Summary and Conclusions
5.0 SUMMARY AND CONCLUSIONS

Product development and value addition is the need of the hour in the seafood processing industry. The consumer's demand for innovative and convenience foods are always on the increase. Buyers increasingly demand food products that are of high-quality, taste, appearance and nutrition and preferably, require minimum preparation time. Potential for ready to serve and ready to eat products in the export and internal trade is on the increase and a large variety of such products have flooded the markets. The affluent lifestyle and purchasing power made consumers choose ready to eat assembled meals than go in for raw food that has to be prepared and cooked before consumption. This trend is increasing with more and more business prospects in the food industry.

Retort Pouches are flexible, laminated containers that can be thermal processed like cans. The materials of these flexible containers can withstand thermal processing temperatures, provide superior barrier properties for a long shelf life and combines the advantages of both metal cans and plastic packages. Advantages of retort pouches over cans are many where the final processed product has a higher consumer appeal than those processed in cans due to the shorter process time and therefore a better quality retention and appearance.

High moisture smoked products are a delicacy in the advanced countries due to their sensory properties like flavour, texture and colour imparted during the smoking process. These highly priced products have to be stored at refrigerated temperatures and have a short shelf life. Smoked seafood that is vaccum packed and thermal processed in retort pouches and stored at ambient temperature had a shelf life of one year.

Fish and shellfish are highly perishable owing to their high water activity, pH and the presence of autolytic enzymes, which cause the rapid spoilage. They should be processed or preserved in the minimum shortest time without any delay.
Low temperature should be maintained from the time of harvesting, through handling up to the thermal processing stage. Tuna is one of the largest internationally traded fish commodity, which has contributed to 9% of the total trade in value terms. The Indian EEZ has rich potential and exploitation of tuna resources is one of the thrust areas for increasing marine products exports. There is an ever increasing demand for tuna products in the sashimi, chilled, frozen, canned and smoked forms worldwide.

It is only appropriate to develop an innovative and acceptable smoked product in the thermal processed ready to eat form in flexible pouches by using the local resources available in the country. This study was undertaken to develop a smoked product from yellowfin tuna with a high moisture content and capable of storage at ambient or room temperature with an extended shelf life. Locally available wood was standardized as the source of smoke flavour imparted to the fish. The smoked tuna was then thermal processed in flexible pouches and stored at ambient (28±2°C) and accelerated temperature (37±2°C) to determine the shelf life. Thermal processing was done in three different forms namely; tuna in brine, tuna in oil and tuna dry pack.

Indigenous see through pouches were made of three layers viz., outer polyester, middle nylon coated with silicon dioxide and inner of cast polypropylene. The silicon dioxide and aluminium oxide are nano particles capable of extending good barrier properties. The fourth pouch used in the study was a two layer see through pouch having layers of 10 μm outer polyester and 140 μm inside cast polypropylene. These pouches were tested for different physical properties to find their suitability for thermal processing. The thickness of different pouches ranged from 101 -118.8 μm for the three layer retort pouches and 131 μm for the two layered pouch. The tensile strength ranged from 450 kg/cm² in machine and cross direction for indigenous opaque pouches to 816 kg/cm² and 488 kg/cm² for the imported see through pouches. For the indigenous retort pouches it was 816 kg/cm² and 717 kg/cm² and for 2 layer pouches it was 316 kg/cm² and 292 kg/cm² respectively. Elongation at break was 20, 95, 78, and 53 for opaque, imported see
through, indigenous see through and two layer pouches. Heat seal strength given as percentage for the four pouches were 310, 504, 538 and 303 in machine direction and 237.9, 224, 412 and 286 in cross direction for opaque imported see through, indigenous see through and two layer pouches. The oxygen transmission rate (OTR) of the imported see through, indigenous see through and two layer pouches were 0.2, 0.6, 2, and 5.5 cc/m²/24 h at 1 atmosphere pressure at 21°C and the water vapour transmission rate (WVTR) were 0.2, 0.2, 0.86 and 1.99 g/m²/24 h at 90 ± 2% RH & 37°C. It was seen from the results that the migration into n-heptane stimulants was higher than distilled water in all the pouches and the values were very low and much below the limits of 10 mg/dm² or 60 ppm. The residual air in the thermal processed pouches was also below 2% for all the four pouches. The pouches were all found suitable for thermal processing.

The raw tuna was of good quality and initial pH was 6.21 which decreased to 6.02 after brining and smoking. The total volatile base nitrogen (TVBN) content increased from 10.82 mg N/100g to 13.53 mg N/100g and the free fatty acid value (FFA) also increased from 5.54 in the raw to 6.89 after smoking. Thiobarbituric acid values which are compounds of secondary oxidation also showed an increasing trend from 0.23 mg malonaldehyde/kg in raw to 0.30 mg malonaldehyde/kg in smoked tuna. The levels of histamine in the raw tuna sample were 4.67 ppm which increased to 5.89 ppm after smoking. The levels of cadaverine and putrescence were very low in the raw (0.23 and 0.10 ppm), brined (0.36 and 0.05 ppm) and smoked tuna (0.26 and 0.17 ppm). This biogenic amine value increased during the smoking process to 3.23 ppm. The level of spermidine in raw tuna was 0.16 ppm which remained the same after smoking the fish. However in the case of spermine the level decreased from 0.22 ppm in raw to 0.13 ppm in smoked. Tyramine levels in raw tuna were found to be 0.18 ppm which decreased to 0.15 ppm after smoking.

The individual PAH compounds in raw, brined and smoked tuna showed that Benzo-a-Pyrene was not detected in the raw and brine tuna taken in this.
study. The tuna which was smoked with coconut husks at 75°C for a period of 60 min was found to contain 1.48 μg/kg of benzo-a-pyrene. Levels of other important polyaromatic compounds were dibenzo-anthracene (1.9μg/kg), benzo-a-anthracene (3.53 μg/kg), benzo-b-Fluoranthene, (2 μg/kg) benzo-k- fluoranthene (0.83 μg/kg) and indeno pyrene (1.9 μg/kg) and pyrene (9.2 μg/kg).

Carbonyl content was 0.14 mg/kg for the raw tuna. Tuna smoked with acacia and teak wood recorded the high value of 2.01 and 1.61 mg/kg followed by cheruteak and coconut husk which gave values of 1.41 and 1.01 mg/kg respectively. maruthu, kolamavu, anjily and cashew had low values of total carbonyls ranging from 0.42 mg/kg to 0.27 mg/kg.

Phenol was not detected in the raw and brine sample. In smoking tuna the phenol content was 0.74 mg/kg. Coconut husk had the highest phenol content of 0.62 mg/kg and cashew had the lowest with 0.31 mg/kg. In tuna smoked with cheruteak it was 0.49 mg/kg, Kolamavu 0.52 mg/kg, Acacia 0.32 mg/kg, maruthu 0.42 mg/kg, anjily 0.39 mg/kg and teak 0.56 mg/kg.

Smoking parameters were standardized based on the sensory scores, polyaromatic, carbonyl and phenolic compounds present in the wood and the availability and cost of the wood. The smoked tuna was then subjected to thermal processing in different pouches in three different forms using filling medium like oil, brine and as dry pack (without any filling medium). Four different types of flexible pouches were used for the thermal processing and storage of smoked tuna. Thermal process evaluation and heat penetration characteristics and changes during storage in various biochemical factors and smoke components were determined at regular intervals at ambient (28 ± 2°C) and accelerated (37 ± 2°C) temperature storage.

Smoked tuna was packed in four different pouches in three different forms and were subjected to heat sterilization in an over pressure autoclave and the data
was recorded. The total process time (TB) and cook value (Cg) for smoked tuna in brine was 32 min and 75.61 min in INOP, 27.77 min and 69.74 min in IMST pouches, 31.91 min and 77.98 min in INST pouches and 33.21 min and 74.14 min in INTL pouches respectively. In the case of smoked tuna in oil packed in INOP TB was 38.02 min and Cg was 86.83. In IMST pouches it was 29.25 min and 75.37 min, 33.25 min and 80.26 min in INST pouches and 36.35 and 79.82 min in INTL pouches. For dry pack tuna the TB values were 42.01, 31.83, 38.04 and 39.1 min and the cook value were 89.31, 77, 84.05 and 88.45 min for tuna processed in INOP, IMST, INST and INTL pouches respectively. All the pouches were found to be commercially sterile.

Effects of rotation on heat penetration parameters were studied. The total process time (TB) in indigenous opaque pouches for smoked tuna in oil processed in a stationary retort to a Fo value of 10 was 38.02 minutes. TB values for 2 rpm was 31.73 min, 31.11 min for 4 rpm, 30.05 min for 6 rpm and 28.92 min for 8 rpm. For smoked tuna in brine medium the TB to reach the same Fo in a stationary retort was 32 min, whereas in the rotary retort it was 25.95 min at 2 rpm, 23.83 min at 4 rpm, 23.20 min at 6 rpm and 21.60 at 8 rpm. The data pertaining to smoked tuna in oil and brine subjected to stationary, 2 rpm, 4 rpm, 6 rpm and 8 rpm showed that total process time and cook value varied significantly between different levels of rotation.

The Changes in polyaromatic hydrocarbon components during storage of smoked and thermal processed tuna in brine, oil and dry pack showed that the initial BaP content for tuna in brine were 1.65 μg/kg which decreased slightly to 1.39 μg/kg after 12 months. Change in other important PAH compounds from initial to final were 0.81- 0.79 μg/kg for dibenzo-anthracene, 3.49 -3.12 μg/kg for benzo-a-anthracene, 1.5 -1.08 μg/kg for benzofluoranthene, 0.55-0.35 μg/kg for benzofluoranthene, 7.40 - 4.61 μg/kg for indeno pyrene and 44.78 - 43.23 μg/kg for pyrene. The BaP content of the smoked tuna in oil was 1.58 μg/kg which decreased to 1.13 μg/kg after storage. The changes in other PAH compounds of
The changes in total carbonyl components during storage of smoked and thermal processed tuna in brine, oil and dry pack gave a decreasing trend. The total carbonyls in smoked tuna in brine have shown a decrease from 0.98 to 0.44 mg/kg in INOP, 0.89 - to 0.53 mg/kg in INST, 0.94 to 0.54 mg/kg in IMST and from 1.01 to 0.61 mg/kg in INTL pouches. The initial carbonyl values of tuna packed in oil medium were 0.93 mg /kg for INOP, 0.92 mg/kg in indigenous see through pouches and 0.95 mg /kg in IMST and 0.94 mg/kg in INTL. In IMST and INST pouches the values decreased to 0.63 mg/kg and 0.64 mg/kg respectively. In INTL pouches the carbonyl content was 0.67mg/kg after storage for 4 months. For smoked tuna dry pack the initial values of carbonyls were 0.85, 0.81, 0.80 and 0.85 mg/kg for INOP, IMST, INST and INTL pouches. The final levels after storage were 0.44, 0.66 and 0.63mg/kg in INOP, INST and IMST pouches. In INTL pouches the value decreased to 0.75 mg/kg after storage for four months.

The changes in total phenols during storage of smoked and thermal processed tuna in brine, oil and dry pack showed a decreasing trend. The initial total phenol values in INOP, IMST, INST and INTL were 0.80, 0.72, 0.83 and 0.76 mg/kg and final values were 0.22, 0.30 and 0.39 mg/kg for the opaque and see through pouches. The changes in phenol content of smoked tuna in oil medium decreased from 0.85 to 0.55 mg/kg in opaque pouches, 0.86 to 0.38 mg/kg in INST pouches and from 0.88 to 0.42 mg/kg in IMST pouches after storage for 12 months. In INTL pouches the values reduced from 0.89 to 0.72 mg/kg after storage for 4 months. Initial values for tuna packed in INOP, IMST, INST and INTL pouches
were 0.78, 0.70, 0.86, and 0.61 mg/kg and final levels were 0.21, 0.20 and 0.15 mg/kg after 12 months of storage. The levels of phenols decreased to 0.40 mg/kg after four months in INTL pouches.

No significant decrease or loss of amino acids was observed between the raw, smoked and thermal processed tuna products. During storage certain amino acids were found to increase and decrease in some cases. This is mainly because of the variations in individual amino acid content. However the total percentage of amino acids remained the same.

The histamine levels of smoked tuna in brine decreased from 7.13 to 4.72 ppm. The initial and final level of putrescine was 0.38 and 0.31 ppm; cadaverine 0.26 and 21 ppm; spermidine 0.17 and 0.11 ppm; tyramine 0.27 and 0.17 ppm and agmatine 0.21 and 0.12 ppm. The level of histamine in tuna in oil was 6.93 ppm and decreased to 4.93 ppm after the storage period of 12 months. In oil packs final levels were putrescine (0.58 ppm), cadaverine (0.64ppm), spermine (0.11 ppm), spermidine (0.13ppm), tyramine (0.11ppm) and agmatine (0.13ppm). The final levels of histamine in the tuna dry pack was 6.43 ppm and others like putrescine, cadaverine, spermidine, spermine tyramine and agmatine were 0.17 , 0.2, 0.11, 0.17 , 0.19 and 0.19 ppm respectively.

Shelf life of the smoked and thermal processed tuna was evaluated during storage at accelerated (37±2 °C) and ambient temperature (28±2 °C).

The initial and final values of pH for tuna in brine in INOP pouches were 6.01 and 5.60, in IMST pouches 6.00 and 5.64 and in INST pouch 6.00 and 5.62. INTL pouches had a pH of 5.70 after 4 months of storage. In the case of accelerated storage the final pH values were 5.63 in INOP, 5.63 in IMST, 5.64 in INST and 5.72 in INTL pouches. The changes in pH for smoked tuna in oil showed a decreasing trend. The final pH levels were 5.6 in INOP, 5.62 in INST, 5.64 in IMST and 5.70 in INTL pouches. In the case of accelerated storage the final values were similar to that of ambient temperature stored tuna. The changes in pH values
of tuna in dry pack varied from 6.00 to 5.50 with an average value of 5.8. During the period of storage there was a decrease in the pH values from 6.03 to a final value of 5.50.

The TBA values of smoked tuna packed in brine had initial value of 0.26 mg/kg which decreased to 0.18 mg malonaldehyde/kg in INOP. In INST and IMST the values increased from 0.32 and 0.12 to 0.33 and 0.22 mg malonaldehyde/kg respectively. In INTL the TBA values were 0.13 which increased to 0.68 mg malonaldehyde/kg after storage for 4 months at ambient temperature. TBA values for smoked tuna in oil medium ranged from initial values of 0.26 to 0.15 in INOP, 0.24 to 0.14 for IMST, 0.13 to 0.33 mg malonaldehyde/kg in INST pouches during twelve months of storage. For INTL pouches the initial value was 0.21 which increased to 0.61 mg malonaldehyde/kg after storage for 4 months. Initial values for tuna dry pack in INOP, INST, IMST and INTL pouches were 0.12, 0.24, 0.13 mg and 0.21 mg malonaldehyde/kg respectively and final values were 0.36, 0.28 and 0.71 mg and 0.78 malonaldehyde/kg. Similar increase was observed in the case of pouches stored at accelerated temperature in all the pouches in different media.

The changes in FFA content of thermal processed smoked tuna in brine medium at ambient and accelerated temperature storage showed a gradual increase in the FFA values during the storage period of 12 months from 1.88 to 4.80 mg % in INOP, 1.94 to 5.96 mg% in INST pouches and 1.42 to 4.94 mg % of oleic acid in IMST. For INTL pouches an increase from 1.46 to 2.82 mg % of oleic acid within a period of 4 months was observed. The changes in FFA of smoked tuna in oil showed that the initial and final values were 1.68 and 5.7, 1.94 and 6.5, 1.23 and 16.3 and 2.17and 4.11 mg % of oleic acid for the INOP, INST, IMST and INTL pouches. The final values for tuna in dry pack were 6.3, 6.9, 6.89 and 4.89 mg % of oleic acid for INOP, INST, IMST and INTL pouches. A similar increasing trend in FFA values was observed for the pouches stored at accelerated temperature.
The CIE $L^*$ $a^*$ and $b^*$ values of smoked tuna in brine, smoked tuna in oil and tuna dry pack showed that $L^*$ values (lightness) decreased in the tuna muscle during the storage period of 12 months. There was a slight decrease in the $a^*$ (redness) value and an increase in the $b^*$ (yellowness) values during storage. For tuna packed in INOP pouches the decrease in $L^*$ values was less in comparison to see through pouches. In the case of tuna packed in INTL pouches the decrease in $L^*$ values were very high after 4 months of storage. The changes were more evident in the case of samples stored at accelerated temperature.

Changes in texture profile during storage of smoked tuna in brine, oil and dry pack observed that in all the three forms of tuna the hardness 1 and hardness 2, springiness and chewiness values were found to decrease during storage. The cohesiveness value remained more or less the same throughout the storage period. Between the storage temperatures also there was no much difference in the textural values.

Sensory score of tuna in brine after twelve months of storage at ambient and accelerated temperature were 7.00 and 6.2 in INOP, 6.8 and 6.0 in INST, 6.8 and 6 in IMST pouches. The values for INTL pouches were 4 and below after 4 and 2 months storage for ambient and accelerated temperature in the entire three media used in the study. Changes in overall sensory score of tuna in oil stored at ambient temperature were 7, 6.8 and 7 in INOP, IMST and INST pouches and 6.8, 6.3, 6.8 and 4 at accelerated temperature. For dry pack tuna the rating was 7, 6.9 and 7 in INOP, INST and IMST after storage for 12 months. In accelerated conditions final scores after 12 months were 6.8, 6.1, and 6.8 for INOP, IMST and INST pouches respectively.

The research findings can be summarized as follows

1. Developed a high moisture smoked tuna product capable of storage at ambient temperature with extended shelf life.
2. Standardised indigenously available wood as the source of wood smoke based on different parameters and coconut was found suitable based on cost, availability, flavour, colour and BaP content.

3. Smoking was found responsible for imparting the characteristic flavour and colour to the tuna product.

4. Indigenous opaque and see through three layered retortable pouches and imported see through pouch were found suitable for thermal processing and storage of smoked tuna in oil, brine and dry pack.

5. Indigenous two layer pouches made of PEST/CPP were found suitable for thermal processing based on physical properties but unsuitable for storage of thermal processed products.

6. Heat penetration rate was faster in brine medium followed by oil and dry pack.

7. Heat penetration rate was quicker in see through three layer pouches compared to opaque retortable pouches.

8. Rotation (2 rpm, 4 rpm, 6 rpm and 8 rpm) increased the heat penetration rate and decreased the total process time. Time to reach Fo 10 for smoked tuna was quicker at higher rpm compared to stationary retort.

9. Smoked and thermal processed tuna in oil medium was found to be more favored organoleptically than tuna in brine and tuna dry pack.

10. Smoke components like PAH remained unchanged during storage in dry pack and showed some decrease in the case of smoked tuna in oil and brine.

11. Total carbonyl values decreased during storage. Browning of the filling media was seen in the case of tuna in brine and oil.

12. Total phenols also showed a decreasing trend during storage of smoked tuna in different media.

13. Changes in colour and sensory properties were more prominent in tuna packed in see through pouches.

14. Tuna stored at accelerated temperature had a lower organoleptic score compared to ambient temperature stored products.
15. Tuna packed in different three layer retortable pouches and stored at ambient and accelerated temperature were acceptable after a period of one year storage.

This study is the first of its kind in India, where in smoked and thermal processed products have been developed using locally available wood as the source of wood smoke and flavoring and a shelf life of one year has been achieved. Retortable pouches of three layers, both imported and indigenous were found suitable to store thermal processed products. Heat penetration rate is quicker in retort pouches due to their thin profile in comparison to cans and hence the total process time is lesser. The nutritional and sensory attributes of the pouch products are better retained during processing. Hence these products are more acceptable than canned products. Indian vegetarian food products and fish curry products are available in the ready to eat form in the markets. Smoked and thermal processed products have not gained an entry to the market and hence this study will pave an opening for such products. Currently trade in tuna products from India is meager compared to the global trade. In India proper utilization of tuna resources is yet to be achieved due to the lack of infrastructure for handling and knowledge of value addition. The raw material cost is also less due to the poor quality of the fish when landed. Hence, the availability of such products will help in the trade of tuna products, improving the quality of raw material landing and ultimately realizing a better value to the fishermen and processors.