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Postmortem autolytic changes of iced stored banded snakehead (*Channa striata*) (Bloch, 1793)

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Abstract
The autolytic changes of iced stored banded snakehead (*Channa striata*) (Bloch, 1793) was assessed through evaluation of the changes in protein solubility, textural attributes and proteolytic enzyme over 18 days storage period. Study revealed that myofibrillar protein decreased in a significant manner while amount of denatured protein increased throughout the entire study. Total collagen content muscle was stable up to 7th day of incubation and a decrease in this variable was observed. A decrease in acid soluble and insoluble collagen fractions were observed throughout the study, while a small increase in the pepsin soluble collagen till 7th day of incubation was decreased thereafter. Collagenolytic and enzymes activity reaches the maximum level within 3rd day of incubation and are directly involved in the postmortem collagen degradation. An increase in free and decrease in bound lysosomal enzyme activity indicated the instability of lysosomal membrane.

Keywords: Iced stored, collagen, myofibrillar, texture

1. Introduction
Postmortem fish musculature are generally susceptible to undergo many biochemical changes, leading to the development of undesirable, un-usual textural changes like soft or mushy texture and this definitely cause problems for the food industry. These physicochemical changes are mainly induced by the influence of endogenous proteases enzymes [1]. The fish muscle textural changes are influenced by ante and postmortem factors, the antemortem factors includes physical and chemical properties of fish species. The postmortem factors include the rate and extent of pH decline, rigor mortis, rate and extent of proteolysis causing breakdown of myofibrils and connective tissue, and storage condition [2]. Hydrolysis of myofibrillar and connective tissue protein by endogenous proteolytic enzymes is important in the early deterioration process [3]. The enzymatic activities are dependent on fish species and its maximum active pH values close to neutrality or higher. Proteins are degraded into large fragments, and this enhances the susceptibility of the proteins to other proteinases [4]. During chilled storage, an attachment between muscle fibers and myocommata, and the whole sarcolemma degraded, muscle fibers in fish muscle detached from the myocommatal sheets [5, 6]. Degradation of collagenous protein due to collagenolytic activity is a crucial problem [3]. Endogenous collagenolytic enzyme may break down the connective tissue in the fish muscle and thereby lead to undesirable textural changes and gaping [5, 7]. Lysosomal proteases are also related to the turnover of the fish during postmortem spoilage. Cathepsins are lysosomal acid proteases synthesized as zymogen form and are released at sites of injury or upon cold storage of postmortem muscle [8]. Upon muscle storage, as a consequence of lysosomes breakdown, cathepsins enzymes are released from the lysosome into the cytoplasmic fluid and intracellular spaces. Due to wide pH range in activity, Cathepsins D and L have an important role in the autolytic degradation whereas other cathepsins are more active at pH values too low than that of physiological significance. Textural and biochemical changes during iced storage of Sutchi cat fish steaks during refrigerated storage have been reported [9]. They investigated that storage in ice seriously influence the textural properties, and therefore triggered an interest to investigate the textural and biochemical properties of banded snakehead. The normal procedure for preserving high quality of fresh fish, was to store the fish in ice. Therefore in this study, the effects of storage conditions on proteolytic enzymes, muscle proteins and textural properties of banded snake Head were investigated.

2. Materials and Methods

2.1 Sample collection

Fish samples were procured from a local fish market, Tiruvalla, Kerala, India. They were caught by the cast net from the nearby paddy fields by fishermen and transported to the market in live condition and later transferred to the laboratory in a water container to immerse the live fish. The banded snakehead was slaughtered (stunned by a blow to the head), kept in plastic boxes with ice (ice: fish is 0.5:1) and stored in a refrigerated chamber (4 ^{0}C) for a period of 18 days. During storage, ice was periodically added and replenished every 12hrs. The day of slaughter was defined as day 0. Fish samples were retrieved at 0, 1, 3, 5, 7, 9, 12, 15 and18 days of storage in ice and used for physicochemical analysis. Muscle between the gills and the dorsal fins were used for analyses in triplicates.

2.2 Measurement of the pH Value

One gram of pooled muscles was homogenized in 10mL of distilled water, and then the pH values were determined with a pH meter (ELICO's pH Meter LI 127).

2.3 Solubility properties of proteins

Proteins were extracted from white muscle by the modified method of Devadasan and Nair [10] and collagen fractionation was carried out according to the method of Zhang *et al.* [11]. The amount of proteins in the extracts were determined in terms of nitrogen using Micro-Kjeldhal method.

2.4 Water holding capacity

Water holding capacity (WHC) was determined according to the method described by Borresen [12] with slight modifications. Homogenized sample (2 g) was centrifuged at 4500 rpm for 15 minute, through a filter that allows the water to be removed from the muscle. After centrifugation, the fillets were weighed again, and the difference before and after centrifugation was calculated. The moisture content was determined, and used to calculate the water holding capacity. Water holding capacity is presented as % remaining sample after centrifugation. WHC was calculated as the ratio of water remaining compared to the water content in the sample before.

2.5 Expressible water content

Expressible moisture content (EWC) was measured according to the modified method of Benjakul [13]. 2 g of test sample was placed between 6 filter papers and a standard weight (5 kg) was placed on the top of the sample and maintained for 2 minutes. The sample was then removed and weighed again. EMC was calculated and expressed as percentage of sample weight.

2.6 Texture properties analysis

Texture Analyzer (Lloyd Instruments, UK, model LRX PLUS) and Nexygen software (Lloyds Instruments) was used for instrumental texture analysis. Flat-faced cylindrical probe of 50 mm diameter equipped with a load cell of 50 N and a test speed of 12mm/min compressed the bite size piece (1 cm^3) twice in a reciprocating motion, imitating the mouth action. Samples were subjected to a double compression of 40%; and probe test speed and trigger force were maintained at 12 mm/min and 0.5 Kgf, respectively. From the force-time plot, hardness 1- after first compression (Kgf), hardness 2- after second compression (Kgf), cohesiveness, springiness(mm) chewiness, adhesiveness(Kgf/ mm) and stiffness (Kgf/ mm) were evaluated.

2.7 Analysis of lysosomal enzymes

The analyses of lysosomal enzymes were done according to the method adopted by Mohanan [14]. The total, free and bound lysosomal specific activity was expressed in terms of acid phosphatase as µg of *p*-nitro phenol liberated/ min/mg protein.

2.8 Analysis of collagenase enzymes

Collagenase enzyme was extracted and measured according to Park [15]. The concentration of the hydrolyzed amino acids was determined by a standard curve with L- leucine.

2.9 Statistical analysis

Microsoft Excel for Windows 7 was used to calculate Statistics mean and standards deviation values for the different quality characteristics. One way ANOVA and regression analysis were conducted using PASW Statistics 18 to analyze the variation within the samples.

3. Results

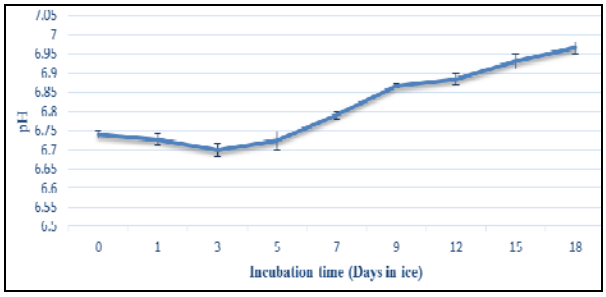
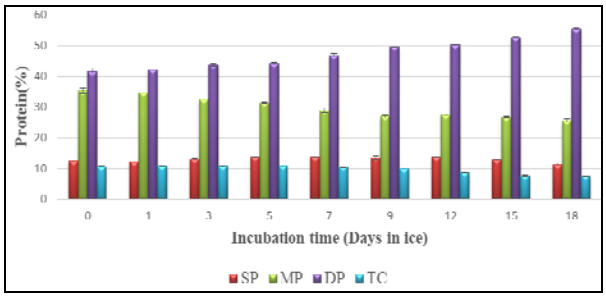


Fig 1: Changes in pH of banded snakehead muscle fraction stored at iced condition

3.1 Change in pH value

Fig.1 showed change in pH value stored in iced condition for 18 days. The pH value in fish muscle immediately after death was 6.94 and was decreased to 6.82 on 3rd day of incubation and thereafter a gradual increase was observed (r^2=0.89, p<0.05).



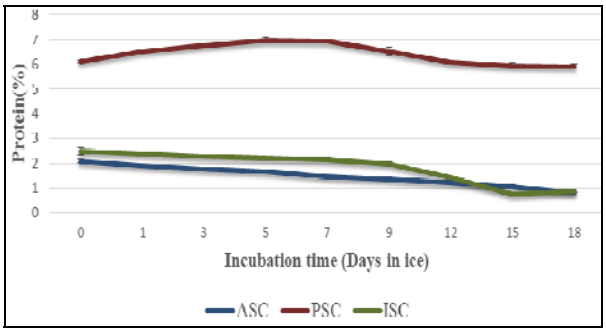
SP: Sarcoplasmic Protein; MP: Myofibrillar protein; DP: Denatured protein; TC: Total collagen

Fig 2: Changes protein solubility changes in banded snakehead stored at iced condition

3.2 Protein solubility changes

Fig. 2 showed the sarcoplasmic, My ofibrillar, denatured protein and total collagen content in banded snakehead during the storage period of 18 days in ice. Snakehead muscle showed no significant variation in the sarcoplasmic protein content (r2=0.04, p>0.05), only up to 9% reduction were observed during the incubation period. The Myofibrillar protein solubility gradually decreased during entire storage period in ice for 18 days. Solubility of Myofibrillar protein decreased from 35.31% to 25.57% (r^2= 0.8838, p<0.05) and denatured

protein concentration increased as incubation time proceeded and increased from 41.59% to 55.48% (r2=0.98, $p<0.05$). The collagen content in the fish muscle was stable up to 7th day of incubation and a decrease in this variable was observed thereafter (r^2=0.89, $p<0.05$).

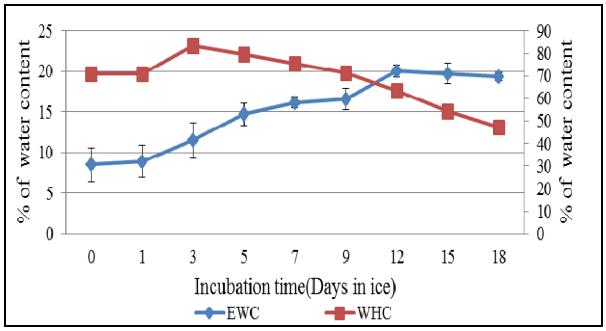


ASC: Acid soluble collagen; PSC: Pepsin soluble collagen; ISC: Insoluble collagen

Fig 3: Change in collagen fractions in banded snakehead stored at iced Condition

3.3 Change in collagen fractions

Fig.3 showed change in acid soluble, pepsin soluble and insoluble collagen fractions of muscle in banded snakehead during the storage period of 18 days in ice. It was observed that the acid soluble and insoluble collagen content decreased throughout the entire study. A steep decrease in insoluble collagen content was noticed after 9th day of incubation on ice. Fish muscle show a small increase in the pepsin soluble collagen content to fifth day of incubation and continued till seventh day and further decreased thereafter throughout the study. Statistical studies showed that there was a significant variation between the days of incubation in ice ($p<0.05$).



WHC- water holding capacity, EWC-Expressible water content

Fig 4: Changes in water holding capacity and expressible water content of banded snakehead muscle fraction stored at iced condition

3.4 Water holding capacity and expressible water content

The changes in WHC and EWC in muscles of banded snakehead during 18 day of incubation is in fig. 4. Upon incubation in ice, the fish muscle showed a variation in water holding capacity and decreased from 70.99 to 47.17 (r^2- 0.67, $P<0.05$) and total expressible water content was increased from 8.52 to 19.36 (r^2=0.87, $p<0.05$). An increase in the expressible water content up to ninth day of incubation time in ice was observed.

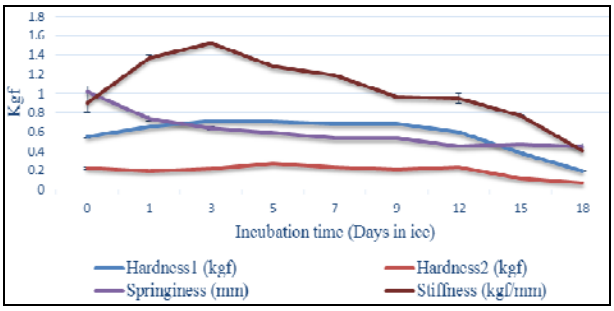


Fig 5: Changes in textural profile of banded snakehead muscle fraction stored at iced condition

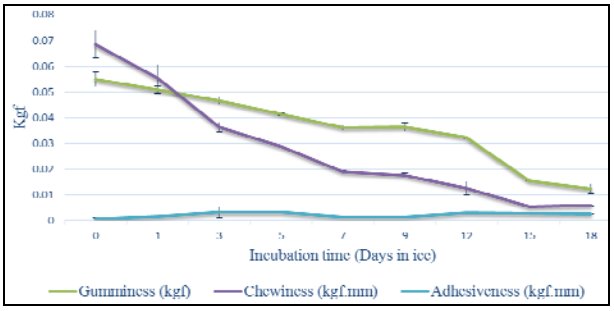


Fig 6: Changes in texture profile of banded snakehead muscle fraction stored at iced condition

3.5 Texture properties analysis

Fig. 5 & 6 showed change in textural profile of banded snake head stored at iced condition for 18 days. Hardness 1(r^2-0.5443) and hardness 2(r^2- 0.496) were found to decrease significantly ($p<0.05$) during storage. The highest value for stiffness noticed on the third day of incubation was 1.37 and gradually decreased to1.52. The springiness decreased throughout the study from 1.016 to 0.45(r^2-0.68, $p<0.05$). The value for chewiness decreased from 0.07 to 0.006 by the end of storage period (r^2-0.83, $p<0.05$). The value for gumminess decreased from 0.06 to 0.01($p<0.05$). Adhesiveness also changed from 0.0008 to 0.0026($p<0.05$).

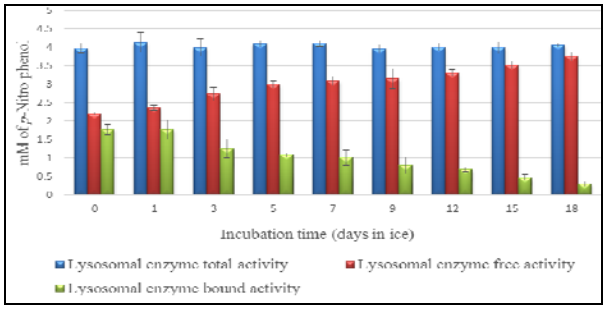


Fig 7: Changes in lysosomal enzyme activity of banded snakehead muscle fraction stored at iced condition

3.6 Change in lysosomal enzyme activity

Fig.7 showed change in the free total and bound lysosomal enzyme activity in banded snakehead muscle stored for 18 days in ice. An increase in the free activity and decrease in bound activity for acid phosphatase were observed for 18 days incubation in fish muscle fraction ($p<0.005$).

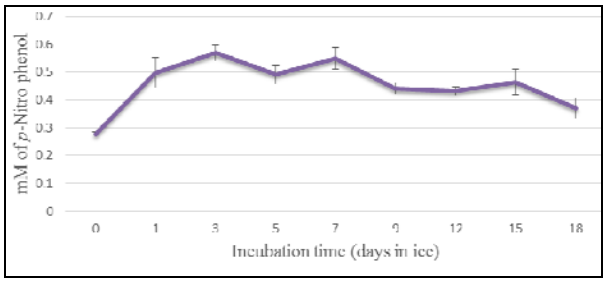


Fig 8: Changes in collagenolytic enzyme activity of banded snakehead muscle fraction stored at iced condition

3.7 Change in collagenase enzyme activity

The fig. 8 showed the collagenase enzyme activity in fish muscle incubated in iced condition for 18 days. It was noticed that the enzyme activity reached maximum level within 3rd day of incubation and thereafter slight variation were observed (p<0.005).

4. Discussion

Postmortem factors influencing the quality deterioration includes the rate and extent of pH decline, rigor mortis, rate and extent of proteolysis causing breakdown of myofibrils and connective tissue, and temperature during storage [2, 16, 17]. Our result showed a slight reduction in pH value on initial incubation period and possibly due to the accumulation of hydrogen ions liberated from the hydrolysis of ATP, moreover equally responsible by the lactate generated by postmortem anaerobic oxidation of glucose. Premortem physiological conditions of fish also had a significant effect on the postmortem pH value changes [18]. The myofibrillar protein solubility gradually decreased during entire storage period in ice, whereas a decrease in the content of denatured protein was observed and towards the 18th day fish was organoleptically unacceptable condition. Gradual decrease in myofibrillar protein solubility were observed in Thai Pangas muscle during the storage period of 25 days in ice [19]. Also, reported that during ice storage, Monterey sardine myofibrillar muscle protein fraction was unstable [20]. In our research, the extraction capacity for myofibrillar protein decreased (p<0.05) to 47% on the 5th day and 81% at the end of the storage period. A contrary characteristics was observed for the alkali- soluble fractions, indicating the denaturation of protein upon storage in ice. Postmortem breakdown of proteins offered an optimum growth and reproduction condition for spoilage causing microflora leading to the formation of amines from small peptides and amino acids, and an increase in the pH value [21]. Sarcoplasmic protein content showed no significant variation and this result was in good agreement with Devadasan and Nair, who studied the sarcoplasmic protein changes in sardine, prawn, and mackerel stored in iced condition [10]. In a study of 10 days storage in ice of shrimp and prawn muscle, a slight decrease was observed in sarcoplasmic protein content [22]. Our result inferred that the sarcoplasmic proteins were not denatured to any considerable extent, or this could be due to the releasing of membrane stored proteins. Water binding capacity is a property of protein functionality that is determined by the fish freshness [23]. The present result indicated that there was significant loss in the fish muscle quality during incubation in ice for 18 days. Decrease in WHC was primarily due to denaturation/aggregation of actin and in particular myosin. The actomyosin cross-bridge formation in rigor mortis possibly reduced the water binding capacity of the myofibrils. An increase in the expressible water content up to 9th day of incubation time in ice was observed. The water loss was primarily due to protein denaturation and resulted in entrapment of small quantities of water within the muscle structure [24]. After the 9th day, there was no variation in EWC, indicating that the fish muscle protein underwent complete denaturation at last stage of incubation. When moisture content level increased the water binding capacity of the proteins weakened and the texture of fish muscle was completely degraded. Water-holding capacity in farmed sea bream was inversely related to the collagen content in it [25]. A significant level negative relationship was found between the expressible water content and the water-soluble collagen in bovine m. infraspinatus during ageing in a vacuum at 3 °C up to 20 days [26]. The collagen content in the fish muscle was stable up to 7th day of incubation and a decrease in this variable was observed thereafter. Stroma protein slightly decreased in shrimp and prawn during ice storage [22]. It reported that collagen content in farmed sea bream muscle diminished slightly over storage time [25]. The structural change in the collagen fibrillar network corresponded to the postmortem tenderization [7]. Also result showed a change in acid soluble, pepsin soluble and insoluble collagen content in banded snakehead during the incubation period. Acetic acid soluble collagen are located on the non-helical telopeptide regions, remain intact, while pepsin soluble collagens are “atelo-collagen” since the pepsin treatment results in cleavage of the telopeptide region. It was observed that the acid soluble and insoluble collagen content decreased throughout the entire study, suggesting the acid soluble collagen fractions easily undergo postmortem degradation than pepsin soluble collagen. A small increase in the pepsin soluble collagen content to 5th day of incubation was observed and continued till 7th day and was decreased thereafter, indicating the muscle softening. Generally Type I and Type V collagen has been identified from the fish intramuscular connective tissues in fish muscle and its proportion very important for the stability and thickness of collagen fibrils. Higher proportion of Type V collagen was found in the form of thinner collagen in fish muscle. Type V collagen became solubilized after day one of chilled storage of sardine with the concomitant weakening of pericellular connective tissue induced by disintegration of thin collagen fibrils with no changes in the type I collagen located in interstitial connective tissue [27]. Additionally, non-helical region of collagen cross link is type V collagen [28]. The heat soluble collagen and pepsin-soluble collagen (PSC) contents in the muscle of both pre- and post-spawned fresh water prawn (*Macrobrachium rosenbergii*) increased markedly after 3 days of storage, while the insoluble collagen content were decreased in both cases [1]. A decline was detected in acid-soluble collagen (ASC) in the first few postmortem hours, perhaps related to the end of rigor mortis that occurs at these stages [25]. It also reported that between the 5th and 10th day of ageing a significant increase was noted in acetic acid-soluble collagen and total soluble collagen content along with a decrease in insoluble collagen content [26]. Pepsin-soluble collagen (PSC) increased, while insoluble collagen (ISC) decreased from 96th hour. A change in pH may both change the properties of the collagen molecules and thereby make them more susceptible to attack by endogenous proteases, and activate the proteases directly [9]. Texture properties analysis also showed a significant level of variation during the incubation period in ice. Hardness 1 refers to the peak force during first compression and hardness 2 during second compression, and were found to decrease significantly during storage, possibly

due to the weakening of connective tissue of fish muscle proteolysis by endogenous and microbial enzymes. The highest value for stiffness noticed at the third day of incubation and gradually decreased thereafter, indicating the whole fish reached in full rigor condition by third day of incubation in ice and then it entered in post rigor stage. Softening phenomenon highly correlated with weakening of pericellular tissue in sardine and collagen content in the fish muscle was directly related to firmness [25, 27]. Springiness is the elastic or recovering property of the fish muscle during compression and lose elasticity during storage was detected. This result was in good agreement of Sutchi cat fish steaks during refrigerated storage [9]. The value for chewiness decreased and it indicated that the meat was softened during storage. Change in gumminess and adhesiveness showed that the fish muscle underwent deterioration and undesirable physical changes as incubation time proceeded in ice. It was noticed that the collagenolytic enzyme activity reached maximum level within third day of incubation, the result strongly pointed out that collagenase enzyme had a significant role on postmortem breakdown of collagen. The collagenolytic enzymes could be partly responsible for the degradation of collagen and other extracellular matrix proteins in fish muscle than myofibrillar proteins followed by texture softening of seafood products [7, 9, 29, 30]. The observed textural changes could therefore be a result of changes in the connective tissue. It also suggested that collagenolytic enzyme participated in postmortem softening by acting on both type I and V collagen [31, 32]. Furthermore, type V collagen greatly participated in postmortem degradation than type I followed by weakening pericellular connective tissue [27, 33]. Preliminary attack on the collagen triple helix has been achieved by specific collagenases. Once the initial cleavage has been achieved, other non-specific proteases can pursue attack [34]. The lysosomal enzyme activity are also involved in postmortem tissue softening. The free lysosomal activity increased till the last day of incubation period, whereas the bound activity decreased, indicating the lysosomal instability and release of membrane bounded enzymes. Additionally, the result proved that the lysosomal membrane was less resistant to postmortem changes. The lysosomal enzymes in increased Myosin heavy chain degradation of Atlantic cod skeletal muscle at lowest pH [35]. Activity of acid phosphatase of muscle lysosomes in beef and pork stored at 4 °C and its activity reaches its maximum between the second and fourth day of postmortem and at that time majority of lysosomes had leaky membranes [36].

5. Conclusion

Our results showed the quality changes and texture difference of the banded snakehead in iced condition. The WHC and EWC were proportional to the total collagen content. When moisture content level increased, the water binding capacity of the proteins decreased markedly and the texture of adversely affected. The study also revealed that both collagenase and lysosomal enzymes were actively involved in tissue deterioration process and undesirably affected the textural properties in fish.

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