QUALITY CHANGES DURING FROZEN STORAGE AND COOKING OF MILK SHARK (SCOLIODON SORRAKAWAH) MUSCLE TISSUE: EFFECT ON STRUCTURAL PROTEINS AND TEXTURAL CHARACTERISTICS

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ABSTRACT

Structural protein characteristics and textural quality of shark muscle tissue during frozen storage and subsequent cooking were evaluated. Myofibrillar protein (45.62 %) and pepsin soluble collagen (13.69 %) contributed to major proportion of total protein (22.01 %) and significantly affect textural characteristics. Urea, contribute to the off-odour in fresh shark muscle and add to the non-protein nitrogen (NPN) fraction that are hydrolyzed during processing conditions. Freezing and frozen storage effect protein extractability through protein aggregation and cooking caused gelatinization of protein molecules. Conformational changes in protein molecules were reflected in SDS-PAGE, TPA and histochemical analysis. Fresh samples showed optimum texture at 70 °C (H1-1.93 kgf) that modified to 80 °C (H1-2 kgf) with frozen storage. Histochemical studies of fresh tissue indicated parallel myotome bundles enveloped by collagenous sheath that undergo gelatinization during cooking. Quantity and distribution of structural proteins contribute considerably to the muscle texture. Myofibrillar protein and collagen significantly affect the textural properties during frozen storage and cooking. Collagenous sheath that envelops the myotomes (myofibrillar protein) lose its integrity during frozen storage and consequent cooking, thereby, allowing muscle bundles to slide over at high cooking temperatures. Due to denaturation, water holding capacity of protein is reduced during frozen storage and cooking.

KEYWORDS: Myofibrillar Protein; Collagen; SDS-PAGE; Texture Profile Analysis; Scoliodon sorrakawah.

1. INTRODUCTION

Fish quality is a multifaceted concept, with key aspects being safety, nutritional quality and availability, freshness, eating quality, species and size, and product type. Variations in flavour, odour, texture and colour directly reflect meat freshness. Nutritional and textural characteristics of fish are the major features that verify the consumer preferences’. Fish
muscle protein and its distribution are the primary determinants of the nutritional significance and commercialization [1]. Structural proteins, comprising of myofibrillar proteins and collagen, account for 70-80% of the total protein; and organize the structure and the specific textural properties of muscle tissue. Arrangement of structural components and the changes occurring in these components during processing affect texture in a significant manner. Hence, textural characteristics are influenced equally by both intrinsic and extrinsic factors. Collagen, though contribute a minor fraction, is vital in determining chemical and organoleptic properties [2]. In raw fish meat, the firmness is closely associated with collagen. The systematic arrangement of collagen fibre plays an important role in determining the tissue properties [3]. Protein functionality, thus, is determined to a large extend by its physiochemical and structural properties [4]. Consequently, these basic functional components considerably determine the nutritional and textural properties of fish tissue. Understanding shark proteins and the effect of protein components on textural quality is needed that could be realized by analyzing protein characteristics and variations caused in the components during frozen storage and cooking.

Sharks are commercially important and valuable source of protein [5]. Shark proteins are highly popular and are appreciated dietary supplement and an alternate protein source. Typically, cartilaginous tissue is used to extract collagen and glycoproteins [6]. Dewi et al. reported that smaller sharks are used as fresh, chilled or as frozen meat, whilst the larger sharks are used to provide fins and hides [7]. Shark by-products, such as, fin, liver, meat, fat and skin find various uses. Shark liver oil and shark fins are reasonably high in demand and are exported in considerable quantity to the Eastern Countries [8]. In addition, shark collagen is of health significance and contains antiangiogenic and antitumor compounds [9]. Shark cartilage is said to be of significant considerable health benefits, which needs scientific confirmation. From economic and nutritional perspective, an appropriate utilization of the shark meat is necessary.

Though, freezing is a widely accepted preservation technique, long-term frozen storage of muscle tissue might lead to marked increase in toughness and dryness of the tissue [10]. Formation of cell compartments, protein coagulation, myofibrillar protein aggregation, dehydration, loss of water-holding capacity after thawing and changes in flavour are the common variations reported to occur during frozen storage. These alterations depend on species, freezing method and time-temperature profile during frozen storage [10]. Cooking also produces a notable change in the muscle components and texture that could be correlated
to flesh quality. Texture of cooked flesh depends on the size of muscle fibres after cooking, the quantity of coagulated proteins in the interstices and the gel formed by collagen and lipids that allow sliding of the muscle fibres and myomeres. Baylan et al. reported that during cooking, secondary and tertiary structures of proteins were lost due to the split in hydrogen bonds, resulting in unfolding of native conformation [11].

The present study investigates the protein characteristics and textural variations in shark muscle tissue (*Scoliodon sorarakawah*) that are subjected to different cooking temperatures. The study attempts to understand the combined effects of the frozen storage and cooking on the physicochemical properties, protein fractions, textural and histochemical properties.

2. **Materials and method**

2.1. **Raw material**

Milk shark (*Scoliodon sorarakawah*), an elasmobranch commonly found in the inshore waters of Kerala coast along the South-West coast of India, were obtained from commercial fishing boats. Samples measuring 70-100 cm in length and weighing 1-1.5 kg were individually wrapped in polythene sheets and were brought to the laboratory within 8 h in iced-stored conditions. They were then headed, cleaned and eviscerated.

2.2. **Sample preparation**

Unfrozen samples (F, 5 kg) were packed in polyethylene bags and kept in freezer for analysis while for frozen-storage study, cleaned-eviscerated samples were individually quick-frozen using air blast tunnel freezer at -40 °C, wrapped separately in polythene bags and stored in 5-ply cartons at -18 °C. After six months (Fr), the samples (5 kg) were randomly selected and enclosed in polythene bags and thawed at room temperature for 30 min, according to CODEX alimentarius, before analysis [12].

During cooking experiments, both unfrozen and frozen samples were cut in small cubical pieces (~2 cm³), wrapped in aluminium foils and subjected to different cook temperatures (45-90 °C) for one minute [13]. The abdominal muscular region was considered for the analysis.

2.3. **Chemicals**

All chemicals used in the study were of analytical grade. Chemicals for electrophoresis were purchased from Genei, Bangalore, India.
2.4. Physicochemical Analysis
Homogenate was prepared by blending 10 g of sample with 90 ml of distilled water for 30 s and pH was measured using a calibrated Cyberscan pH 500 (digital pH meter, MERCK). Expressible moisture (EM) was measured according to Ng [14]. For determining expressible moisture, pressure of 10 kg/cm² was applied on tissue (1 g) placed between two pre-weighed filter papers for 10 s and the weight differences was expressed as the percentage of sample weight. For water binding potential, the tissue was homogenized (Yorco Micro Tissue Homogenizer, India) with water (1: 30) in pre-weighed polycarbonate tubes, heated at 60 ºC for 30 mi, cooled and centrifuged (4000 rpm for 10 min) at room temperature. Water binding potential (WBP) was calculated as the weight difference and accounted for the amount of water held per gram sample [15]. Proximate composition comprising of moisture 934.01 (4.1.03), protein 955.04 (2.4.03), fat 920.39 (4.5.01) and ash 942.05 (4.1.10) were evaluated according to Official Methods of Analysis of the Association of Official Analytical Chemists [16]. All measurements were performed in triplicate and were expressed on wet weight basis.

2.5. Protein fractionation
Muscle proteins were fractionated according to the methodology of Hashimoto et al. [17] and Mizuta et al. [18], which was standardized in our laboratory [19]. 10 g of tissue was homogenized with 10 v (100 ml) of 0.05 M phosphate buffer (pH 7.5) and supernatant was collected after centrifugation (4000 rpm for 10 min, MB-20 Superspeed Refrigerated Centrifuge). Sarcoplasmic fraction (SP) was precipitated from the supernatant using 10 % trichloroacetic acid. Residual tissue was further homogenized with 10 v (100 ml) 0.6 M NaCl-phosphate buffer (pH 7.5) and supernatant containing myofibrillar protein fraction (MY) was collected after centrifugation (4000 rpm for 10 min, MB - 20 Superspeed Refrigerated Centrifuge). SP and MY were fractionationed at 4 ºC. The residual tissue were rigorously extracted overnight with alkali and washed in distilled water, and were suspended in 10 v (100 ml) of 0.5 M acetic acid, digested at room temperature for 48 h using enzyme pepsin (1: 20 w/w, SIGMA Co. Ltd., USA). Supernatant collected were pepsin soluble collagen fraction (PSC). The fractions were evaluated for nitrogen content by micro-kjeldahl and converted to protein using the conversion factor 6.25 (AOAC, 2000). All analyses were done in triplicates.

2.6. SDS-PAG Electrophoresis
Samples with equal protein content of 30 µg were subjected to discontinuous polyacrylamide gel electrophoresis (Large Vertical Model Electrophoreses unit, Genei, Bangalore, India), as
described by Laemmli [20], with slight modifications. Electrophoretic separation was performed at 20 °C for 6 h, using 10% polyacrylamide gel concentration for sarcoplasmic and myofibrillar fraction and 7% gel concentration for pepsin soluble collagen using discontinuous Tris-HCl/ glycine buffer system. Stacking gel strength was 4%. The electrophoresis was initially adjusted to 3 mA/well that was increased to 10 mA/well towards the end of the gel run. Protein bands were stained with Coomassie Brilliant Blue R-250 (0.05% w/v) in 15% methanol (v/v) and 5% acetic acid (v/v). De-staining was performed in an aqueous solution of 5% methanol and 7.5% acetic acid, and the samples were stored in a solution of 10% glycerol and 7.5% acetic acid.

Molecular mass calibration kits of broad range and high range (Genei, Bangalore, India) consisting of myosin (205 kD), phosphorylase b (97 kD), bovine serum albumin (66 kD), ovalalbumen (43 kD), carbonic anhydrase (30 kD), soybean trypsin inhibitor (20.1 kD), lysozyme (14.3 kD), aprotinin (6.5 kD) and insulin (3 kD), were used as standards for molecular mass determination.

2.7. **Texture Profile Analysis (TPA) and Sensory Evaluation**

Texture Analyzer (Lloyd Instruments, UK, model LRX PLUS) and Nexygen software (Lloyds Instruments) was used for instrumental texture analysis of unfrozen and frozen shark muscle tissue, cooked at various temperatures. Flat-faced cylindrical probe of 50 mm diameter compressed the bite size piece (~2 cm³) 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 (H1, kgf), hardness 2- after second compression (H2, kgf), cohesiveness (C), springiness (S, mm) and stiffness (SF, kgf/mm) were evaluated [21].

A six-member panel performed the sensory evaluation using 7-point scale with scores being 7-like extremely or much more, 4-like or dislike or neither more or less and 1-dislike extremely or much less. The selected characteristics were tested as defined by Jowitt [22]. Wateriness, firmness, elasticity, cohesiveness, juiciness and hardness were the attributes evaluated. The sensory panel also recorded appearance, colour, odour, texture and flavour and finally giving overall organoleptic scores. Replicates of five were measured for each sample.
2.8. Histochemical Analysis

Samples (1 cm thick) were fixed overnight in Bouin’s fixative containing picric acid, 40% formalin and glacial acetic acid (15:5:1) and were subjected to serial dehydration using ethanol of various dilutions (70% for 24 h and 80-96% for 30 min each and absolute alcohol for 20 min). Finally, the dehydration was completed with the samples being dipped in acetone. The dehydrated samples were embedded in paraffin wax with ceresin (congealing point-60 °C) and sectioned (8 μm, thickness) using rotary microtome (SIPCON SP 1120 Rotary Microtome, India) and fixed on slides. Double staining technique using Weigert’s Haematoxylin stain for 5 min followed by acid: alcohol for 30 s and Van Geison’s stain for 5 min were used to stain collagen fibrils (red) and myofibrillar proteins (yellow). The stained slides were passed through alcohol series for 10 min, followed by xylene for another 10 min and were mounted using DPX mountant. The sections were photographed using Nikon Eclipse E-200 compound light microscope fitted with Nikon DN 100 Digital Net Camera.

2.9. Statistical Evaluation

Results are expressed as means ± standard error of means. Statistical difference among the results were analyzed using one way analysis of variance (ANOVA) using a Minitab software (version 14.0, Minitab Inc, State College, PA, USA). P values of <0.05 were considered significant. Automated surface fitting analysis was performed using TableCurve 3D (Systat Software Inc., Bangalore, India) to analyse the affects of temperature and protein fractions (sarcoplasmic protein, myofibrillar protein and collagen) on muscle hardness.

3. Results and discussion

3.1. Physicochemical properties

Variations in expressible moisture (EM) and water binding potential (WBP) of shark muscle tissue with frozen storage and subsequent cooking are shown in Table 1. Fish muscle pH is considered as a shelf-life and spoilage index. Fresh samples upon cooking showed pH ranging between 6.3 and 6.4. Ali reported that pH in seafood varied from 5.8-7.2 depending on the struggle at the time of harvest although normal variation was between 5.8 and 6.5 [23]. In frozen stored-cooked samples, pH showed a significant reduction and varied between 5.7 and 5.8. This could probably be due to ante-mortem stress, protein degradation and loss of hydration property during freezing and subsequent cooking. The reduced pH with frozen storage makes tissue soft, thereby, the tissue losing its inherent water binding property, which is evident from increased EM. The pH affects water binding properties and subsequent, gel-
forming ability. There was no significant difference in the pH with frozen storage and subsequent cooking. Ante-mortem stress and post-mortem glycolysis lead to the accumulation of lactic acid in the muscle tissue possibly contributing to the lower pH in frozen samples [24].

Expressible moisture (EM) is lowest at 55 °C, for fresh (67.1 %) and frozen stored sample (73.28 %), whilst water binding potential (WBP) is observed to be high at 65 °C (2.07 g) and 50 °C (2.69 g) for fresh and frozen samples, respectively. EM and WBP showed significant difference between fresh and frozen-stored samples. Hydration property is a function of protein functionality and vice-versa; and protein and water binding potential possibly have positive correlation; and varies with temperature and frozen storage period [25]. It could be possible that irreversible bond formation in actomyosin, presence of charged groups on proteins and physical configuration of myofibrillar proteins and collagen, together with sarcolemma, affect the water retaining capacity of the muscle tissue. Foh et al. reported that amino acids, protein conformation and surface hydrophobicity/ polarity influence the hydration properties of muscle protein [4]. Interaction of muscle proteins together with water and lipid are also vital determinants in food texture.

Proximate composition of shark muscle tissue show moisture 72.46 %, crude protein 22.04 %, ash 2.36 % and lipid 0.90 %. Non-protein nitrogen fraction (NPN) could be a contributing factor towards the total nitrogen content, thus adding to the crude protein content. Protein content showed significant decrease with frozen storage (P<0.05). The variations showed in protein content in fresh and frozen-stored samples with cooking temperature could be attributed to protein denaturation associated with temperature variations [26]. Lipid content is higher in fresh samples that reduced with frozen storage, which could be attributed to fat oxidation [26]. Increase in ash content with frozen storage could be credited to the drip loss and dehydration associated with frozen-storage and cooking that lead to moisture loss [27]. The effects of cooking on proximate composition has been previously reported and were comparable with results obtained in atlantic sharpnose, lemon shark, scalloped hammerhead shark and tiger shark [28].

3.2. Quantitative and qualitative fractionation of protein fractions

Fractionation of sarcoplastic protein fraction (SP), myofibrillar protein fraction (MY), total collagen (TC) and pepsin soluble collagen (PSC) during frozen storage and cooking are shown in Fig. 1. In fresh uncooked samples, sarcoplastic protein content was 16.07 % that reduced during frozen storage (14.31 %). Reduced pH during frozen storage may affect the extractability of sarcoplastic protein by protonizing ionisable carboxylic acid side chain.
leading to hydrophobicity and consequently, the aggregation and accumulation of these proteins in the interfibrillar spaces [29]. Non-protein nitrogen including urea also contributes to SP in fresh samples. High concentration of urea and trimethylamine-oxide (TMAO) in the tissue offers an off-odour to fresh samples that are lost during frozen-storage. This may also counteract as protein-destabilizing force and affect the texture during frozen-storage [30]. The ammonical odours of fresh samples are lost during frozen-storage possibly due to its hydrolysis. Myofibrillar protein contributes the major fraction of total protein (45.62 %).

With frozen storage and cooking, the extractability of myofibrillar proteins showed significant decrease possibly due to aggregation and gelatinization, respectively. Collagen content was higher (13.69 %) that was in accordance with the earlier findings [31]. More than 80 % of the total collagen were solubilised by enzyme pepsin and collected as pepsin soluble collagen. PSC showed similar trend in both fresh and frozen-stored samples. Cooking result in the shrinkage of collagen due to the breakdown of hydrogen bonds in the protein and could be the reason.

Frozen storage reduced protein extractability possibly modifying the proteins by oxidation. The aggregation/ denaturation of proteins were more severe during frozen storage. Protein denaturation induced during cooking lead to the loss of hydration property and possibly contributing to the cooking loss. Association-dissociation-denaturation of proteins during frozen storage and subsequent cooking is the main contributing factor for the reduced extractability of protein fractions in the shark muscle tissue. Weakening of fibrous linkages in muscle structure during initial stages of processing could also contribute to the reduced extractability of protein fractions [32]. Gelation, emulsification, water binding and lipid binding specify the conformational status of protein system. SDS-PAGE indicates the disintegration and conformational changes in protein molecules during frozen storage and cooking (Fig. 2). SP included protein bands ranging between 40 to 60 kD and might include myoalbumin, globulins and enzymes. The band between 29 and 43 kD could be glyceraldehyde-3-phosphate dehydrogenase. Presence of additional bands with frozen-storage (65 °C) and cooking could be due to the disintegration and conformational changes in this fraction. Low molecular weight enzymes are probably lost with frozen storage. Sarcoplasmic proteins contribute to thermal gelation of structural proteins, thereby, affecting the textural characteristics of the muscle tissue [33]. MY showed myosin (200 kD), α-actinin (105 kD) and thick fused band at 29 kD that could be overlapped actin, tropomyosin and troponin [34]. With frozen storage, the myosin heavy chain molecules (MHC) became intense and new band
was observed at the top of the gel possibly due to aggregation that were too large to enter the gel. It could be due to covalent bond formation. Cooking cleaved MHC and actin into smaller polypeptide chains. Protein bands with low molecular weight (<6.5 kD) were not separated distinctly in PAGE. Collagen bands in the lane 1 (F-c) indicate type I collagen consisting of two α-chains (α1 and α2) and one β-chain. The estimated molecular weights for the α1 and α2 chains were approximately 120 and 112 kD, respectively. The β-chain is could be a dimer with high molecular weight (205 kD). The SDS-PAGE patterns of collagen were similar to other fish species [35]. With frozen-storage, low molecular weight bands fade while aggregation cause accumulation cause formation of protein band at the top of the gel and with subsequent cooking, high molecular weight components disappeared due to hydrolysis into small molecular weight proteins [6]. Functional properties of the proteins are highly influenced by molecular weight distribution. Aggregation and gelatinization of protein molecules were observed to hinder the electrolytic mobility of proteins. These conformational changes in the protein fractions significantly affected the muscle texture. Cooking cause solubilisation of proteins and hence, leads to loss of proteins.

3.3. Texture Profile Analysis

Frozen storage and cooking contribute to denaturation and conformational changes in protein molecules that affect the fish muscle texture as evident from TPA results (Fig. 3). Fresh samples indicated first phase of hardening (H1) at 50 °C (4.33 kgf) and the second phase of hardening (H2) at 70 °C (1.93 kgf) with maximum juiciness. The variations in hardening observed during frozen storage and subsequent cooking may possibly be due to the differential freeze denaturation of the structural proteins. In frozen-stored sample, optimum texture was observed at 80 °C (H1, 2 kgf). Three dimensional surface plot analyses were done to analyse the role of temperature on protein fraction and textural parameter together. The data for protein fractions, hardness (H1) and cooking temperature for fresh samples were fitted into surface plot to simulate the response surfaces to optimize and indicate the interrelationship between these three parameters. Surface plot analysis also emphasized that sarcoplasmic protein (P<0.05), myofibrillar protein (P<0.001) and pepsin soluble collagen (P<0.05) in fresh samples showed significant variation with cook temperature with optimum texture being obtained at 70 °C. It is observed that the affect of MY on texture with respect to temperature is significantly higher (R²= 0.89) compared to SP (R²= 0.58) and PSC (R²=0.65) (Fig. 4). Hardness and springiness were low at 60 °C possibly because collagen fibres become irreversibly solubilised and encourage textural changes due to sliding of myotomes
that are related to heat denaturation. Hardness 1, hardness 2, springiness and stiffness showed highly significant variation (p<0.01) between temperatures. High cohesiveness at 50 °C could also be due to denaturation of collagen. At 70 °C, myofibrillar proteins affected cohesiveness and are associated with the loss water molecules and hydration property of the tissue. An increased opacity of fish flesh during cooking is observed probably due to the precipitation of thermally denatured sarcoplasmic proteins that commence at 45 °C [33]. In raw condition and at 45 °C, the textural parameters were high and decreased considerably with increase in temperatures probably due to dissociation of actin-myosin complex and denaturation of myosin tail. Heat induced gelation of myofibrillar proteins is an important functional property and gives desirable texture and stabilization of lipids and water during processing [36]. Sensory analyses determine consumer preferences’. Cooked muscle tissue showed second phase of hardening during later stage of cooking that concur with the second phase of hardening as observed in TPA results (Fig. 3). Overall scoring indicated first phase of hardening for unfrozen samples at 50 °C and second phase of hardening at 70 °C with maximum juiciness. These conditions varied in frozen stored mantle probably due to differential freeze denaturation.

3.4. Histological analysis
The arrangement of structural proteins and the changes brought in these with frozen storage and subsequent cooking effect texture and it’s evident in histological analysis (Fig.5). The myotomes in shark muscle tissue are arranged parallelly and enveloped by collagenous sheath that are fibrous in nature. During frozen storage and subsequent cooking, sarcolemma disintegrates and plasmolemma splits-off from fibre and large spaces containing fragmentary and precipitated material are formed. Apart from contraction, the predominant change observed is the gradual increase in permeability of membranous structures in frozen storage. On freezing, the ammoniacal odour in the tissue is lost considerably making it more acceptable. The cell detachment during cooking could be due to the loss of intercellular integrity through loosening of collagen fibres. The firmness of cooked muscle is observed to be weak and constant. The specific influences of connective tissue on texture depend on their thickness, amount of collagen, the density and type of cross-linkages between fibrils. Shark muscle with its higher collagen content would contribute significantly to the muscle texture. Gelation of shark collagen is also a contributing factor in effecting the texture during frozen storage and cooking. Nomura et al. observed that shark collagen denature at 15 °C or lower temperatures [37]. With progressive cooking in both fresh and frozen stored samples, the
collagen undergoes gelatinization and myotomes disperse in the gel. Due to low denaturation temperature of shark collagen, this gelation could be more prominent in frozen samples and myotomes fragment and feebly scatter in the gel. This is due to the structural deterioration of myofibrillar proteins and collagen, and loss of inherent water holding capacity by the protein fractions [38]. In fresh samples, myotomes are compact and restrained within the collagen gel. Additionally, freezing and frozen storage cause formation and development of ice crystals in the tissue that causes lyses and disintegrate the tissue integrity. However, this phenomenon is not distinguished in shark probably because of low temperature denaturation and gelatinization of protein molecules.

4. Conclusions

Shark has high protein content (22.01 %) of which myofibrillar protein (45.62 %) and pepsin soluble collagen (13.69 %) contribute significantly to the textural variations. Non-protein nitrogen including urea provides off-odour to shark meat and contributed to the sarcoplasmic protein fraction, which was hydrolysed during frozen storage and cooking giving no odour in the fish tissue. Water binding potential and pH influence protein functionality during frozen storage and cooking. Frozen storage and subsequent cooking affect myofibrillar protein and collagen and affect muscle texture of shark muscle tissue. Frozen storage affect extractability through aggregation while cooking caused gelatinization of protein molecules. Histochemical studies indicated parallel arrangement of myotomes enveloped by collagenous sheath. Optimum cook temperature for fresh shark tissue samples was 70 °C while it shifted to 80 °C with frozen storage.

Acknowledgement

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List of figure captions

Figure 1. Variation in protein fractions: (a) Sarcoplasmic (SP) and myofibrillar fractions (MY), (b) Total collagen (TC) and pepsin soluble collagen (PSC) in fresh (F) and frozen-stored samples (Fr), (Mean±SE, n=3)

Figure 2. SDS-PAGE of protein fractions of shark muscle tissue, F-Fresh, Fr-Frozen-stored, a-sarcoplasmic protein, b-myofibrillar protein, c-pepsin soluble collagen.
Figure 3 Texture profile analysis: (a) Hardness 1 and Hardness 2, (b) Cohesiveness, (c) Springiness, (d) Stiffness and (e) Organoleptic score

Figure 4 Automated surface fitting analysis using TableCurve 3D. 5 (a) three dimensional plot of sarcoplasmic protein and hardness, 5 (b) myofibrillar protein and hardness, and 5 (c) collagen and hardness, at varying cook temperatures

Figure 5 Histological sections of fresh and frozen samples of shark muscle tissue, *Scoliodon sorrokawah*, Fresh sample: (a) uncooked, (b)-50°C, (c)-70°C and (d)-90°C, Frozen stored samples: (a') uncooked, (b')-50°C, (c')-80°C and (d')-90°C

Table 1. Physical characteristics: expressible moisture and water binding potential of fresh and frozen-stored shark muscle tissue cooked at various temperatures (Mean±se; n=3)*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>EM (%)</th>
<th>WBP(g)</th>
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<tr>
<td></td>
<td>F</td>
<td>Fr</td>
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<tr>
<td>UC</td>
<td>74.2±0.2C</td>
<td>87.5±0.0Ba</td>
</tr>
<tr>
<td>C1</td>
<td>71.7±0.0B</td>
<td>88.2±0.0Ba</td>
</tr>
<tr>
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<td>75.4±0.0C</td>
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<tr>
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<td>85.8±0.0Ba</td>
</tr>
<tr>
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</tr>
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</tr>
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<td>C9</td>
<td>75.2±0.0C</td>
<td>75.6±0.0Aa</td>
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F- Fresh, Fr- Frozen stored six months

Means with different uppercase indicate significant differences within the condition (A-P<0.001, B-P<0.01, C- P<0.05); Means with different lowercase indicate significant differences between the conditions (a-P<0.05)
Fig. 1

![Graph 1]

Fig. 2

![Graph 2]

Fig. 3

![Graph 3]
Fig. 4.

Fig. 5.
References


