Summary
VI. SUMMARY

The present investigation was aimed at isolation and characterization of transglutaminase (TGase) enzyme from four different fish species. The setting ability of four chosen fish species with its thermal gelation characteristics has been studied. The effect of activators like CaCl$_2$ and inhibitors like EDTA, NH$_4$Cl and lysine-HCl on the isolated TGase enzyme activity have been established both *invivo* and *invitro*. The salient features of the investigation have been presented in this section.

- The proximate composition of four fish species revealed the fat content of oil sardine was higher, while that of bigeye snapper, tilapia and common carp was less than 3%. The moisture content of oil sardine was lower among the four species studied and it is mainly due to higher fat and protein content.

- The crude TGase enzyme activity from tilapia was higher followed by oil sardine, common carp and bigeye snapper. With successive purification the specific activity of TGase from all the fish species increased. However, the yield of enzyme obtained was higher in bigeye snapper and common carp followed by tilapia and oil sardine.

- The molecular weight of TGase from different fish species varied from 73-95 kDa as estimated by gel filtration technique. The temperature optimum of the TGase was found to be 37°C for bigeye snapper, oil sardine and common carp while 50°C for tilapia.

- The effect of CaCl$_2$ at different concentrations on the specific activity of TGase revealed higher activity in the range of 50-100 mM of CaCl$_2$. The effect of inhibitors such as EDTA, NH$_4$Cl and lysine-HCl on the specific activity of TGase from different fish species was found to be concentration dependent. The TGase from bigeye snapper showed a specific activity of 8.82 units / mg at a concentration of 0.25 M lysine-HCl while, those from oil sardine, tilapia and common carp did not show any activity. The TGase from different fish species clearly showed homology in its molecular weight, requirement of activators and inhibitors for its activity.
The gel forming ability of mince from four different fish species as assessed by dynamic viscoelastic behavior (DVB) in the temperature range of 30°-90°C revealed higher gel forming ability for tilapia, washed oil sardine, common carp and bigeye snapper. The viscoelastic nature of heat induced gel was further assessed by torque sweep, wherein, the gel from tilapia was stronger followed by common carp, washed oil sardine and bigeye snapper. The gel forming ability of oil sardine mince was carried out after washing in chilled water to reduce the fat content, while that of bigeye snapper, tilapia and common carp was used in unwashed condition. Among the four species studied, tilapia had higher gelling ability and the bigeye snapper had lower gelling ability.

The setting ability of four fish species was temperature dependent and the optimum temperature for all the fish species was found to be 40°C. At a setting temperature of 40°C tilapia yielded higher storage modulus (G’) values during setting. The setting ability of other three species was slightly lower than tilapia. When the set meat was further subjected to heating in the temperature range of 40°-90°C, the elastic component developed was higher (∼725 KPa) for tilapia, common carp and oil sardine while it was lower (515 KPa) for bigeye snapper. The strength of the gel network as assessed by torque sweep indicated higher value of stress at fracture for tilapia, common carp and oil sardine (washed), whereas, that of bigeye snapper was marginally lower. The results of setting and DVB measurements correlated well with the TGase activity measurement done at 37°C which is closer to setting temperature followed in the study. Though the setting ability of bigeye snapper and common carp was good the corresponding TGase activity was much lower in these species compared to the TGase activity from oil sardine and tilapia. This indicates that apart from catalysis reaction by TGase other interactions like hydrophobic and formation of disulphide bond may also be involved.

Use of CaCl₂ at different concentrations during setting of mince from four different fish species proved to be beneficial in getting higher elastic structure. Incorporation of CaCl₂ during setting and further heat processing resulted in a gel of higher strength as revealed by torque sweep. The concentration of CaCl₂
at which improvement of setting ability and gel forming ability of set meat of all the fish mince was found to be in the range of 10-20 mM. Addition of Ca$^{2+}$ to the fish mince has favored cross-linking of myosin molecules as demonstrated by SDS-PAGE pattern.

- Addition of inhibitors like EDTA, NH$_4$Cl and lysine-HCl to the fish mince during setting reduced the structure build up reaction as revealed by G’ maxima at the end of setting reaction. The reduction in setting ability in presence of inhibitor was found to be highly concentration dependent. Among the three inhibitors it is NH$_4$Cl at 1.0 M concentration which had maximum inhibitory effect on setting ability of all the fish species studied. This was in confirmation with the study of inhibitors with pure isolated enzyme. The effect of inhibitors was also seen during thermal gelation (when set meat was heated from 40$^\circ$-90$^\circ$C) wherein, the increase in G’ value was significantly reduced during heating regime.

- The effect of setting process on the properties of proteins from four fish species was evaluated with reference to solubility in different solvents, electrophoretic mobility under reduced condition and free sulphydryl content. Setting of fish mince from four fish species at 40$^\circ$C reduced the solubility in extraction buffer by 55-66% depending on the fish species. The maximum reduction in the solubility was recorded in oil sardine mince and the minimum reduction was in bigeye snapper. A similar trend was obtained when the solvent used was tris buffer containing 1% SDS. The presence of CaCl$_2$ at 10 mM further reduced the solubility indicating the formation of larger aggregates. Higher protein solubility was recorded in set meat with inhibitors like EDTA (0.03M), NH$_4$Cl (1.0 M) and lysine-HCl (0.25 M). The higher solubility in presence of inhibitors both in extraction buffer and tris containing SDS buffer confirms that aggregates are not being formed as the activity of the TGase enzyme has been reduced / blocked.

- The SDS-PAGE pattern of set meat of four fish species revealed reduction in the 205 kDa component. This reduction was significant in the presence of CaCl$_2$ indicating cross-linking of myosin heavy chain during setting process. The SDS-PAGE pattern of set meat with inhibitors revealed the presence of
205 kDa component indicating inactivation of TGase enzyme and no cross-link formation.

- The effect of setting without and with activators and inhibitors on the free sulfhydryl content of the mince was evaluated. The increase in free sulfhydryl content of all fish species was observed as a result of setting at 40°C. This is mainly due to opening up of polypeptide chain during setting and exposing the buried sulfhydryl residues. Presence of CaCl₂ during setting showed decrease in sulfhydryl content (than that of set at 40°C) indicating possible formation of disulfide linkages. This was more predominant in tilapia and common carp mince than that of bigeye snapper and oil sardine mince. The free sulfhydryl content of set meat in the presence of inhibitors showed a lower value than control sample. However, this reduction is marginal and can possibly linked to the formation of disulfide bonds.

- Addition of isolated TGase from four chosen fish species to flat fish mince was attempted in order to ascertain the modification of gelling behaviour of fish mince. The flat fish mince was mixed with TGase (2 units/ g mince) and 10 mM CaCl₂. The setting ability of flat fish in presence of TGase improved considerably and it was higher when TGase from oil sardine and tilapia was added. This was further confirmed by dynamic viscoelastic behavior in the temperature range of 40°C-90°C and the torque sweep of the heat set gel.

In conclusion, fish tissue TGase can be isolated in substantial quantity and can be used as a source for modification of gelling ability of other fish species.