

ISSN 2454 - 5538

Vol. 5 No. 2

July - December 2019



# FISHTECH

## REPORTER

**भा कृ अनु प - केंद्रीय मात्स्यिकी प्रौद्योगिकी संस्थान**  
**ICAR - CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY**

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## From the Editorial Board...

**T**he current issue of FishTech reporter covers 11 articles for conveying the recent research findings of ICAR-Central Institute of Fisheries Technology.

An unconventional fishing practice with rescue boats which offer better stability and endurance at sea compared to the conventional wooden or FRP boats is reported from Northern Saurashtra coast of Gujarat.

In the fish processing domain, the current issue discusses articles on the development of protein concentrate from *Squilla* sp., use of slurry ice for shelf life extension of Bombay duck, extraction and characterization of gelatin from *Lutjanus* sp. and thermal behavior of gelatin extracted from reef cod. ICAR- CIFT give more importance to seaweed utilization. Recently, the research interest is directed towards development of nutraceuticals from seaweed. In this area, an article describing the antimicrobial properties of extracts prepared from a brown seaweed is included in this issue. This issue also features an article studying the emerging drivers and barriers to fish consumption in Kerala on the ground of increasing health consciousness among the seafood consumers.

ICAR-CIFT is always in the forefront in detection of seafood pathogens and this issue covers an article describing the specificity of Real time PCR for detecting listeriolysin O gene in *Listeria monocytogenes*. The current issue discusses study on the screening of antibiotic residues in commercial probiotics used in India which suggests urgent requirement for stringent regulations on the labelling with regard to the actual ingredients of the probiotic. A new initiative taken up by CIFT on the utilization of fish market waste for converting into feed and other agricultural inputs is also dealt with in this issue.

We hope our interventions will result in better harvesting and utilization of fishery resources of the country. On the ground of Covid-19 crisis we request all our stakeholders in the fisheries sector to follow the guidelines issued by the Govt. of India to facilitate a smooth functioning of fisheries sector and also to safeguard your health.



# Contents

<b>Converting fish market waste into feed: an initiative of ICAR-CIFT</b>	1
Zynudheen A. A., Binsi P. K., Geethalakshmi V. and Ravishankar C. N.	
<b>Fishing with modified rescue boats: Report from Jamnagar, Gujarat</b>	2
Prajith K. K. and Ejaz A. R. Parmar	
<b>Quantitative PCR for the detection of listeriolysin O gene of <i>Listeria monocytogenes</i> in seafood</b>	4
Pankaj Kishore, Satyen K. Panda, Minimol V. A. and Zynudheen A. A.	
<b>Slurry Ice: An alternative preservation medium - A study on Bombay duck (<i>Harpadon nehereus</i>)</b>	6
Jeyakumari A., George Ninan, Narasimha Murthy L. and Visnuvinayagam S.	
<b>Extraction and characterization of gelatin from the scales of blubber lip Snapper (<i>Lutjanus rivulatus</i>)</b>	8
Moe Theingi Hlaing, Elavarasan K., Parvathy U. and Joshy C. G.	
<b>Consternation on the safety of commercial aquaculture probiotics in India</b>	10
Lakshmi T. R., Bibindas K. S., Niladri Sekhar Chatterjee and Toms C. Joseph	
<b>Development of protein concentrate from squilla by foam mat drying</b>	11
Viji P., Jesmi Debbarma and George Ninan	
<b>Thermal behaviour of gelatin extracted from reef cod (<i>Epinephelus diacanthus</i>) skin waste</b>	12
Renuka V., Noby Varghese K.A., Ravishankar C.N. and Toms C. Joseph	
<b>Aquaculture fishers: transformation of traditional fishermen from wild fish hunters to farmed fish harvesters</b>	14
Madhusudana Rao B., Sreedhar U. and Raghu Prakash R.	
<b>Antimicrobial activity of seaweed extracts of <i>Padina gymnospora</i> extracted by supercritical and conventional method</b>	16
Anupama T. K., Lekshmi R.G. Kumar and Suseela Mathew	
<b>A review of drivers and barriers to fish consumption based on Theory of Planned Behaviour</b>	18
Sajeev M. V., Mohanty A. K., Sajesh V. K. and Rejula K.	



## Converting fish market waste into feed: an initiative of ICAR-CIFT

**Zynudheen A. A., Binsi P. K., Geethalakshmi V. and Ravishankar C. N.**  
ICAR- Central Institute of Fisheries Technology

**F**ishery waste which forms nearly 50% of the total weight of fish landed is an environmental issue in the present scenario. Huge quantity of fish cutting waste is generated in urban markets throughout the country. In this context, ICAR-CIFT has developed a simplified method for the production of feed by directly using the mixed wet waste from markets. Under the Swatchtha Abhiyan initiative of Central Government, a series of demonstration programmes were conducted on fish waste utilisation in urban fish markets and fishing harbours for the benefit of fishermen, fish vendors, Government officials, fish farmers, poultry farmers etc. Since fish market waste is of mixed nature with different parts of fish, emphasis was given to convert the wet waste into fish and poultry feed.

Currently, aquaculture accounts for 40.33% of the world's fish production and feed is the main input in fish culture which accounts about 50-60% of the variable costs of production. Fish frames and other discards contain significant amounts of muscle proteins and have a better balance of the dietary essential amino acids compared to all other animal protein sources. Converting fish discards into feed for aquaculture and poultry is an important option for utilization of unsorted waste from industry as well as fish markets. There is a growing demand for pelleted feeds, due to the intensification of aquaculture activities. Choosing the right feed, using a correct feeding method, calculating the feed cost for ensuring the cost effectiveness are important in scientific and successful management of fish farms.

A total of 26 demonstration programmes were conducted for over 950 people in various fish markets and landing centres of Kerala, Tamil Nadu, Andhra Pradesh, Maharashtra, Gujarat, Lakshdweep etc. and on many occasions elected representatives of Block Panchayat and Grama Panchayat presidents were the chief guests for the training programme. Smt. J. Mercykutty Amma Hon. Minister for Fisheries, Kerala was the chief guest for the programme conducted at Kollam fish market.

Field studies conducted by ICAR-CIFT have demonstrated the high nutritional value and good acceptance among test animals when compared to other commercial feeds. The protein content of the feed developed from fish waste ranged from 32.5 to 45%.

This technology is simple and cost effective which can be practiced by small scale vendors and self-help groups with a machinery cost of nearly Rs. 1.5 lakhs, whereas for handling large quantity of waste bigger capacity machines are required. In effect the programme has evoked huge response from various sectors, including farmers, fishermen, traders and entrepreneurs for converting fish waste into useful products.

The training programmes were well perceived by the stakeholders and many new entrepreneurs have started manufacturing fish feed following CIFT technology. They opined CIFT technology as a simplified process which can be easily adapted at farm level and found to be economical as well.



To support the industry, ICAR-CIFT is entertaining visit to production facilities, training, pilot level production, machine line details

and DPR for the interested entrepreneurs and farmers on converting fish waste into feed and other agricultural inputs



## Fishing with modified rescue boats: Report from Jamnagar, Gujarat

**Prajith K K and Ejaz A.R. Parmar**  
ICAR- Central Institute of Fisheries Technology

The State of Gujarat is located on the western coast of India. Geographically the state is categorized into Kutch, Saurashtra, North Gujarat, Central Gujarat and South Gujarat. The state occupies about 33% (1,64,000 Km<sup>2</sup>) of the total continental shelf area and 10% the total Exclusive Economic Zone of the country. Peculiar geographical landscape of the state offers scope for exploitation of several types of finfish and shellfish resources by both traditional and mechanized fishing. The fishing activities of the state are mainly confined to the Kathiawar Peninsula (Saurashtra) region. Trawl is the

major fishing gear in this region which mainly target ribbon fish, perches, croakers, cuttle fishes etc. whereas in the Kutch and upper part of the Saurashtra, gillnets and bag nets, are the predominantly operated gears.

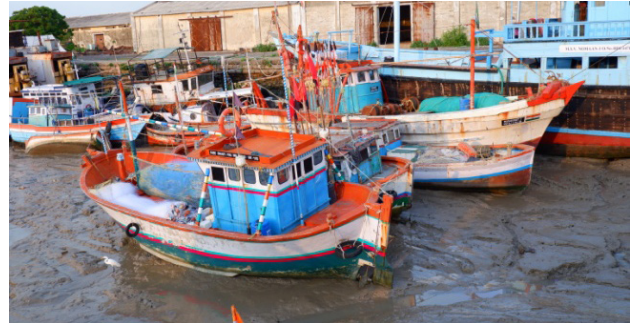
Jamnagar district is located on the northern part of the Saurashtra and is one of the important fishing areas of the state. A recent survey conducted at Jamnagar, revealed that fishing units and type of fishing in this region is totally different from that of other part of the state.

Fishing boat building in Gujarat state is





*Polyethylene multifilament gill nets (Jaada Jaal) at Jamnagar harbour*



*Fig 5. Modified fishing boats berthed at Jamnagar harbour*

mainly concentrated in the Gir Somnath and Junagadh districts. Fishing is one of the major businesses and livelihood activities of these districts. Gear specific wooden/FRP boats of various sizes are designed and fabricated in these locations. However in South Gujarat and northern Saurashtra and other parts of Gujarat, intensified fishing systems are absent due to the factors like higher tidal fluctuation, particular geographical conditions etc. Fishing in these regions is done with minimal investment and active fishing method like trawling is absent. According to Marine fisheries Census 2010, 47% of the mechanized gillnetters of the state is concentrated in Jamnagar (24%), and Kutch (23%) districts (CMFRI,2012). As the tidal fluctuations are very high in the northern Gujarat, the fishing in these locations are purely based on the tidal influence. In some part of Kutch, a traditional tide based fishing is exists, which is locally known as *Pagadiya fishing* (Dash et al 2012, Anjani and Prajith 2017) .

In south Gujarat, northern Saurashtra and Kutch region use of rescue boats for fishing has been observed (Prajith et al 2017). The rescue boats are mainly procured from Alang, which is one the major ship breaking yard in the state. The cost of such small rescue boats will be approximately rupees one to three lakhs and an extra amount of rupees two to three lakhs is invested for modification. The rescue boats have

a long capsule shape and the major structural modifications carried out is the construction of bow (front beak like part of the boat), wheel house cabin and providing other storage facilities. The boats are registered with the Department of Fisheries after the modifications. According to fishermen the modified rescues boat provides better stability and endurance at sea and is cheaper compared to the conventional wooden or FRP boats.

The length of the boat varies from 6-9 meter. The large boats with a size of 9 meter carryout 4-5 days fishing. The modified rescue boats used for fishing usually conduct fishing with 4-5 crew members lasting 4-5 days. They usually carry 3-4 blocks of ice each weighing 80 kg. The fishing trip is usually planned depending on the tidal fluctuation. Similar types of boats (with or without modifications) were reported from Narmada, Baruch, Bhuj district of the state where it is mainly used for the operation of bag nets (Prajith et al 2017).

Large meshed (200mm) polyethylene mono and multifilament gillnets are the major gear used for fishing There is a targeted fishery for the high valued fishes like ghol, seer, tuna, shark etc. The large meshed nets are locally known as *Jaada jaal*. The length of the net which varies from 5000 to 10000m and depends on the factors like area of operation, availability of catch, physical

condition of the sea etc. The entire net consists of 50-70 panels, each having a length of 100-200m. The number of panels used in a fishing operation depends on physical and biological conditions at the sea. The operation depth of the net is usually 15-25m. and soaking time is 12 hours. Yellow and brown coloured plastic and Ethylene Vinyl Acetate (EVA) (200-250mm diameter) each costing rupees 20-25 are used as floats. Whereas specially fabricated round cement blocks are used as sinkers (approximately 250-300g/piece). Life span of the fishing net is usually 2 years.

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## Quantitative PCR for the detection of listeriolysin O gene of *Listeria monocytogenes* in seafood

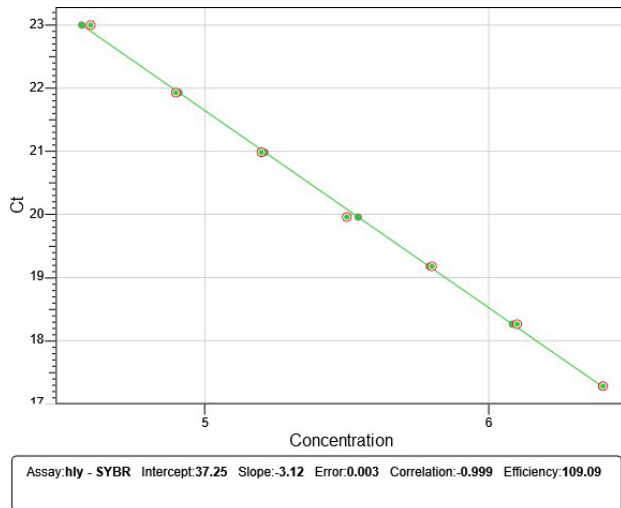
Pankaj Kishore, Satyen K. Panda, Minimol V. A. and Zynudheen A.A.  
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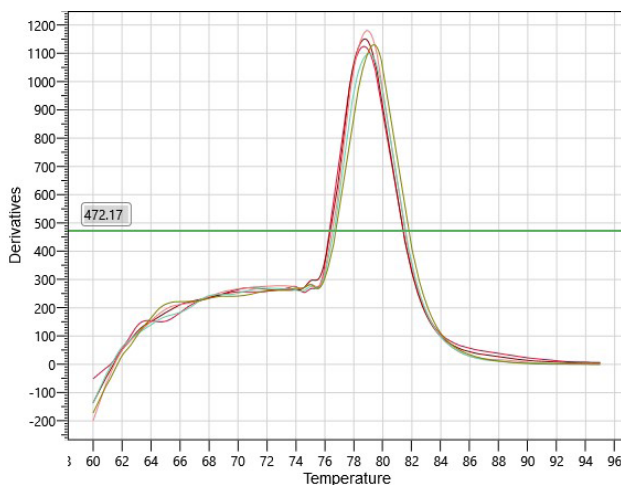
**L***isteria monocytogenes* is a significant foodborne pathogen that causes systemic listeriosis with severe symptoms that may lead to fatality. This microorganism has wide distribution in the environment and has shown relatively high frequency of isolation in foods. The qualitative and quantitative determination of *L. monocytogenes* in food matrix requires series of biochemical and molecular tests. The molecular detection of *Listeria* species in food industry with species-specific genetic markers

provides more accuracy than conventional detection tools. Hence, identification of *Listeria* species using molecular detection methods can have considerable impacts on food industry in relation to safety.

The prevalence of *L. monocytogenes* in seafood is quite low (1-2.5%) as determined by the conventional identification protocol (Leong *et al.*, 2015). The use of rapid enumeration techniques for the identification of *L. monocytogenes* in seafood with the co-occurrence of competing



**Fig1: Amplification plot for the Real time detection of listeriolysin O gene in *L. monocytogenes***



**Fig 2: Melt curve analysis of real time PCR amplification products of listeriolysin O gene**

micro flora is very useful for both the qualitative and quantitative way for the analysis of this pathogen. Reports on the efficiency of real time PCR (qPCR) in the detection of *L. monocytogenes* in seafood is scanty. With this view, Efficacy of Real time PCR targeting 106 bp segment of listeriolysin O gene using, Forward - GGGAAATCTGTCTCAGGTGATGT and Reverse - CGATGATTTGAACTTCATCTTTTGC primers (Guilbaud *et al.*, 2005) was assessed by artificially inoculating (1:5 of  $10^7$  CFU/g) pure culture of *L. monocytogenes* (ATCC 13239) in shrimp matrix. Sterile distilled water was used

as negative control. The PCR reagents used in this study were procured from Origin lab and Diagnostics, India. The qPCR amplification was performed on Himedia Quest 96 with program of 40 cycles at 95°C, 61°C and 72°C for 30 sec for each step. Melting curve was obtained from 60°C to 95°C with increment of 0.5°C with hold of 10 sec. Agarose (2%) electrophoresis was used to visualize the amplified products. The linearity was assessed over 7  $\log_{10}$  CFU/g for the diluted sample for determination of count (Fig 1). Melting curve analysis confirmed the presence of a single amplicon and uniform melting temperature of 79°C (Fig 2). The observations of the present study indicates that the SYBR Green based Real Time PCR targeting listeriolysin O gene may have high specificity for the routine detection and analysis of *L. monocytogenes* in shrimp. Further research on the feasibility of the procedure under different pre-PCR conditions as well as different seafood matrices will give more insight of this PCR assay for the accurate identification of *L. monocytogenes* in seafood to the meet regulatory compliances.

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## Slurry Ice: An alternative preservation medium - A study on Bombay duck (*Harpadon nehereus*)

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**B**ombay duck (*Harpadon nehereus*) is one of the major marine species abundant in North West coast of India. Traditionally the demand of fresh fish is limited and major portion of landings were converted to dried product. Bombay duck is prone to spoilage due to its high moisture content. Traditionally, block ice or flake ice is used for preservation. Moreover due to less demand and lower price these fishes are discarded into sea or kept under insufficient ice onboard. There is a need for alternate method for the preservation of fish onboard. It has been reported that slurry ice could maintain the quality of fish and shell fish better onboard when compared to block ice. The major advantages of slurry ice include: (a) it provides rapid chilling rate than other chilling methods due to large heat transfer surface area created by its numerous particles (b) it cover the fish surface completely which reduce the dehydration of samples (c) it can be prepared onboard with required condition. Slurry ice contains homogenous mixture of small ice particles and carrier liquid. The liquid can be either pure freshwater or a binary solution consisting of water and a freezing point depressant (eg. Sodium chloride, propylene glycol, ethanol and ethylene glycol). Several authors reported that fish and shell fish stored under slurry ice had extended shelf life (Mugica et al., 2008; Zhang et al., 2015; Narasimha Murthy et al., 2018; Jeyakumari et al., 2018). Very few studies have been carried out on quality of Bombay duck stored under conventional flake ice. Hence, the present study was undertaken to evaluate the quality of Bombay duck fish stored in slurry ice and it was compared with the conventional flake ice.

Fresh Bombay duck (*Harpadon nehereus*) (200-250g size;  $20 \pm 0.5$  cm) were procured from fish market at Vashi, Navi Mumbai and brought to laboratory in iced condition (fish to ice ratio=1:1). A prototype system was used to prepare the slurry ice in this work (Chirag, Navi Mumbai, India). Fresh Bombay duck fish were divided into two sets. 1) The first set of fish was kept under flake ice (Control) 2). The second set of fish was kept under slurry ice. The ratio of fish to ice (flake ice/slurry ice) was maintained 1:1. The melted ice was drained and compensated with required ice at a regular interval. All the samples were kept in insulated ice boxes and samples were taken at known intervals for analysis until overall acceptability rejection. Biochemical parameters such as pH, TVB-N, TMA-N, PV and TBA showed an increasing trend during storage. It was observed that TVB-N and TMA-N content were within the acceptable limit in both samples during storage. Result indicated that TVB-N and TMA-N content were found to be poor indicator and did not show substantial increase when fish was found spoiled. Peroxide value crossed the acceptable limit ( $20 \text{ meq.O}_2/\text{Kg}$ ) on 13<sup>th</sup> day in

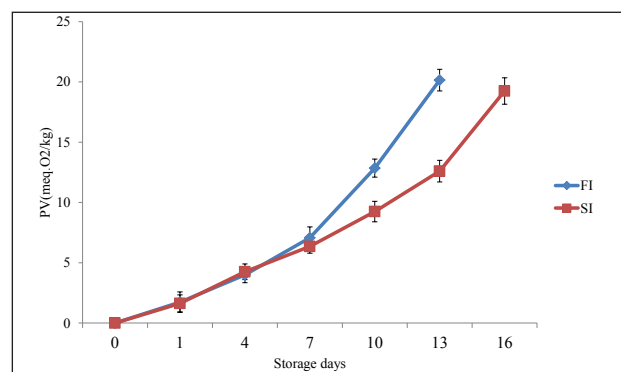


Fig. 1. Changes in PV of Bombay duck during storage



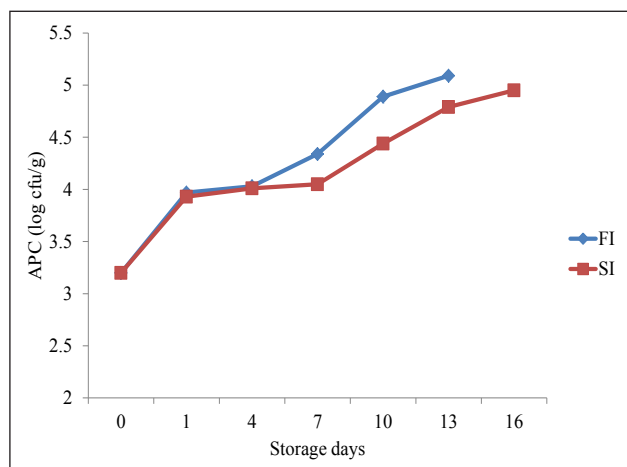


Fig. 2. Changes in APC of Bombay duck during storage

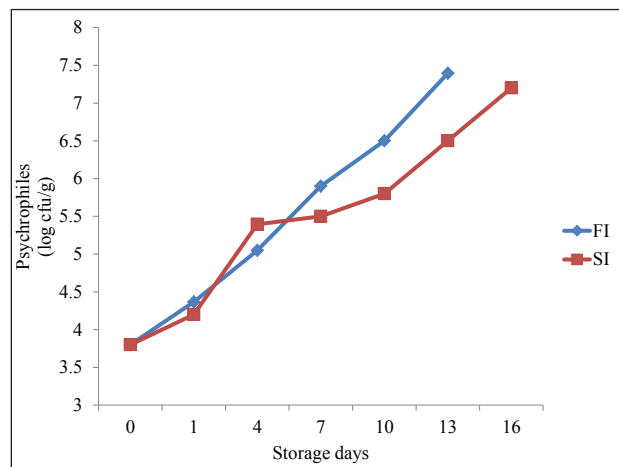


Fig. 3. Changes in psychrophilic count of Bombay duck during storage

control. However, fish stored under slurry ice had peroxide value of  $19.25 \text{ meq.O}_2/\text{Kg}$  on 16<sup>th</sup> day (Fig.1). In both the samples TBA values were within the limit throughout the storage. Salt content varied from 0.15-0.28% in control. However, fish stored under slurry ice showed increasing trend (0.15- 2.2%) in salt content during storage. Since, the slurry ice is prepared from filtered seawater, it might have influenced the salt uptake in fish samples during storage. However, it did not affect the sensory quality as indicated by higher score for overall acceptability. Microbial analysis showed a gradual increase in aerobic plate count (APC) during storage. Control reached acceptable limit of APC ( $5.09 \log_{10}$ ) on 13<sup>th</sup> day. However, fish stored under slurry ice had APC of  $4.95 \log_{10}$  on 16<sup>th</sup> day (Fig.2). Psychrophilic bacteria showed gradual increase during storage and reached  $7.39 \log \text{ cfu/g}$  on 13<sup>th</sup> day,  $7.20 \log \text{ cfu/g}$  on 16<sup>th</sup> day for fish stored under flake ice, slurry ice, respectively (Fig.3). Results indicated psychrophilic bacteria count can be considered as good microbiological quality indicator than TPC for the Bombay duck stored under slurry ice. The increase in psychrophilic bacteria count was coincided with sensory analysis. Based on the microbial and sensory and analysis, fish stored in slurry ice had an extended shelf life up to 16 days than control (13 days) when compared control.

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## Extraction and characterization of gelatin from the scales of blubber lip Snapper (*Lutjanus rivulatus*)

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Most of the commercial gelatins are derived from skin, scale, bone, ligament and tendon of porcine or bovine. However, due to religious reasons as well as health concerns, gelatin extracted from fish source is gaining popularity. Hence, identified as an alternative to the mammalian origin finding suitability in food, pharmaceutical and cosmetic industries. The fish processing industries generate large amounts of secondary raw materials as processing discards every year and its disposal is an economic concern for the industry. The best way to utilize these secondary raw materials is to develop valued added byproducts. Fish scales and bones are more preferable for extraction of gelatin as it yields large amount of gelatin due to high content of amino acids (proline) compared to fish skin. In the present study, gelatin was extracted by standard protocols using acid and alkali treatments from the scales of Blubber lip (*Lutjanus rivulatus*), one of the commercially available discards from seafood industry.

The gelatin was prepared as per the methodology of Nagai et al. (2004) with modifications. Cleaned and dried fish scales were initially treated with 0.4% (w/w) NaOH solution for two hours followed by treatment with 1% (w/v) HCl for two hours. This was followed by an additional treatment with 1% (w/v) HCl for two hours and further with 2% (w/v) HCl for another two hours. Treated samples were rinsed with water thoroughly till it reached near neutral pH between each treatment. The pretreated fish scales were taken in flasks for gelatin extraction with 4%(w/v) of deionized water. The extraction was carried out in a water bath for two hours at 70 °C. The gelatin solutions were filtered and spray dried (referred to as Gelatin 1). Previously 1<sup>st</sup> round extracted

fish scales were further treated with 2% HCl for two hours followed by treatment with 0.4% (w/w) of NaOH solution for two hours and gelatin was extracted as similar to 1<sup>st</sup> extracted gelatin to get 2<sup>nd</sup> extracted gelatin (referred to as Gelatin 2). The proximate composition of extracted gelatins were determined as per AOAC (2016). The functional properties viz., foaming properties (Cho et al., 2004), fat binding capacity (FBC) (Cho et al., 2004) and emulsifying properties (Pearce and Kinsella, 1978) were determined. The proximate composition and functional properties of extracted gelatins were compared with commercial gelatin powder (Sigma, life science) sourced from bovine skin having gel strength 225 g bloom (Table 1, 2).

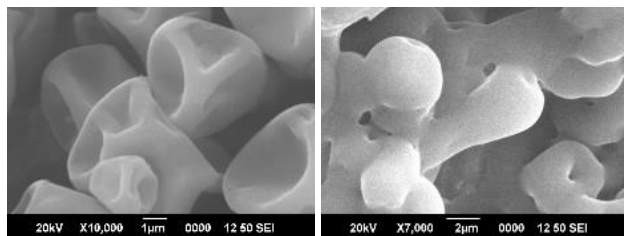
The Blubber lip scales comprised of 10.62 % moisture, 0.09 % fat, 42.74 % ash and 38.85 % protein. The yield of gelatin extracted by 1<sup>st</sup> and 2<sup>nd</sup> extraction method was 3.27 and 5.20 %, respectively. The proximate composition of extracted gelatin and commercial gelatin is given in Table 1. The protein content of gelatin extracted by 2<sup>nd</sup> extraction (Gelatin 2) was comparable to commercial gelatin sample. However, the fat and ash content of extracted gelatin samples were higher compared to commercial sample.

### Proximate composition of commercial and extracted gelatins

Parameters	Commercial Gelatin	Gelatin 1	Gelatin 2
Moisture	10.05	8.37	9.43
Fat	0.23	5.52	5.42
Ash	0.42	10.63	4.62
Protein	81.89	70.47	76.49

**Table 2. Functional properties of commercial and extracted gelatins**

Functional Properties	Commercial Gelatin	Gelatin 1	Gelatin 2
Foam Formation Capacity (FA)	1.45	1.60	1.60
Foam Stability(FS)	1.40	1.05	1.04
Fat Binding Capacity (%)	191.10	255.55	377.19
Emulsifying Activity Index (EAI)(m <sup>2</sup> /g)	3.30	1.88	4.15
Emulsifying Stability Index(ESI)(min)	29.57	25.98	23.51



*Fig 1. SEM image of spray dried (a) Gelatin 1(b) Gelatin 2*

The morphology of spray dried gelatin were analysed in SEM (Jeol, JSM 6390LA). The gelatin obtained by first extraction showed uniform non-spherical shrivelled particles with surface dents and cavity. The observed surface dents of particles can be attributed to the rapid expansion during the final stages of drying (Jafari et al., 2008). The second extracted sample (Gelatin 2) showed porous, floc-like structures made up of small clusters. In general, the morphology of spray dried particles is affected by spray drying process parameters as well as solution properties of gelatin.

The functional properties of gelatin extracted by two methods were determined and given in the Table 2. Foaming capacity of both samples were similar and comparatively higher than the commercial one and foam stability were also at par with the standard gelatin sample. The fat binding capacity of gelatin 2 was superior to other samples. Emulsifying activity index of gelatin 2 was also higher than that of the standard gelatin sample, however emulsifying stability index of extracted samples were slightly lower than standard gelatin. Hence, with regard

to the nutritional as well as functional aspect, gelatin 2 was found to be superior than first extracted one (gelatin 1) and was comparable to the commercial grade. Further optimization studies can facilitate improvement in yield as well as associated properties of the gelatin extracted from this potential source.

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## Consternation on the Safety of Commercial Aquaculture Probiotics in India

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Due to intensification of aquaculture, aquatic species are exposed to high-stress situations, increasing the incidence of diseases resulting in reduction of productivity. The need for improved disease resistance, growth promotion, enhanced nutrient digestion, better water quality and stress tolerance has resulted in the use of probiotics in aquaculture. Probiotics are regarded as live microbial feed supplement, which beneficially affects the host by improving microbial balance of its environment, intestine or both. Research on aquaculture probiotics worldwide has projected it to be a safe and efficient prophylactic health measure for attaining sustainable intensification in aquaculture. The wide popularity gained by aquaculture probiotics has broadened their market. There is an increasing trend in the use of commercial probiotics in shrimp aquaculture. Currently, commercial aquaculture probiotic products claim to contain various microbes such as bacteria under the genera *Bacillus* sp. and *Pseudomonas* sp., lactic acid bacteria like *Lactobacillus* sp., *Pediococcus* sp. and *Bifidobacterium* sp., autotrophs such as *Thiobacillus* sp., nitrifying and denitrifying bacteria, fungus *Aspergillus* sp., the yeast *Saccharomyces cerevisiae* and many more. Farmers are increasingly dependent on a proliferating range of commercial probiotics, often of uncertain composition, efficacy and safety. The market of aquaculture probiotics used for shrimp farming in India is estimated to be more than \$100 million. Even though the aquaculture probiotics are highly commercialized and widely used, lack of regulations to access the quality of aquaculture probiotics is absent in most of the countries.

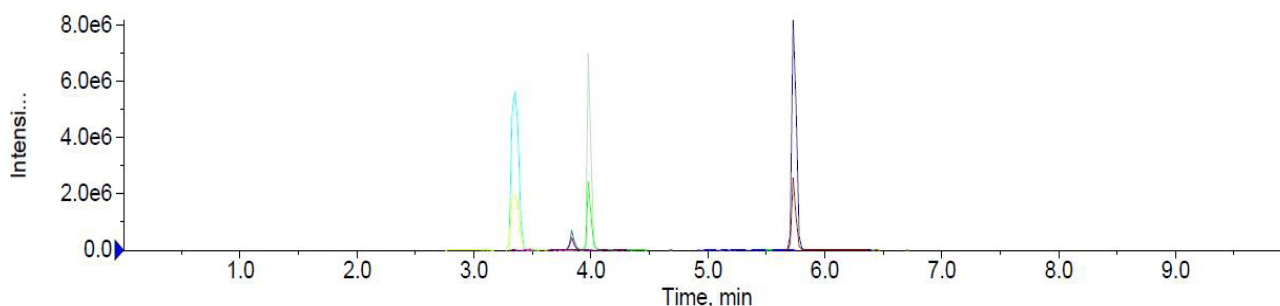
In India, the only government regulation that exists for probiotic is the certificate of standards for antibiotic free aquaculture inputs issued by Coastal Aquaculture Authority (CAA). The aquaculture input manufacturers or distributors register their products by applying directly to CAA with the antibiotic test result statement obtained by them. Randomized sampling of aquaculture inputs from different market locations for monitoring its quality and content is currently lacking. Although many of the feed supplements declared to have live microorganisms, were registered as either probiotic, feed additive or immunostimulant. As such there are no criteria for assigning the aquaculture inputs into a specific product category at the time of registration.

In the present study, sixty commercial aquaculture probiotics; 53 indigenous, 5 imported from USA and 2 imported from Thailand, procured from markets of the state of Andhra Pradesh were analysed for the presence of antibiotics. Five products included in our study does not have CAA registration and were still available in the market. An initial screening by microbiological three plate method with *Bacillus subtilis* revealed the presence of antimicrobial substance in two of the probiotics. Both the products were from the same manufacturer and shall contain *Lactobacillus* and yeast based on the label. Microbiological analysis revealed that *Lactobacillus* was not present in both the probiotics. Both the products were analysed for the presence of antibiotics using LC-MS/MS (SciexQTrap) in ESI+ mode. Multiple Reaction Monitoring technique was used to identify and quantify the antibiotics. The ground sample was extracted with acidified acetonitrile and buffered water (50:50), following



C18 dispersive cleanup and injected into LC-MS/MS for analysis. Quantifier ion of the compound and qualifier/quantifier ion ratio was used for further confirmation of identity. Both probiotics were observed to contain multiple antibiotics; trimethoprim, sulfamethoxazole and ciprofloxacin and an antiparasitic drug albendazole. Since the aquaculture probiotics are directly applied to farms and further get released to aquatic environment including natural water bodies, presence of any hazardous material including antibiotic is a matter of concern. A candidate probiotic proposed for commercialization should assure high level of protection to human and animal and should be regarded as GRAS. Economic burden spared

by small holder aquaculture farmers for the commercial probiotics with the perception of better productivity will be justified only if they have a significant positive effect in practice. The actual quality and safety of the commercial aquaculture probiotics sold are under question. Most of the brands do not fulfil the basic requirement of labelling the exact contents of the probiotic product. Moreover presence of antimicrobial substances is an added concern to the farmers, seafood processors and consumers. Therefore, development and implementation of standardized assessment procedures and strict regulations over labelling, content, safety and health statements of commercial aquaculture probiotics are urgently required.



*Fig: LC-MS/MS chromatogram showing antibiotic residues in commercial aquaculture probiotic*

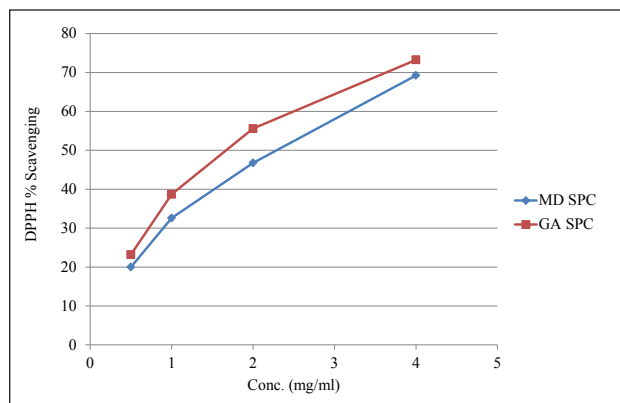
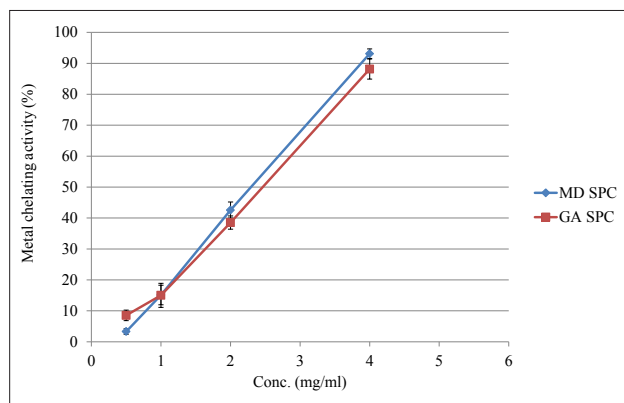


## Development of Protein concentrate from *Squilla* by foam mat drying

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**F**oam mat drying is a recent technique adopted by the food industry for drying high moisture foods like fruit juice or vegetables. It involves the formation of stable foam by adding a whipping agent into liquid or semi liquid foods and subsequent whipping. The foam facilitates rapid drying and retention of nutritional quality of

heat sensitive food materials. *Squilla* is a major component of shrimp by-catch and utilization of *Squilla* sp. is limited to the preparation of squilla meal, silage and manure apart from chitosan production. *Squilla* spp. contains 13-15% protein, however, higher moisture content and hygroscopic characteristic of its protein confines



squilla's processing. So, little attention has been paid on utilization of squilla for the development of edible products.

An attempt has been made to prepare protein concentrate from this underutilized crustacean, by foam mat drying technique. Squilla was thoroughly washed; head removed and cut into smaller pieces. Squilla protein was collected by hot water extraction method. For this, the squilla pieces were dipped in potable water (1:1 ratio) maintained at 60°C for 30 min with intermittent stirring. The muscle portion got extracted into the water which was then filtered by cheese cloth and centrifuged at 5000 rpm for 15 min. The residue was ground with whipping agents (Gum Acacia and maltodextrin) and made into foam by adding the filtrate obtained after centrifugation. The stable foam was poured as a mat (2-3 mm) in aluminum tray and dried at 55°C in a hot air oven for 16 h. Proximate composition, emulsion properties and antioxidant activities of the squilla protein concentrate (SPC) was evaluated.

Yield of SPC was 12% from raw material. The moisture and protein content of squilla meat was 82.94 and 13.2%, respectively whereas the moisture and protein content of SPC prepared by foam mat drying process was 21.55 and 36.2%, respectively. The protein concentrate displayed good emulsion properties while the emulsion activity index and emulsion stability index of Gum Acacia added and maltodextrin added SPC were 148.2 and 150 m<sup>2</sup>/g and 26.26 and 34.08 min, respectively. However, the foaming capacity of the protein concentrate was poor (18-21%). Both the protein concentrate had excellent metal chelating and DPPH scavenging activities. Source of whipping agent didn't influence the properties of dried protein concentrate considerably. The dried protein concentrate had very good solubility and can be used as a protein/flavouring agent in food formulations such as soup, sauce or snacks. Further studies need to be focused on the formulation of food products using squilla protein concentrate.



## Thermal behaviour of gelatin extracted from reef cod (*Epinephelus diacanthus*) skin waste

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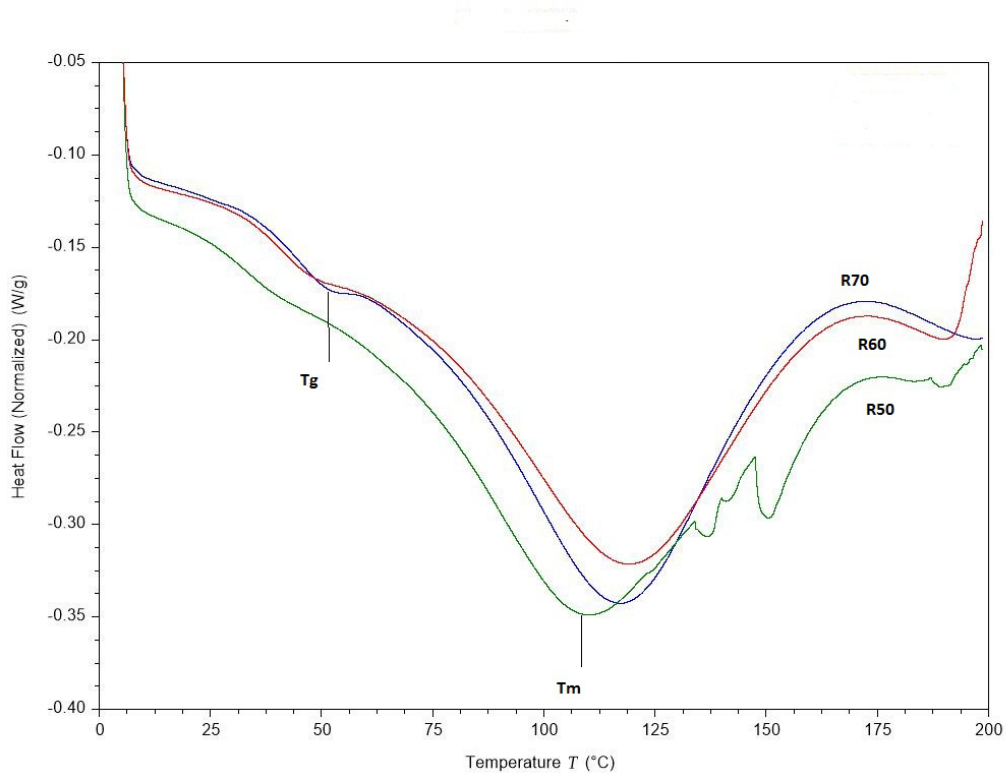
Differential scanning calorimetry (DSC) analysis was employed to study the thermal behaviour of gelatin extracted from the skin of

reef cod collected from seafood establishments of Veraval, Gujarat. In India, an estimated 4,319 tons of grouper was caught during the year 2017

**Table 1. DSC thermal profile of gelatins extracted from reef cod skin**

Sample	$T_{gi}$ (°C)	$T_{gp}$ (°C)	$\Delta H_g$ (Jg <sup>-1</sup> )	$T_{mi}$ (°C)	$T_{mp}$ (°C)	$\Delta H_m$ (Jg <sup>-1</sup> )
R50	30.32	37.05	0.53	73.66	106.25	31.59
R60	32.99	43.00	1.51	77.54	118.92	71.59
R70	36.44	50.12	1.46	79.00	117.84	81.00

$T_{gi}$  -Onset glass transition temperature;  $T_{gp}$  -Peak glass transition temperature;  $T_{mi}$  -Onset solid-melting temperature;  $T_{mp}$  - Peak solid-melting temperature;  $\Delta H_g$  -Enthalpy change for glass transition;  $\Delta H_m$  - Enthalpy change for solid-melting.

*Fig. 1: Thermal profile of gelatin extracted from reef cod skin*

(CMFRI, 2018). Reef cod is mainly exported as whole, whole cleaned and fillet to the EU and Non-EU countries. The percentage of waste generated due to fillet processing was almost 70-72% and the waste is generally used for fish meal production.

The present study was carried out to extract gelatin from the skin of reef cod by using vacuum oven drier. Gelatin was extracted from the skin of reef cod with different oven drying temperatures of 50, 60 and 70°C and were given code R50, R60 and R70 respectively. DSC gives the details

about the temperature and enthalpies associated with phase transition when the gelatin is heated. Glass transition and solid melting properties of reef cod skin gelatin with reference to different drying temperature are shown in Fig. 1 and Table 1. Gelatin produced using different oven drying temperatures had followed more or less similar thermal behavior pattern, which revealed that there was no impact of drying temperature on thermal behavior properties of gelatin.

The onset of glass transition temperature in reef cod skin gelatin was lowest at 30.32°C in

R50. Highest glass transition onset and peak temperatures (36.44°C and 50.12°C) were observed in R70. Increase in the glass transition temperature with respect to oven drying temperature might be due to the evaporation of crystallisable water in the gelatin (Reutner et. al, 1985). Moreover, gelatin has the tendency to absorb water from the environment and this water is partially crystalizable. Fish skin gelatin had lower glass transition, gelling point and gel strength when compared to terrestrial animal gelatin because of a lesser amount of imino acids in fish gelatin (Rahman & Al-Mahrouqi, 2009; Rahman et al, 2008).

Onset solid melting temperature was high in R70 and lowest in R50. Higher solid melting temperature leads to higher enthalpy change during solid melting. Compared to other samples R70 had higher solid melting enthalpy change (Hm) of 81.00 Jg<sup>-1</sup>. This phenomenon may be due to the presence of higher bound water in the sample. The moisture present in the sample play a vital role in the solid melting phenomenon. Moreover, solid melting peaks of gelatin are associated with helix-coil transition of gelatin, breakdown of hydrogen bonds and triple helix into a random arrangement (Bigi et. al 2004). Similar thermal behavior trend was also reported in the gelatin extracted from small tooth emperor skin (Al-Saidi et. al., 2011).

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## Aquaculture fishers: Transformation of Traditional Fishermen from Wild Fish Hunters to Farmed Fish Harvesters

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**A**quaculture fishers are fishermen engaged in harvest of fish and shrimp from aquaculture farms. In Andhra Pradesh, there are an estimated 6.05 lakh marine fishermen living

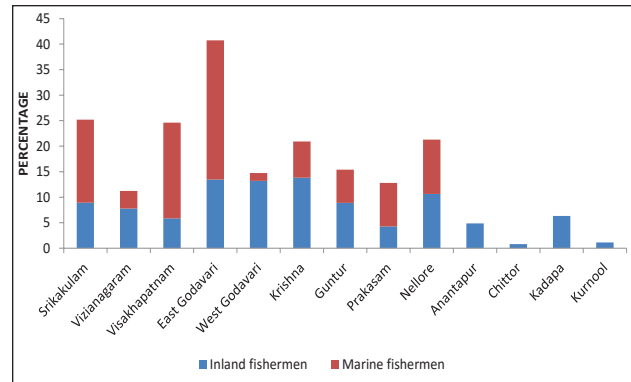
in 555 coastal villages along the 974 km long coast line. Traditionally, fishermen were engaged in the capture of wild fish either from the seas or inland water bodies. They have honed their skills



*Aquaculture fishers harvesting *Penaeus vannamei* shrimp (Brackish water Aquaculture)*

and developed fishing gear aimed to capture demersal and pelagic fishes in the rough seas, fast flowing rivers and huge reservoirs. However, in recent times dwindling catches in both marine and inland water bodies has drastically affecting the livelihood of fishermen. Fishermen are slowly but steadily opting to move away from capture fisheries within the state or migrating to work as fishing crew in other states. However, the flourishing aquaculture sector in the state is providing opportunities for fishermen to hone their harvesting skills in the aquaculture sector.

The inland sector of the Andhra Pradesh is bestowed with 25,067 water bodies with an effective water spread area of 2,97,517 hectares. The state has been contributing significantly to the fish basket of the country through freshwater and brackish water aquaculture. Andhra Pradesh has 1.96 lakh hectares under aquaculture of which 1.04 lakh hectares is under freshwater aquaculture and 0.92 lakh hectares is under brackish water aquaculture. The total inland fishermen population in Andhra Pradesh is 1.67 lakhs. Andhra Pradesh is the leading state in farming of *Penaeus vannamei* shrimp in brackish water which is mainly exported to USA, EU, SE Asia, China and Japan. The state also grows significant quantities of freshwater fish namely India major carps (*Labeo rohita* and *Catla catla*), pangasius (*Pangasionodon hypophthalmus*), pacu (*Piaractus brachypomus*) which are mainly intended for the domestic markets; both within



*Fig. 1. Distribution of marine and inland fishermen in Andhra Pradesh*

and outside the state.

Fishermen are integral to the aquaculture operation mainly at the time of harvest. A short survey was conducted among fishermen (n=25) in Andhra Pradesh to elicit information on the fishing operations and practices followed in finfish farms (n=10) and shrimp farms (n=15) and the results are presented in Table 1. The harvesting of finfish was done by operating the drag nets. These nets were pulled manually by the fishermen. Harvesting from big fish ponds (>25 acres) was done by pulling the net from the tank bunds with the help of tractors and fishermen guiding the net by moving in the pond water. Shrimp harvesting was done by using either cast nets and/or drag nets. Although final harvest of shrimp was done after 120 days of farming, now-a-days shrimp farmers opt for mid-harvest after the end of the 3<sup>rd</sup> month of stocking. Fishermen were engaged, continuously throughout the year, in the mid-harvest and final harvest operations. Harvesting of one hectare shrimp farm requires 6 fishermen and each is paid Rs 600 per day. The biomass in shrimp farms ranges from 3 to 6 tonnes per hectare. The harvesting operation starts in the early morning and ends by noon. Shrimp farmers pay additional amount to the fishermen who bring their own fishing nets. The nets were sanitized in potassium permanganate solution before casting them in the pond water. Sanitization of fishing nets was practiced more in shrimp harvesting and more specifically during



**Table 1. Details of fishing nets and charges of fish harvest operations in aquaculture farms in Andhra Pradesh**

Fishing operation/handling	Observation
Type of fishing nets used	Drag Nets and Cast Nets
Gear material	Nylon multifilament
Drag Net Specifications	20mm mesh size, 350 m length, 9-10m width
Cast Net specifications	20 mm mesh size, 4.4 m length, 4 kg weight
Number of fishermen engaged for aquaculture harvesting (per pond)	6
Wages paid to each fishermen for harvesting (per day)	Rs. 500/-
Fishing Gear hiring charges (per day)	Rs 100 per 100 m length of drag net; Rs 200/- per cast net

the mid-harvest operations (to avoid spread of shrimp diseases). However, fishermen fear that the disinfectants/ sanitizers have adverse impact on the durability of the fishing nets.

Majority of the inland fishermen of Andhra Pradesh (Fig. 1) are distributed in Krishna (13.8%), East Godavari (13.5%), West Godavari (13.2%) and Nellore (10.7%) districts and it is pertinent to note that these districts viz., West Godavari (37%), Krishna (36%), East Godavari (9%) and Nellore (6.7%) have relatively higher

area under aquaculture. This clearly indicates a direct relationship between farm spread area and the number of inland fishermen. The potential area for brackish water aquaculture is 1.74 lakh hectares and 8 lakh hectares for freshwater aquaculture indicating scope for further increase in brackish water and freshwater aquaculture from the existing farm spread area in the state. This opens more opportunities to the fishermen as harvesters of aquaculture produce.



## Antimicrobial activity of seaweed extracts of *Padina gymnospora* extracted by supercritical and conventional method

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The interest in replacement of synthetic food preservatives with natural ones received much attention in recent years due to the concern on toxicity and safety. The extended use of synthetic preservation leads to adverse health effects such as liver damage and suspected to be mutagenic and neurotoxic. Hence, most of the consumers prefer additive free foods or natural preservatives from plants, algae and microalgae.

Nowadays, seaweeds are of emerging interest in both food and pharmaceutical industry due to the presence of bioactive compounds with economic relevance including polysaccharides, iodine organic products, macro and micro-elements, vitamins, and unsaturated fatty acids (FAs). Several studies have shown that seaweed or its extracts possess physiological such as antioxidants, antimicrobials, anti-coagulants,

anti-thrombotics, and anti-inflammatory for the treatment of several diseases, in addition to their anti-tumor and anti-viral properties.

The extraction of bioactive compounds from seaweeds is usually performed using organic solvents but these methods have major drawbacks; they use large quantity of organic toxic compounds, labour intensive, long extraction time and low extraction yields. Supercritical fluid extraction (SFE) is an alternative extraction technique over the traditional solvent extraction. SFE is considered as environmental friendly technology, since concentration step is most often eliminated after the extraction process. Moreover, supercritical CO<sub>2</sub> has low viscosity, low surface tension, high diffusivity and good density and is also non-toxic, non-flammable, cheap, widely available, chemically inert under several conditions, and gaseous at normal pressure and temperature (Goto *et al.*, 2015). This technology is well-known today and is considered as a green process. Comparing with the conventional means, SFE exhibited great potential in extracting bioactive compounds especially in terms of yield, phenolic compounds and other antimicrobial compounds. The advantage of supercritical CO<sub>2</sub> extracts over conventional extracts is that supercritical CO<sub>2</sub> extracts can be directly used by the food industry since no toxic solvents are present.

*Padina gymnospora* is a brown seaweed and is commonly found in the tropical countries, and is distributed along Indian coast. It has demonstrated significant antimicrobial activity against several pathogens and used for the treatment of cutaneous diseases (Baliano *et al.*, 2016). The present study was carried out to compare the antimicrobial efficiency of *Padina gymnospora* extracts, prepared using supercritical and conventional methods. The SFE conditions used was temp 40°C, pressure 225 bar and time 45 min with ethanol as co solvent. Conventional extraction was carried out

by solvent based extraction using 40% methanol and 40% ethanol.

The antimicrobial activity of extracts of *P. gymnospora* were assessed using the micro titer-plate-based dilution method against bacterial strains including *Pseudomonas aeruginosa* ATCC 27853, *E.coli* ATCC 25922, *Salmonella enterica* sub sp. *enterica* serovar Enteritidis ATCC 13076, *Listeria monocytogenes* ATCC 19112 and *Staphylococcus aureus* ATCC 25923. All these cultures were grown in Brain Heart Infusion (BHI) broth for 18-24 hr. After centrifugation at 8000rpm for 15min, the cultures were washed twice in sterile phosphate buffer and suspended in same buffer. The turbidity of the suspension was adjusted to 0.5Mc Farland standard corresponding to 10<sup>8</sup> cfu/ml. A 1:10 dilution of cell suspension (10<sup>7</sup>cfu/ml) was used for the study.

The results indicated that, the supercritical CO<sub>2</sub> extract had strong antimicrobial activity against all the five bacterial strains studied (Table.1). Highest activity was found against *L. monocytogenes* (31.2 µg/ml), followed by *S. aureus* and *P. aeruginosa* (62.5 µg/ml), *S. enteritidis* (125 µg/ml) and *E.coli* (250 µg/ml) respectively. Generally antimicrobials are less effective against gram negative bacteria because of their more complex multilayered cell wall structure which makes it difficult for the active components to penetrate. However, the supercritical CO<sub>2</sub> extract obtained from *P. gymnospora* showed excellent antimicrobial activity against gram negative as well as gram positive bacteria. Methanol extracts also showed a noticeable activity against all the bacterial strains tested, but lesser active than supercritical CO<sub>2</sub> extracts. Ethanolic extracts showed activity only against *S. aureus*, *P. aeruginosa* and *L. monocytogenes* (250 µg/ml) and no activity was reported against *S. Enteritidis*, a major food borne bacteria.

Overall results indicated that the supercritical extracts of brown seaweed *P.*

Extract	MIC ( $\mu\text{g/ml}$ )				
	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. Enteritidis</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>
Supercritical CO <sub>2</sub> extract	62.5	250	125	31.2	62.5
40% methanol	125	250	500	250	250
40% Ethanol	250	1000	>1000	250	250

*gymnospora* exhibited high antimicrobial activity followed by methanol and ethanol extracts. The study showed the extraction efficiency of SFE for extraction of antimicrobial compounds and was found superior than the conventional extraction. Employing a moderate pressure and temperature might have helped in extracting compounds of high antimicrobial activity. Similar results were reported by Mendiola et al (2007). The present study indicates that SFE extracts of *P. gymnospora* have promising applications in enhancing the food safety

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## A review of drivers and barriers to fish consumption based on Theory of Planned Behaviour

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Most Keralites have a positive attitude towards seafood and consider it as an important part of healthy and balanced diet. The annual per capita consumption of fish for the entire Indian population is estimated at 5-6 kg whereas for the fish eating population it is found to be 8-9 kg. Average annual per capita fish consumption is highest in Kerala state at 30 kg which is very high when compared to that of other states of India

(Shyam, *et al.* 2015). Issues of fish adulteration have been widely discussed by media and have created an increased health, safety and quality consciousness among consumers. These issues have created new drivers and barriers to fish consumption with consumers changing their fish purchase behaviour and market choices. The article studies the emerging drivers and barriers to fish consumption using 'Theory of Planned



Behaviour' (TPB) as a theoretical base. Later, the factors identified were consolidated into a framework of fish consumption.

### Theory of Planned Behaviour

The theory of planned behavior (TPB) was introduced as an extension of Theory of Reasoned Action (TRA). The underlying concept of TPB says that person's intention to perform certain behaviour, which is defined as people's motivation, including the willingness to perform; is a latent variable and this variable is dependent on attitude and subjective norms (Ajzen, 1991; Petrovici, *et al.*, 2004; Saba & Vassallo, 2002, Bonne *et al.*, 2007).

The perceived behavioural control (PBC) as the third construct is the extension of the TRA model to develop the TPB model. The unique nature of TPB model is that it considers the noneconomic factors, which are overlooked in traditional economic models (Petrovici, *et al.*, 2004).

### Drivers and barriers to fish consumption

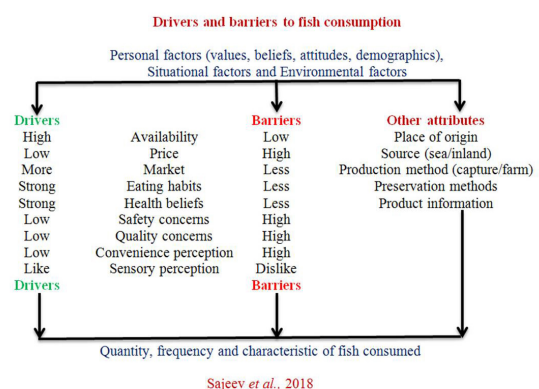
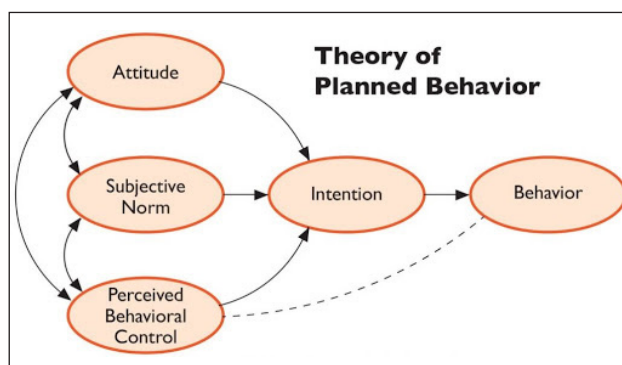
Empirical evidence shows differences in the use of information sources by consumers depending on the food product, the communicated information and the potential health or safety risk (Gutteling and Wiegman, 1996; Jungermann *et al.*, 1996). With respect to fish, consumers mostly use personal sources of information, such as fishmongers and family and friends (Pieniak *et al.*, 2007).

Pieniak *et al.* (2010 a,b) identified knowledge

as a relevant determinant of fish consumption. Consumers with a higher level of knowledge about fish were found to eat fish more frequently. Knowledge studies focused mainly on production aspects, whereas consumer information and education campaigns have mainly been focused on the health and nutritional benefits of fish, as well as on convenience issues acting as barriers to consumption (Olsen, 2003; Verbeke and Vackier, 2005).

Olsen (2004) identified four salient beliefs reasonable in forming seafood/food consumption attitude as: taste, distaste (negative affect), nutrition (Steptoe *et al.*, 1995) and quality / freshness. After the taste issues, the nutritional aspects are the second prominent factor that affect consumer's food attitude, which is directly related to health and healthy eating behaviour (Olsen, 2001). The quality of the fish/seafood freshness is another prime determinate. In this regards, frozen fish are treated as "non-fresh" "bad quality" "tasteless" "watery" "boring" (Olsen, 1998). A study by Olsen (2004) infers that price, value for money and household income are not barrier in seafood consumption, while Verbeke & Vackier, (2005), reported that price negatively affect the fish consumption.

The review of the drivers and barriers to fish consumption using 'Theory of Planned Behaviour' as a theoretical base (Sajeev *et al.*, 2018) provided the following framework for quantity, frequency and characteristics of fish consumed.



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