VI. SUMMARY

With increase demand for fish and fishery products World over, supply of acceptable quality fish to increasing population is being accorded top priority by planners. About 20-25% of total fish catch is lost due to spoilage because of its inherent characteristics and also due to lack of required infrastructure for its preservation. While several species of by-catch, non-conventional and underutilized species are equally important from nutritional point of view; its utilization in fresh form is being limited because of poor consumer acceptance. In order to utilize by-catches, which are normally of low economic value, attempts are being made for the development of novel processing techniques so as to reach the consumer at affordable prices. Extrusion processing is one such technology where it is being expanded to fish mince based cereal products so as to achieve supply of nutritious food. Extruder plays several important functions in the processing of these foods. Among the most important are precooking and gelatinization of the starch, providing a desired shape to the product and giving the product an expanded, crisp and pleasing characteristics. Addition of fish meat to cereal based extruded products will proportionally add to the nutritive value. Utilization of low economic value fish for extrusion processing is another step stone in managing the optimum utilization of under utilized fish and by-catches.

Utilization of fish processing waste like skin and bones is receiving attention by various researchers with twin objectives avoiding environmental pollution and to derive value added products for better economic viability. Gelatin is one such product, which can be obtained from skin of various marine fish species. The conventional source of raw material for gelatin preparation is from the skin of bovine and porcine sources. With the recent outbreak of mad cow disease (Bovine Spongiforme Encephalopathy) the industry is looking for alternate raw material for gelatin preparation.

With this rationale the objective of the present investigation was to

- Utilize low economic value fish like ribbonfish and bull’s eye fish for extrusion process with cereal flours (rice flour, wheat semolina and ragi flour) using twin-screw extruder.
• To characterize fish proteins and starch from cereal flours used for extrusion process by thermal and optical analysis.

• To optimize the process variables like barrel temperature, screw speed and die diameter of the extruder and the quantity of fish meat.

• To assess the composition and properties of fish mince-cereal based extruded product.

• To prepare and assess the properties of gelatin from skin of bull’s eye fish.

Ribbonfish (*Trichiurus* spp.) and bull’s eye fish (*Priacanthus* spp.) caught by trawl along West coast off Mangalore were used in the present study. Fish mince without skin, scales and bones were used for the extrusion process. The fish mince was mixed with individual cereal flours either at 10% or 20% and after proper preconditioning subjected to extrusion process. Both the fish belong to low fat fish having fat content of below 2%. The protein content of ribbonfish and bull’s eye fish meat was 17.91% and 16.71% respectively. The amino acid profile revealed that both the fish were having a higher proportion of alanine, glutamic acid, lysine and leucine. The lysine content of ribbonfish and bull’s eye fish meat was 10.57 and 9.79 (as percentage amino acids) respectively. The fatty acid profile revealed that the major fatty acid present in ribbonfish was erucic acid or cetoleic acid (C22 : 1n9) and that of bull’s eye fish were heptadecanoic acid (C17 : 0) and arachidic acid (C20 : 0). The EPA and DHA content of ribbonfish and bull’s eye fish were 11.83 & 7.86 and 6.99 & 8.96% respectively.

The protein content in wheat semolina (11.88%) was higher than rice and ragi flour. Higher ash content in ragi showed the presence of high amount of calcium and soluble fibers.

The thermogram (DSC profile) of the meat from both the fish showed three transitions. For ribbonfish meat, the peak temperatures for the denaturation of myosin, sarcoplasmic protein and actin were at 33.17°C, 48.85°C and 60.96°C and the corresponding transitions for bull’s eye fish meat were at 38.35°C, 47.72°C and 63.02°C. The DSC profile of flours/semolina revealed that the peak gelatinization temperature of rice flour, wheat semolina and ragi flour were at 64.73°C, 58.16°C and 69.72°C respectively.
FT-Raman spectra of proteins from ribbon and bull’s eye fish showed a strong band at 1005 cm\(^{-1}\) indicating phenylalanine compound. The sharp peak at 760 cm\(^{-1}\) in both ribbonfish and bull’s eye fish is due to indole ring of tryptophan. The ratio of the bands \(I_{855}/I_{830}\) for ribbonfish was 0.64 and that of bulls eye was 0.80, indicating that tyrosine residues are not exposed.

The FT-Raman spectra of native rice flour, wheat semolina and ragi flour revealed a conspicuous peak at a wavenumber of 480 cm\(^{-1}\) due to presence of \(\alpha-(1\rightarrow4)\)-glycosidic linkage. In the spectra of wheat semolina there was two additional peaks between 1663-1630 cm\(^{-1}\) and another between 1595-1528 cm\(^{-1}\), which corresponds to Amide I and Amide II respectively. In wheat semolina and ragi flour after gelatinization the intensity of all bands decreased except the bands at 1633 cm\(^{-1}\) and 3213 cm\(^{-1}\), which were increased indicating the irreversible absorption of water. In the spectra of rice flour the spectral changes after gelatinization was marginal.

Dynamic rheological properties of flour samples (10% w/v solution) revealed that the maximum \(G'\) (storage modulus) for rice flour, wheat semolina and ragi flour were 6029 Pa, 118 Pa and 544 Pa respectively. The temperature at which cross over (gelling point) has taken place for rice flour, wheat semolina and ragi flour during dynamic rheological testing was found to be at 61.63\(^{\circ}\)C, 60.22\(^{\circ}\)C and 52.68\(^{\circ}\)C respectively.

Phase contrast microscopy revealed that the shape of starch granules was irregular varying from round to polyhedral. All the granules were found to be intact and there was no disruption in shape. In the present investigation phase contrast microscopy revealed that the granule size of wheat semolina was higher than rice or ragi flour. The phase contrast microscopic photograph of the flours in wet state showed swelling of the granules. After gelatinization the granules swell to a greater extent and the swelling was greater for wheat semolina as compared to rice and ragi flour.

**Extruded products**

Extruded products were prepared from a mixture of rice flour, wheat semolina and ragi flour with and without addition of fish meat (ribbonfish/bull’s eye fish) as a function of barrel temperature, screw speed and die diameter. As a function of screw speed and die diameter, samples were prepared only with ribbonfish meat at a concentration of 10% and their respective controls.
The moisture content of all the extruded products decreased with increase in barrel temperature, indicating higher temperature during extrusion process could reduce the moisture content of final product. However with increase in fish mince content, higher moisture content was recorded in the final product.

With increase in screw speed the moisture content of the products showed an increasing trend. This reveals that residence time will be shortened with increase in screw speed, hence the moisture content of final products increased.

The moisture content of all the extruded products increased with increase in die diameter. This is mainly related to the pressure developed inside the barrel wherein, it progressively reduces with increasing die diameter.

The protein content of the extruded products did not show any significant difference with respect to variables such as barrel temperature, screw speed and die diameter. However, the fat content did show some variation as the function of different variables and was related to moisture content.

Aminoacid composition of extruded products from a mixture of rice flour-fish mince (ribbonfish and bull’s eye fish meat) mixture showed a higher proportion of glutamic acid, alanine, tryptophan, leucine and lysine. With increase in percentage of fish meat from 10% to 20%, the percentage of essential aminoacids especially lysine increased.

Expansion ratio

The expansion ratio of extruded products from flour-fish mince (ribbonfish/bull’s eye fish) increased with increase in barrel temperature. The highest expansion ratio of 375% was obtained for the products extruded at 120oC with rice flour and ribbonfish at 10% level. Products with ragi flour-fish mince mixture showed the least expansion ratio at different temperatures tried.

The effect of screw speed on the expansion ration of extruded products revealed no significant difference between control and fish mince based products. The extent of decrease in expansion ratio in relation to screw speed was higher in samples incorporated with fish mince.

The expansion ratio of different extruded products as a function of die diameter indicated a decreasing trend with increase in die diameter. The extent of decrease was
minimum in case of ragi flour and ribbonfish mixed extrudates. This could be related to higher moisture content and improper gelatinization in the final product.

**Water absorption capacity (WAC)**

The water absorption capacity of the extruded products increased with increase in barrel temperature. At 10% fish mince incorporation (either ribbonfish or bull’s eye fish) to rice flour yielded a water absorption capacity of 7.0 g water/g dried material. When the fish meat proportion was increased to 20% there was significant decrease in WAC values in all the samples.

The increase in WAC of extruded products as a function of screw speed revealed divergent results for different products. For wheat semolina-10% ribbonfish mince significant increase in WAC values with screw speed was recorded. WAC values of products with rice or ragi flour and fish mince recorded a marginal increase. The WAC values of samples are mainly dependent on the nature of protein and the extent of exposure of hydrophilic group upon processing.

The increase in WAC values with incorporation of 10% ribbonfish mince was more in case of rice flour than with wheat semolina and ragi flour products. Wheat semolina – fish mince based products registered a decrease in WAC value with increase in die diameter for which no convincing reason could be given.

**Colour**

The color of the extruded products was assessed by Hunters colorimeter with L*, a* and b* co-ordinates. Higher L* values indicates lighter color of the product. The L* values of the products obtained from rice flour only (control) showed a decreasing trend with increase in temperature indicating darkening of the products. However, the L* values of the products obtained from wheat semolina and ragi flour revealed an increase in L* value with increase in barrel temperature. This could be attributed to the reactions in the pigments of the respective flours with increase in temperature. Incorporation of fish mince at 10% level could significantly increase the L* values with increase in temperature in all the extruded products. There was an inverse relationship between L* and a* or b* values in all the samples studied. The color of the ragi flour based extruded products with respect to L* values were consistently lower compared to rice flour or wheat semolina products.
The L* values of the control samples (without fish meat) obtained from different flours revealed increasing trend with increase in screw speed. However this increase was not significant. The extrudates from rice flour and 10% ribbonfish mince showed a significant increase in L* value as a function of screw speed over that of respective control. The extrudates obtained from mixture of wheat semolina-10% ribbonfish mince and ragi flour-10% ribbonfish mince registered a decreasing L* value with increase in screw speed. Thus it is evident that addition of ribbonfish mince to wheat semolina and ragi flour registered a dark color with increase in screw speed while the extruded products with rice flour-10% ribbonfish mince had a light color.

The L*, a* and b* values of extruded products obtained as a function of die diameter did not show any appreciable change. From the results it is evident that barrel temperature and screw speed had influenced the development of desired color.

Texture analysis of extruded products

The texture of extruded products with respect to crispness and breaking strength has been analyzed. The products prepared with 10% fish mince (ribbonfish/bull’s eye fish) at 90oC were crispier irrespective of the flour or fish mince. Higher crispness was recorded in the extruded products obtained from rice flour – fish mince and wheat semolina – fish mince as compared to ragi flour – fish mince. The crispness was related to expansion ratio of the product and as products from ragi flour had lesser expansion, the product was less crispy. The crispness was assessed only with products prepared at a screw speed of 350 RPM and die diameter of 4 mm.

The breaking strength of the extruded products obtained from flour/semolina (control samples) decreased with increase in barrel temperature. Addition of 10% ribbonfish or bull’s eye fish to the flour/semolina did not affect breaking strength significantly as a function of temperature. When the fish meat proportion was increased to 20% higher breaking strength was recorded in all the extrudates especially when the barrel temperature was 70oC. Breaking strength is an indicator of the force required for fracture and higher breaking strength means improper gelatinization and lesser expansion ratio. An inverse relation could be focused between crispness and breaking strength values obtained under identical conditions.
The effect of screw speed on the breaking strength of the extrudates obtained from rice flour-fish mince and ragi flour – fish mince showed an increasing trend with increase in screw speed. However wheat semolina-fish mince mixture the breaking strength reduced considerably as compared to the control at any screw speed in the experimental regime. Though explanation could be found with reference to rice and ragi extrudates with respect to improper gelatinization, the same could not be true with wheat semolina based extrudates. This could be perhaps due to behaviour of gluten in wheat to different shear condition as induced by screw speed.

The effect of different die diameter in extrusion process on the breaking strength of extrudates revealed ragi flour-fish mince mixture had a significant effect in increasing the breaking strength of the final products.

The study revealed that good quality extruded products can be prepared using rice flour-ribbonfish at 10% level with extrusion processing conditions like barrel temperature of 90°C and die diameter of 4 mm. Quality analysis of extruded products from wheat semolina-fish mince mixture revealed that better products can be prepared using wheat semolina with 10% bull’s eye fish mince at a barrel temperature of 120°C and screw speed of 350 RPM using a 4 mm die diameter. Optimum extrusion variables for ragi based extruded products were a barrel temperature of 90°C and screw speed of 350 RPM using a 4 mm die diameter. The most compatible fish species for ragi flour based extruded products was found to be bull’s eye fish mince at a concentration of 10%.

**Gelatin**

Gelatin has been prepared from the skin of bull’s eye fish (Priacanthus spp.) and its physicochemical and rheological properties were assessed. The proximate composition of bull’s eye fish skin showed that the moisture content was 52.79% and a protein content of 25.19%. The yield of gelatin obtained in the present investigation was found to be 3-4%.

The protein content of freeze-dried gelatin was found to be 94.6% and 93% of protein was found to be soluble in phosphate buffer containing 0.3 M NaCl. The moisture content of freeze-dried gelatin was 2.2%. Bloom strength values of the gelatin prepared recorded a value of 108 g.
The aminoacid composition of gelatin from the skin of bull’s eye fish revealed higher proportion of glycine. The other major aminoacids present in the gelatin were alanine (19.45 %) and hydroxyproline (13.45%).

SDS PAGE pattern revealed the presence of bands at 120 KD and 170 KD revealing the presence of α-chains and β-chains respectively.

Setting index data revealed that with increase in concentration of gelatin solution, time taken for complete solidification reduced considerably. At a concentration of 10 mg/ml 100% solidification occurred after 180 minutes; when the concentration was increased to 40%, time taken for complete solidification was 105 minutes.

Flow profile of gelatin solution of various concentrations as a function of temperature has been assessed. Irrespective of the concentration of sample at temperatures of 25°C, 40°C, 50°C and 60°C, shearing caused the impairment of the sample as evident from the down curve data. Flow profile of gelatin solution (30 mg/ml) at 10°C showed minimum thixotropic area between up curve and down curve indicating least damage to the structure as affected by shearing. The flow profile data of gelatin solution indicated non-Newtonian behavior with pseudoplastic behavior at all concentrations and temperatures.