

# Research Highlights

## 2015-2016



**Central Institute of Fisheries Technology**

(Indian Council of Agricultural Research)

Willingdon Island, CIFT Junction, Matsyapuri P.O., Kochi - 682 029



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# Preface



Another successful and fruitful year of active research goes to the credit of ICAR-Central Institute of Fisheries Technology. During the year under report also, the Institute continued to serve the fishing and fish processing industries by extending all technical guidance and help in the context of their changing needs. The impact of ICAR-CIFT on the development of fishing, seafood processing, seafood safety and quality control in India is well recognized. This year too our priorities in research were centered around the following main topics:

- ❑ Environmental protection and eco-friendly technologies for harvest sector
- ❑ Harvest technologies for responsible fishing in traditional / mechanized and inland fisheries sectors
- ❑ Post harvest technologies for culture and inland sectors / preparation of products and byproducts and packaging technologies
- ❑ Eco-friendly technologies for waste utilization
- ❑ Engineering and instrumentation technologies / processing equipments
- ❑ Fundamental studies on the Biochemistry of fish spoilage and preservation/ Microbiology of fish spoilage and preservation
- ❑ Fisheries extension

A quick glance at the salient research achievements of the Institute stands testimony to this. I am happy to present before you this concise publication on the major research achievements of the Institute during 2015-16.

A handwritten signature in black ink, appearing to read 'Ravishankar C.N.', written in a cursive style.

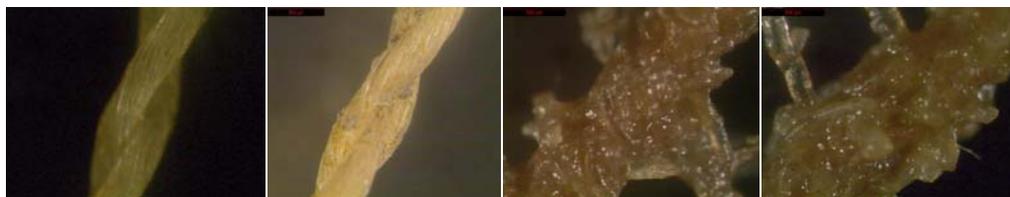
**(Ravishankar C.N.)**  
Director

Kochi  
30 August, 2016

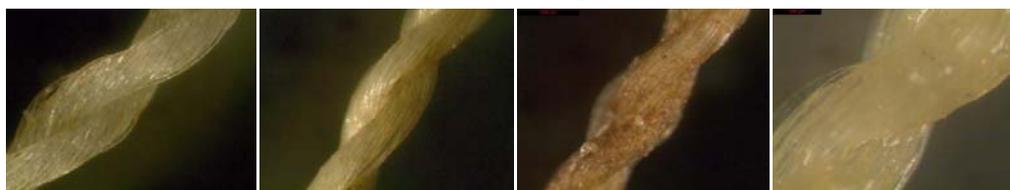


## Fishing Technology

- Netting material treated with 0.002% each of nano titanium and copper oxide hydrogel showed excellent bio-fouling resistance.



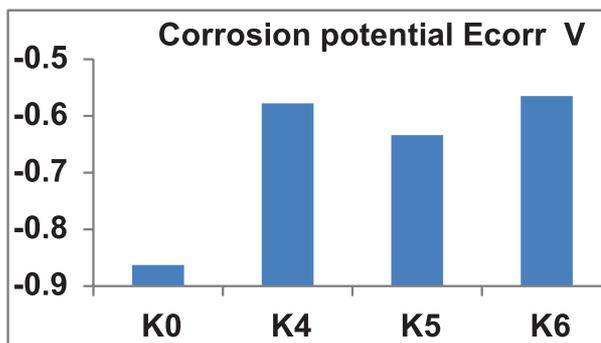
Untreated nylon netting material



0.002% each of nano CuO and TiO<sub>2</sub>

The untreated nylon nettings and hydrogel incorporated with 0.002% each of nano copper oxide and nano titanium oxide treated netting material after exposing in the Kochi estuary for 7, 15, 30 and 60 days

- In a surface modification study of boat building steel using multi-walled carbon nanotube (MWCNT) and nano titanium dioxide composites, it was revealed that steel treated with 0.02% MWCNT and 0.01% nano TiO<sub>2</sub> showed good corrosion resistance compared to untreated control.



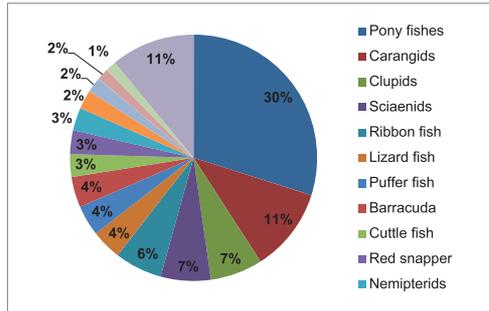
Linear sweep voltammetric data of MWCNT-nano titanium oxide composite treated boat building steel (K0 - untreated steel, K4 - 0.01% MWCNT and 0.005% TiO<sub>2</sub>, K5 - 0.01% MWCNT and 0.01% TiO<sub>2</sub>, K6 - 0.02% MWCNT and 0.01% TiO<sub>2</sub>)

- The average CPUE in trawls fitted with square mesh codend operated along Sindhudurg coast (Maharashtra) by private vessels was 18.00 kg/h and the escapement was 3.18 kg/h with a normalized value of INR 7.0.
- The total tension offered by the gear increased from 8 KN to about 15 KN, when the speed was increased from 2.0 to 3.4 knots, while operating the 33.0 m shrimp trawl attached with BRD.

- In field trials with Juvenile Trash Excluder Device (JTED) with 1 cm spacing it was observed that 27 commercial species were retained in the codend and 12 species had escaped into the cover codend.



FV Sagar Harita



Percentage composition of catch during JTED operations

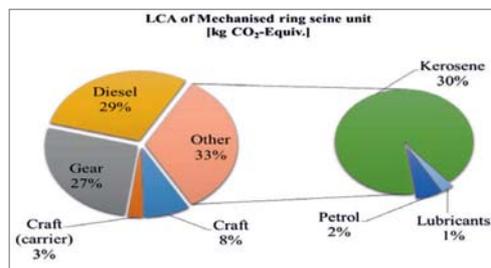
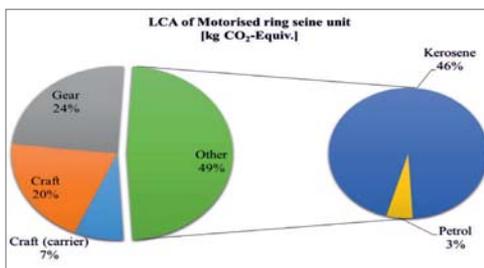
- The construction of the 19.75 m energy efficient fishing vessel, christened as *FV Sagar Harita*, was completed at the Goa Shipyard Ltd., Goa.

- An innovative collapsible fish trap of 1.5 m x 0.8 m size with two entrance funnels on both sides was designed and fabricated.



Collapsible fish trap

- Life cycle analysis showed that motorized ring seine fleet had higher impact when compared to mechanized ring seine fleet with a 24% higher value for Global Warming Potential.

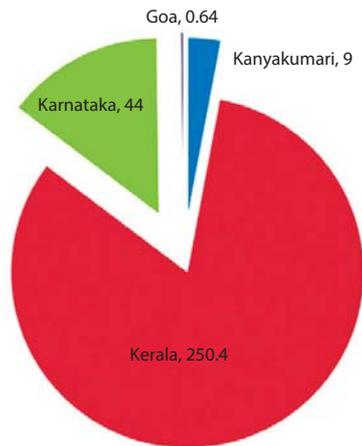


Percentage contribution to carbon emission of motorized and mechanized ring seine fishing system in operation

- The total quantity of coconut wood available for fishing boat construction is estimated to be 37.16 lakh m<sup>3</sup> in south-west coast from the total 123.86 lakh palms.



Coconut wood canoe under field testing



Total number of >60 year palms (In lakhs)

- Two canoes of dimensions 9.0 m L<sub>OA</sub>, 1.50 m breadth and 0.70 m depth were constructed using treated coconut panels and is being used for gillnetting, seining etc. in the artisanal sector.
- The contribution of CDOM was less than 25% towards total absorption which increased to 25-100% during the post-monsoon season along the coastal waters off Kochi.
- Significant correlation was observed ( $p < 0.05$ ) as concentration of chlorophyll and temperature of water and correlated with the abundance of *Acetes* sp. along the Veraval coast of Gujarat.

## Fish Processing

- Fibre-rich seaweed (*Caulerpa racemosa* and *Ulva lactuca*) incorporated semi-sweet biscuits and extruded snack product was developed.



Seaweed (*Caulerpa racemosa*) and seaweed-incorporated semi-sweet biscuits

- ❑ Thermal processed ready to eat squid masala in polypropylene and EVOH based multi-layered containers had a shelf life of one year at ambient storage temperature ( $28 \pm 2$  °C).



Squid masala in multi-layered container



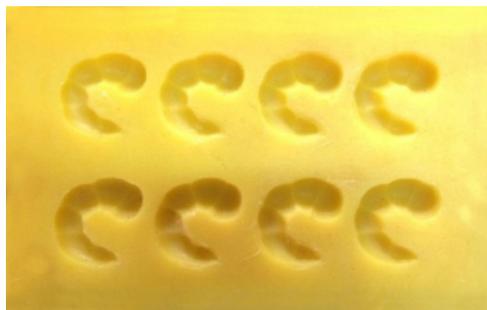
Restructured nuggets from Pangasius-mussel

- ❑ A protocol was standardized for preparing restructured nuggets from Pangasius fish in combination with green mussel mince.

- ❑ Incorporation of nano-chitosan in biodegradable polylactic acid based films enhanced antimicrobial activity.
- ❑ DHA fortified nutritional supplement based on wheat flour, soy flour, gram flour, ground nut and cashew nut was prepared.



DHA fortified nutritional supplement

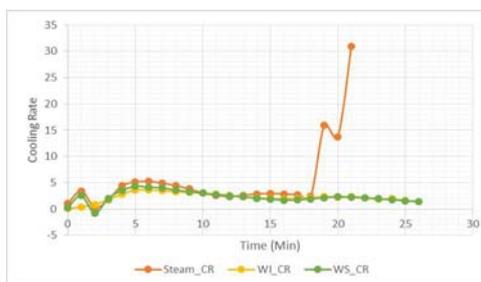
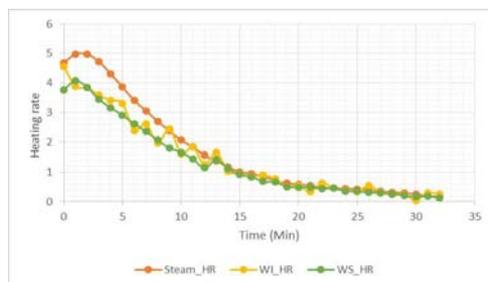


Plastic mould for preparing shrimp analogues

- ❑ Shrimp analogue product from fish mince was prepared using the prototype mould.

- ❑ The average annual energy consumption and energy cost for seafood processing units in Kochi was 42,137.3 KW units and ₹ 7,84,258.50/-, respectively.
- ❑ A reduction of 13.3 and 8.4% process time was observed in thermal processing

of fish using in steam-air and water spray retorts, respectively compared to water immersion retorts.



Heating and cooling rate of thermal processed dolphinfish

- ❑ Crystallin protein isolated from tuna eye ball had higher thermal stability at freezing and above ambient temperature.
- ❑ Antioxidant properties and amino acid composition of squilla protein hydrolysates were characterized.



Squilla

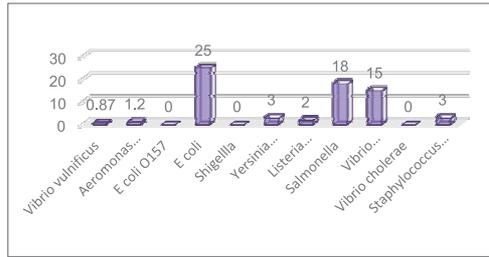
Squilla protein hydrolysate

- ❑ Extracted sulphated polysaccharides with rich antioxidant properties from seaweed.
- ❑ Microencapsulated fish oil with fish protein hydrolysate exhibited improved oxidative stability.
- ❑ Tuna protein hydrolysate-incorporated health drink was formulated. Up to 10% hydrolysate incorporation was sensorily acceptable with improved functionality.
- ❑ Addition of maltodextrin and gum arabic in fish protein hydrolysates (FPH) effectively masked the bitter taste of FPH in sweet corn vegetable soup.

## Quality Assurance and Management

- ❑ Exclusive presence of enterohaemorrhagic *Escherichia coli* O157:H7 and *Yersinia enterocolitica* in seafood sourced from local fish markets revealed cross contamination from animal meat.

- Among pathogens in seafoods from local markets, highest prevalence was recorded for *E. coli* (25%), followed by *Salmonella* (18%), *Vibrio parahaemolyticus* (15%), *Listeria monocytogenes* (2%), *Aeromonas hydrophila* (1.2%) and *V. vulnificus* (0.87%).



Food-borne pathogens prevalence in seafoods



Collection of sample from landing market

- Progressive hazard profiling in the value chain of oil sardine (*Sardinella longiceps*) indicated one log increase in bacterial load (4.49-5.57 log cfu/g) subsequent to landing from the boat, through transportation and till it reaches the retail level in the market.

- In salted and dried fishery products, *Staphylococcus aureus* was detected as the major contaminating pathogen. A moderate level (23.8%) of the isolates were capable of producing enterotoxin.
- Hazard profiling of milkfish (*Chanos chanos*) from aquaculture grow-out ponds indicated high risk of histamine formation in temperature abuse conditions.
- Ciguatera Fish Poisoning (CFP), a human intoxication caused by the consumption of fish which bio-accumulate ciguatoxins was analyzed. The study revealed the suspicious occurrence of CFP along the south-west coast of India.



*Lutjanus bohar* fish sample collected from Vizhinjam, Thiruvananthapuram for CFP analysis

- ❑ Recontamination potential of *Salmonella* in cooked and raw shrimp products was assessed. Higher recovery (67.04%) and higher survival (95-100%) of *Salmonella typhimurium* was recorded in cooked and frozen shrimp (*L. vannamei*) compared to raw product.
- ❑ Risk assessment of commercially available heat shucked clams indicated *E. coli*, *S. aureus* and *Salmonella* as potential hazards and suitable mitigation measures were developed.
- ❑ LC-MS/MS method for determination of 160 pesticides in fish and fishery products was developed. Methods for MRM parameters and chromatographic separation of these pesticides were also developed.
- ❑ A method for multi-residue analysis of persistent organic pollutants in seaweeds was developed by ethylacetate extraction, solid phase dispersive cleanup followed by GC MS/MS analysis. The micro-pollutants had 65-120% recovery by this method.
- ❑ An LC-MS/MS method was developed for simultaneous analysis of tetracycline and sulphonamide antibiotic residues in shrimp.
- ❑ Surveillance sampling and root-cause analysis of export rejections was carried out for hazards like *Salmonella*, Coliform and *L. monocytogenes* for various seafood establishments and mitigation measures were suggested.
- ❑ Biogenic amine build up in high pressure treated tuna was modelled for predictive analysis during subsequent chilled storage conditions.

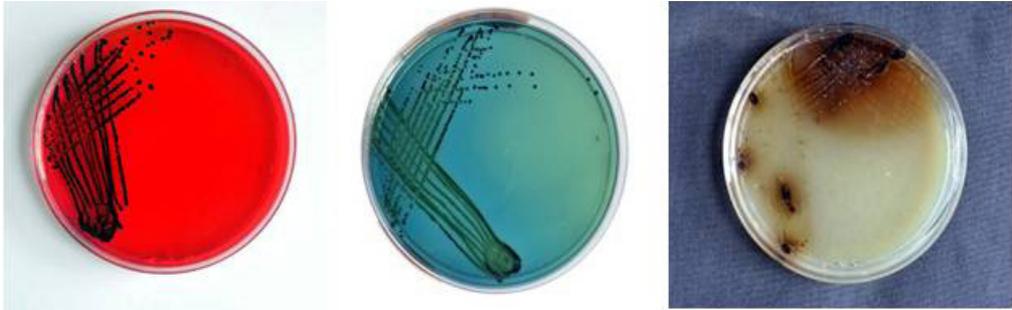
## Microbiology, Fermentation and Biotechnology

- ❑ Occurrence of *Clostridium botulinum* was seen in 7% of the canned, ready to cook and fresh fish and fish products.
- ❑ Incidence of *V. parahaemolyticus* that carry *tdh* gene, a marker for virulence was seen in commercial fish, water and sediment samples from aquaculture farms.



Fish and fish products screened for *C. botulinum*

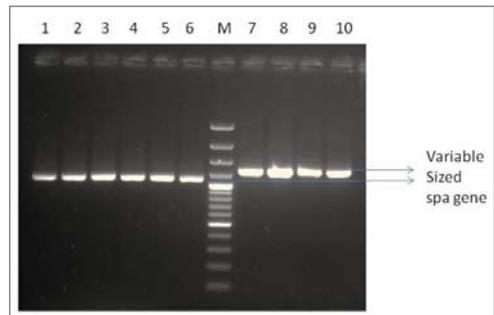
- ❑ *V. parahaemolyticus* strain causing Early Mortality Syndrome was not detected in any of the shrimp samples screened from Andhra Pradesh.
- ❑ *Salmonella* was detected in 13 percent of the fish samples from Ernakulam District.



*Salmonella* spp. isolated from the samples in XLD agar, HEA agar and BSA

- ❑ About 62.5% of *V. cholerae* isolates were resistant to ampicillin (10µg), 37.5% to cefotaxime (30µg), and 12.5% to ceftriaxone (30µg) and nitrofurantoin (300µg). Thyme oil was effective in inhibiting cholera toxin gene positive *V. cholerae*.
- ❑ A protocol was standardized for Pulse Field Gel Electrophoresis of *Vibrio parahaemolyticus* strains isolated from fish and fishery environments.

- ❑ MLST data of Multidrug Resistant *Staphylococcus aureus* (MRSA) revealed that the strain SA635, MRSA7 and MRSA9 belonged to ST5.



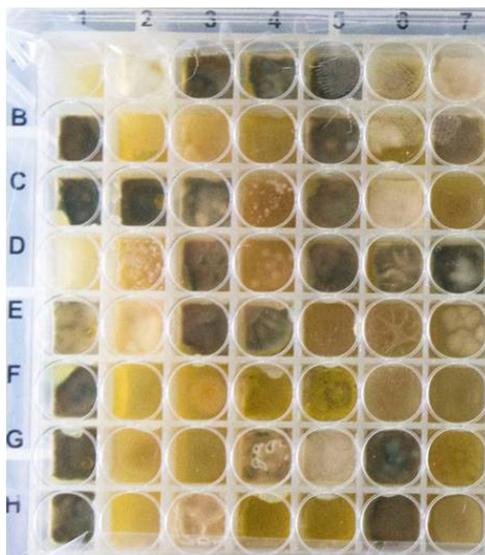
Whole gene spa of non-assignable MRSA

- ❑ A new spa type of MRSA was identified and was assigned spa type t15669 in the RIDOM server.

- ❑ Genotyping of eight isolates of *Aeromonas* spp. from a fish disease outbreak in Mangalam dam, Thrissur District revealed genotypes of *Aer*<sup>+</sup>, *Alt*<sup>+</sup>, *Ast*<sup>+</sup>, *Act*<sup>+</sup>, *Ahp*<sup>-</sup>, *ascV*<sup>-</sup> and *aopB*.

- ❑ ISP-2 medium along with sample treatment of dry heat and benzalkonium chloride and SDS with 6% yeast extract was best suited for isolation of Actinobacteria from marine sediments.

- Antibiotic sensitivity profile of 12 MRSA cultures revealed that all the strains were multi-drug resistant (MDR). MDR of the strains ranged from three to eight classes of antibiotics. Two out of 18 Actinomycetes strains isolated from marine sediments were active against MRSA ATCC 43300 by agar-overlay method.



High throughput culturing of Actinobacteria

- LD<sub>50</sub> value of 15 strains of *Aeromonas hydrophila* and nine strains each of *A. jandaei* and *A. veronii* isolated from farms with disease outbreak revealed that majority of the *A. hydrophila* strains are highly virulent in rohu fingerlings.
- Nineteen strains of *Bacillus* sp. showed the ability to inhibit fish and shrimp pathogens; *A. hydrophila*, *E. tarda*, *V. cholerae* and *V. vulnificus* by well diffusion test for use as probiotic in aquaculture farms.

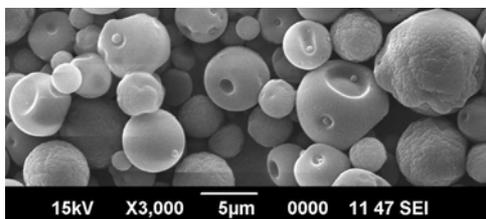


Shidol - A fermented fish product and its microbial diversity

- Microbial profiling of Hentek and Shidol, two fermented fish products was carried out.
- V. cholerae* belonging to the pathogenic O139 serogroup was isolated for the first time from commercial sold freshwater fish, *Catla catla* from a fish market in Visakhapatnam, Andhra Pradesh.
- Fish protein hydrolysates enhanced antimicrobial activity of chitosan against bacterial strains of *E. coli* and *B. cereus*. Similarly green tea extract exhibited high antimicrobial activity against *Brochothrix thermosphacta*, *Pseudomonas* sp. and *B. cereus*.

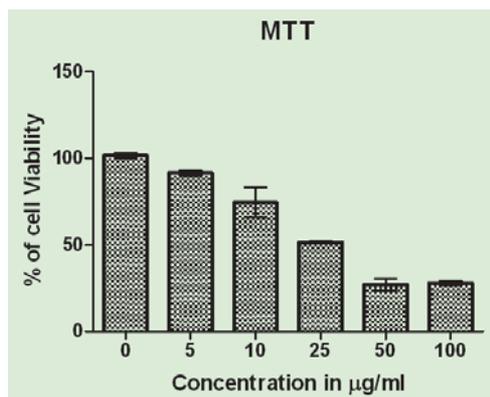
## Biochemistry and Nutrition

- A combination of maltodextrin and whey protein was found to be good for microencapsulation of squalene taking into consideration the high encapsulation efficiency (92%) and oxidative stability for which a process was developed.



SEM image of microencapsulated squalene particles

- Fruit juice was enriched with 30% Sargassum extract without affecting the sensory properties of the juice. Macroporous adsorbent resin was used to remove the seaweed-like smell the juice without affecting the nutritional quality.
- Iron and calcium-fortified fish soup powder was prepared by employing response surface methodology by varying the concentrations of starch and fish meat (protein content) and nutritional evaluation was carried out.

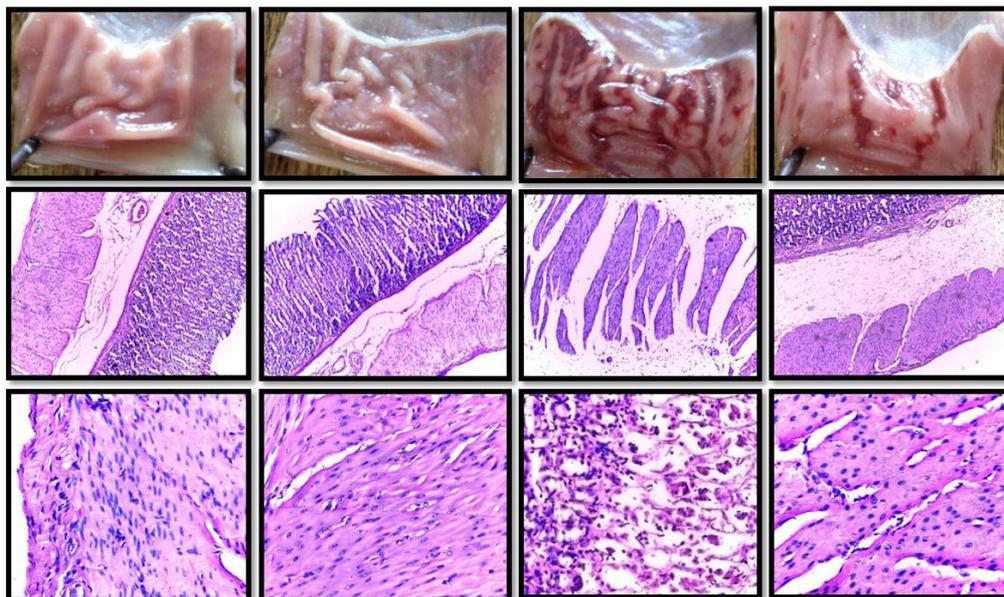


Cell cytotoxicity assay of proteoglycan isolated from deep sea shark (*Echinorhinus brucus*) cartilage against MCF-7 cell lines

- Proteoglycans extracted from *Scolidon* spp. cartilage was characterized and evaluated for their anti-proliferative activity against MCF-7 cell lines. Significant cytotoxic effect of 73% cell death was observed for 100 µg/ml treated sample.

- Proteases were extracted from visceral waste of fish species from three different habitats. Marine water fish (little tuna) proteases exhibited higher specific enzyme activity and recovery compared to other habitat species.
- Anti-ulcer activities of fish collagen against ethanol-HCl induced peptic ulcer in rats were studied. In ulcer-induced collagen treated group of rats, partial resistance to denudation of the mucosal layer was observed which could be due

to the protective effect of collagen supplementation.



A. Normal control      B. Collagen control      C. Ulcer      D. Ulcer + Collagen  
Gross pathology and histopathology of gastric lesions in peptic ulcer-induced in experimental rats

## Engineering

- ❑ In order to establish and quantify significant variables (related to design and construction practices) affecting quality of fishing vessels, 12 aspects were finalized for detailed study based on an initial study conducted on 24 boats constructed from six boat yards in Kerala.
- ❑ Data base on varying size and power of boats, propeller fabrication and skill level were made.
- ❑ Designed and fabricated a prototype of dryer (10 kg capacity) with electrical backup.
- ❑ Fabricated a prototype of cutting machine for fresh and frozen fish.



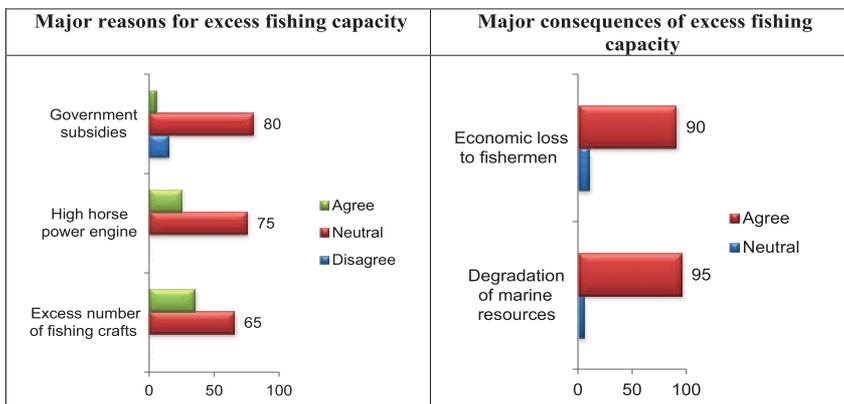
Small prototype of dryer with electrical backup

## Extension, Information and Statistics

- ❑ Input and output indicators (quantitative and economical aspects) of trawl fisheries in Kerala were collected, covering three selected districts viz., Kozhikode, Ernakulam and Kollam from north, central and south regions, respectively.
- ❑ Fish supply chain analysis of domestic fish markets in Ernakulam revealed that the inter-market efficiency ranged from 3.11 to 3.46 and intra-market efficiency from 2.04 to 4.87.
- ❑ Constraint analysis of domestic fish markets showed that poor road connectivity and absence of separate assemblage area for display are the major constraints.
- ❑ Perception analysis of mechanized trawl owners of Visakhapatnam showed that majority of the fishermen had a neutral perception on availability of excess fishing fleets in the coast (75%) and increasing fishing pressure over the years (70%). The reason for excess capacity was the entry of large number of fishing fleets into marine fisheries (35%) and indiscriminate use of high power engines (25%).



Interaction with the retailer at the Chambakkara fish market, Ernakulam, Kerala



- ❑ Product-wise and activity-wise energy consumption of the seafood processing units showed that shrimp was the major product which consumed high energy followed by cephalopods. The energy consumption is high for the cold storage activity (40%).



हर कदम, हर डगर  
किसानों का हमसफर  
भारतीय कृषि अनुसंधान परिषद

*Agr<sup>✿</sup>search with a <sup>♫</sup>human touch*