5.0 Summary and Conclusions....
The present study has evaluated the suitability of the skin of the freshwater fish as a raw material for the production of gelatin, optimized the process parameters for the gelatin extraction and studied the physico-chemical properties of gelatins & gel based products. The raw materials for the study were the skins of three cultured freshwater carps viz., Rohu (*Labeo rohita* – Hamilton Buchanan), Common carp (*Cyprinus carpio*) and Grass carp (*Ctenopharyngodon idella*). To summarize the work, the following conclusions are drawn from the study:

The gelatin extraction process from the skin of carps was optimized by adopting Response Surface Methodology which consisted of a preliminary screening and Response Surface Modelling to optimize the process parameters.

A two level fractional factorial screening design was employed to identify the critical independent variables that had significant influence on the gelatin extraction based on the responses for dependent variables viz., gel strength and yield. Alkali pretreatment concentration, Acid pretreatment concentration, Pretreatment time (min) and Extraction temperature (°C) were identified as critical independent variables that had influence on two responses studied in carp skin gelatin extraction.

The Response Surface Model was built on the basis of these factors as a four factor, five level Central Composite Design where the experimental values were compared with predicted values. A total of 31 experimental runs using the Central Composite Design were carried out to study the effect of the selected independent variables on the responses. The Analysis of Variance showed that the predicted models were statistically valid.

The optimization solutions for the extraction of gelatin from the skin of carps gave a composite desirability value above 0.8 based on the set parameters. The responses predicted by the solutions are within the range of the experimental values obtained in the response surface model.
concentration was found to be the most important factor affecting the gel strength and yield in the gelatin extraction process from carp skin under a given set of optimization parameters.

Verification experiments were conducted under optimal conditions to compare predicted values and actual values of responses and similar results were obtained. Therefore, the estimated response surface model was adopted for optimization of gelatin processing from the skins of Rohu, Common carp and Grass carp.

Physical properties of the carp skin gelatins were studied. The gelatin yield was significantly lower in Grass carp than the other two sources. The maximum yield was observed for Rohu (12.9%) followed by Common carp (12%) and Grass carp (10.5%). High viscosity gelatin was obtained from Rohu, Common carp and Grass carp, which is suited for film forming applications. The viscosity value was in the range of 5.96 – 7.07 and was significantly higher for Grass carp gelatin followed by Rohu and Common carp gelatins.

Melting point of gelatin obtained from carps (28.13 -29.1 °C) was comparable to gelatin from mammalian sources. The comparatively high amount of imino acid content can be a contributory factor for the high melting point characteristics of gelatins from these species. The setting temperature observed for the gels from Rohu, Common carp and Grass carp skins were in the range of 17.9 ⁰C – 20. 5⁰ C. Common carp had the lowest setting temperature and the highest was for Grass carp. Grass carp gel showed a significantly faster setting time of 68.6 seconds when compared to the other two gels. The setting and melting temperatures observed for gelatins from the skin of Rohu, Common carp and Grass carp are similar, if not better than many of the gelatins from animal sources and can possibly substitute the same in many applications without extensive modifications. This offers future scope for developing binary blends of these gelatins with animal gelatins that are completely compatible and commercially useful in many applications.
Gelatins from the skins carps were found to have a mild but easily perceivable odour, had a snowy white appearance and were light-textured. This could be a positive attribute, since it is easier to incorporate these gelatins into any food system without imparting any strong colour.

A study on the functional properties of the carp skin gelatins showed that the gels had medium gel strengths in the range of 181 – 230 B which are of commercial significance, considering the potential applications in food and edible film preparations. Gel strengths of carp skin gelatins were comparable to that of gelatins obtained from tropical fish species. Foam formation ability is an important functional property of gelatin which has significance in food applications viz., in the preparation of products like marshmallows. Foam formation ability of Common carp gelatin was 2.44, significantly lower than Rohu (2.51) and Grass carp (2.83) gelatins. Foam stability of Common carp was 1.90, significantly less than Rohu (1.86) and Grass carp (1.78) gelatins, demonstrating the lower stability of Common carp gelatin. Rohu skin gelatin had the highest fat-binding capacity (457.3%) and Common carp skin gelatin had the lowest water-holding capacity (176%). The high amount hydrophobic amino acid, tyrosine is probably responsible for the high fat binding capacity of Rohu skin gelatin. Grass carp gelatin had the maximum content of hydrophilic amino acid hydroxyproline among the carp gelatins and a correspondingly high value for water holding capacity.

Texture Profile Analysis of carp skin gelatins showed that hardness and cohesiveness values were maximum for Grass carp gelatin. Springiness index was significantly lower for Common carp gelatin than the other two gels. Grass carp gel showed significantly higher values for Gumminess, chewiness and fracture force than the other gels. Gelatins from Rohu, Common carp and Grass carp had very low adhesiveness, implying their chewability. The texture attributes of the gelatins of Rohu, Common carp and Grass carp gives an indication that these are useful in food applications for the preparation of the products like fruit gums where gelatin helps in thermo reversible gel formation, provide taste and color neutrality, gives easy pouring ability due to
low viscosity and excellent clarity. In addition gelatin gives unique texture and excellent mouth feeling, chewability and attractive appearance.

Chemical analysis of carp skin gelatin indicated that the moisture content of the samples was below 10%, which is less than the limit prescribed for edible gelatin i.e., 15% (GME, 2005). The ash content in gelatin samples were in the range of 1.10 - 1.18%, much less than the recommended maximum limit (2%) set for edible gelatin (GME, 2005). The pH varies between 4.05 - 4.42. Grass carp gelatin shows significantly higher values for pH. The values of pH for gelatin samples are outside the range prescribed for Type A Gelatin (pH 6.0 - 9.5) and Type B Gelatin (pH 4.7 - 5.6). This is because the pretreatment method employed during the extraction process involves both alkaline and acid treatments.

The amino acid composition of Gelatins from the skin of Rohu, Common carp and Grass carp was higher than gelatin from many other tropical and coldwater fish species with respect to the imino acid and other important amino acids content. This could be the reason for the compatibility of these gels with mammalian gels in respect of physico-chemical properties. Carp gelatin samples had high content of imino acids (Proline + Hydroxyproline) in the range of 19.49 - 19.86% of protein. High content of imino acids improves the rheological properties of gelatin as it is involved in formation of triple helical regions that immobilize water. Imino acids also impart considerable rigidity to the gelatin structure. Grass carp skin gelatin contained the lowest proline (8.2%) and highest hydroxyproline content (11.66%) among the three gelatins. However, maximum gel strength was observed for Grass carp gelatin which shows that Hydroxyproline is the major determinant of stability due to its hydrogen bonding ability through its hydroxyl group, although proline is also important. Carp skin gelatin samples had significant amounts of serine and threonine (4.88 - 9.1%) with free hydroxyl groups which can contribute to the gel strength by the generation of hydrogen bonds and helical structures. Significantly higher values for alanine was observed for Grass carp skin gelatin (8.3%). A higher content of this amino acid can be one of the reason for higher viscoelastic properties Grass carp.
skin gelatin than the other carp skin gelatins. The amino acid composition of Gelatins from the skin of Rohu, Common carp and Grass carp was higher than gelatin from many other fish species with respect to the imino acid and other important amino acids content.

Molecular weight distribution pattern of carp skin gelatins was studied which showed that Grass carp skin gelatin had predominantly β-chains with molecular weights of 200 kDa and less intensive α-chain bands with molecular weights in the range of 116 KDa and 97 KDa and sub α-units of molecular weights 55 to 66 kDa. Rohu gelatin had predominantly α-chains with molecular weights in the range of 116 KDa and 97 KDa and a wide range of sub α - units of molecular weights 6.5 to 66 kDa. In the case of Common carp skin gelatin, the β- chains with molecular weights of 200 kDa are also present, but their bands are less intensive than the α-chain bands. Grass carp skin gelatin with higher concentration of β-chain peptides and lower amounts of low molecular weight α - chains and sub α- units showed superior functional properties and high values for bloom, viscosity, melting point, setting point and a faster setting time than the other carp skin gelatins.

The FTIR spectra of carp skin gelatins were found to be dependent on the extraction temperature. Grass carp and Rohu gelatins with lower extraction temperatures at 40 and 49 °C respectively showed the low intensity amide A, I and II bands and the amide III band was not fully distinguished. These changes are indicative of greater disorder in gelatin and are associated with loss of triple helix state. This is consistent with changes expected as a result of denaturation of collagen to gelatin. The Common carp skin gelatin extracted at higher temperature exhibited a much broader amide A than was observed for the low temperature extracted Rohu and Grass carp gelatins. High temperature-extracted Common carp skin gelatin may contain a significant amount of intermolecular crosslinks. This can produce FTIR spectra showing a higher degree of molecular order.
Gelatins from the carp skins are safe with respect to heavy metals. The quantum of arsenic, lead, copper, zinc, cadmium and chromium in the samples were below the limits prescribed. Bacteriological analysis showed that the microbiological quality and conforms to the standards of edible gelatin. The total plate counts were well below the allowable limit of 1000/g. Other bacterial groups viz., *Coliforms*, *E. coli*, *Salmonella*, *Clostridial spores*, *Staphylococci*, & *Pseudomonas* were not detected in the samples.

A comparative study of mammalian skin gelatins and carp skin gelatins showed that mammalian skin gelatins (bovine & porcine) showed significantly higher viscosity, melting & setting temperature and faster setting time when compared to carp skin gelatins. The odour scores were higher for mammalian gelatins, indicating that they had a distinguishable odour and hence can be considered as inferior to carp skin gelatins in organoleptic qualities.

Gel strengths of Grass carp and bovine skin gelatins were comparable. Foam formation ability was similar for mammalian and Grass carp skin gelatins and mammalian skin gelatins exhibited significantly better foam stability than carp skin gelatins. No significant differences were observed in the water holding and fat binding capacities of Grass carp and mammalian skin gelatins.

Studies on the physical and mechanical properties of mammalian and carp skin gelatin based films showed that the mechanical properties of Grass carp skin gelatin based film was superior to Rohu and Common carp skin gelatin films and comparable to films made from commercial bovine hide and pork skin gelatin. Carp skin gelatin films had significantly lower water vapour permeability than mammalian skin gelatin films. Low oxygen permeability was noticed for carp skin gelatin films than mammalian skin gelatins which indicate that carp skin gelatin based films have superior gas barrier property than mammalian skin gelatin films.

In the gel dessert formulations, the highest bloom was observed for pork skin gelatin based dessert, followed by bovine skin, Grass carp, Rohu
and Common carp skin gelatin desserts. Desserts from Common carp & Rohu skin gelatin had similar gel strength. No significant difference in gel strength observed in the case of desserts from Grass carp and Bovine skin gelatin desserts. Melting points of the fish skin gelatin water desserts showed lower values than the mammalian skin gelatins desserts. The lower melting point of the fish gelatin helps in better flavour release in dessert preparations. The fishy odour was not prominent in fish gelatin based desserts. In mammalian gelatin based desserts, the characteristic odour was easily detectable. Hence the fish gelatin based desserts were rated higher in organoleptic evaluation. Grass carp skin and bovine skin gelatin based desserts had similar values for gel strength, cohesiveness and springiness which indicated the compatibility in textural properties.

Future Outlook & research Needs

Increasing demand for fish gelatin may pave the way for further research and exploration of fish gelatin as an alternative for mammalian gelatin, as it fulfills the majority of consumer needs and complements the increasing global demand for gelatin. The current production of fish gelatin may not increase significantly in near future, as the availability of raw material, coupled with the relatively low yield will be limiting factors in fish gelatin production. However, though fish gelatin will be unable to completely replace mammalian gelatin, it might become a niche product offering unique and competitive properties to other biopolymers, as well as meeting the demand of global halal / kosher market.

Carp skin gelatin has superior physico-chemical and functional properties when compared to other gelatins of fish origin and hence assumes commercial significance. Utilization of carp skins for gelatin extraction can alleviate the problems of waste generation during commercial processing of carps, besides the production of an important biopolymer for food and industrial applications. The future emphasis on research in the utilization of freshwater fish skins for gelatin production should be on scaling up the extraction and production process and securing control of the extraction conditions during this process. As Good Manufacturing Practices (GMP) and HACCP (Hazard Analysis and Critical Control Point) are becoming
increasingly important in food manufacturing, future research has to be
directed towards the development of low cost and high quality fish gelatins
with minimal or no contaminants. Detailed investigations are to be carried out
to standardize the purity of samples / raw material used to ensure uniformity.
The use of physical (ultrasound and ionizing radiation), enzymatic, and natural
(plant phenolics and genipin) crosslinking agents to enhance the gel strength
and other functional properties of freshwater fish skin gelatin is an emerging
area in gelatin research.