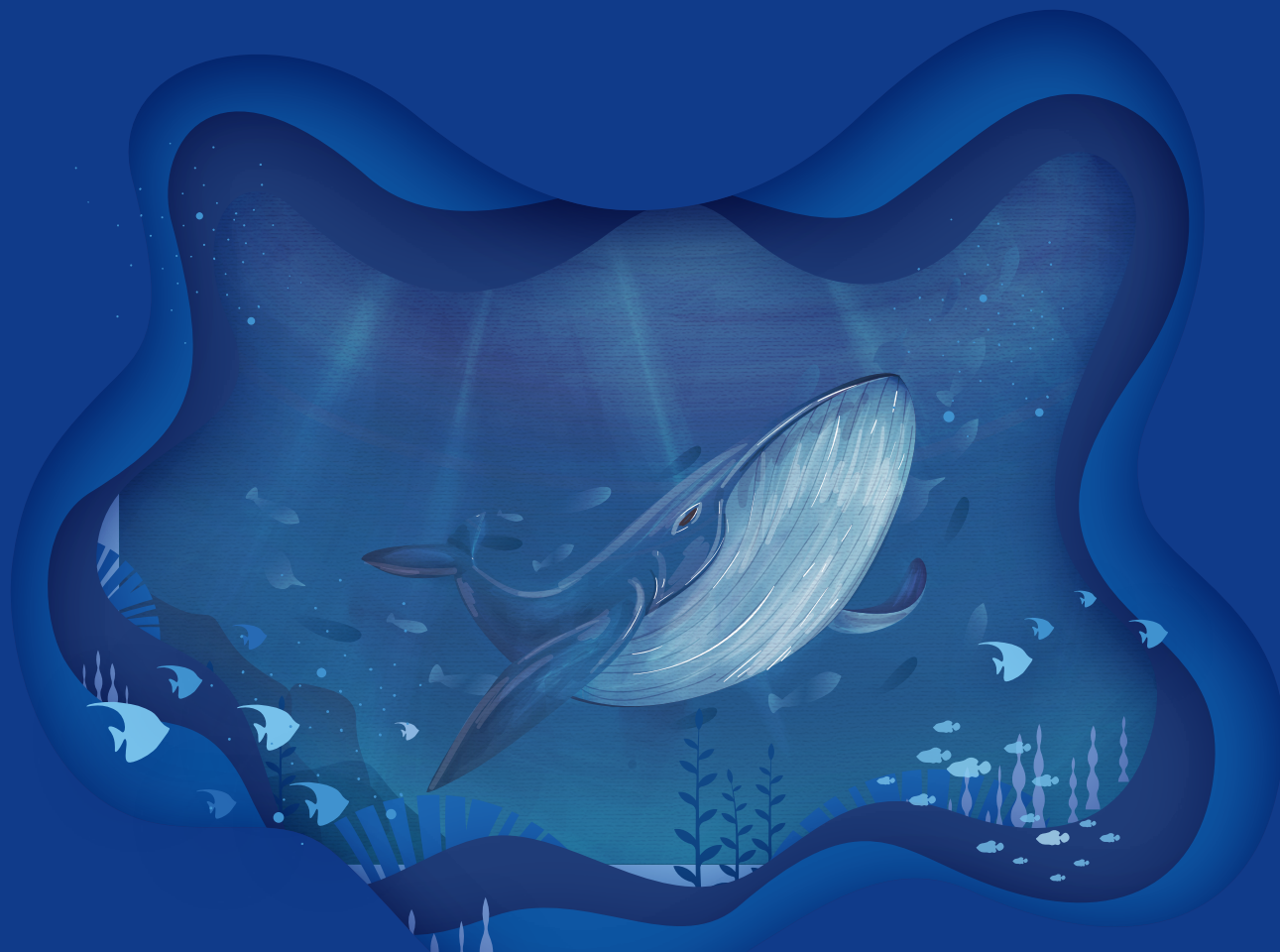




# FISHTECH REPORTER

VOL.09 (2) JULY - DECEMBER 2023



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

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# FishTech Reporter

Vol 09(2) July - December 2023

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## From the **Editorial** Board

Indian seafood exports are expected to reach \$12 billion by 2025, with the highest contribution from frozen shrimp, the topmost export item among traded goods. Within the Sustainable Development Goals framework, there is particular emphasis on the sustainability of fish resources, seafood value chains, and seafood trade. Therefore, overall fisheries development should encompass both the domestic and international seafood sectors in the value chain, employing an integrated, inclusive, sustainable, and holistic approach.

While marine fisheries resources have generally reached a level of stagnation, there remain certain underutilized or untapped species. Addressing this issue, the potential to utilize swimming crab in the southwest coast of India has been highlighted.

Shrimp stands as the primary commodity in seafood exports. However, the process of shrimp peeling is a laborious task for women workers in seafood processing units. An article discussing a specialized method of shrimp peeling using enzymes is included, showcasing how it can significantly reduce the effort required for peeling, thereby maximizing productivity in shrimp processing units.

Product development from secondary raw materials can be highly efficient and effective in utilizing fish resources. Chitin and chitosan can be derived from various unconventional sources. This issue features a unique extraction method for chitin and chitosan from squid pens.

With the increase in aquaculture production, there has been a heightened focus on reducing the cost of feed, which constitutes a major expense in fish production. Research on better utilization of fish waste, both nationally and internationally, is being pursued. An article on the biogenic amine content of fish feed prepared from Tilapia waste highlights the importance of the quality of fish waste used in feed preparation. Furthermore, there is coverage on the potential to utilize the largely underutilized myctophid reserve for fish feed preparation. Additionally, the effect of microwave power on the biochemical quality of tuna during microwave vacuum heating is explored.

This issue also presents a green method for chitin extraction using microbial fermentation of shrimp shell waste. Furthermore, molecular insights into the DNA gyrase of *V. cholerae* have been included to stimulate readers' interest.

Drying of brown seaweed through infrared and electrical oven drying methods is featured, as seaweed value addition through drying offers a simple means of maximizing profit. Various drying methods for sole fish, including open sun, solar-LPG, and infrared dryers, are also covered.

In recent times, the rise of online fish marketing companies due to lifestyle changes is notable. An article on online fish markets is included in this issue. Additionally, an article focusing on the establishment of a database on the import and export of fish oil and fish meal is featured.

In conclusion, this issue prioritizes modern, advanced, and updated technologies/methods for the overall development of fisheries, ensuring maximum benefits for all actors in the fish value chain.

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## **Mass landing of swimming crab (*Charybdis smithii*) along the coastal waters of the Southwest coast of India and potential for utilisation**

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Crustaceans are an economically valuable resource in Indian fisheries and in the last two decades, they contributed nearly 15% to the total landing. Among them, crabs are an important group which significantly contributes to the fishery after the penaeid and non-penaeid prawns. More than 60% edible marine crab fishery in India is contributed by three species, *Portunus sanguinolentus* (28.2%), *Portunus pelagicus* (25%) and *Charybdis feriata* (7.7%). Besides this, *C. lucifera*, *C. natator*, *C. smithii*, *C. annulata*, *Monomia gladiator*, *Podophthalmus vigil*, *Scylla serrata* and *S. olivacea* also contributed to the fishery on a small scale (Jose, 2022). Crabs are harvested using a variety of craft and gear combinations, among which trawl is the major one.

Among the above species, *C. smithii* (Fig:1) is endemic to the Indian Ocean (Bernd and Boetius, 2000) and this species is one of the very few swimming crabs that exhibits swarming in the open ocean. (Sankarankutty and Rangarajan; 1962 Croce and Holthuis

1965; Losse, 1969; Rice, 1969). Romanov et al (2009) reported that swimming crabs are dispersed by the monsoon current throughout the equatorial Indian Ocean and feed mainly on mesopelagics and *C. smithii* is an important prey for more than 30 species. Aggregation of the crabs on the continental shelf of the Indian Ocean precedes their breeding during North East monsoon from October to January (Van Couwelaar et al 1997). These crabs have a predatory impact on the upper water column and there are reports on the depredation of catch in gill nets and bait in the long-line fishery (Renjith et al 2023, Van Couwelaar et al 1997). Despite their swimming capabilities, many of the crab species live at the bottom of the sea. Due to the light and weakly calcified, non-granulated carapace, *C. smithii* actively swims in the open ocean and does not need any substrate to survive. This ability to remain and swim in the water column allows this species to form a swarm on the ocean surface. There are various observations on the occurrence of *C. smithii*

in the world's oceans. Balasubramanian and Suseelan (1990) reported the occurrence of this species along the west coast of India. Swarms occur throughout the year except during May and June. However, a significant difference in the average catch was reported during pre-monsoon, monsoon and post-monsoon. Swarming of *C. smithii* was reported during intra-monsoon (October to December) and NE monsoon from east Africa and off Oman respectively (Daniel and Chakrapany, 1983).



Fig. 1. *Charybdis smithii*

Recently, unusual landing of *C. smithii* was reported from trawlers operating along the coast of Kerala. Two months of onboard observations (October-November 2023) from RV Matsyakumari II of ICAR-Central Institute of Fisheries Technology, reported a total of 10.83 tons of *C. smithii* caught in 24 fishing operations and the average catch per day was about 400 kg (Fig. 2). The along off Kochi operations were conducted at a depth of less than 40 m, using bottom trawls, having cod-end mesh size of 25 mm



Fig. 2. *C. smithii* landed onboard Matsyakumari II

A survey was also conducted along the major fishing harbours of Kochi, to record the landing. Commercial trawls operating along the Kochi from various depths targeting *Heterocarpus gibbosus*, *H. woodmasoni*, (200-250m depth) cephalopods (30-50m), *Metapenaeus dobsoni*, *M. affinis* and *M. brevicornis* (15-25m) reported heavy landing of *C. smithii* as bycatch. Similar findings were observed for the last five to six years along the Kerala coast. The swarming usually commences by the end of August / beginning of September and will last till December. The catch depends on the area and depth of the operation, with *C. smithii* forming more than 60% of the catch during peak season. Dineshbabu et al. (2020) reported massive landing of *C. smithii* from the Mangalore coast during January to March 2020. The catch rate was in the range of 200-1600 kg per trawler, which formed more than one-fourth of the multiday trawl boat landing. Dineshbabu et al. (2020) have reported that these crabs are often sold at the rate of INR 10-13 rupees/ kilogram from the Mangalore coast, however, no such market exists for this crab in Kerala.

The higher number of low-value species in a targeted fishery causes several operational and economic problems for the fishery. Trawlers usually avoid or change the ground wherever such landing occurs. Fishers opined that nowadays distribution of this species extends up to the shallow waters and causes

damage to the coastal gillnets. Deep setting of the net by providing more sinkers is a strategy used by gillnet fishers to avoid net damage.

Yogesh et al (2019) reported that the nutritional profile of *C. smithii* is comparable with other edible crabs. Since the meat content of this crab is very low, the chances for utilization as seafood are negligible. As the seasonal swarming of *C. smithii* is regular along the coast, it is recommended that the resources can be effectively utilised for the preparation of fish meal, chitosan etc. and value addition of this species may be attempted. This resource can also be screened for biologically active compounds.

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## ***Biogenic amine content in fish feed prepared from Tilapia fish waste***

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**F**ish waste, which is a by-product of fish markets/fish processing industries, generally represents 30-70% of the original weight of fish, depending on the processing method employed (Ahuja et al., 2020). The large quantity of fish waste thus produced can become a source of environmental pollution. Converting this waste into useful products is one of the means of increasing the profitability of the seafood industry in addition to reducing environmental impact. Several fish waste-derived biomolecules with excellent chemical, structural and functional properties have found applications in food and pharmaceutical industries. Among various applications, converting fish waste into feed is an emerging area of research with considerable commercial interest, which will also help to attain the Sustainable Development Goals (SDGs) by 2030 (Thirukumaran et al., 2022), primarily Goal 2 (Zero Hunger), Goal 12 (Responsible Consumption and Production), Goal 14 (Life Below Water) and Goal 8 (Decent Work and Economic Growth). As in the case of fish meal, quality of fish waste is very important as a raw material for feed making. The freshness of

the raw materials significantly impacts the growth performance when utilized as a feed ingredient in fish diets. The content of biogenic amines serves as an indicator of the freshness of raw materials.

Biogenic amines (BAs) are low molecular weight organic bases that are generated through the microbial decarboxylation of specific amino acids or the transamination of aldehydes and ketones by amino acid transaminases. Improper storage conditions or temperature abuse of fish waste can lead to the formation of BAs due to microbial enzymatic action. The most commonly encountered BAs in fish are histamine, tyramine, putrescine, and cadaverine. Low levels of BAs in fish meal were reported to contribute to improved protein digestibility (Jasour et al., 2018). Conversely, higher levels of BAs in fish diets can lead to reduced fish growth and diminished feed intake (Mundheim et al., 2004).

In the present study, floating fish feed was prepared from Tilapia (*Oreochromis mossambicus*) fish waste along with other ingredients listed in Table 1.

Table 1. Ingredients used for fish feed preparation

Sl. No.	Ingredients	Quantity
1.	Rice bran	1000 g
2.	Corn starch	1500 g
3.	Soya flour	500 g
4.	Wheat flour	900 g
5.	Sunflower oil	50 ml
6.	Tilapia Fish waste	1300 g

The Bureau of Indian Standards (BIS) has published five standards specifically for aqua feed; 1) IS 16150 (Part 1): 2023 fish feed - specification, part 1 carp feed, 2) IS 16150 (Part 2): 2014 fish feed - specification, part 2 catfish feed, 3) IS 16150 (Part 3): 2014 fish feed - specification, part 3 marine shrimp feed, 4) IS 16150 (Part 4): 2014 fish feed - specification, part 4 freshwater prawn (*Macrobrachium rosenbergii*) feed and 5) IS 16150 (Part 5): 2023 fish feed — specification, part 5 pangasius feed. All these standards provide the requirements for aquafeed.

The biogenic amine content of the fish feed was analyzed by high-performance liquid chromatography (HPLC) as per ISO 19343: 2017 (E). The results are given in Table 2. Cadaverine (417 ppm) was the most prevalent BA in the fish feed, followed by Tyramine (222 ppm). Histamine was present at a concentration of 30 ppm. According to a previous study, when choosing fish meal for shrimp diets, especially for very young juveniles and carnivorous species, it is crucial to consider the freshness of the raw materials (Ricque-Marie et al., 1998). As

suggested by the authors, the freshness can be determined by assessing the TVN (Total Volatile Nitrogen) levels in the raw material, which should be below 30 mg N/100 g. Another important quality parameter is the sum of amine contents in the final product, which should be less than 2000 mg/kg (Ricque-Marie et al., 1998). In the present study, the sum total of BAs in the fish feed was 788 ppm, which is way less than 2000 ppm indicating the good quality of the raw material. Although the BIS standards for aqua feed do not specifically address the presence of BAs, including histamine, they still assume importance as indicators of the quality of raw materials used in the preparation of fish feed. Also, elevated levels of these amines can adversely impact fish by affecting their feed intake and growth rate.

Table 2. Biogenic amine content of feed prepared from Tilapia fish waste

Sl. No.	Biogenic amine	Value (ppm)
1	Putrescine	85
2	Cadaverine	417
3	Spermidine	22
4	Spermine	12
5	Histamine	30
6	Tyramine	222

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## ***Enzyme Assisted Peeling of Shrimp: A promising pre-processing intervention to minimize drudgery and maximize productivity in shrimp processing units***

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**F**rozen shrimp is the major item of fisheries export from India in 2022-23, both in terms of quantity (7,11,099 MT; 41%) and value (Rs. 43,136 crores; 67%) (mpeda.gov.in). Peeled shrimp products namely peeled-deveined (PD), peeled-undeveined (PUD), peeled-deveined tail on (PDTO), Butterfly cut-PDTO, cooked-peeled shrimp, etc. (Fig. 1) are common shrimp products exported from India.

Peeling of shrimp is a pre-processing step

wherein exoskeleton (shell) is separated from abdominal meat portion. Structurally, three layers viz., shell, epidermis and muscle are tightly interwoven. The shrimp shell and the epidermis are strongly connected by intra-cuticular fibers and on the other hand the epidermis and shrimp muscle are firmly attached by extensive interdigitation (Talbot et al., 1972). The shell is removed from the meat portion by exerting significant amount of manual force.



*Peeled-shrimp (Butterfly Cut-PDTO)*



*Peeled-shrimp (Cooked)*

**Fig. 1. Peeled-shrimp products**

Females form 63% of the total personnel in shrimp processing units and peeling activity is the major activity performed by the women work force (Rao *et al.*, 2022). The peeling activity is laborious and time-consuming activity wherein the women continuously peel the shrimp for 4-6 hours during a shift. Generally, each person can peel about 40 kg of shrimp per shift. Pre-treatment process can assist in easy peeling which would help in reducing the physical effort during the peeling step.

A pre-treatment process called 'maturation' aids in the loosening of the crosslinks between the muscle and shell prior to peeling. The common maturation practices employed in the pre-processing of shrimp are the use of ice or brine solution (sodium chloride, with or without phosphates) for several days (Dang *et al.*, 2018a, 2018b) but such long maturation times adversely affect shrimp meat quality. After the harvest of shrimp, the post-mortem changes due to the shrimp's intrinsic enzymes and microbial enzymes are responsible for shell-loosening. However, external addition of enzymes derived from microorganisms, plants or animals to augment maturation is a promising approach to accelerate the loosening of shell. Pre-treatment process that assists in loosening of shrimp shell

while maintaining the organoleptic quality is needed for the shrimp processing industry. In this context, the present study aims to introduce a pre-processing intervention that minimizes the effort involved in peeling (easy peeling) by using proteolytic enzymes.

Headless shrimp (*Penaeus vannamei*) procured from a shrimp processing unit, Visakhapatnam, Andhra Pradesh was used for enzymatic maturation study. Endo-protease (from *Bacillus licheniformis*) and exo-protease (from *Aspergillus oryzae*) sourced from Sigma-Aldrich Chemicals Private Limited, were tested for their efficiency to augment shell-loosening of the headless shrimp. The endo-protease and exo-protease enzymes were used at 0.5% concentration (v/v) at three ratio of enzymes; endo-protease: exoprotease [1:1 (T1), 2:1 (T2) and 1:2 (T3)]. The headless shrimps were dip treated in enzymes solution at 1:1 ratio (w/v). All the enzyme treatments were performed at 5°C for 1 h with continuous stirring at 150 rpm. Control shrimp were not exposed to enzymes but other conditions of temperature, time and stirring were maintained. The control (C) and treated shrimp (T1, T2 and T3) were analysed at 15 min interval for peelability, texture and, organoleptic changes and the results are presented in Table 1.

Table 1. Effect of Endo- and Exo-protease enzyme treatment on peelability of shrimps

Time (min)	Control (C)	Treatments		
		T1 (1:1)	T2 (2:1)	T3 (1:2)
0	<ul style="list-style-type: none"> <li>• Very difficult to peel</li> </ul>	<ul style="list-style-type: none"> <li>• Very difficult to peel</li> </ul>	<ul style="list-style-type: none"> <li>• Very difficult to peel</li> </ul>	<ul style="list-style-type: none"> <li>• Very difficult to peel</li> </ul>
15	<ul style="list-style-type: none"> <li>• Difficult to peel</li> <li>• Last abdominal segment and telson difficult to peel off.</li> <li>• Shell and muscle are connected with connecting tissues</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson easy to peel off.</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson easy to peel off.</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson easy to peel off.</li> </ul>
30	<ul style="list-style-type: none"> <li>• Difficult to peel</li> <li>• Last abdominal segment and telson difficult to peel off.</li> <li>• Shell and muscle are connected with connecting tissues</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson easy to peel off.</li> <li>• Soft shell</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson easy to peel off.</li> <li>• Softer shell than T1</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson easy to peel off.</li> <li>• Softer shell than T1</li> </ul>
45	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson easy to peel off.</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson easy to peel off.</li> <li>• Abdominal shell, telson and pleopods separated from muscle.</li> <li>• Very soft shell</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson easy to peel off</li> <li>• Abdominal shell, telson and pleopods separated from muscle</li> <li>• Softer shell than T1</li> <li>• Soft meat</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson easy to peel off</li> <li>• Abdominal shell, telson and pleopods separated from muscle</li> <li>• Softer shell than T1</li> <li>• Soft meat</li> </ul>
60	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson easy to peel off.</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson become easy to peel off</li> <li>• Abdominal shell, telson and pleopods are separated from muscle</li> <li>• Very soft shell</li> <li>• Soft meat</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson become easy to peel off</li> <li>• Abdominal shell, telson and pleopods are separated from muscle</li> <li>• Softer shell than T1</li> <li>• Soft meat</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to peel,</li> <li>• Last abdominal segment and telson become easy to peel off</li> <li>• Abdominal shell, telson and pleopods are separated from muscle</li> <li>• Softer shell than T1</li> <li>• Soft meat</li> </ul>

\*T1: Shrimp treated with endo- protease and exo-protease at the ratio of 1:1; T2: Shrimp treated with endo- and exo-protease at the ratio of 2:1 and T3: Shrimp treated with endo- and exo-protease at the ratio of 1:2



The results indicate that the proteolytic enzyme [0.5% concentration (v/w)] treatment effectively improved the peelability of the shrimp. A combination of endo- and exo-protease at 1:1 ratio resulted in the best peeling of shrimp followed by endo- and exo-protease at 1:2 ratio after 30 min of enzyme maturation. However, shell became very soft after 45 min of enzyme maturation in all the enzyme treated shrimp. It was pertinent to note that the enzymatic maturation did not affect the texture, colour and taste of the shrimp as compared to the control.

Ease-of-peeling would benefit the female predominant workforce in shrimp pre-processing areas by way of reducing their unit effort required for peeling. Easy peeling would also enable the workers to peel relatively higher quantities of shrimp. Easy peeling also helps in progressing towards mechanical peeling. Further studies are needed to optimize the enzyme concentration, enzyme combinations, maturation times for easy peeling of different grades and species of shrimp.

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## Effects of microwave power on the biochemical quality of dark and white muscle of tuna during microwave vacuum heating

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Microwave processing has been receiving great attention by the food processors and researchers worldwide in the recent years due to its higher heat transfer efficiency. Microwave heating is superior to conventional heating methods due to higher energy efficiency, shorter processing time and retention of nutritional components. Microwave vacuum heating is a hybrid technology, making use of high heat transfer property of microwave and controlled temperature rise aided by vacuum. Microwave vacuum drying has been successfully demonstrated for drying of various food products including seafood. Superior product quality can be achieved by microwave vacuum drying in terms of sensory attributes and rehydration properties (Viji et al., 2019). Like any other thermal processing method, drying induces many physicochemical changes in the fish during dehydration and it is necessary to evaluate and standardize the process conditions to minimize those changes. Eastern little tuna (*Euthynnus affinis*) is an abundantly caught tuna species in Indian coast. It has good demand in the Indian market and is mostly consumed locally as fresh, dried or pickled products. Fishes belonging to the group tuna are characterized by high content of dark muscle. The present research was aimed

to evaluate the physico- chemical changes to dark and white meat of Eastern little tuna during microwave vacuum heating.

Fresh Eastern little tuna (average weight 1 kg) procured from Visakhapatnam fishing harbour was brought to the laboratory in iced condition. After washing the tuna thoroughly, skinless fillets were prepared and the fillet was cut into uniform chunks manually. The chunks were dehydrated using a laboratory model microwave vacuum oven (RagaTech, Pune, India) at different power (600 W -T1, 700 W -T2 and 800 W -T3) for 2 h. Proximate composition of tuna chunks dehydrated at different microwave power was analysed by AOAC method (AOAC 2000). The dark muscle and white meat of dehydrated tuna chunks were separated manually from each sample and packed in polythene pouch for further analysis. Solubility of dark and white muscle proteins was estimated by measuring salt soluble (SSN) and water soluble nitrogen (WSN) content (Winton and Winton, 1958; Ironside and Love, 1958). Lipid oxidation was measured by determining thiobarbituric acid reactive substances (TBARS) (Tarladgis, Watts and Youthan 1960) and the result was expressed as mg malonaldehyde/kg sample. The color of dark and white muscle was evaluated by measuring  $L^*$ ,  $a^*$  and  $b^*$  values of the

ground muscle using Hunter's colorimeter (ColorFlex EZ, Hunter Lab, USA).

Microwave power had a significant influence on the proximate parameters of tuna muscle. Moisture content of fresh fish muscle was (76.85)%, which was reduced to 60.76, 53.67 and (47.32)%, in tuna chunks dried at 600 W, 700 W and 800 W, respectively. Consequently, protein content increased from (19.63)% in fresh tuna to 33.02, 39.51, and (45.02)%, respectively in T1, T2 and T3 samples. The results indicate that increasing microwave power had

ash content. Moisture removal during microwave drying is primarily dependent on the power of microwave applied. Many reports suggest that moisture removal increases with microwave power (Chahbani et al., 2018; Pankyamma et al., 2021).

Extractability of water soluble and salt soluble nitrogen fractions of dark and white muscle significantly decreased with increase in microwave power. White muscle registered higher loss of WSN and SSN compared to dark muscle. The observed loss in WSN and SSN could be attributed

Table 1. Proximate composition of Tuna chunks

Sample/parameter	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Fresh tuna	76.85±1.60 <sup>d</sup>	19.63±1.05 <sup>a</sup>	1.85±0.17 <sup>a</sup>	1.15±0.26 <sup>a</sup>
T1-600 W	60.76±1.11 <sup>c</sup>	33.02±1.12 <sup>b</sup>	2.89±0.19 <sup>b</sup>	1.86±0.59 <sup>a</sup>
T2-700 W	53.67±1.39 <sup>b</sup>	39.51±0.53 <sup>b</sup>	3.43±0.47 <sup>c</sup>	2.12±0.85 <sup>b</sup>
T3-800 W	47.32±1.28 <sup>a</sup>	45.02±0.44 <sup>c</sup>	3.88±0.34 <sup>c</sup>	2.46±0.78 <sup>b</sup>

significantly reduced the moisture content while increasing the percentage of protein and fat. Nearly 30% reduction in moisture was achieved by vacuum-microwave heating for 2 h at 800 W. Since, the fresh fish had a negligible level of ash, the processing parameters didn't pose an influence on

to the protein denaturation that occurred during microwave heating. Mostly, sarcoplasmic proteins contribute to water soluble fractions in fish. It has been proven that microwave treatments accelerate protein degradation by altering the tertiary structure (Dong et al., 2021).

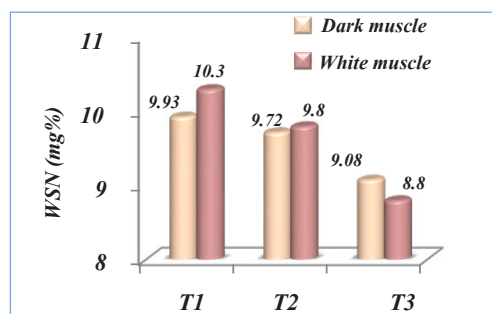
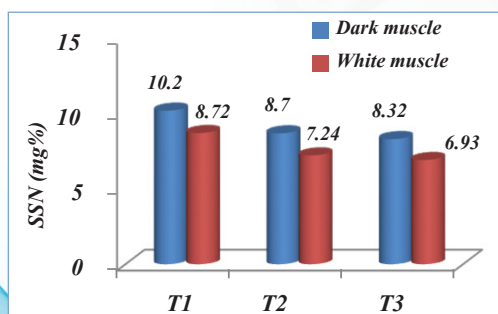


Fig. 1. Salt soluble and water soluble nitrogen content of the samples



Lightness of the muscles reduced with increase in power, but the redness value ( $a^*$ ) increased from 5.7 to 8.8 in dark muscle and 2.71 to 5.04 in white muscle on increasing microwave power level from 600-800 W. Rate of reduction in  $L^*$  value and the rate of increase in  $a^*$  value was higher with dark muscle compared to white muscle, which could be due to the presence of more myoglobin in dark muscle and its degradation during microwave heating. Lipid oxidation of both dark and white muscle increased significantly with increase in microwave power with higher values in dark muscle than white muscle. Researchers suggest that a high electromagnetic field separates fat cells from the muscle and hence, it becomes exposed to oxidation (Yarmand and Homayouni, 2009). So, with the increase of microwave power, more lipids get oxidized. Since the dark muscle of tuna is reported to have high lipid content than white muscle, the TBARS value of former (1.162-1.662 mg MDA/Kg sample) was significantly higher to that of later (0.771-0.889 mg MDA/Kg sample).

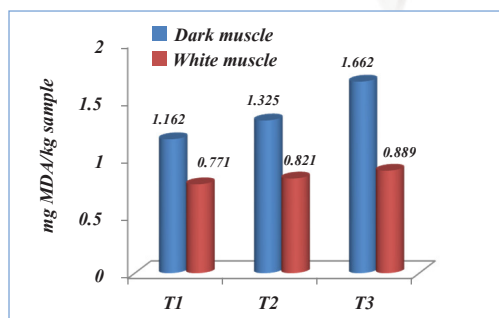


Fig. 2. TBARS values of dark and white muscles of Tuna after microwave heating

The study demonstrated that the dehydration rate increases with an increase in microwave power. Solubility of protein reduced when microwave power was increased from 600-800 W, and white muscle was more susceptible to protein denaturation during microwave heating compared to dark muscle. On the other hand, dark muscle lipids were more oxidized compared to those of white muscle with an increase in microwave power. The results of the study suggest that microwave heating can be employed for the rapid drying of seafood while the changes in protein and lipid qualities need to be addressed.

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## Extraction and characterization of $\beta$ -Chitin and Chitosan from Squid Pen

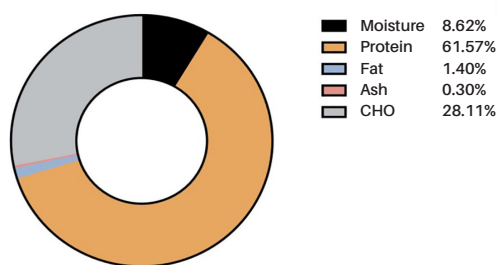
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India holds a significant position as one of the largest global exporters of frozen squid items. Frozen squid industry plays a vital role in earning foreign exchange through an export contribution of 83,846 metric tons in quantity, valued at 3593.75 crore rupees (USD 454.61 million), accounting for 4.83 per cent share in quantity and 5.62 per cent in dollar earnings (MPEDA, 2022-23) in 2022-23. However, this thriving industry generates enormous quantity of squid pen, as secondary raw material which represents an untapped resource of bioactive compounds. Squid pen is an internalized shell with a unique composition of non-mineralized skeletal elements comprised of  $\beta$ -chitin and protein, which are discarded as waste material after industrial processing. Unlike  $\alpha$ -chitin isolated from crustaceans,  $\beta$ -chitin has parallel arrangement with weak intra-sheets hydrogen bonds (Cuong et.al., 2016). An average 100 metric tons of squid pen waste is generated annually from our country after the industrial processing, which actually can be utilized as a source for bioactive compounds. The primary component in squid pen is chitin, a versatile biomaterial having numerous applications. So the present study was aimed to develop a suitable protocol for chitin extraction from industrial generated squid pen waste.

Squid pen was collected as waste from the seafood industries of Veraval region. It was thoroughly cleaned, dried and ground into a fine powder before characterization. The average moisture content of the pen powder was 8.62%. The proximate composition of squid pen powder showed a high protein of 61.57% and a low lipid and ash of 1.4% and 0.3% respectively.



Extraction of  $\beta$ -chitin was carried out with both hot extraction and cold extraction methods at different treatment time and temperature. The protocol of cold extraction with extended deproteinization step (5% NaOH; 55°C; 24h) was found to give better results when compared with hot extraction (90°C for 3h). Average yield of chitosan obtained was 28%. Comparative analysis revealed that the cold extraction method yielded better results, resulting in higher chitin-chitosan recovery.

Deacetylation is a critical step in chitosan production, where chitin is chemically modified to remove acetyl groups. Sodium

hydroxide (NaOH) was used at varying concentrations (20-50%) to achieve deacetylation at different time durations (24hrs, 48hrs and 72 hrs). Observations showed that 50% NaOH concentration led to a maximum degree of deacetylation (DDA-92%) and wide differences were not observed in chitosan composition with duration of treatment except in the whiteness of all samples.

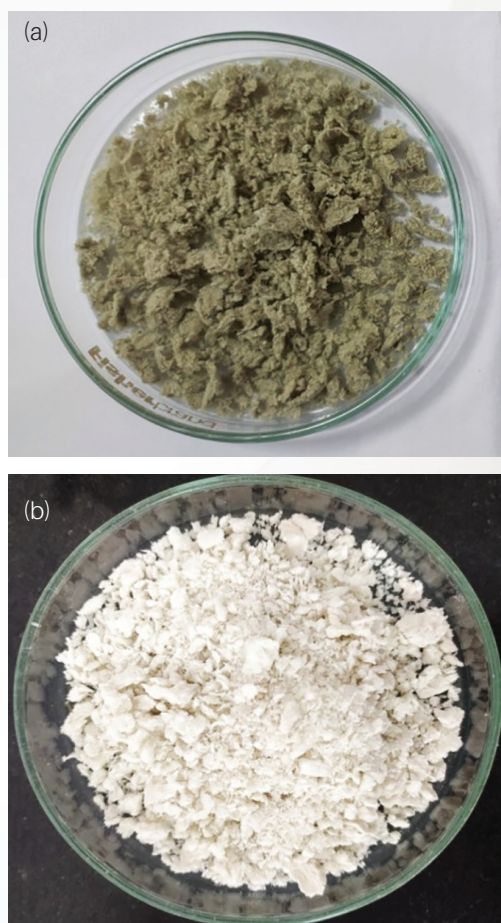
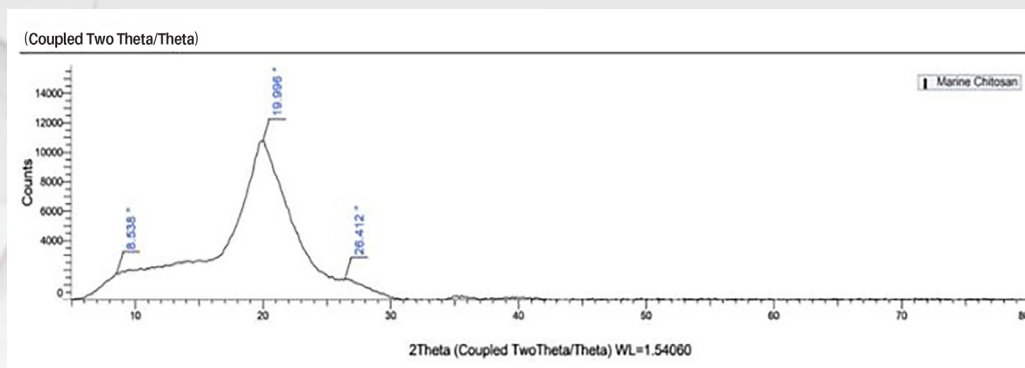


Fig. 1. Chitin obtained through (a) hot extraction  
(b) cold extraction process

The chitin and chitosan extracted from the squid pen waste were characterized to assess their properties and quality. Chitin yield of 32% of the total weight of dried squid pen powder and 25% chitosan was observed. After deproteinization, the protein content in the chitin was in the range of 16-18% and fat 0.3-0.6%. The purity of the product obtained was measured by Fourier Transform Infrared (FTIR). FTIR spectrum showed similar peaks obtained in the squid pen chitosan samples when compared with standard chitosan, indicating the presence of organic functional groups such as out-of-plane bending, C-O-C stretching, and CH<sub>2</sub> stretching. The chitin and chitosan exhibited desirable properties, including a characteristic white color. For further characterization, X-ray diffraction was employed to provide a more comprehensive analysis of their structural and morphological characteristics. X-ray diffraction (XRD) is used for microstructural analysis and identification of crystalline material structure, including atomic arrangement, crystalline size, crystallinity of polymers, recognition of crystalline phase i.e polymorphism and orientation of polymers. The diffractogram of the sample exhibited a typical peak of chitosan i.e  $2\theta \sim 20^\circ$  which is in congruence with the JCPDS No. 039-1894. The XRD data reveals that the X-ray diffraction peak is at  $19.9960^\circ$ , interplanar spacing corresponding to the XRD peak is  $4.43683 \text{ \AA}$  and Full Width at Half Maxima (FWHM) is  $0.160^\circ$ . The smaller FWHM values typically indicate more ordered and crystalline structure of the chitosan particles.





Index	Angle	d Value	FWHM	Rel. Intensity	Intensity
1	8.538 °	10.34811 Å	0.160	16.4%	1774.698
2	19.996 °	4.43683 Å	0.160	100.0%	10812.900
3	26.412 °	3.37186 Å	0.160	13.0%	1410.216

Fig. 2. XRD spectra of the squid pen chitosan

Since the mineral content is low in pen, demineralization can be avoided unlike in conventional chitin extraction protocol for crustaceans, which is a cause for decrease in molecular weight (Chaussard and Domard, 2004). Utilization of squid pen is a waste-to-resource approach that reduces environmental impact, optimizes resource utilization, and provides additional revenue streams for the industry. So, harnessing the potential of squid pen waste, not only contributes to the value addition of the industry but also aligns with sustainable practices for effective waste management

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## Effect of infrared (IR) drying and electrical oven drying on antioxidant potential of brown seaweed

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Seaweeds in dried and powdered form have gained popularity as an ingredient in nutraceutical and dietary supplements. Usually, fresh seaweeds have 75 to 85% water and drying of seaweeds is a common post-harvest method for their preservation (Neoh, Matanjun and Lee, 2016). Drying seaweeds prior to their processing also prevents microbial attack and serve the purpose of easy handling and storage. In India, one of the most popular drying methods is open-air sun drying. Though economical, open-air sun drying requires large area, and poses the risk of getting contaminated with dust, insects or animal droppings. Open-air sun drying can result in uneven drying and a significant loss in quality (Santoshkumar, Yoha and Moses, 2023). Thus, drying in a hygienic and controlled environment is more favored. Oven drying can overcome most of the limitations of open-air drying. Retention of phytochemicals and other bioactive compounds was reported in brown seaweeds dried in an oven dryer (Uribe et al., 2020). But the process is less energy efficient as longer drying time is required. Modern drying processes, such as vacuum drying, freeze drying, microwave-assisted

drying, and infrared drying, are replacing traditional drying methods because they are much more efficient and takes lesser time. Though each method has its own advantages, it is crucial to select the right drying conditions and process for superior results. However, little is known about how various drying methods impact the phytochemical and nutrient content of seaweeds.

*Turbinaria conoides* (J. Agardh) Kuzing, a brown seaweed, was collected from the Gulf of Mannar in Mandapam region of Tamil Nadu. It was dried separately using an infrared dryer (IR) at 50°C and a conventional electrical oven dryer at 50°C for 3 hours and 28 hours, respectively and pulverized. Samples were extracted with ethanol and electrical oven-dried samples showed a higher percentage of extract. Crude protein content of IR dried sample was 9% while that of electrical dried sample was 12%. Water activity for both samples was below 0.6, which is lower than the required level for microbial growth (Jayasinghe, Pahalawattaarachchi and Ranaweera, 2016). DPPH radical scavenging activity, ABTS activity, and total antioxidant assay were used to assess the antioxidant activity

of ethanolic extract for IR and electrical oven-dried seaweed powder and results are given in Table 1. In addition, the total phenolic and flavonoid levels were determined. Phenolic content of IR dried sample ranged from 12.4 to 15 mg Gallic acid equivalent/g and for electrical oven dried sample it ranged from 17.2 to 21 mg Gallic acid equivalent/g, respectively. In addition, flavonoid content in IR dried sample ranged from 38 to 40 mg Quercetin equivalent/g and in electrical oven dried sample, it ranged from 40.6 to 42 mg Quercetin equivalent/g, respectively. Significant difference in phenolic content

was observed between the two drying methods ( $p < 0.05$ ) though not much difference was found in flavonoid content.

IR dryer significantly reduces the drying time which compensates its higher costs. In addition, IR dryer offers more precise temperature control and thus improves the quality of final product. For bulk preparation of hygienic dried seaweeds, IR dryer showed more potential than a conventional electrical oven dryer without compromising the antioxidant potential of seaweed.

Table 1. Results of antioxidant assays for the two drying methods

	mg Trolox Equivalent / g of extract		
	DPPH Assay	ABTS Assay	Total antioxidant Assay
IR dried sample	$2.26 \pm 0.95$	$17.86 \pm 0.24$	$30.78 \pm 0.25$
Electrical oven dried sample	$2.53 \pm 0.70$	$13.74 \pm 0.33$	$30.38 \pm 0.62$



Fig. 1. *Turbinaria conoides* dried using (a) IR dryer (b) Electrical oven dryer.



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## ***Probing the potential of myctophid silage in aqua-feed formulation***

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**M**yctophids are the most abundant and diverse mesopelagic fishes in the Southern Ocean (Saunders et al., 2019). It contributes to about 65% biomass among mesopelagics with an estimated global biomass of 550-660 million tonnes. Though myctophid fishes are one of the most abundant marine organisms, they are least studied and underutilized group. Moreover, reports states that they are usually considered as by-catch from the shrimp trawlers and discarded back to the sea (Hidalgo and Browman, 2019). Considering the large standing stocks of myctophids in some parts of the world ocean and its fishery potential, a strategy should be devised to exploit the biomass rather than dumping as by-catch. It has been reported that myctophids have considerably good amount of protein ranging from 11-23% and lipid content of 0.5-26% (Zhang et al., 2023). However, inspite of its nutrient richness, they are often being neglected. Only a small portion of lanternfishes is being utilized in the form of sun-dried fish products, fishmeal, fish oil, fish silage etc. (Shaviklo and Rafipour, 2013). The myctophids can be a source of protein and lipid if processed and extracted appropriately.

Proteins are often reported as one of the high-priced ingredients in aquafeed formulations adding to nearly 70% of the total operational cost. Hence, to make the global aquaculture industry more economical and sustainable, one important aspect is to use cost-effective feed ingredients. Fish meal remains as one of the important protein sources in aquafeed formulations taking into account its unique essential amino acid composition, micronutrient content and high protein digestibility. However, the high price along with the irregularity in supply have fostered researchers to screen affordable replacements for fish meal, whether wholly or partially. (Howlader et al., 2023). Considering the high protein content in myctophids, its potential as an aquafeed protein source needs be explored.

Fish silage has recently emerged as an affordable alternative for fish meal. It is defined as a liquid product obtained from the whole fish or parts of it, by resorting to different production methods such as chemical, biological and enzymatic silage. Silage prepared by any of the above methods are reported to be proteins, enzymes, organic acids, amino acids,

and even biologically active secondary metabolites qualifying it as a sustainable, low cost feed ingredient. In this regard, the present study was designed with an objective to utilize the myctophid biomass as an aqua feed ingredient, specifically as a protein source by resorting to silaging. Around 86 - 90% of total lipid in myctophids are contributed by wax esters (Noguchi et al., 2004).

Two myctophid species, viz, *Neoscopilus microchir* and *Diaphus watasei* were selected for the present study (fig 1a,1b). They were mixed in a ratio of 1:1 and accordingly, a mince was formulated. Nutrient profiling of the mince thus developed indicated that it had fairly good amounts of protein, lipid and ash with values of 77.14, 6.13 and 8.91 % ash respectively on dry weight basis. Myctophid silage was prepared by adding commercial grade formic acid at different concentrations of 2.5% (T1), 3% (T2) and 3.5% (T3) (fig 2a, 2b). The pH was checked regularly which was maintained below 4 and after five days it was found to be stable. The silage thus prepared was evaluated for biochemical changes for 10 days with sampling on every alternative day. Changes in degree of

hydrolysis and protein was studied. It was observed that on the 7<sup>th</sup> day of silaging, an intense putrid smell was recorded for the T1 treatment indicated its spoilage. However, such adverse changes were not observed for T2 and T3 treatments. Further, the lowest degree of hydrolysis was recorded for T1 and the values were almost similar for T2 and T3 with values of 53.57 and 51.23% respectively. The degree of hydrolysis is important indicator in determining the functional properties of hydrolyzed proteins. Several factors can affect the degree of hydrolysis such as time of silaging, method of silaging, the nature of raw material employed etc. However, in this case all these parameters have remained constant with only difference in the concentration of formic acid. It was clear from the data that employing a lower concentration of 2.5% formic acid has resulted in a lower degree of hydrolysis which is not preferred. The degree of hydrolysis was found to increase upto 53.57% when 3% formic acid was employed. Interestingly, it was observed that a further increase in formic acid concentration to 3.5% resulted in slightly lowering the degree of hydrolysis to 51.23% indicating 3% concentration was more ideal.



Fig. 1. (a) *Neoscopilus microchir*



Fig. 2. (a) Silage mixed with formic acid



Fig. 1 (b). *Diaphus watasei*



Fig. 2 (b). Myctophid Silage

From the point of degree of hydrolysis, T2 treatment can be considered as better. Furthermore, by employing a weak organic acid such as formic acid at concentration of 3%, myctophid silages with better assimilation could be achieved. This study points out the significance of converting myctophid into valuable aquafeed ingredient, specifically as a protein source. However, further scientific evaluation in terms of growth performance has to be carried out for validating the above findings and efficacy of the developed feed with myctophid silage has to be evaluated by studying its growth and physio-metabolic changes in a fish model study.

## Acknowledgments

The authors duly acknowledge the funding received from SAARC for pursuing the PhD programme (SAC No. 749/2020/Award letter/2, dated 08.11.2020). The authors also duly acknowledge ICAR-CIFT for providing all the necessary facilities for carrying out the research work.

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## ***Bioprocessing of shrimp shell for extracting chitin using *Bacillus licheniformis* and *Lactobacillus fermentum****

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**W**aste generated during the processing of shellfish has been increasing worldwide over the years. Shrimp waste generally comprises, the head, shell and tail portions accounting for 40-50% of the total weight. Some of the shrimp waste is utilized as feed for aquaculture but the majority is dumped openly on landfills or in the ocean which seriously pollutes the environment. The shrimp waste is heterogeneous in composition and contain 20–40% protein, 30–60% minerals (calcium carbonate), 20–30% chitin, and 0–14% lipids (muscle residues and carotenoids) (Shahidi and Synowiecki, 1991; Kaur and Dhillon, 2015). However, the presence of rich sources of calcium, protein, chitin, and pigments in the shrimp shell waste paved the way for the researchers to utilize shrimp shells for manufacturing expensive medicines and nutritional food, which creates significant importance in the shrimp processing industry (Zhang et al., 2022).

Chitin, an insoluble linear homopolymer of  $\beta$ -(1 $\rightarrow$ 4)-linked-N-acetyl-D-glucosamine, is the second-largest carbohydrate polymer in nature, next to cellulose (Duan et al., 2012; Sharp, 2013). This natural biopolymer is abundant in crustaceans, insects, and

microbes and mostly utilized as a base material for making chitin derived materials like chitosan produced by deacetylation. This biopolymer is well known for its uses in many fields such as pharmaceuticals, cosmetics, food, agriculture, paper and packaging industry, wastewater treatment and textile industry (Akar, San, and Akar, 2016; Choo et al., 2016). Traditionally, chitin has been extracted chemically from shrimp shells, but this process produces toxic wastewater produced that is incompatible with environment protection. In chemical method, two harsh chemical treatments are used for deproteinization (DP) and demineralization (DM). Hydrochloric acid is used for removing minerals and strong alkali is used to remove protein and other organic compounds under high temperature (Hongkulsup, Khutoryanskiy, and Niranjana, 2016). HCl used for DM can cause adverse effect on the intrinsic property of chitin which decreases the final quality (Percot, Viton, and Domard, 2003). Therefore, one of the alternative methods used to replace these harsh chemicals is the use of a biotechnological method which is environmentally friendly and has higher degree of acetylation (DA) compared to the

other methods (Mao, Guo, Sun, and Xue, 2017; Zhang et al., 2022:).

The chitin extraction through microbial fermentation has emerged as a promising method because it is environmentally safe, technically adaptable, and commercially viable. Microbial fermentation can be performed in 4 ways: One-step, Two-step, Successive and Co-fermentation. In one-step, only one bacterial strain is used for the process. The chitin extraction using single strain is simple and inexpensive but it has relatively low DM and DP efficiency. In two-step fermentation, one protease and one acid producing bacterium (Zhang et al., 2012 ; Liu et al., 2020) are employed and highly purified chitin can be obtained in this method compared to one-step fermentation. *Bacillus pumilus*, *B. subtilis*, *Pseudomonas aeruginosa* (Sini, Santhosh, and Mathew, 2007; Ghorbel-Bellaaj et al., 2013; Sedaghat, Yousefzadi, Toiserkani, and Najafipour, 2017) etc., are used for DP process and *Lactobacillus plantarum*, *B. coagulans*, *Streptococcus thermophilus* and *Gluconobacter oxydans* (Mao et al., 2013; Liu et al., 2014, Zhang, et al., 2017; Dun et al., 2019) were commonly used for DM process. One of the disadvantages of two-step fermentation is the protease-producing bacteria must be removed before the DM process, due to the competitive inhibition and interference existing between protease and acid producing microorganisms (Zhang et al., 2021). The requirement of re-sterilization, change of medium and collecting residue

between the DM and DP process extends the processing time and also affects the efficiency.

Successive fermentation is sequential execution of DP and DM and Co-fermentation is simultaneous execution of DP and DM process. In this method, reesterilization process can be omitted and thereby reducing the cost. Zhang et al., (2012) performed successive fermentation of shrimp shell powder using *L. plantarum* ATCC 8014 and *Serratia marcescens* B742 to extract chitin and results of two-step fermentation showed higher efficiency than one-step fermentation. Liu et al., (2021) studied both one-step and successive co-fermentation and found that successive co-fermentation with *B. licheniformis* and *G. oxydans* have better DM and DP process than individual fermentation. However, many factors such as inoculum volume, pH, type and concentration of carbon source, temperature and reaction time affects the efficiency of fermentation. Table 1 provided the summary of different methods used for chitin extraction using microbial fermentation.

A study was conducted by utilizing microbial fermentation approach to obtain chitin from *Penaeus vannamei* shell waste. In the present study, successive co-fermentation technique was employed for chitin extraction from shell using *Bacillus licheniformis* and *Lactobacillus fermentum*. 5g of shrimp shell waste was added to 100ml

of water supplemented with 5% glucose and inoculated with 5% *B. licheniformis* at concentration of 7-8 log cfu/g. after incubation in shaker incubator at 30°C for 72 hours, 5% *L. fermentum* was added with 5% of glucose and further incubated under similar conditions for 144hrs. The DP and DM value reached to 90.21±0.07% and 87.47±0.33% respectively after 144 hrs. of

fermentation. The study also found that *B. licheniformis* and *L. fermentum* can be utilized to develop a fermentation system that will extract chitin from shrimp shells and produce chitin that is of better-quality. Therefore, it was found that the microbial fermentation approach for chitin extraction from shrimp shells is a simple and feasible technology which can serve as a substitute for traditional chitin extraction.

Table 1. Chitin extraction by different microbial fermentation methods

Shrimp shell source	Fermentation methods	Incubation Conditions	DP	DM	Yield of Chitin (%)	References
<i>Penaeus merguensis</i>	Fermentation using <i>Pseudomonas aeruginosa</i>	Incubation 30 °C for 6 days	92%	82%	47%	Sedaghat et al, 2017
<i>Metapenaeopsis dobsoni</i>	Fermentation by <i>B. subtilis</i>	30 °C for 15 days	84%	72%	ND	Sini et al., 2007
<i>P. vannamei</i>	Fermentation using <i>Halobacterium salinarum</i> and <i>Halococcus dom browskii</i>	37 °C for 16 days	95%	ND	ND	Dayakar et al., 2021
<i>Litopenaeus sp.</i>	Successive fermentation using <i>Lactobacillus brevis</i> and <i>Rhizopus oligosporus</i>	30 °C for 8 days	96%	66.45%	ND	Aranday et al., 2017
Shrimp shells	Successive two-Step fermentation using <i>Exiguobacterium profundum</i> and <i>Lactobacillus acidophilus</i>	Room temperature for 5 days	85.9%	95%	16.32%	Xie et al., 2021



<i>P. vannamei</i>	Successive fermentation using <i>B. licheniformis</i> followed by <i>Gluconobacter oxydans</i>	30 °C for 4 days	87%	93.5%	ND	Liu et al., 2014
Shrimp shells	Successive co-fermentation using <i>Bacillus subtilis</i> and <i>Lactobacillus plantarum</i>	37 °C for 6 days	94.1%	96.3%	21.2%	Zhang et al., 2022

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## ***Molecular insights into DNA gyrase subunit A of an environmental El Tor variant of V. cholerae O1***

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**V***ibrio cholerae* is globally disseminated gram negative zoonotic pathogen and is mainly found in the aquatic flora and fauna where it resides mainly in chitinous aquatic invertebrates. It causes fatal cholera disease in human and mainly occur through the ingestion of contaminated water as well as the consumption of partially cooked or uncooked seafoods. Recently, there have been numerous reports of cholera outbreaks worldwide accounting one lakh death annually with Middle East and Southern Asia contributing to more episodes of endemic cholerae diseases. Pathogenic strain possesses various virulence factors such as genes encoding cholera prophage region (*ctx*, *ace*, and *zot*) and type IV pilli (*tcp* gene clusters) which are essential for the bacteria to colonize and infect in human host. In addition to this, pandemic strains found to possess various other defence and secretory systems including pandemic islands.

Recently, several outbreak cases were reported due to multidrug resistant *V. cholerae* globally and among these, the reduced susceptibility to ciprofloxacin is major concern. The emergence of

the fluoroquinolone resistant strains are attributed to the presence of mutation in fluoroquinolone resistance determinant element such as *gyrA*, *gyrB*, *parC* and *parE* genes. The mutations in bacterial enzymes such as DNA gyrase and DNA topoisomerase IV can change or modify the drug targets or lessen the activity of drug either by reducing its accumulation or deleterious lethal action on cells. The major target for quinolone resistance in gram negative bacteria is DNA gyrase whereas in gram positive bacteria it is *parC* gene. The mutation is due to the amino acid substitution event in quinolone resistance determining region and this region is recognized as DNA binding region of the enzyme with the drug. The mutation in 83<sup>rd</sup> and 87<sup>th</sup> position of the amino acid in *gyrA* is considered as the hot spot region where as in *parC* gene, the hot spot region for mutation is positioned at 85. It was reported that the rate of mutation has a strong positive correlation for quinolone resistance in bacteria. In addition to target-enzyme mechanism of resistance, plasmid mediated resistance termed *qnr* is also common in many gram-negative bacteria. However, in *V. cholerae* the most common mechanism of resistance is enzyme

mediated efflux system where the mutated *gyrA* impair the target binding site of the DNA-enzyme complex thereby altering the membrane permeability of the cell.

The research study conducted to monitor the aquatic environment of Cochin, India for the presence of new variants of *V. cholerae* over a period of 05 years (2018-2022) revealed ctx negative O1 positive characteristics in one isolate from coastal water near to a retail seafood market of Thoppumpady fishing harbour, Cochin and the isolate was identified as *V. cholerae* O1 El Tor variant. Antimicrobial susceptibility testing showed that the isolate was resistant to Ampicillin, Chloramphenicol, Cotrimoxazole, Ciprofloxacin and Streptomycin. The isolate possessed several virulence and antimicrobial resistance genes such as *VgrG*, *mshA*, *ompT*, *toxR*, *ompU*, *rtxA*, *als*, *VasX*, *makA*, *hlyA*, *gyrA*, *dfrA1*, *strB*, *parE*, *sul2*, *parC*, *strA*, *VC1786ICE9-floR*, and *catB9* genes. The protein sequence of the gene coding for DNA gyrase A of the isolate was predicted using NCBI BLAST. The protein sequence of the *gyrA* gene of the isolate showed 99.89% identity to the protein sequences of *gyrA* of *V. cholerae* O1 serotype, ATCC 39315/El Tor Inaba N16961 (Uniprot id Q9KSJ81). The sequence alignment of protein showed mutation in *gyrA* sequence of the isolate at 83<sup>rd</sup> position (Serine to Isoleucine)

(Fig. 1). The SWISS MODEL template search followed by alignment (ProMod3) and Model quality search (QMEAN Scoring Function) resulted 99.89% sequence identity with AlphaFold DB model of C3LLV4\_VIBCM (*gyrA* protein of *V. cholerae* serotype O1 strain M66-2). Active binding site prediction using Biovia Discovery studio Visualizer (Version 21.1.0.20298) revealed the potential drug target of the enzyme (Fig 2). The effect of mutation on the flexibility of the protein needs to be investigated further by employing drug-protein interaction via docking and molecular stimulation and will be undertaken in the future research. It was reported that, global emergence and spread of new variants of *V. cholerae* is mainly attributed due to certain changes in their genetic makeup particularly in genes associated with cholera toxin production and antimicrobial resistance. There are several drug-protein interaction methods such as molecular docking analysis, bio inspired microwell array etc, that were developed to study the interaction between the drug resistant mutation on protein function and also on drug resistance level of the strain. These experimental studies will give better understanding on the underlying mechanism of native and mutated forms of protein on drug and also will give valuable information for the exploration and discovery of new drugs.





Fig. 1. Sequence alignment of gyrA protein sequence (EMBOSS 001\_1- environmental EI Tor variant V. cholerae O1; Q9KSJ8:Domain- gyrA protein sequence of V. cholerae O1 serotype O1 strain ATCC 39315/EI Tor Inaba N16961(Uniprot id Q9KSJ81).

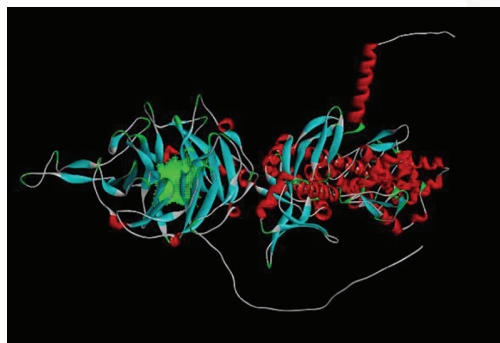


Fig. 2. Predicted 3D structure of gyrA enzyme of environmental EI Tor variant V. cholerae O1. The active binding site shown in green grid box with coordinates (X=-45.0889, Y=-7.579, Z=36.3016)

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## **Comparative analysis of drying properties of sole fish (*Cynoglossus malabaricus*) dried using open sun, solar-LPG and infrared dryer**

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Drying is one of the most commonly used energy-intensive preservation methods in post-harvest processing of food materials. Food materials, such as various fruits, vegetables, herbs, and other agricultural products are dried to reduce their water activity to a microbiologically safe level. Reducing the water activity of food products down to a level that prevents bacterial growth, enzymatic reactions, and other deteriorative processes, extends their shelf life. The high moisture content of fish makes it extremely perishable. It is therefore dried to extend its shelf life and make it available all year round. As per FAO (2020), 17 million metric tons of fish, accounting for 12% of global fish production, were consumed in dried, salted, and smoked forms (Neranjala, Eranga, and Dissanayake, 2022). Dried fish export from India increased from 7,506 tons in 1995 to a commercial scale of 88,997 tons in 2017 (Madan et al., 2018). The nutritional value and quality of the dried fish are also retained after drying (Al Banna et al., 2022). Traditionally, fish is sun-dried in the open space. It is the cheapest method of drying that exposes fish to sunlight and natural wind movement. The effectiveness of the drying is determined by prevalent weather conditions, moreover hygiene of the process is not ensured.

Solar drying method that eliminates the drawback of open-sun drying is drying using a solar-LPG hybrid dryer which harnesses thermal energy and stores it for a few hours by using the flat plate water collectors. This method allows fish products to be dried hygienically, and under controlled conditions, producing high-quality products. In comparison to conventional open-sun drying, the drying period is also shortened (Murali, Amulya, Alfiya, Delfiya, and Samuel, 2020). Advanced drying methods such as infrared (IR) drying, which uses IR radiations to penetrate the product increase the rate at which moisture is removed from the product, shorten the drying time and enhances the quality of the dried product (Prashob, Aniesrani Delfiya, Murali, Alfiya, and Samuel, 2022).

The current study examines how drying method affect the quality and drying characteristics of *Cynoglossus malabaricus*, also called tongue sole fish. Sole fish is widely consumed in the form of dried fish in Kerala. Sole fish was dried by three methods; a batch-type infrared dryer, a solar-LPG hybrid dryer, and open sun drying. Sole fish purchased from local fish landing centre was thoroughly cleaned, and was subjected to drying. The initial moisture content of the fish was measured using the

gravimetric method. Weight loss during drying was measured at an interval of 60 minutes. The chamber temperature of the solar LPG dryer and IR dryer was set at 60°C. The sample was kept under direct sunlight for open sun drying. Temperature and humidity readings ranged between 32.55 to 40.9°C and 59.18 to 29.04% respectively when measured every 60 minutes using

a datalogger (EMCON, Kerala). Vernier calipers were used to measure the initial and final dimensions of the sample to find out the percentage of shrinkage. The method described by Doymaz and Ismail (2011) was used to determine the rehydration ratio of dried sole fish. The different methods employed for drying is depicted in Figure 1.

Table 1. Drying and quality characteristics of sole fish

Drying method	Drying time (h)	Final moisture content (%)	Average drying rate (g/solids.h)	Shrinkage (%)	Rehydration ratio
Open sun drying	19	16.77	0.15	24.85	1.66
Solar-LPG hybrid dryer	11	14.82	0.26	17.77	1.77
Batch type infrared dryer	8	12.78	0.36	16.05	1.95



(a) Open sun drying



(b) Solar-LPG hybrid dryer



(c) Batch type IR dryer

Fig. 1. Different drying techniques employed for sole fish drying

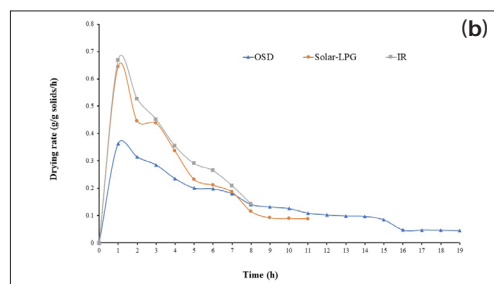
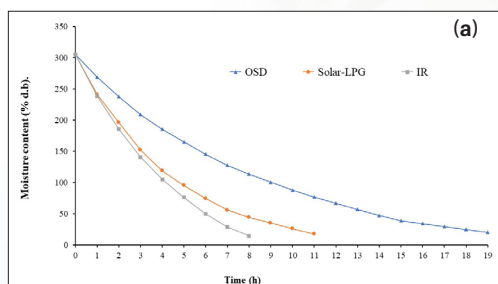


Fig. 2. Change in moisture content (a) and drying rate (b) of sole fish against time in different drying methods



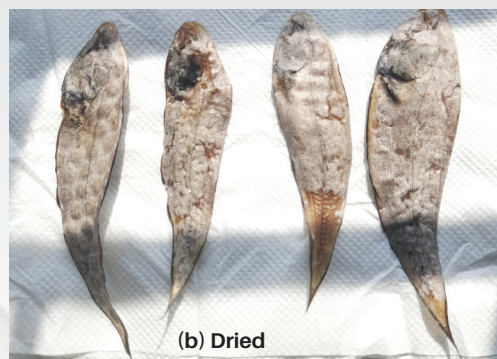


Fig. 3. Sole fish before and after drying in Batch-type IR dryer

The sole fish sample had an initial moisture content of 75.32 (% w. b.) which was reduced to 16.77(% w. b.) in open sun-dried fish, 14.82 (%w. b) in Solar-LPG dried fish, and 12.78 (% w. b.) in batch type infrared dryer (Fig. 2a). The sample dried in Batch type infrared dryer dried in 8 h. The samples dried under an IR dryer showed the highest drying rate (0.36 g/g solids/h) followed by a Solar-LPG hybrid dryer (0.26 g/g solids/h) and open sun drying (0.15 g/g solids/h) (Fig. 2b). The quality characteristics of dried sample is given in Table 1. Batch-type infrared dryer had the least shrinkage (16.05 %) after drying, followed by Solar-LPG hybrid drying (17.77 %) and the maximum shrinkage of 24.85% was observed in open sun drying. The rehydration ratio was found highest for sole fish dried in batch type infrared dryer, followed by solar-LPG dryer and open sun drying.

From the study, it was concluded that, batch type IR dryer was found to be most efficient for drying sole fish. IR drying takes only less time to dry the sample. Moreover, IR drying improved the drying rates when compared to solar-LPG hybrid drying and open sun drying with less shrinkage and better rehydration.

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## Exploring the strategic landscape of online fish markets in Ernakulam, Kerala

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The digital transformation of fish marketing has revolutionized the marketing channels. Online fish marketing platforms have emerged alongside local markets by expanding access to fresh seafood and providing unprecedented convenience (Sajeev, 2021). Leveraging Kerala's vast coastline and rich marine biodiversity, these online platforms have become vital for consumers seeking high-quality seafood (Sajeev et al., 2021). This digital shift not only enhances accessibility but also supports local fisheries, promotes sustainability, and strengthens the

connection between fishers and consumer. To comprehensively explore online fish markets in Ernakulam, Kerala, this study employed a purposive sampling approach, focusing on five prominent platforms. Data collection involved digital scouting, examining critical attributes such as platform choice, advertisement channels, payment options, delivery logistics, fish variety, and product range. The study aims to provide insights into the strategic landscape of online fish vending, shedding light on how these platforms navigate challenges and optimize opportunities. The profile of online fish vending portals is presented in table-1.

Table 1. Profile of online fish vending portals in Ernakulam, Kerala

Portals	Choice of platforms		Advertisement channels		Payment options		Delivery options	Variety of fish		Product Channel
	Platforms used	Numbers	Medium used	Numbers	Options	Numbers	Numbers	Average number	Maximum number	Numbers
I	W, A	2	SM	1	CC/DC/UPI/WLT/NB/COD/CAOD/PL	8	3	28	40	90
II	W, A	2	SM, NP	2	CC/DC/NB/UPI/COD/CRCY	6	3	16	55	48
III	W, A	2	SM, NP, BB	3	CC/DC/NB/UPI/FC/GC	6	2	12	18	15
IV	W, A, SM	3	SM	1	CC/DC/NB/WLT/UPI/COD	6	4	20	45	80
V	W, A	2	SM, NP, BB, TV, RD	5	CC/DC/UPI/NB/WLT/EMI/PL	7	3	36	60	180

W: Website, A: Mobile App, SM: Social Media, NP: Newspaper, BB: Billboard, TV: Television, RD: Radio, CC: Credit Card, DC: Debit Card, UPI: Uniform Payment Interface, INB: Internet Banking, Wlt: Wallet [Paytm, Licious Wallet, Simpl, Ola Money, Airtel Money], PL: Pay Later [Simpl/Lazypay/ICICI/Amazon Pay], CRCY: Cryptocurrency, FC: Food Card, GC: Gift Card, EMI [Zestmoney], COD: Cash On Delivery, CAOD: Card On Delivery

The fish vending portals in Ernakulam exhibited a diverse selection of platform choices, incorporating websites (W), mobile apps (A), and social media (SM) to establish a robust online presence. Some portals adopt a hybrid approach, strategically utilizing a combination of these platforms to implement a comprehensive outreach strategy aimed at a broader audience. This selection of platform choices carries various implications for both the portals and their customers. While portals benefit from an extended reach and caters to different demographics, it poses challenges such as resource allocation and maintaining a consistent brand image.

The advertisement channels used demonstrate variability, while focusing on brand promotion and audience engagement. Utilizing components such as social media (SM), newspapers (NP), billboards (BB), television (TV), and radio (RD) channels to differing extents allows these portals to tailor their marketing mix to appeal to a diverse audience. The maximum number of channels utilized was five. The use of multiple channels allows targeted outreach, enhances visibility, and increases brand awareness. However, challenges include resource allocation and potential information overload for customer.

Fish vending portals provided customers with an extensive array of payment options, reflecting a commitment to catering to

diverse financial preferences. The range includes credit cards (CC), debit cards (DC), net banking (NB), uniform payment interface (UPI), wallet (WLT), pay later (PL), cryptocurrency (CRCY), food cards (FC), gift cards (GC), and instalment options (EMI). This diverse selection is designed to enhance customer convenience and accessibility, with the maximum number of payment options offered being seven. This benefits both portals and customers, although challenges include integration difficulties and potential higher transaction costs for portals. Customers may experience decision complexity due to the wide array of options.

The portals in Ernakulam were found to adopt a customer-centric approach by providing flexible delivery options, offering immediate delivery, scheduled deliveries for the next day, and specific time slots. This diversity aligns with operational efficiency, allowing customers to choose the delivery option that best suits their needs. The maximum number of delivery options offered is four, reflecting a balanced strategy that considers both customer flexibility and operational efficiency. While this enhances customer satisfaction, challenges include logistical coordination and potential decision-making complexity for customers.

Fish vending portals also showcased a commitment to diversity in their offerings, providing a variety of fish that ranges from



an average of 12 to 36, with the maximum varieties reaching up to 60. This broad spectrum caters to the preferences of customers with specific culinary choices, reflecting a dedication to providing diverse fish options. This diverse variety sets portals apart from competitors by providing a wide variety of fish, contributing to market differentiation. This, in turn, attracts a diverse customer base, enhancing overall customer satisfaction. Challenges include inventory management and ensuring freshness.

The portals displayed a significant variation in the number of products offered, ranging from 15 to 180. This diversity in product range allows some portals to present an extensive selection, offering numerous products, while others opt for a more limited selection. For portals, offering a broad range of products enhances their market presence and attracts a wider customer

base, contributing to overall visibility and competitiveness. Additionally, a diverse product offering enables portals to tap into multiple revenue streams, providing financial flexibility. While a broad range enhances market presence, it poses challenges in inventory management.

Fish vending portals strategically navigated the online market, balancing customer satisfaction and operational efficiency across various attributes. Despite challenges, their commitment to differentiation positions them well in the competitive online fish vending landscape. Based on the findings, the strategic landscape of online fish vending portals was mapped as in Figure 1. Ultimately, customer preferences will play a crucial role in determining the portal of choice (Sajeev, Joshy, Jesmi and Abhay, 2023). In conclusion, Kerala's digital fish markets present a diverse landscape with unique

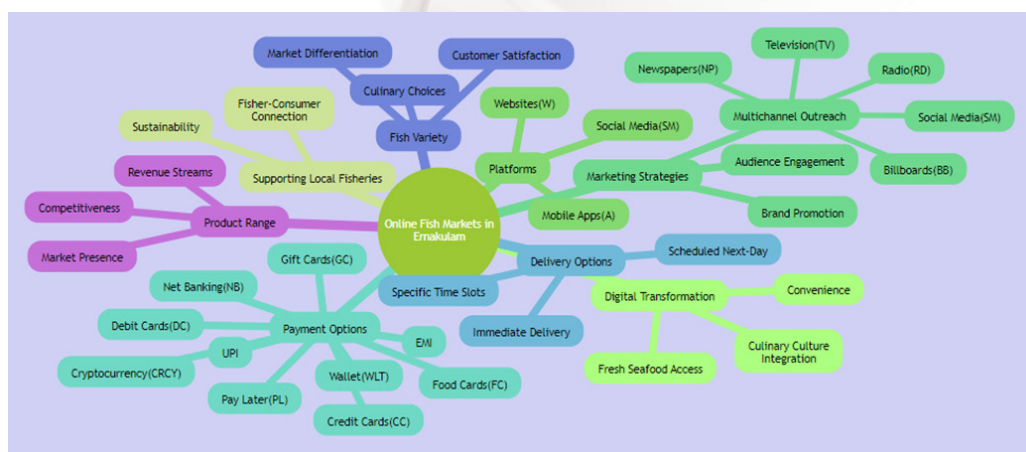


Fig. 1. Strategic landscape of online fish vending portals

strengths and areas for improvement. As consumers increasingly turn to online platforms for seafood, understanding these nuances empowers both businesses and consumers to make informed choices.

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## ***A customized database on import and export trade of fish oil and fish meal in India***

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**M**ining of trade data is an important part of any development programme for formulating and implementing policy related matters for researchers and policy makers. It is noted that trade data in any specified field may be scares which is difficult to collect and also retriev meaningful information for the researchers, academicians and policy makers. The best option to overcome this issue is to bring the scares data into a customized database, so that it can be accessed, used and analysed at any point of time. By keeping this in mind, a customized database was developed on trade statistics of import of fish oil and fish meal to India from other countries and export of fish oil and fish meal from India to the world in terms of quantity and price under specified harmonized system (HS) code using Microsoft Office Access (MS Access). The design of the databases includes creation of different types of tables, queries, forms and reports and these objects are intended to store data, write search queries for retrieving data, add, edit or delete data records from the table and to generate compiled and formatted outputs.

The aim of the relational database is to provide customized trade statistics to the user with respect to the contents integrated into the system with specific HS codes and categories.

The data were arranged in nine tables in the database. The rows defined in the database contains the entries such as the year, country, HS Code, weight (in kgs), price (in US dollars) and Mode is a record. The data fields, year, HS Code, weight in kg and price in US \$ are defined as numbers. In the database, the data field, year is defined as a primary key and it uniquely identifies each record from the export and import statistics of fish fat oil and fish meal.

Queries are defined to retrieve data on year-wise export, year-wise import, commodity-wise export, commodity-wise import, period-wise export and period-wise import and export of fish oil and fish meal under specified HS codes.

Forms were designed to allow users to interact with the database through navigating and interacting with the data stored in the database. Report forms were



used to present data in a customized and predefined format, which allow users to generate reports with specific criteria, such as export or import data of a particular HS code (e.g., 1504) or other conditions. These

the criteria mentioned, such as export and import of different commodities and specific time periods.

To enhance the utility of these reports, buttons have been added to allow users

HS CODES	COMMODITY DETAILS	Export	Export - Year
1504	Fats and Oils and their fractions, of fish or marine mammals, whether or not refined, but not chemically modified	Import	Import - Year
150410	Fish-liver oils, fractions, not chemically modified	Export & Import	Export & Import - Year
150420	Fish oils except liver, not chemically modified	Export - Commodity	Export - Period
150430	Marine mammal fats, oils, etc. not chemically modified	Import - Commodity	Import - Period
230120	Flour or meal, pellet, fish, etc. for animal feed	Export & Import - Commodity	Export & Import - Period

Source: UNComtrade

report forms can be very useful for end-users to extract meaningful information from the database without needing to know the underlying database structure or run complex queries.

Reports in a database application, such as Microsoft Access, are powerful tools for presenting, analysing, and summarizing selected data from the database. They offer a structured and customizable way to convey information to users. Reports will be generated based on various criteria, including data from tables or queries, and they help users gain insights from the database. These reports are designed to recollect customized database queries. This means that user can create reports to display specific sets of data based on

to export the report contents to external document formats, such as PDF, Microsoft Excel, or Microsoft Word. This feature enables users to save and share the reports locally on their devices, print or incorporate the same into presentations. Reports in Microsoft Access is designed with a user-friendly interface, including labels, headers, and formatting to make the information easy to read and better understanding.

A readily accessible database on the trade statistics on import and export of fish meal and fish oil has been designed and developed in MS Access. The database provides information on year-wise and country-wise import and export of fish oil and fish meal to and from India.

## Export of All Items

Year	Country	HS Code	Commodity	Weight in kg	Price in US \$
2000	France	1504	Fish, marine mammal fat or oil not chemically modified	485	596
2000	Nigeria	1504	Fish, marine mammal fat or oil not chemically modified	106690	940478
2000	South Africa	1504	Fish, marine mammal fat or oil not chemically modified	1000	1758
2000	United Arab Emirates	1504	Fish, marine mammal fat or oil not chemically modified	4400	6682
2000	United Kingdom	1504	Fish, marine mammal fat or oil not chemically modified	1000	3581
2000	Viet Nam	1504	Fish, marine mammal fat or oil not chemically modified	3250	26408
2000	World	1504	Fish, marine mammal fat or oil not chemically modified	132290	1051284
2001	France	1504	Fish, marine mammal fat or oil not chemically modified	8140	15457
2001	Japan	1504	Fish, marine mammal fat or oil not chemically modified	3520	5287
2001	Malaysia	1504	Fish, marine mammal fat or oil not chemically modified	95	667
2001	Nigeria	1504	Fish, marine mammal fat or oil not chemically modified	73725	463224
2001	United Arab Emirates	1504	Fish, marine mammal fat or oil not chemically modified	1550	3166

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