Fish is the cheapest source of good quality animal protein readily available to the masses throughout the world with increasing demand. Proteins are functional components in process food, where they contribute to texture and sensory characteristics besides the nutritional properties. Generally, fish are kept at low temperature to minimize the protein degradation and spoilage. Muscle protein degradation is primarily a major problem associated with fish. Myofibrillar protein present in the fish muscle is the important protein fraction responsible for the physicochemical properties of the protein in the food system. These properties of protein undergo changes during different processing conditions to which the protein is exposed.

In India Pangasius hypophthalmus is cultured in inland sector, mainly Andhra Pradesh and West Bengal. Now days it is also cultured in other states of India. The annual production is presently around 0.7 million tones. The Pangasius hypophthalmus has white or pale pink colour flesh. Unlike cod fillets, which are substituted because of their white flesh, advantages of Pangasius fillets are characterized by absence of fishy odour, small bones and thin skin. The cooking process results in delicate and firm texture, allowing a wide range of culinary preparations. These characteristics, together with their availability in market in standard size, leads preparation of value added sutchi catfish fillets, particularly to meet the consumer demands/preferences of the food service industry and restaurant. Presently consumer demands are increasing for ready to eat or cook fish and meat products with smaller portion size, less fat and salt as well as being easy and fast cook, finally leading to value addition. Value added fish products can be made using muscle restriction technology by utilizing low cost fish species. Fish mince also contributes some role in ready to cook products. At present, a large quantity of Pangasius is produced in India and is mainly consumed fresh. Preservation of this fish in various forms and development of value added products, particularly mince and mince based products will further help in providing more options for its proper utilization.

Refrigerators are very common and house hold means of short term storage for fish and fishery products. It is very well established fact that whole fish can be stored longer than the minced at low temperature. Cryoprotectants are commonly used during frozen storage of surimi to prevent freeze denaturation of proteins. However during refrigerated storage of fish mince, the extent of protein denaturation will be less but there is no reports on the use of cryoprotectants in mince at refrigeration storage. Therefore attempts were made in the present investigation to study the refrigerated temperature storage stability of Pangasius mince added without and with cryoprotectants.

MATERIAL AND METHODS

Raw material preparation: Fish sutchi/ basa (Pangasius hypophthalmus), were purchased from cage culture in Dhasai reservoir, Thane District, Mumbai. Fish kept in ice with 1:1 ratio (fish: ice) and transported to Post Harvest Technology

NAAS Rating (2016)-4.20
Laboratory, CIFE within 3 hrs. Mince were separated from fish fillets, using deboning machine (Baader 694, Lubek, Made in Germany). Cryoprotectants i.e. 4% sucrose, and 4% sorbitol with 0.2% STPP were mixed with mince. Prepared mince packed in polythene bags and then kept in refrigerator (3-5°C) for further analysis of mince quality.

**pH measurement:** 10 g mince samples and control were homogenized with 90 ml distilled water in a homogenizer (Polytron system PT 2100, Kinematica, AG, Germany) for 30s and pH value of homogenate mince was measured by a digital pH meter (Hanna instruments, HI 2211, USA) standardized by buffer at pH 4.8 and 9.2.

**Proximate composition:** Moisture, protein and ash content in prepared mince samples were measured, by using methods from standard<sup>8</sup>. Crude Fat content was measured as per the method given by Folch<sup>9</sup>.

**Biochemical analysis:** Non Protein Nitrogen (NPN) content, free fatty acid (FFA) content, Peroxide Value (PV) of stored samples was analyzed by A.O.A.C. standard method<sup>8</sup>. Total volatile basic Nitrogen (TVBN) content was carried out by Conway method (1962)<sup>10</sup>. Thiobarbituric acid reactive substances (TBARS) content was determined according to Tarladgis<sup>11</sup>.  

**Mince gel preparation:** Prepared mince samples were placed in a mixer and 2.5% salt was added in that. Then mixture was chopped for 1 minute at 4°C to obtain a homogeneous sol. The sol was then stuffed into polyvinylidine casing with a diameter of 2.5 cm and both ends of the casing were sealed tightly. Sols were incubated at 40°C for 30 minutes, followed by heating at 90°C for 20 minutes in water bath<sup>12</sup>. All gels were cooled in iced water for 20 minutes and stored overnight at 4°C prior to analyses.

**Whiteness:** Whiteness was measured using Lab scan XE- Colorimeter (Hunter Lab scan XE, U.S.A.) which gives acceptable level of mince sample based on L*, a* and b* values. Whiteness was calculated as per the following formula;

$$\text{Whiteness} = 100 - [(100 - L*)^2 + a^2 + b^2]^{1/2}$$

**Expressible drip:** Expressible moisture content was measured according to the method of Benjakul<sup>13</sup> et al. (2001) with slight modifications. Gel samples were cut to a thickness of 5 mm, weighed (X) and placed between 3 sheets of whatman paper No. 4 at the bottom and 2 sheets on the top of the sample. The standard weight (5 kg) was placed at the top and held for 2 min. The sample was then removed from the papers and weighed again (Y). Expressible moisture content was calculated using the following equation:

$$\text{Expressible moisture content (\%) } = \frac{(X - Y)}{X} \times 100$$

**Gel strength:** Textural analysis of gels was performed using a texture analyzer - Model TA-XT2 (Stable Micro Systems, Surrey, UK). Gels were tested at room temperature. Prepared mince gels were cut into five cylindrical shaped pieces of 2.5 cm in length. The breaking force (gel strength) and deformation (elasticity/deformability) were measured for each sample by keeping the pieces of each sample into the texture analyzer equipped with a spherical plunger (5 mm diameter); pre-test speed : 1.0 mm/s; post test speed: 10.0 mm/s; distance : 4.0 mm; time : 10s) with 50 kg load cell. The probe was pressed into the cut surface of gel perpendicularly at a constant speed, until puncture occurred. The force in gram (g) required to puncture into the gel (breaking force) and the distance (in mm) at which the probe punctured into the gel (deformation) were recorded. Gel strength for each mince gel was measured from respective breaking force and deformation.

**SDS-PAGE protein pattern:** Protein patterns of prepared mince was analysed by SDS-PAGE according to the method of Laemmli<sup>14</sup>. To prepare the protein samples, 27 ml of 5% (w/v) SDS solution were added to the sample (3 g). The mixture was then homogenized using a homogeniser (Polytron, Kinematica, Switzerland) at a speed of 11,000 rpm for 2 min and incubated at 85°C for 1 h to dissolve total proteins. The samples were centrifuged at 3500×g for 20 min to remove undissolved debris. Protein concentration of the supernatant was determined by the Biuret method (Robinson and Hodgen, 1940)<sup>15</sup> using bovine serum albumin as a standard. The sample was then mixed with sample buffer (4 ml of 10% SDS, 2 ml of glycerol, 1 ml of β-mercaptoethanol, 2.5 ml of 0.5 M Tris-HCl (pH 6.8), and 0.03 g Bromophenol blue at 1:1 ratio (v/v)). The samples (20 μg proteins) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Hoefer Mini Electrophoresis (San Francisco, USA). After separation, the proteins were stained with 0.02% (w/v)
Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and distained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

Statistical analysis: Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan’s multiple-range tests. Analysis was performed using a SPSS package16.

RESULTS AND DISCUSSION

Changes in proximate composition: The results of changes in proximate composition of mince with and without cryoprotectants during refrigeration storage are given in Table-1. From the results, it was observed that moisture content of mince increased significantly (p<0.05) with increased refrigerated storage period up to 6 days irrespective of cryoprotectants treatment. The moisture content at zero day significantly differed from that of 3rd and 6th day of refrigerated storage (p<0.05). The increase in moisture during storage may be due to the absorption of water from the environment. With increased storage time, moisture content also increased, while crude protein, fat and ash were decreased significantly in almost all samples. Similar results were observed by Somboonyarithi17 and Yathavamoorthi et al.18 where in they reported increase in moisture content. Protein is the major constituent in any fish and its products. Nevertheless higher protein values were observed in mince without cryoprotectants compared with treated mince. The less protein content in treated mince samples may be attributed to the addition of 8% cryoprotectants. However addition of cryoprotectants resulted in reducing the moisture content in treated mince samples.

Changes in pH: pH of treated samples and control mince at zero day was 6.5. The pH of mince was different during refrigerated storage. After six days of storage, pH of the samples and control were 4.8 and 5.6 respectively. Benjakul et al.19 reported that the pH of ice stored P. tatenus mince remain relatively constant for up to 6 days and gradually increased thereafter. Changes in pH can affect the properties of connective tissue20. According to Matsumoto and Noguchi20, pH affects the denaturation rate of myofibrillar proteins, which is important in gel forming ability of minced fish. Myofibrillar protein is unstable and rapidly loses their ATPase activity with drastic change in pH. Rodger et al.22 reported that the declining of pH value during low temperature storage might be due to formation of lactic acid from glycogen.

Biochemical changes: During refrigerated storage, values of NPN and TVBN were progressively increased (p<0.05) in samples and control as shown in Table-2. Suwanich et al.23 and Somboonyarithi27 were reported that NPN and TVBN values are increased with increased refrigerated storage period of channel cat fish and tilapia washed mince respectively. According to Suwanich et al.23 the value of TVBN increased in unwashed mince stored at 5 and 0°C due to bacterial decomposition. However, Al-kahtani et al.24 reported lower levels of TVBN in warm-water tilapia (19.5 mg/100g) and Spanish mackerel (25.2 mg/100g) at rejection.

Free Fatty Acids (FFA), peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were analyzed in samples and control mince during refrigerated storage. Fat degradation products like FFA, PV and TBARS increased at refrigeration temperature and deteriorate the product. Lipid oxidation, corresponding to the oxidative deterioration of polyunsaturated fatty acids in fish muscle, leads to the production of off-flavors and off-odors, thereby shortening the shelf-life of food25. The TBA value and peroxide value are both well established methods for determining oxidation products26. TBARS and peroxide values of grass carp washed mince were increased at 4°C within 15 days27. According to Somboonyarithi27 TBARS value increased during ice storage of tilapia washed mince. The values of fat degradation products like FFA, PV and TBARS are higher in Pangasius mince as compared with those reported above. This might be attributed to the higher fat content in Pangasius mince.

Changes in whiteness, expressible moisture and gel strength: Whiteness of control mince did not vary during refrigerated storage but whiteness of mince treated with cryoprotectants increased significantly during 6 days of refrigerated storage (Table-3). The increase in whiteness in treated mince samples may be due to addition of cryoprotectants. Expressible moisture is an indicator of water holding capacity of mince. In the present investigation, expressible moisture increased significantly throughout the storage of 6 days irrespective of treatments. Water holding capacity has a direct correlation between myofibrillar protein content and gel strength of the product28-29. The gel strength of
Table 1: Proximate Analysis.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Days</th>
<th>MWOC</th>
<th>MWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>0</td>
<td>74.79±0.36a</td>
<td>70.67±0.03a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>76.38±0.13a</td>
<td>72.19±0.97a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>78.41±0.05a</td>
<td>74.39±0.43a</td>
</tr>
<tr>
<td>Protein</td>
<td>0</td>
<td>18.85±0.36a</td>
<td>16.89±0.19a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>17.31±0.43a</td>
<td>16.08±0.41a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>16.51±0.19a</td>
<td>14.04±0.37a</td>
</tr>
<tr>
<td>Fat</td>
<td>0</td>
<td>4.34±0.17a</td>
<td>3.28±0.20a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.94±0.03a</td>
<td>2.51±0.19a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.37±0.09a</td>
<td>2.25±0.31a</td>
</tr>
<tr>
<td>Ash</td>
<td>0</td>
<td>1.36±0.19a</td>
<td>1.31±0.31a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.09±0.05a</td>
<td>1.32±0.05a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.07±0.03a</td>
<td>1.22±0.01a</td>
</tr>
</tbody>
</table>

Table 2: Biochemical changes

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Days</th>
<th>MWOC</th>
<th>MWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPN (mg%)</td>
<td>0</td>
<td>0.14±0.04a</td>
<td>0.12±0.02a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.23±0.04a</td>
<td>0.34±0.06a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.31±0.01a</td>
<td>0.39±0.03a</td>
</tr>
<tr>
<td>TVBN (mg %)</td>
<td>0</td>
<td>14.59±1.58a</td>
<td>17.36±1.58a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>25.77±0.00a</td>
<td>38.37±0.24a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>41.52±4.17a</td>
<td>38.70±4.79a</td>
</tr>
<tr>
<td>FFA (% oleic acid)</td>
<td>0</td>
<td>0.08±0.00a</td>
<td>0.07±0.10a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.11±0.02a</td>
<td>0.15±0.01a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.29±0.03a</td>
<td>0.21±0.01a</td>
</tr>
<tr>
<td>PV(milli-equivalent peroxide O₂/kgfal)</td>
<td>0</td>
<td>2.16±0.28a</td>
<td>2.15±1.15a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.83±0.54a</td>
<td>3.65±1.81a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>11.82±3.72a</td>
<td>18.42±4.73a</td>
</tr>
<tr>
<td>TBARS (mg MDA/kg)</td>
<td>0</td>
<td>0.13±0.04a</td>
<td>0.09±0.02a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.48±0.09a</td>
<td>2.35±0.01a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.33±0.03a</td>
<td>1.01±0.02a</td>
</tr>
</tbody>
</table>

Table 3: Changes in whiteness, expressible moisture, and gel strength of mince with and without cryoprotectants at refrigerated temperature (3-5°C)

Note: 1. Different small letters in the same column of each particular indicate significant difference with storage period (p<0.05), Values are mean±SD (n=3)
2. MWOC = mince without cryoprotectants, MWC = mince with cryoprotectants decreased during ice storage. Table 3 Changes in whiteness, expressible moisture, and gel strength of mince with and without cryoprotectants at refrigerated temperature (3-5°C)

control mince and treated mince were 207.86 and 245.43 g.cm respectively at day zero. However, it decreased significantly during further storage up to 6 days irrespective of cryoprotectant treatment. This might be due to the enzymatic activity which might have degraded myofibrillar protein in the mince. The same is evident with increased TVBN and NPN values. Further the microbial load was higher in the mince which might have affected adversely with cryoprotectants like sugar and sorbitol in it and this may not exist its effect in protecting the protein at refrigerated stored. Decrease in gel strength is associated with increase in expressible moisture throughout the refrigerated storage. Mehta et al. found that the gel strength of ice storage IMC continuously decreased throughout the storage. According to Matsumoto and Noguchi, pH affects the denaturation rate of myofibrillar protein, which is important in gel forming ability of minced fish. Myofibrillar protein is unstable and rapidly loses their ATPase activity. According to Xiong et al. surimi of grass carp had decreased in gel strength and water holding capacity during refrigerated storage. Gel strength of tilapia surimi...
Changes of Texture Profile Analysis: Texture profile analysis of the mince gels added without and with cryoprotectant and stored at refrigerated temperature revealed that at day zero, there was not much difference in most of the textural parameters among all samples tested, except hardness and adhesiveness (Table-4). However, hardness, springiness and chewiness values of treated mince decreased after 6 days of storage. The results indicated that softening of texture occurred after 6 days of storage, which was probably due to the proteolytic action promoted by muscle endopeptidases (Calpains I and II and Cathepsins B, D, H and L) and microbial (bacteria and yeasts) proteinases. According to Yathavamoorthi et al., hardness decreased, chewiness also changes in response to storage condition of tilapia washed mince and cohesiveness remained same.

SDS-PAGE protein pattern of sample and control: Changes in protein pattern of MWOC during refrigerated storage are depicted in Fig.-1. From the figure it was observed that MHC and actin band were visible at day zero in MWOC. However the band intensity of MHC and actin become invisible at day 3 and 6 of refrigerated storage indicating severe degradation of myofibrillar proteins. Further disappearance of MHC was observed in surimi gels prepared from refrigerated store MWOC during day 0, 3, and 6. This disappearance of MHC might be partially due to polymerization and partially due to degradation. Almost similar trend was observed in MWC and MWOC (Fig.-1 and Fig.-2)

These results are in accordance with reduced gel strength and increased expressible moisture content. Proteolysis may be the main reason for degradation of MHC during refrigerated storage. Tolstra reported the proteolytic action promoted by muscle endopeptidases. Maqsood et al. also reported the softening of surimi texture during increased refrigerated storage. The results obtained in the present investigation are in agreement with above findings.

CONCLUSION

Deboned Pangasius mince was mixed with cryoprotectants i.e. 4% sucrose, and 4% sorbitol with 0.2% STPP and mince kept along with sample as control. From the results i.e, pH, proximate composition, biochemical, textural, SDS-PAGE, and sensory analysis, it was observed that Pangasius mince degraded quickly under refrigerated storage condition irrespective of addition of cryoprotectants and can be stored...
only up to 3 days. It can be concluded that further investigations are required to know why the cryoprotectants were not effective on Pangasius mince during its refrigeration storage and possibility of using alternative cryoprotectants.

ACKNOWLEDGEMENTS
The authors gratefully acknowledge to the Director of Central Institute of Fisheries Education (CIFE) for granting permission to carry out the work and for providing necessary facilities.

REFERENCES