Biochemical, textural and sensory analysis of paneer developed from mince of *Pangasianodon hypophthalmus* (Sauvage, 1878)

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**Abstract**

Fish paneer was developed utilizing low cost fresh water fish pangas (*Pangasianodon hypophthalmus*). Fish mince was mixed with salt, potato starch, soya protein isolate and starch soluble at 1.5%, 2%, 2.5% and 3% concentration respectively. Paneer was prepared by thermal gelation initially at 45 °C for 30 minutes followed by 90 °C for 20 minutes. Paneer preparation was standardized using texture profile analysis, colour and sensory characteristics (Hedonic scale rating). The highest score for sensory characteristic was found for each of the ingredients at 2% concentration and can be recommended for paneer and similar type of products. The quality analysis such as biochemical, microbial and sensory attributes was done to evaluate the quality of raw material and fish paneer. All the biochemical, microbial sensory parameters were found within the acceptable range for raw fish and paneer. Thus less demanded Pangas can be utilised and marketed as highly demanded fish paneer.

**Keywords:** fish paneer, fish mince, texture, thermal setting, *Pangasianodon hypophthalmus*

**Introduction**

Fish is an important dietary constituent of several population groups and it has significant nutritional value such as high quality proteins, vitamins, minerals and lipids, and the largest source of ω-3 series polyunsaturated fatty acids (especially the EPA and DHA) which bring numerous benefits to human health (Limin *et al.*, 2006; Visentainer *et al.*, 2007) \[35, 52\]. A large proportion of total landed fish remains unused due to basic problems related to unattractive color, flavor, texture, small size and high fat content. Recovery of flesh by mechanical deboning and development of value-added products are perhaps the most promising approaches. There are various possibilities for product development using mince from low cost fishery resources. Pangas (*Pangasianodon hypophthalmus*) is an exotic catfish species (Family: Pangasiidae) which is gaining importance for aquaculture in India especially due to its great potential for value addition, faster growth rate and high yield.

Fish meat in minced form is a foundation material for a wide variety of ready-to-eat value added convenient products. A fish mince or muscle portion or flesh of fish offers an opportunity to exercise control over flavour, appearance and keeping quality by the incorporation of additives (Rodger *et al.*, 1980) \[38\]. The unique characteristic of minced fish is its texture forming ability and so it is an excellent base material to manufacture a variety of ready-to-eat seafood products such as fish finger, cutlet, patties, burger, sausages, fish balls etc. Fish or meat muscle forms a viscous sol on grinding with salt and turns to an elastic gel upon heating. Thermal gelation of the sol provides the elasticity of comminuted fish meat gel products (Sano *et al.*, 1994) \[41\]. Thermal setting of meat involves a cooking which causes cross-linking of amino acid residues, thus forming a tight protein network and entrapping water. This technique also helps to produce a variety of products such as fish cakes, fish balls, fish ham, fish sausages, fish nuggets, fish crackers of Malaysia etc. Development of a product applying thermal gelation was attempted here utilizing mince from low cost fresh water fish pangas with incorporation of different additives. The developed product named fish paneer was optimized based on texture, colour and sensory characteristics and its quality characteristics were evaluated.
Material and Methods
Collection of fish
Thai pangas fish (*Pangasianodon hypophthalmus*) was procured from local fish market located at Agartala, West Tripura and brought to the laboratory under iced condition in plastic poly styrene insulated containers within 1 hr. of collection and used for this study. The average length and weight of fish were 45.5±5.27 cm and 2500.3±17.60 g respectively.

Preparation of minced meat
Immediately after reaching laboratory of the Department of Fish Processing Technology & Engg. (College of Fisheries, Lembucherra, Tripura) the pangas fish were washed with ice cold potable water to remove dirt, sand and unwanted material. Immediately the fishes were gutted, dressed, filleted by hand and minced by employing a mechanical meat mincer with a 3 mm hole plate.

Development of fish paneer from mince
Minced meat prepared from *Pangasianodon hypophthalmus* was optimized by incorporating additives like salt at different concentration and potato starch, soya protein isolate, starch soluble at 1.5%, 2%, 2.5% and 3%. The meat mixture was packed in rectangular shaped aluminium foil packs and thermal setting was done at 45°C for 30 minutes followed by 90°C for 20 minutes (Fig 2). The thermally set meat was cut into small cubes (such as milk paneer) and tested for texture profile, color & sensory characteristics. Based on the analysis of texture profile (hardness, springiness, gumminess, cohesiveness, adhesiveness and cutting strength), colour (L*, a*, b* and whiteness) and evaluation of sensory characteristics (appearance, colour, flavour, texture, odour, taste & overall acceptability) the ingredient combination was optimized for development of fish paneer (Fig2). The flow chart of fish paneer preparation is given in Fig 1.

Biochemical and microbial analysis
Moisture, ash, crude protein lipid and non-protein-nitrogen content of the mince were determined according to AOAC (2000) [7]. Total volatile base nitrogen (TVBN) was estimated by Conway’s micro-diffusion method (Conway, 1947) [11]. The Peroxide value (PV) was determined according to the methods suggested by Jacob (1958) [25]. Thiobarbituric acid (TBA) value was determined by the titrimetric method of Tarladgis et al. (1960) using thiobarbituric acid standard in 90% glacial acetic acid. For analysing pH, 10 g of sample was blended with 10 ml CO$_2$ free water. The temperature of the prepared sample was adjusted to 25°C and pH was measured using a digital pH meter (Sartorius). Salt soluble protein (SSP) was extracted by homogenising 10 g of minced fish with Dyer’s buffer (Dyer et al., 1950) [14]. The supernatant containing salt soluble protein, i.e., myofibrillar fraction of muscle, was estimated through Kjeldahl distillation unit following standard method (AOAC, 2000) [7]. Results were expressed as g SSP per 100 g minced fish. Water holding capacity of minced meat was determined as expressible moisture content following the method of Suvanich et al. (2000).

The total plate count (TPC) was estimated by the spread plate technique (Hitching et al., 1995) [17]. Ten grams of the sample were weighed aseptically into a sterile sample dish and transferred to a sterile polythene pouch containing 90 ml normal saline. The sample was blended in a Stomacher (Seward, West Sussex, UK) for 60 s at normal speed. Using a sterile pipette, 1 ml of the supernatant was aseptically transferred into a 9 ml saline tube and mixed well using vortex mixer. Similarly, further dilutions were prepared. A total of 0.1 ml each of the appropriate dilution was pipetted out on to sterile agar petri dishes, taken in duplicates for each dilution. The plates were incubated at 37 °C for 48 h in an inverted position. After incubation, the individual bacterial colonies were counted. The average number of colonies were calculated and expressed as log CFU/g of the sample.

Instrumental analysis of texture profile
Texture properties of fish paneer were determined using a texturometer (TA-XT2 Stable Micro Systems, Surrey, England, UK). Paneer were taken out from the aluminium foil pack and equilibrated to room temperature for 30 min in a plastic bag to avoid dehydration before the mechanical properties were measured. Cutting strength (N) was performed using a knife blade probe at a test speed of 2 mm s$^{-1}$. Texture profile analysis (TPA) was performed using an aluminium cylindrical probe (P/50) with 50 mm diameter. Samples were compressed to 40% of the initial height using pre-test speed of 1 mm s$^{-1}$, test speed of 5 mm s$^{-1}$ and post-test speed of 5 mm s$^{-1}$. Hardness, springiness, adhesiveness, cohesiveness and gumminess were reported for each treatment. Three samples were analysed for each treatment at room temperature (25–27°C).

Instrumental analysis of colour
Colour of fish paneer was determined in triplicate using spectrocolourimeter (Colourflex EZ, Hunter Associates Laboratory, Inc, Reston, VA) with illuminant of D 65/10°. This instrument was calibrated with black and white reference tiles before analysis. A horizontal section of paneer measuring approx. 5 mm was placed above the light sources and post processing L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) values were recorded. The CIELAB (L*, a*, b*) colour scale was used for the study. Whiteness was calculated as described by Lanier et al. (1991):

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

Sensory Analysis
The changes in the sensory characteristics of the fish paneer samples were evaluated by a panel of 10 researchers from the Institute, who have previously participated in the evaluation of similar products, on a 10-point scale (IS: 6273 [II] 1971; Vijayan, 1984) [50]. A sensory score of 4 was taken as the limit of acceptability. The panelists were asked to assign a score of 1 to 10 (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = light slightly, 7 = like moderately, 8 = like very much, 9 = like extremely, 10 = excellent) for appearance, color, flavor, odor, taste, texture and overall acceptability, as described by Vijayan (1984) [50].

Statistical Analysis
The Statistical Package for Social Sciences (SPSS, version 16.0 for windows) was used for analysis of the experimental results. The results were expressed as mean ± standard deviation.

Results and Discussion
Optimization of concentration of additives
Based on the texture profile (hardness, springiness,
gumminess, cohesiveness, adhesiveness and cutting strength), colour (L*, a*, b* and whiteness) and scores of sensory characteristics (appearance, colour, flavour, texture, odour, taste & overall acceptability), paneer was standardized. Result for textural profile, colour and sensory of fish paneer with different concentration of additives are presented in Table 1, 2 and 3. Minced meat of pangas was incorporated with additives like salt, potato starch, soya protein isolate and starch soluble at different concentration of 1.5%, 2%, 2.5% and 3%.

The function of salt is to help solubilize myofibrillar proteins which forms a continuous matrix and then undergo thermal aggregation, cross-linking and develop into fine three dimensional solid-like networks resulting in elastic gel (Aguilera, 1995) [3]. Protein denaturation and aggregation, induced by heating under the proper conditions, drives gelation of surimi (Lanier et al., 2005). Paneer developed with 2% salt concentration was found to give better cutting strength, hardness & other texture characteristics (Table 1) compared to other salt conc. The same sample showed an optimum whiteness (Table 2) score. Score for all the sensory characteristics (Table 3) was higher for paneer at 2% salt conc. Fish paneer optimized with 2% salt are further treated with soya protein isolate at different concentrations (1.5%, 2%, 2.5% and 3%) for further improvement in texture, colour and sensory characteristics. Paneer with 2% soya protein isolate gave better cutting strength, whiteness and sensory quality compared to other compositions. Akesowan (2008) and Adisak (2008) reported increased moisture content, cooking yield and colour of the pork sausage after addition of 2% soya protein isolate.

![Table 1: Textural profile of fish paneer with different concentration of additives](image)

![Table 2: Colour of fish paneer with different concentration of additives](image)

![Table 3: Sensory score of fish paneer with different concentration of additives](image)
Carbohydrates, such as gums and starches, promote the formation of the continuous matrix by interacting with water and proteins in the fish paste and improving the water holding capacity (WHC) (Hunt et al., 2009). It increases firmness and gel strength (Lee et al., 1992). Addition of carbohydrates into a formulation could modify the capacity of salt to solubilise myofibrillar proteins, which would affect the mechanical and functional properties of gels. In the present study, potato starch and starch soluble (Himedia) were incorporated separately with optimized salt and SPI at different concentration. It showed that 2% potato starch gave better result in regard to cutting strength, gumm properties of gels. In the present study, potato starch provided softer texture to sausage compared to other fresh water fishes (Akande, 2001; Dhanpal et al., 2006). The total protein of the fish paneer was found to be 78.6% moisture, 16.5% protein and 2.26% fat as 78.6% moisture, 16.5% protein and 2.26% fat (Table 4). Higher moisture content (75.47%) in fish paneer than raw fish (74.25%) may be due to addition of water during preparation of paneer. Moisture content is an important factor that determines gelation properties of minced meat (Lanier & Lee, 1992) [27]. Uddin et al. (2006) [48] suggested that the standard water content of surimi is 78%. In present study, the protein content of fish paneer was higher than raw fish. This could be due to addition of soya protein isolate to minced meat during preparation of fish paneer. In a similar study, increase in protein level of milk paneer was attributed to incorporation of soya protein during preparation (Kanawjia et al., 1990) [28]. The fat content of paneer was found to be 4.89% which is lower than fat content of raw fish 6.20%. This was due to loss of fat during thermal setting of fish mince. Loss of fat due to spreading during heat treatment was also observed by Larsen et al. (2010) [32] in boiled king salmon fillets. Study revealed that ash content in raw fish was superior to fish paneer. This may be attributed to addition of additives during preparation of fish paneer. Jindal and Bawa (1988) [26] also reported an increase in ash content of cooked sausages with an increase in additive levels. Ashgarzadeh (2010) [31] found 78.6% moisture, 16.5% protein and 2.26% fat in silver carp (Hypophthalmichthys molitrix) mince.

### Proximate composition

The proximate composition of raw fish (Pangasianodon hypophthalmus) and fish paneer are given in the Table 4. Moisture, protein, fat, and ash contents in fresh pangas were found to be 74.25%, 16.5%, 2.26%, and 1.05% respectively which are within acceptable limit. Ejaz (2013) also reported an increase in ash content of cooked fish mince from 4.79 to 57.51% in ventral and 2.95 to 5.54% in dorsal portion. et al.

### Biochemical and microbial analysis

Biochemical qualities of raw fish and fish paneer are presented in Table 5. All biochemical and microbial parameters (TVBN, TBA, PV, pH, NPN, SSP, WSP, WHC and TPC) were well below the rejection limit as per the food standards. Amongst the biochemical quality attributes, the total volatile basic nitrogen (TVBN) indicates the breakdown of amino acid into mixture of ammonia, DMA and TMA along with other amines. According to Connell (1975), 35-45 mg100 g-1 of TVB-N content is the limit of acceptability for fish and fishery products. The TVBN value of raw fish and fish paneer were 5.07 mg100 g-1 and 4.20 mg100 g-1 respectively which are within acceptable limit. Eijaz (2013) found TVBN value of 6.38 mg100 g-1 in fish burger developed from pangasius mince.

### Table 4: Proximate composition of Raw fish (Pangasianodon hypophthalmus) and fish

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw fish</th>
<th>Fish paneer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (%)</td>
<td>15.18±0.33</td>
<td>15.66±0.25</td>
</tr>
<tr>
<td>Total lipid (%)</td>
<td>6.20±0.37</td>
<td>4.89±0.25</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>74.25±0.37</td>
<td>75.47±0.71</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.05±0.08</td>
<td>1.79±0.28</td>
</tr>
</tbody>
</table>

*Data expressed as mean±SD, n=3.

The proximate composition of fish paneer was found to be moisture 75.47%, protein 15.66%, lipid 4.89% and ash 1.79% (Table 4). Higher moisture content (75.47%) in fish paneer than raw fish (74.25%) may be due to addition of water during preparation of paneer. Moisture content is an important factor that determines gelation properties of minced meat (Lanier & Lee, 1992) [27]. Uddin et al. (2006) [48] suggested that the standard water content of surimi is 78%. In present study, the protein content of fish paneer was higher than raw fish. This could be due to addition of soya protein isolate to minced meat during preparation of fish paneer. In a similar study, increase in protein level of milk paneer was attributed to incorporation of soya protein during preparation (Kanawjia et al., 1990) [28]. The fat content of paneer was found to be 4.89% which is lower than fat content of raw fish 6.20%. This was due to loss of fat during thermal setting of fish mince. Loss of fat due to spreading during heat treatment was also observed by Larsen et al. (2010) [32] in boiled king salmon fillets. Study revealed that ash content in raw fish was superior to fish paneer. This may be attributed to addition of additives during preparation of fish paneer. Jindal and Bawa (1988) [26] also reported an increase in ash content of cooked sausages with an increase in additive levels. Ashgarzadeh (2010) [31] found 78.6% moisture, 16.5% protein and 2.26% fat in silver carp (Hypophthalmichthys molitrix) mince.

### Table 5: Biochemical and microbial quality of raw fish and fish paneer

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw fish</th>
<th>Fish paneer</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVBN (mg%)</td>
<td>5.07±0.51</td>
<td>4.20±0.50</td>
</tr>
<tr>
<td>PV (meq O2 kg-1 fat)</td>
<td>2.79±0.41</td>
<td>3.34±0.59</td>
</tr>
<tr>
<td>TBA (mg malonaldehyde kg-1)</td>
<td>0.09±0.01</td>
<td>0.13±0.04</td>
</tr>
<tr>
<td>pH</td>
<td>6.33±0.39</td>
<td>6.21±0.29</td>
</tr>
<tr>
<td>NPN (g%)</td>
<td>0.38±0.01</td>
<td>0.37±0.94</td>
</tr>
<tr>
<td>SSP (%)</td>
<td>10.28±0.47</td>
<td>11.14±0.01</td>
</tr>
<tr>
<td>WSP (%)</td>
<td>4.02±0.87</td>
<td>3.78±0.51</td>
</tr>
<tr>
<td>WHC (%)</td>
<td>71.19±0.84</td>
<td>77.34±2.23</td>
</tr>
<tr>
<td>TPC log cfu/g</td>
<td>5.19±0.04</td>
<td>4.83±0.05</td>
</tr>
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</table>

*Data expressed as mean±SD, n=3.
PV is the indicator of primary oxidation of lipid. In the present study, PV for raw fish and fish paneer were 2.79 and 3.34 meq.O₂ kg⁻¹ fat respectively. Mechanical deboning and mincing accelerates the oxidative changes due to the separation of fat from tissue and skin during deboning (Siddaiah et al., 2001) [40]. According to Connell (1995) [49], peroxide value exceeding 10 meq.O₂ kg⁻¹ fat of fish meat is regarded as unfit for human consumption. PV for both raw fish and paneer remained within the ranges of acceptability. Viji et al. (2014) [51] reported a lower PV value of 0.90 meq.O₂ kg⁻¹ fat in pangasius fish steak.

The TBA value is widely used as an indicator of the degree of secondary lipid oxidation. The TBA value of raw fish and fish mince were 0.09 mg malonaldehyde kg⁻¹ fat and 0.13 mg malonaldehyde kg⁻¹ fat respectively (Table 5). Rancidity appears in fish when TBA becomes greater than 1-2 mg malonaldehyde kg⁻¹ fat (Connell, 1975). So, the raw fish and fish mince in the study showed good quality. Debbarma & Majumdar (2013) [12] found TBA value of 0.79 mg malonaldehyde kg⁻¹ fat and 0.56 mg malonaldehyde kg⁻¹ fat in unwashed and washed pangas mince respectively. Raw fish and fish mince had pH of 6.33 and 6.21 which indicated the freshness of fish. Huss (1995) reported that the post mortem pH for most fish is 7 or slightly lower than 7 immediately after catch. The low pH is an indicator of stress which the fish might have encountered during harvesting (Mohan et al., 2008). pH of 6.59 in unwashed mince and 6.93 in washed mince of pangas were reported by Debbarma & Majumdar (2013) [12]. Viji et al. (2014) [51] reported a pH value of 6.35 for pangasius fish steak.

NPN can serve as the measure of freshness of sea food. Change in concentration of NPN after catch of fish is due to enzyme and microbial degradation of muscle. This NPN-fraction (non-protein nitrogen) constitutes 0.2 to 0.4 % in freshwater fishes (Sen, 2005) [42]. In the present study, NPN value of raw fish and fish mince were 0.38% and 0.37% respectively. Hossain (2004) [15] reported 0.35% and 0.27% NPN in unwashed and washed pangas mince respectively. Debbarma & Majumdar (2013) [12] also reported similar result of 0.39 % NPN in pangas mince and 0.35 % NPN in surumi prepared from pangas mince. Siddaiah et al. (2001) found an NPN value of 0.38 % in kamaboko prepared from silver carp mince.

The salt soluble protein (SSP) and water soluble protein (WSP) of raw fish were 10% and 4.02% and for fish mince 11.14% and 3.78% respectively. The lower salt soluble protein of fish mince than mince could be due to the loss of protein during cooking. Leander et al. (1980) also observed that when meat is cooked, water, soluble proteins and fats are expelled from the tissue. Debbarma & Majumdar (2013) reported SSP of 10.15% in unwashed mince and 12.84% in washed mince of pangas. Siddaiah et al. (2001) reported 10.67% SSP and 4.54% WSP in silver carp (Hypophthalmichthys molitrix) mince. 11.17% SSP and 4.36% WSP were recorded in rohu fish mince by Sankar (2000).

Water holding capacity (WHC) of fish mince was 77.34% and raw fish was 71.19% (Table 5). Higher WHC of mince could be due to partial unfolding of myofibrillar protein and formation of three dimensional structures during thermal gelation of fish mince (Kamah et al., 1992). Salt concentration also affected the WHC of restructured products. (Uresti et al., 2004) [49]. Akahane & Shimizu (1989) reported that water holding capacity (WHC) of Alaska pollock surimi increased in salt ground surimi paste. WHC has shown to depend on the physicochemical properties of proteins such as hydrophobicity, solubility and dispersion capacity (Sikorski and Kolakowska 1994). In addition, water-holding capacity of fish mince is directly correlated with gel product quality (Honikel & Hamm, 1994) [18].

TPC was done to estimate microbial load on raw fish and fish mince and was found to be 5.19 log cfu g⁻¹ and 4.83log cfu g⁻¹ (Table 5) respectively. Heat treatment given during thermal gelation of fish mince decreases the microbial load from raw fish. Abdelrahman (2014) reported decrease in TPC of raw cutlet from 3.20 log cfu g⁻¹ to 2.70 log cfu g⁻¹ in cooked cutlet. The total bacterial count (TPC) of 5.32 log cfu g⁻¹ in pangasius fillets was reported by Ikasari & Suryaningrum (2015). As recommended by International Commission on Microbiological Specification for food (ICMSF, 1986), an increase of total plate count (TPC) up to levels exceeding the value of 7 log cfu g⁻¹ is regarded as microbiologically spoiled fish muscle not fit for human consumption. The total plate count of raw fish and fish paneer was within the acceptable limit given by ICMSF (1986). Elysi (2010) reported total plate count (TPC) of 6.1 and 1.2 log cfu g⁻¹ in raw fish finger (without fried) and fried fish finger prepared from common Carp.

**Conclusions**

Fish paneer was developed with 2% salt, 2% potato starch and 2% soya protein isolate concentration based on texture profile, colour, sensory profile. Fish paneer is a new type of fish product developed by utilizing low value fish pangas. Since, fresh Pangasius fetch low price in market due to some inherent non-appealing muscle quality, its abundant catch can be utilized as an alternative source of raw material for development of fish paneer. This paneer product is new to value addition of fish and is expected to fetch a good market value and will enhance opportunities for entrepreneurship development.
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