenases enzymes showed highest degree in the post-rigor stage than the pre-rigor stage. The degradation pattern of pepsin soluble collagen in muscle tissue is related to the activity of collagenase enzymes. It was reported that the matrix metalloproteases and collagenase enzymes will degrade the helical region in collagen fibrils [31]. Furthermore, the telo-collagen present in the non-helical region of collagen are rich in acetic acid soluble collagens, while pepsin soluble collagens are atelo-collagen, the helical region of collagen [32,33]. Additionally, the serine proteases and Cathepsin L will hydrolyses the acid soluble collagen present in telo-collagen (Yamashita & Konagaya, 1991). Thus, the high proteolytic activity of collagenase enzyme in post-rigor stage and degradation pattern of pepsin soluble collagen pointing out their involvement in postmortem muscle softening in stinging catfish.

4 Conclusion

Assessment of physico-chemical and functional attributes of stinging catfish pointed out that there should be an ultimate post-harvest management practices pivotal for hindrance of endogenous quality deterioration. Present study concluded that total hardness and stiffness of the stinging catfish is more related to myofiibrillar protein than collagen, while cohesiveness is a functionality of collagen than myofibrillar protein. It was also confirmed that the firmness of stinging catfish is due to high pepsin soluble collagen. Additionally, post-rigor softening is highly influenced by pepsin soluble fractions than acid soluble and insoluble collagen fractions. But histochemistry and enzymology of post-mortem fish muscle may also to be included in the study in order to get a clear and complete picture regarding the postmortem degradation of stinging catfish muscle tissue.

References

1) Shahadat H, M, Sarker S, Sharifuzzaman SM, Chowdhury SR. New species of stinging catfish Heteropneustes nani (Siluriformes: Heteropneustidae) from Noakhali. *Vertebrate Zoology*. 2013;63(3):259–267. Available from: https://www.senckenberg.de/wp-content/uploads/2019/08/03_vertebrate_zoology_63-3_hossain_259-267.pdf.
2) Ghaly AE, Budge DD, Brooks S, S M. Fish Spoilage Mechanisms and Preservation Techniques: Review. *American Journal of Applied Sciences*. 2010;7(7):859–877. Available from: https://doi.org/10.3844/ajassp.2010.859.877.
3) Prabhakar SPK, Vatsa, Sant S, Pathak. A Comprehensive Review on Freshness of Fish and Assessment: Analytical Methods and Recent Innovations. *Food Research International*. 2020;133:109157. Available from: https://doi.org/10.1016/j.foodres.2020.109157.
4) Suárez MD, Abad M, Ruiz-Cara T, Estrada JD, García-Gallego M. Changes in muscle collagen content during post mortem storage of farmed sea bream (Sparus aurata): influence on textural properties. *Aquaculture International*. 2005;13(4):315–325. Available from: https://doi.org/10.1007/s10499-004-3405-6.
5) Sato K, Ando M, Kubota S, Origasa K, Kawase H, Toyohara H, et al. Involvement of Type V Collagen in Softening of Fish Muscle during Short-Term Chilled Storage. *Journal of Agricultural and Food Chemistry*. 1997;45(2):343–348. Available from: https://doi.org/10.1021/jf9606619.
6) Koohmaraie M, Geesink GH. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science*. 2006;74(1):34–43. Available from: https://doi.org/10.1016/j.meatsci.2006.04.025.
7) Ahmed Z, Donkor O, Street WA, Vasiljevic T. Calpains- and cathepsins-induced myofibrillar changes in post-mortem fish: Impact on structural softening and release of bioactive peptides. *Trends in Food Science & Technology*. 2015;45(1):130–146. Available from: https://doi.org/10.1016/j.tifs.2015.04.002.
8) Bito M, Yamada K, Mikumo Y, Amano K. Studies on rigor mortis of fish: Differences in the mode of rigor-mortis among some varieties of fish by modified cuttings method. *Bulletin of Tokai Regional Fisheries Research Laboratory*. 1983;109:89–96. Available from: https://agris.fao.org/agris-search/search.do?recordID=JP19840076545.
9) Ryder JM. Determination of adenosine triphosphate and its breakdown products in fish muscle by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*. 1985;33(4):678–680. Available from: https://doi.org/10.1021/jf00064a027.
10) Saito T, Arai KI, Matsuyoshi M. A New Method for Estimating the Freshness of Fish. *Nippon Suisan Gakkaish*. 1959;24(9):749–750. Available from: https://doi.org/10.2331/suisan.24.749.
11) Bremner HA, Hallett IC. Muscle Fiber?Connective Tissue Junctions in the Fish Blue Grenadier (Macruronus novaezelandiae). A Scanning Electron Microscope Study. *Journal of Food Science*. 1985;50(4):975–980. Available from: https://doi.org/10.1111/j.1365-2621.1985.tb12993.x.
12) Borresen T. New methods for determination of liquid holding capacity, salt water holding capacity and cook loss in fish muscle. Tromso, Norway. FTFI. 1980.
13) Benjakul S, Visessanguan W, Tueksuban J. Changes in physico-chemical properties and gel-forming ability of lizardfish (Saurida tumbil) during post-mortem storage in ice. *Food Chemistry*. 2003;80(4):535–544. Available from: https://doi.org/10.1016/S0308-8146(02)00339-4.
14) Devadasan K, Nair MR. Observations on changes in the major protein nitrogen fraction of prawns and sardines during ice storage. *Fishery Technology*. 1970;7(2):195–197. Available from: https://core.ac.uk/download/pdf/33720228.pdf.
15) Nishimoto M, Sakamoto R, Mizuta S, Yoshinaka R. Identification and characterization of molecular species of collagen in ordinary muscle and skin of the Japanese flounder Paralichthys olivaceus. *Food Chemistry*. 2005;90(1-2):151–156. Available from: https://doi.org/10.1016/j.foodchem.2004.03.034.
16) Warrier S, Ninjoor V, Sawant PL, Kumta US. Lysosomal enzymes in drip & muscle of Harpodon nehereus. *Indian Journal of Biochemistry and Biophysics*. 1972;9(2):207–209. Available from: https://www.osti.gov/biblio/4384276.
17) Moore S, Stein W. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *Journal of Biological Chemistry*. 1954;211(18):71178. Available from: https://www.osti.gov/biblio/4384276.
18) Daskalova A. Farmed fish welfare: stress, post-mortem muscle metabolism, and stress-related meat quality changes. *International Aquatic Research*. 2019;11(2):113–124. Available from: https://doi.org/10.1016/S0021-9258(18)71178-2.
19) Li T, Li J, Hu W. Changes in microbiological, physicochemical and muscle proteins of post mortem large yellow croaker (Pseudosciaena crocea). *Food Control*. 2013;34(2):514–520. Available from: https://doi.org/10.1007/s40071-019-0230-0.
20) Li J, Zhou G, Xue P, Dong X, Xia Y, Regenstein J, et al. Spoilage microbes' effect on freshness and IMP degradation in sturgeon fillets during chilled storage. *Food Bioscience*. 2021;41:101008. Available from: https://doi.org/10.1016/j.fbio.2021.101008.
21) Adoga IJ, Egila J, Samuel OF. Storage life of tilapia (Oreochromis niloticus) in ice and ambient temperature. *Researcher*. 2010;2(5):39–44. Available from: http://www.sciencepub.net/researcher/research0205/06_2727_research0205_39_44.pdf.
22) Ali H, Nawaz K, Amoa, Qi Y, Leng, Jia H, et al. Poultry Response to Heat Stress: Its Physiological, Metabolic, and Genetic Implications on Meat Production and Quality Including Strategies to Improve Broiler Production in a Warming World. *Frontiers in Veterinary Science*. 2021;8:1–16. Available from: https://doi.org/10.3389/fvets.2021.699081.
23) Lin SB, Chen LC, Chen HH. The change of thermal gelation properties of horse mackerel mince led by protein denaturation occurring in frozen storage and consequential air floatation wash. *Food Research International*. 2005;38(1):19–27. Available from: https://doi.org/10.1016/j.foodres.2004.08.001.
24) Ochiai Y, Ozawa H. Biochemical and physicochemical characteristics of the major muscle proteins from fish and shellfish. *Fisheries Science*. 2020;86(5):729–740. Available from: https://doi.org/10.1007/s12562-020-01444-y.

25) Guan F, Chen Y, Zhao S, Chen Z, Yu C, Yuan Y. Effect of slurry ice during storage on myofibrillar protein of Pseudosciaena crocea. *Food Scicience & Nutrition*. 2021. Available from: https://doi.org/10.1002/fsn3.2355.
26) Modzelewska-Kapituła M, Kwiatkowska A, Jankowska B, Dąbrowska E. Water holding capacity and collagen profile of bovine m. infraspinatus during postmortem ageing. *Meat Science*. 2015;100:209–216. Available from: https://doi.org/10.1016/j.meatsci.2014.10.023.
27) Moreno HM, Montero MP, Gómez-Guillén MC, Fernández-Martín F, Mørkøre T, Borderías J. Collagen characteristics of farmed Atlantic salmon with firm and soft fillet texture. *Food Chemistry*. 2012;134(2):678–685. Available from: https://doi.org/10.1016/j.foodchem.2012.02.160.
28) Liliana G, Fidalgo JA, Saraiva SP, Aubourg M, Vázquez JA, Torres. Enzymatic activity during frozen storage of atlantic horse mackerel (Trachurus trachurus) pre-treated by high-pressure processing. *Food and Bioprocess Technology*. 2015;8(3):493–502. Available from: https://doi.org/10.1007/s11947-014-1420-9.
29) Hernández-Herrero MM, Duflos G, Malle P, Bouquelet S. Collagenase activity and protein hydrolysis as related to spoilage of iced cod (Gadus morhua). *Food Research International*. 2003;36(2):141–147. Available from: https://doi.org/10.1016/S0963-9969(02)00129-1.
30) Shigemura Y, Ando M, Tsukamasa Y, Makinodan Y, Kawai T. Correlation of type V collagen content with post-mortem softening of fish meat during chilled storage. *Fisheries Science*. 2003;69(4):842–848. Available from: https://doi.org/10.1046/j.1444-2906.2003.00696.x.
31) Alderton AL, Means WJ, Kalchayanand N, Mccormick RJ, Miller KW. Bovine metalloprotease characterization and in vitro connective tissue degradation1. *Journal of Animal Science*. 2004;82(5):1475–1481. Available from: https://doi.org/10.2527/2004.8251475x.
32) Ali AMM, Benjakul S, Kishimura H. Molecular characteristics of acid and pepsin soluble collagens from the scales of golden carp (Probarbus jullieni). *Emirates Journal of Food and Agriculture*. 2017;29(6):450–450. Available from: https://doi.org/10.9755/ejfa.2016-09-1316.
33) Nalinanon S, Benjakul S, Visessanguan W, Kishimura H. Use of pepsin for collagen extraction from the skin of bigeye snapper (Priacanthus tayenus). *Food Chemistry*. 2007;104(2):593–601. Available from: https://doi.org/10.1016/j.foodchem.2006.12.035.