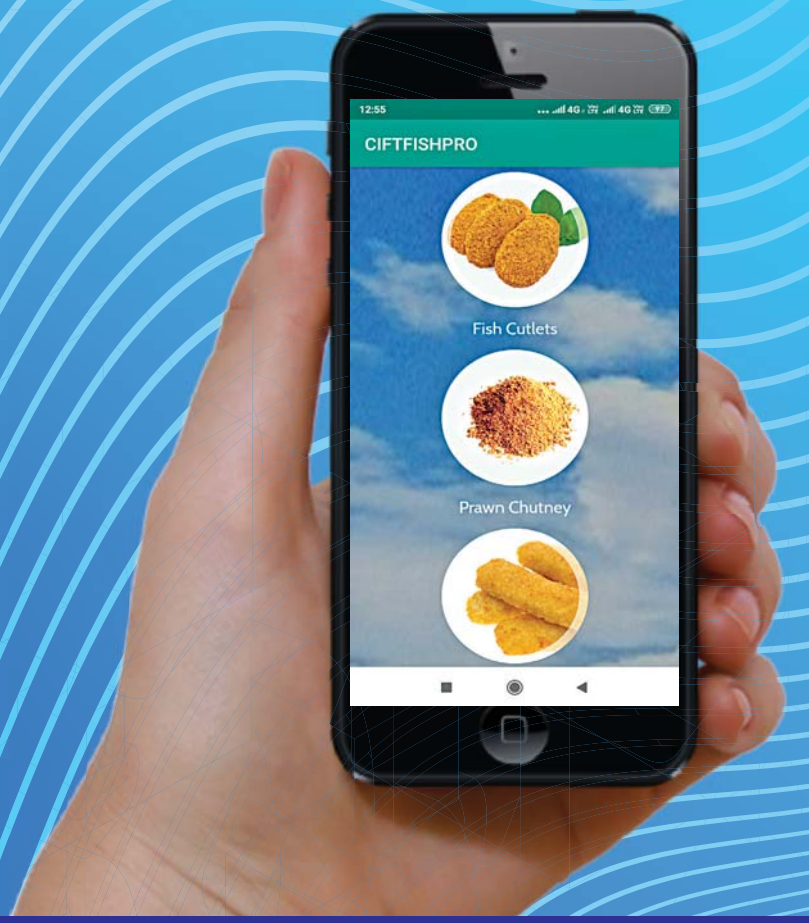


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# FISHTECH reporter

ICAR-CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY
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# From the Editorial Board.....

The July –December 2018 issue of fish tech reporter features 13 articles for introducing the recent research developments carried out at ICAR-Central Institute of Fisheries Technology in different fishery science streams. Use of proteomics approach in food industry is still in its infant stage. In this issue, we report ICAR-CIFT's initiative in proteomics studies in albino rats to analyse the Influence of Squalene on lipid Metabolism. Rare occurrence of yellow boxfish *Ostracion cubicus* from coastal waters off Cochin is reported in this issue. ICAR-CIFT is always dynamic in addressing the food safety concerns. The current issue reports the improved efficacy of chiotsan coated activated carbon for removing excess iron from drinking water. Presence of *tdh* gene, the virulent pathogenic factor in *Vibrio parahaemolyticus* was confirmed in coastal water of North Western Mumbai using southern blotting and hybridization. The molecular characterization of diarrheagenic *E.coli from* seafood of Gujarat coast is also appearing in this issue.

This issue features the latest interventions in seafood processing such as infrared dryer for rapid dehydration, fucoidan supplemented biscuits, preparation of gravads from Nile tilapia, quality of Jawala mince under refrigerated storage etc. The article on preparation and application of water-soluble chitosan will be of great interest to fishery byproducts industry. The features of the recently introduced mobile APP by the Institute CIFTFISHPRO – for android systems on CIFT Value Added Fish Products is also discussed in this issue.

We hope the current issue will update researchers as well as industry stake holders about the latest research developments in the sector.

# Rare occurrence of yellow boxfish *Ostracion cubicus* in trawl operated off Cochin, Kerala

**Chinnadurai S., Renjith R.K., Paras Nath Jha and Rithin Joseph**

ICAR-Central Institute of Fisheries Technology, Kochi, Kerala - 682 029

Boxfishes belong to the family Ostraciidae. As the name reveals, they are box-shaped. These species are characterized by appendicular skeleton, and by the presence of a carapace formed by enlarged and thickened scale plates with transverse square sections. This is a demersal species found in coral reef associated areas and rarely seen in coastal waters. It feeds mainly on algae, but will also feed on sponges, crustaceans, molluscs and small fish. When the fish become stressed or injured it releases poisonous proteins from its skin that may prove lethal to any fish in the surrounding waters (Indumathi et al., 2013). The bright yellow colour and black spots are a form of warning coloration (Aposematism) to any potential predators.

A specimen of yellow boxfish *Ostracion cubicus* (Linnaeus, 1758) was caught in the trawl net during experimental trawl operations on-board R. V. Matsyakumari-II, in Arabian sea along coastal waters off Cochin ($10^{o}$05.912'N and $076^{o}$08.481'E) using off-bottom trawling system (OBTS) in depths range of 12 to 15 m on 18$^{th}$ November 2016. The specimen with 28 mm TL, was bright yellow in colour. The species can grow up to 45 cm. The brightness fades with age and very mature fish will have blue-grey coloration with faded yellow (Yennavar and Tudu, 2010). The species is very rarely encountered in trawl net and hence morphometric counts were taken (Table 1).

Table. 1 Morphometric characters of Box fish

| S.No. | Traits | unit |
|---|---|---|
| 1 | Total length | 28.21mm |
| 2 | Standard length | 21.70mm |
| 3 | Tail length | 7.10mm |
| 4 | Carapace length | 19.29mm |
| 5 | Carapace width | 11.01mm |
| 6 | Eye diameter | 4.62mm |
| 7 | Caudal fin rays | 6 nos. |

## Taxonomy

Kingdom : Animalia
Phylum : Chordata
Class : Actinopterygii
Order : Tetraodontiformes
Family : Ostraciidae

**Synonym (s)**

*Ostracion argus* Ruppell, (1828)
*Ostracion tuberculatus* Linnaeus, (1758)
*Ostracion cubicum* Linnaeus, (1758)

**Distribution**: Indo-Pacific: Red Sea and East Africa to the Hawaiin and Tuamoto islands, north to Ryukyu Islands, south to Lord Howe Island, Maldives, Andaman Islands, Sri Lanka and Australian coast (Smith, 1986).

According to Santini et al. (2013) the family has 14 genera and 37 species found in the Atlantic, Indian and Pacific Oceans. Out of these 37 species under Ostraciidae family, five species are reported in Indian waters (Yennavar and Tudu, 2010). This particular species is widely distributed in Atlantic, Indian and Pacific Oceans. But, in India it has a very restricted distribution. The occurrence of yellow boxfish *O. cubicus* in the Indian sea was first reported by Francis Day (1967). Furthermore, Rao et al. (2000), Devi & Rao (2003) reported *O. cubicus* from Andaman Islands and Murthy (2002), reported in Lakshadweep islands. This species was also

reported from Digha coast, Orissa by Yennavar and Tudu, (2010) and Gulf of Mannar by Varghese et al. (2011). Extensive literature review on the occurrence records of this species along Indian coasts shows that this is the first report of this species from South-west coast of India.

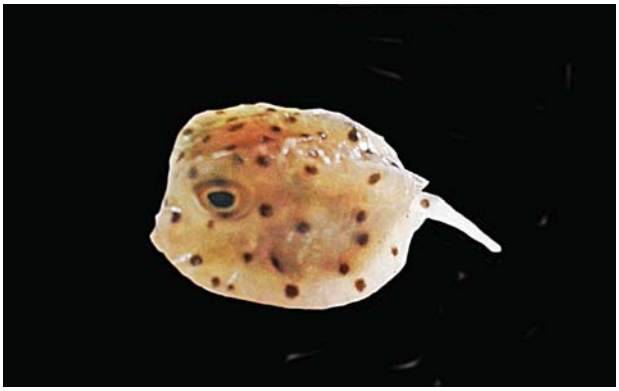

*Fig.1 Boxfish caught in the trawl net*

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# Microbial quality of *shidal* - an ethnic traditional fermented fish product of North-East India


**Ahamed Basha K.[1], Madhusudana Rao B.[1], Ranjith K.N.[2] and Toms C. Joseph[3]**

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[3]ICAR-Central Institute of Fisheries Technology, Veraval Research Centre, Gujarat


Fermented fish products as a dietary source are extensively popular across the globe as they are rich in nutritive value, and their sole preparation process materialized these products as distinctive

ethnic foods. Besides longer preservation ability, it enhances digestibility, flavor, and aroma to the consumers. The North-East India is bestowed with fermented foods such as *utong-ngari* in Nagaland and Assam, *Bordia*, *Sepaa /Shidal/ Hidol*, *Namsing*, *elisa-ngari*, *Iona-iliish* in Assam and Tripura, *ngari* and *hentak* in Manipur, *Gnuchi*, *Sidra*, *Sukuti* in Darjeeling and Sikkim, *ngawum* in Mizoram, and *tungtap* in Meghalaya (Singh et al., 2014). *Shidal*, a salt-free, solid ready to eat fermented fish product, is prepared from freshwater fish mainly *Puntius* spp. (Muzaddadi and Basu, 2003a). *Shidal*, forms a large portion of daily food intake in North-eastern states of India as it holds unique organoleptic properties. However, during the production of *shidal*, safety and hygiene aspects of the product is affected through repeated handling during the process of fermentation. The present study examined the microbial diversity of *Shidal* collected from retail market of Shillong, Meghalaya India. Aseptically, ten gram of *Shidal* was chopped into small pieces and was aseptically blended in 90 ml of sterile phosphate buffer saline (pH 7.0) and suspensions were processed following serial dilution method. Total aerobic count, total anaerobic count, Lactobacillus, Enterobacteriacae, *Staphylococcus aureus*, *Escherichia coli*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Clostridium botulinum*, *Shigella* spp., Methicillin-resistant *Staphylococcus aureus* (MRSA) were examined following USFDA-BAM (online access). The isolates from total plate count and anaerobic plate count was identified as per Surendran, et al., 2013. The details of microbial parameters are mentioned below table *Escherichia coli* were not detected from the sample. *Shidal* was reported negative for the presence of food borne pathogens such as *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Clostridium botulinum*, *shigella* spp., Methicillin-resistant *Staphylococcus aureus* (MRSA). Based on the biochemical identification tests, dominant aerobic plate count were *Micrococcus spp.*, *Staphylococcus spp.* whereas *Clostridium spp* and *Bacillus thuringiensis* emerged as a dominant anaerobic bacteria in shidol. *Bacillus* isolates were identified as *Bacillus licheniformis, Bacillus brevis, Bacillus pumilus*. This study also demonstrated the high counts of lactic acid bacteria in shidal indicating its probiotic potential. The shidal production appears to have been processed under hygienic conditions indicating improvements in the production process as previous studies reported high levels of faecal indicator bacteria (Kakati

*Fig.1 : Shidal, ready to eat fermented fish product of India*

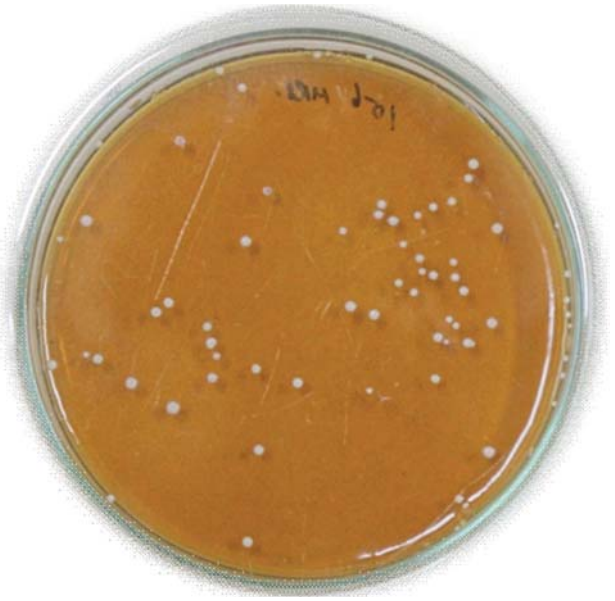

*Fig. 2 : Lactic acid bacteria in Shidal*

and Goswami, 2013). The absence of pathogenic bacteria makes the shidal safe for human consumption. Further studies are needed to ascertain the protective and functional attributes of the shidal associated microorganisms.

Table 1. Microbial quality of shidal

| Microbial parameters | Count cfu/g |
|---|---|
| Total aerobic count | $6.98 \times 10^7$ |
| Total anaerobic count | $1.17 \times 10^8$ |
| Lactobacillus spp. | $1.4 \times 10^8$ |
| Bacillus spp. | $4.76 \times 10^4$ |
| Yeast & mould | $8.7 \times 10^4$ |

**Keywords:** Shidal, Fermented Fish, Microflora

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# Molecular characterization of diarrheagenic *E.coli* from seafood of Gujarat

**G.K. Sivaraman[a], Bibek Ranjan Shome[b] and Mark Adrian Holmes[c]**

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[b]ICAR - NIVEDI, Ramagondanahalli, Yelahanka, Bangalore

[c]University of Cambridge, Veterinary Medicine, Madingley Road, Cambridge CB3 0ES, UK.

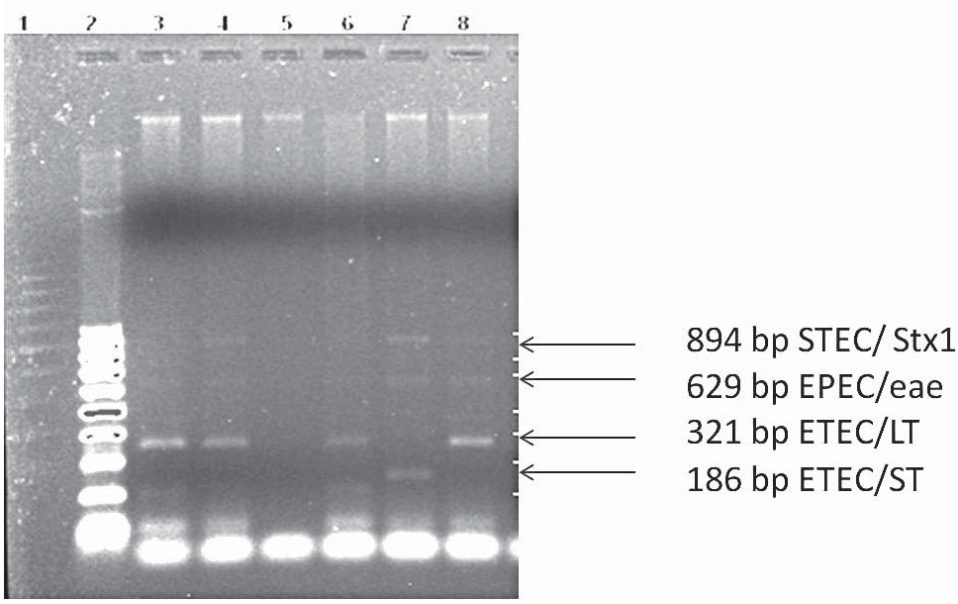
*Escherichia coli* are member of normal microbial flora of human gut. *E.coli* can be pathogenic and cause various infections (Nataro and Kaper 1998). Typically in developing countries *E.coli* is considered as the main etiologic agent of diarrhoea posing a major threat to public health (WHO, 2017). A group of Diarrheagenic *E coli* (DEC) plays an important role in causing enteric and diarrheal diseases. Based on distinct clinical and epidemiological features, associated with certain serotypes and virulence determinants, DEC can be further sub-categorized into six major pathotypes *viz*., Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Shiga toxin producing *E. coli* (STEC), Enteroaggregative *E. coli* (EAggEC), Diffusively aggregated *E. coli* (DAEC) (Feng et al., 2011).

Due to increase in human population the amount of sewage released in water bodies have increased exponentially (Vermeulen *et al*., 2015). The released sewage is mostly untreated along with mixture of various domestic as well as clinical waste lead to contamination of water bodies and coastal areas. This also contaminates the food production chain and may lead to disease outbreaks. Contamination of seafood with patho-

genic *E. coli* strains is mainly due to sewage contamination of water and also due to handling of seafood in processing plants by infected workers (Sivaraman et al., 2017). Detection of pathogenic *E. coli* by conventional methods is rudimentary, time consuming and often more expensive. Therefore molecular methods such as DNA hybridization and PCR have been developed and utilized in recent years for identification of pathogenic *E. coli* (Tobias and Vutukuru 2012). Multiplex Polymerase Chain Reaction (PCR) is a very efficient method that simultaneously detects several genes related to pathogenicity of DEC *E. coli.* The present study was aimed to monitor the incidence of diarrheagenic *E. coli* strains from seafood samples by multiplex PCR method (CDC, 2009).

A total of 120 fresh and processed fish samples were collected in and around Veraval region (Gujarat) and brought to laboratory immediately with suitable sterile polythene bags for enumeration of *E. coli* by ISO, 9308-1 method and 20E BioMerieux identification kit was used for further confirmation (ISO, 1990) as per the manufacturer directions. Deoxyribonucleic acid (DNA) was extracted from the confirmed *E.coli* isolates with GenElute™ Bacterial Genomic DNA Kit and a set of 8 PCR primer pairs were employed for the detection of virulence genes such as *eaeA* for EHEC and EPEC, *bfpA* for EPEC, vt1 and/or vt2 for shiga toxins 1 and 2 of EHEC, ST and/or LT enterotoxins for ETEC, ia for EIEC and pCVD432 of EAEC.

Out of 120 seafood samples screened 54 of isolates from 12 samples were confirmed as *E. coli* and these strains were subjected to pathotyping. A multiplex PCR was standardized for pathotyping of diarrhoeagenic *E. coli* isolates to screen for ETEC, EPEC, EHEC and EAEC. 14 *E. coli* strains (25.92%) were identified as Entero Toxigenic *E. coli* due to the presence of LT and ST genes with PCR band at 321 bp and 186 bp, respectively. 3 *E. coli* strains (5.54%) were confirmed to be Entero Pathogenic *E. coli* (EPEC) the presence *eae* gene (629 bp). Two isolates were positive for Shiga toxin producing *E. coli* (STEC). It was also noted that none of the *E. coli* strains showed presence of pCVD432 plasmid gene from seafood products. Hence no EAEC were present in seafood. The present comprehensive evaluation of pathogenic *E. coli* in seafood from Veraval region revealed that ETEC type was highly prevalent among seafood with highest percentage of 25.92%.




*Multiplex PCR for pathotyping of E.coli isolates from seafood*

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# Sequence variant *tdh* gene in environmental strains of *Vibrio parahaemolyticus*

**Minimol V. Ayyappan, Pankaj Kishore, Mandakini H. Devi and Satyen K. Panda**

ICAR-Central Institute of Fisheries Technology, Cochin

*V. parahemolyticus* is a natural inhabitant of coastal-marine environment. Food poisoning due to *V. parahaemolyticus* is associated with the consumption of raw or partially cooked seafood, especially the shellfish such as clams and oysters. Among the different virulence factors described in *V. parahaemolyticus*, chromosomal *tdh* and *trh* genes are well studied pathogenic determinants and their presence poses serious health risk to humans. The presence of these virulence factors has been found to correlate with the hemolytic toxins as evidenced in various mouse bioassays. Studies have shown that the detection rate of *trh* gene varies between 0% to 59.3% and *tdh* gene from 0% to 8.4% in different seafood and marine samples. Moreover, the detection rates of these genes were higher in clinical samples compared to the environmental and seafood samples. A Study was carried out to ascertain the pathogenic characteristics of *V. parahaemolyticus* from 140 samples comprising fish (40), shellfish (32), coastal sediment (23) and coastal water (45) from different fish markets and landing centers located in and around North Western Mumbai, Maharashtra, using *tdh* and *trh* targeted polymerase chain reactions (Honda et al., 1991; Okura et al., 2003). The *tdh*-specific PCR yielded non-specific amplifications visible as strong bands in the agarose gel and did not produce the expected amplicon size of 263 bp

(Fig. 1). To confirm the presence of *tdh* gene sequences in these amplicons, Southern blotting and hybridization was done using a biotin-labeled polynucleotide probe (Brown, 2001). The probe was prepared by PCR targeting *tdh* gene (263 bp)

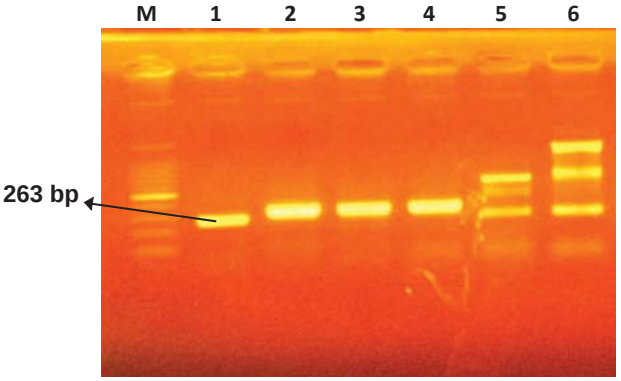


*Fig. 1: Detection of tdh gene of V. parahaemolyticus*
(Lane M: GeneRuler 100 bp (Fermentas, USA); Lane 1: Positive control; Lane 2-6: Amplification pattern obtained for *V. Parahaemolyticus* isolates)

from the reference strain of *V. parahaemolyticus* O3:K6. DNA bands were transferred onto nylon membrane by Southern blotting. Positive hybridization signals were obtained with three products confirming that these products were indeed from the *tdh* gene (Fig. 2).

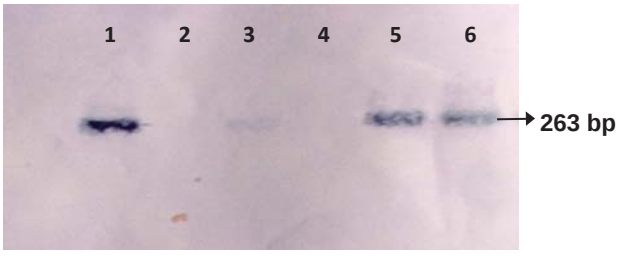


*Fig. 2: Southern blotting and hybridization of* **tdh***-gene products*
(Lane 1, 3, 5, 6: Positive for *tdh* gene; Lane 2, 4: Negative for *tdh* gene)

The *tdh* gene-specific primers are usually very specific and do not cross react with genes other than the *tdh* gene. Among the five isolates tested, three were found positive for *tdh* gene by southern blotting followed by hybridization using biotin-labeled polynucleotide probe. Lee and Pan (1993) have used this technique for the confirmation of *tdh* gene in *V. parahaemolyticus* strains. Sequence variant *tdh* gene of *V. parahaemolyticus* has been previously reported. Nishibuchi and Kaper, (1995) reported five *tdh* alleles in *V. parahaemolyticus,* namely, *tdh1* to *tdh5* with >96.7% sequence identity. Bhowmik et al. (2014) conducted phylogenetic analysis of 52 *tdh* and *trh* genes submitted to the GeneBank and suggested that there was a high level of sequence diversity in *tdh* and *trh* among *V. parahaemolyticus* strains.

The result suggested that seafood may harbor pathogenic *V. parahaemolyticus* possessing variant *tdh* genes which may go undetected by routine PCR using *tdh* gene-specific primers. Several recombination events such as insertion, deletion and duplication can result in alteration of sequences of a particular gene. Such events can inactivate a gene making the bacterium less pathogenic or result in the over expression of gene leading to enhanced pathogenicity. Altered nucleotide sequences alter the amino acid sequences and eventually the structure-function relationships of the protein in question, and these can have several implications on the activities of the protein and the physiology and virulence of the bacterium. It is necessary to further characterize the *tdh* gene in these isolates to understand the nature of sequence variation and their pathogenic significance.

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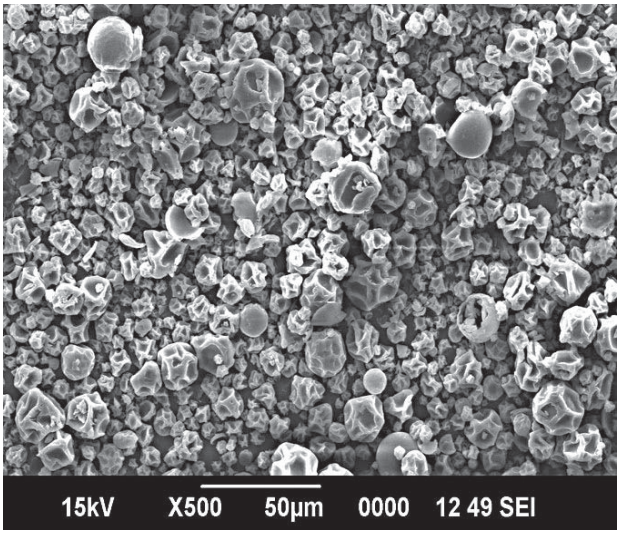
# Carboxymethyl chitosan (CMCH): A water-soluble derivative of chitosan

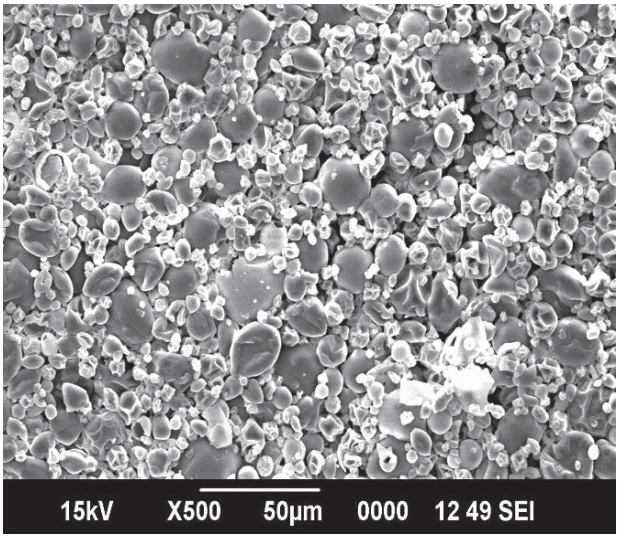
**Binsi P.K. and Zynudheen A.A.**

ICAR-Central Institute of Fisheries Technology, Cochin

The structural uniqueness of chitosan facilitates the modification of its functional moieties which permits flexible manipulation of its biological and engineering properties. However, the poor solubility of chitosan at neutral pH poses practical difficulties in applications. To overcome this technological demerit, various chemical modifications have been suggested. Among these, carboxymethylation has often been applied to improve water solubility to chitosan. Fish Processing Division of ICAR-CIFT has standardised a cost-effective protocol for the production of Carboxymethyl chitosan at pilot scale. CMCH holds several bioactive and physicochemical properties, which are evensuperiorto those of native chitosan. Hence, itis considered as a promising candidate for biomedical applications such as drug carriers, antimicrobial material, gene delivery systems and tissue regeneration devices. As per our laboratory developed method, the properties of CMCH can be tailor-made to fit the requirements of these wider range of applications. In order to derive standardized combinations of process parameters to yield CM-chtosan with a defined set of properties for specific applications, a series of 18 trials were conducted. The properties of CM-chitosan incubated at various reaction temperatures ranging from 10-60ºC for different durations of 1-3 hrs were evaluated. The results indicated a distinct reduction in the solubility of CMCH below 40ºC, whereas only a marginal difference in solubility was observed between the samples incubated at




*nCH*




*CMCH*

higher temperatures for longer durations. The prepared CMCH was found to have good adjuvant properties when incorporated in foliar spray. The unique properties of prepared CM-chitosan such as high viscosity, large hydrodynamic volume, gelling ability, besides its excellent solubility at neutral pH, make it an attractive option for its use as functional ingredient in processing and preservation of wet and dry commodities. The excellent hydrophilic characteristics of CMCH along with itsresemblances with the components of extracellular matrix, antibacterial and antioxidant properties, surface- active and gel-forming capabilities, all can be well exploited and emphasized for cosmetic applications.

***Comparison of microencapsulation efficiency** of CMCH against native chitosan*

The suitability of CMCH to use as wall polymer for encapsulating squid extractives was evaluated. The encapsulates were characterised for structural and storage stability and the results were further compared against the properties of encapsulatesprepared with native chitosan (nCH). In order to maintain the hydrophilic-lipophilic balance, omega 3 fish oil was added before emulsification. The microscopic images indicated certain extent of coagulation in nCH emulsion, whereas CMCH emulsion remained stable for more than 24 hrs without any evidences of phase separation. The SEM images indicated the formation of spherical encapsulates in CMCH powder, whereas a large number of shrunken capsules were visible in spray dried nCH encapsulate powder.

The film forming properties of water-soluble chitosan was compared against that of native chitosan. The tensile analysis of the films indicated a 20 fold higher tensile strength value for nCH chitosan film compared to that of CMCH chitosan. However, the elongation at break value registered for water-soluble chitosan film was 10 fold higher than that of nCH film. The vapour barrier properties of CMCH film was found to be inferior as indicated by the higher water vapour transmission value.

# Jawala Shrimp Mince: Changes in quality under refrigerated storage

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Demand for Ready-to-cook and ready-to-eat fish meat based products is rising. These products having advantages like convenience and remarkable increase in the shelf life (Brennan et al, 2013). The market for chilled fish food items witnessed a steady growth over the period. Thriving sustainability in fish processing is possible when the underutilized fishery resources find its maximum utility.

In 2017 annual marine fish landings of Gujarat was 7.86 lakh tonnes. The major prominent group was non penaeid prawn contributed 1.48 lakh tonnes followed by penaeid prawn 0.35 lakh tonnes of total landing (CMFRI, 2017). Jawala shrimp, *Acetes indicus* is one of the non penaeid, contributed 90% of total non penaeid prawn landing as a seasonal catch, serves as a major raw material for the fish meal industry of Veraval coast, Gujarat. Jawala can be better utilized by converting them in to ready to cook mince form as an intermediate product in the process line of value added products. With this background, present study was undertaken to evaluate the shelf life of Jawala shrimp mince under refrigerated storage.

In brief, to prepare mince, Jawala shrimp (Figure 1A) was stirred in chilled distilled water (4ºC) gently for 10 min then flash cooked using water immersion for 10s and filtered with a layer of nylon screen. In the previous work, the cooking method and time was standardized based on the organoleptic acceptance (Data not shown). The flash cooked meat was mixed with cryoprotectants like sorbitol, STPP followed by packed in trays

*Fig. 1A Raw Jawala shrimp*

and kept under refrigerated temperature 5±1ºC (Fig. 1B). Moisture, crude fat, crude protein and ash content were estimated according to the

*Fig. 1B Minced Meat*

method of AOAC (2000).

Proximate composition was analysed before and after flash cooking the sample. The protein content found to increase from 8.92 to 15.39%. The moisture content decreased by 10% after flash cooking (Fig. 2). The refrigerated storage study was conducted for raw and final product

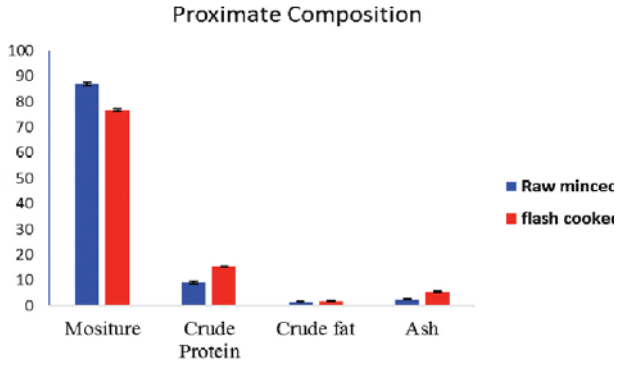


*Fig. 2: Proximate Composition of raw and cooked meat*

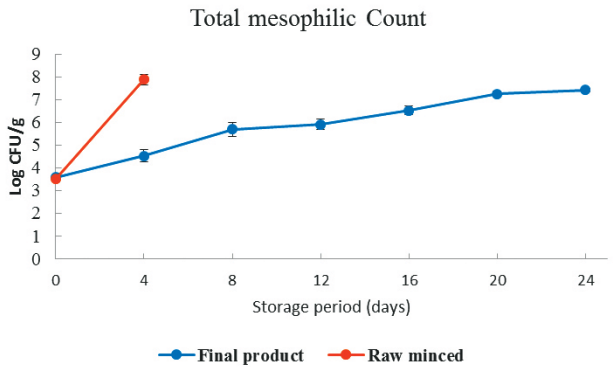


*Fig. 3: Total Mesophilic count*

by analysing the biochemical and microbiological parameters. Sampling was carried out at 4 days interval. At the end of 20 days of refrigerated storage, the total bacterial count increased by 3 log compared to the initial sample (Fig. 3). Pathogenic strain was not detected during the storage period. The Jawala mince prepared in the study had the shelf life of 16 days under refrigerated storage against 4 days of raw minced meat.

## Conclusion

The quality changes of jawala is extremely important due to its smaller size and highly perishable nature. The present study revealed that the jawala mince prepared with cryoprotectants extend the quality and shelf life of the product.

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# Gravad (Sugar-salted) Tilapia: A value added ready to cook product with longer shelf life

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ICAR - Central Institute of Fisheries Technology, Cochin.

Gravad technology is a mild processing technique, in which the fish is filleted, rubbed with salt-sugar mix and marinated over a period of 1-4 days at refrigerated temperature. After the ripening process, the moisture content is reduced by 10%. Presence of salt reduces the microbial activity and thereby increases the shelf life and sugar imparts a desirable taste by counteracting the unkind salty flavour. The salt content varies from 3-6%, and is generally considered as a ready to eat product in Scandinavian countries. However, since raw fish products are not eaten

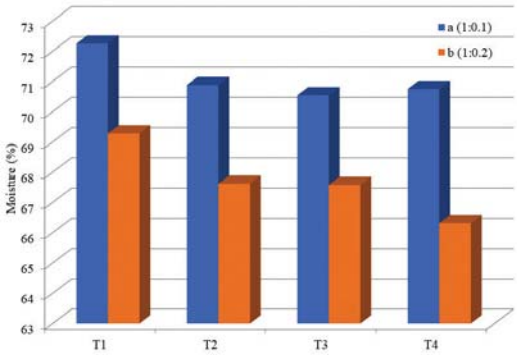
by Indian population, development of gravad fish can be considered an approach to make a value added ready to eat low moisture fish product with extended shelf life. Farming of Nile tilapia is widely expanding in India because of its fast growth rate and good meat quality. Hence, Nile tilapia was widely accepted and became a good table fish in India in the past few years. White colored meat, low fat and absence of pin bones makes this fish an ideal candidate for value added products.

A process has been standardized for preparing gravad loins and steaks from Nile tilapia. For gravad loins, gravading mixture was formulated with salt, sugar and spices. Ratio of loins to gravading mixture was varied from 10:1, 10:2, 10:3 and 10:4. The loins were marinated for 3 days at $2^0$C. After marination, drip loss varied from 1.28% (v/w) in 10:1 to 17% (v/w) in 10:4. Moisture content was 74.26% , 67.19% 63.70% and 58.78%, in gravad loins prepared with 10:1, 10:2, 10:3 and 10:4 ratio, respectively. Moisture of control was 82%. Yield of gravid loins from raw material varied from 25 to 31%. Gravad prepared with a ratio 10:1 (loin to Salt-sugar mix) was highly acceptable by the panelists.

*Gravad steaks of Nile Tilapia*

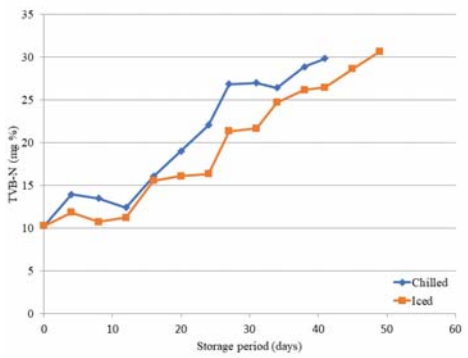
*Gravad loins of Nile Tilapia*

For gravad steaks, fillets were cut into steaks of 2.5 cm width. Four combinations of salt: sugar was selected for preparing the gravad mixture ($T_1$ 2:1, $T_2$ 1:1, $T_3$ 1:1.5 and $T_4$ 1:2). Steaks were mixed with gravad mixture in two ratios; for each treatment, gravads were prepared with a ratio 10:1 (loin to Salt-sugar mix) and packed in polyethylene pouches. Maturation was done for 3 days at $2^0$C. After the maturation period,




*Moisture content of Gravad steaks prepared with different salt-sugar combination (T1-T4) and different Steaks to Gravad mixture ratio (a and b)*

gravads were analysed for its moisture content, salt content, weight loss and organoleptic score. Increasing sugar level in the gravad mixture didn't have a significant role in reducing the moisture content; whereas, moisture content of gravdas prepared with a ratio of 10:1 steak to gravad mixture (w/w) was significantly lower than those prepared with 10:1 ratio. However,




*Total volatile base nitrogen content of gravads stored at chilled and iced condition*

for both the samples, salt content significantly decreased with increasing sugar levels in the gravad mixture. Gravading with more sugar was found to be more acceptable than that with less sugar concentration. In addition, 10:1 ratio of steak to salt-sugar mix was better acceptable than 10:2.

Changes in biochemical and sensory quality of most acceptable gravad (a combination 1:2 salt-sugar in the gravad mixture and a ratio of 10:1 steaks to gravad mixture) stored at different temperature ($0^0$C and $6^0$C) was monitored over a period of 50 days. All the biochemical parameters including TVB-N, PV, FFA, TBARS, WSN and SSN were higher with chilled sample compared to those stored in ice. The shelf life of gravad is determined as 40 days at $0^0$C (iced) and 45 days at $6^0$C (chilled). Reduced moisture content (65%) due to gravading process and presence of salt and sugar as preservatives might have lead to longer shelf life of gravads under refrigerated storage.

# Influence of Squalene on Metabolism in Rats: A Proteomics-Based Study

**Asha K.K. and Suseela Mathew**

ICAR-Central Institute of Fisheries Technology, Cochin - 682 029

Proteomics, is the all-inclusive study of all the proteins in a cell, tissue or biological fluid at a certain time and their interactions. Rapid advances in proteomics have been made possible by meteoric rise in the use of techniques of mass spectrometry, which facilitated the identification of large biomolecules. The past three decades saw proteomics develop from the process of presentation of protein moieties to an interface, which provides evidences to function, through means such as description of protein modifications and interactions and by means of quantitative proteomics, i.e. the total and comprehensive assessment of protein quantities between two distinct biological conditions. Proteomics has quickly become a key and integral part of the all too familiar -omic branches of science that include genomics which is analysis of genes, transcriptomics which is analysis of gene expression and metabolomics which is metabolite profiling. Proteins in a proteome range from being extremely simple to being most complex and have a wide dynamic range of concentration up to the tune of several orders of magnitude making proteomics the most challenging of -omic disciplines which requires the most advanced and complex analysis regime. Whereas, the pharmaceutical industry has heavily relied on proteomics for biomarker discovery and drug target detection, the use of proteomic approaches to address nutrition and health concerns and its application in research in food industry is just beginning to emerge.

**Liver proteome analysis in rats fed with squalene**

Substantial research evidence establishes a link between diet and the status of health or disease in human beings. Nutrients induce metabolic and cellular responses and in the process elaborate/ modulate metabolite levels that can assume the role of biomarkers for predicting the outcome of food-based studies. Based on a four week intervention study in albino rats, the effects of intake of squalene versus high fat diet on liver protein profiling was investigated. The proteomic protein profiling and relative quantification analysis was performed by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) as detailed below.

### In-solution trypsin digestion

Approximately 100 µg of proteins from each sample, normalised to a concentration of 1µg/µL, was subjected to in-solution trypsin digestion to generate peptides. The digested peptide solutions were centrifuged and the supernatant was stored at -20$^0$C until LC-MS/MS analysis. The tryptic peptides were separated by reversed-phase chromatography. Water was used as solvent A and acetonitrile was used as solvent B. Each sample was injected in triplicate with blank injections between each sample. MS analysis of eluting peptides was carried. All analyses were performed using positive mode ESI using a NanoLockSpray™ source. Calibration set the analyzer to detect ions in the range of 50 - 2000 *m/z*. The acquired ion mobility enhanced MSE spectra was analysed using Progenesis QI for Proteomics V3.0 for protein identification and quantification. The protein identifications were obtained by searching against the **human protein database downloaded from UniProt**.

### Results

The results showed that squalene intake in high fat diet (HFD)fed group induced post-prandial changes in proteins (Table) linked to the biological processes like fatty acid transport, fatty acid metabolism, mitochondrial ß-oxidation of fatty acids, lipid transport and lipid metabolism, cholesterol biosynthesis, reverse transport of cholesterol from tissues to the liver, receptor-mediated endocytosis, lipoprotein metabolism,

| Retention time | Neutral mass | Sequence | Accession | Description | Max fold change | Highest mean condition | Lowest mean condition | Anova |
|---|---|---|---|---|---|---|---|---|
| 43.71 | 2552.304 | LKPECLGDISVGNV LEPGAGAVMAR | P21775 | 3-ketoacyl-CoA thiolase A | 2.13 | SQ2 (HFD) | SQ3 (HFD+Sq) | 0.010970 |
| 22.40 | 848.4457 | GATPYGGVK | P17764 | Acetyl-CoA acetyl transferase | 1.09 | SQ2 (HFD) | SQ3 HFD+Sq | 0.022834 |
| 65.09 | 3797.888 | ALNALCNGLIEELNQALET FEEDPAVGAIVLTGGEK | P14604 | Enoyl-CoA hydratase | 3.94 | SQ2 (HFD) | SQ3 HFD+Sq | 0.000100 |
| 42.10 | 1610.717 | MSPEEFTEIMNQR | P22791 | Hydroxymethylglutaryl-CoA synthase | 2.48 | SQ2 (HFD) | SQ3 HFD+Sq | 0.000689 |
| 32.62 | 1453.660 | DKAVFGCHETYK | Q3T940 | Apolipoprotein H (Fragment) | 5.27 | SQ2 (HFD) | SQ3 HFD+Sq | 0.011840 |
| 25.53 | 954.5058 | GQIGAPMPGK | P52873 | Pyruvate carboxylase | 2.47 | SQ2 (HFD) | SQ3 HFD+Sq | 9.28E-05 |
| 51.06 | 1223.683 | GFLVFAGCLLK | P29147 | D-beta-hydroxybutyrate hydrogenase | 4.56 | SQ2 (HFD) | SQ3 HFD+Sq | 0.006669 |
| 53.31 | 1298.800 | LLVPYLIEAIR | Q9WVK7 | Hydroxyacyl-coenzyme A dehydrogenase. | 2.51 | SQ2 (HFD) | SQ3 HFD+Sq | 0.014994 |
| 39.25 | 1459.691 | DYVSQFESSTLGK | P04639 | Apolipoprotein A | 2.63 | SQ2 (HFD) | SQ3 HFD+Sq | 0.032607 |
| 39.49 | 1879.912 | EDTQTGYLADEIIVGTR | A0A0G2K7M4 | Apolipoprotein B receptor | Infinity | SQ2 (HFD) | SQ3 HFD+Sq | 1.70E-06 |
| 32.73 | 1598.780 | ELEEQLGPVAEETR | P02650 | Apolipoprotein E | 2.47 | SQ2 (HFD) | SQ3 HFD+Sq | 0.003769 |

very-low-density lipoprotein particle clearance, binding, internalization, and catabolism of lipoprotein particles, triglyceride metabolism, gluconeogenesis (liver, kidney) and lipid synthesis (adipose tissue, liver, brain) glycolysis and cell respiration, coagulation and antioxidant defense. Most of the proteins that exhibited a fold change in response to squalene feeding exerted a direct influence on lipid metabolism.

**Conclusions**

Our study employed proteomic approaches and the results confirm that squalene feeding caused significant ($p<0.001$) alterations in proteins of lipid metabolic pathways that are likely to have profound effects on serum and liver lipid content. These proteins may function as potential targets to develop therapeutic strategies against lipid-related disorders.

**Table** Change in fold expression of select proteins/enzymes related to lipid metabolism. Here the changes in enzyme levels in liver of rats fed high fat diet (SQ2--HFD) is compared to enzyme levels in livers of rats fed with high fat diet and squalene (SQ3--HFD+Sq) are shown.

# Fucoidan and its application in baked food products

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ICAR-Central Institute of Fisheries Technology, Cochin - 682 029

Brown seaweeds are rich source of bioactive sulphated polysaccharides. Fucoidan is one of the polysaccharides possessing diverse health benefits. This fucose-rich-sulphated polysaccharide is found largely in brown seaweeds. In terms of health benefit, fucoidan has been found to possess varied bioactivities such as anti-cancer, anti-thrombotic, anti-virus, antioxidant, anti-inflammatory, anti-diabetic, and neuro-protective. Hence, fucoidan has emerged as potential functional ingredients in food products. Fucoidan was extracted from the brown seaweed *Sargassum wightii*. The extracted crude fucoidan was used for developing new fucoidan supplemented biscuit.

Biscuits are the popularly consumed bakery items in India and other parts of the world. Their wide popularity is due to their ready to eat (RTE) nature, affordable cost, nutrition, easy availability and high shelf life. The effect of fucoidan enrichment on the physical, biochemical, and sensory characteristics of biscuits was evaluated. The diameter

*Fig.1. Fucoidan extracted from Sargassum wightii*

and thickness of the biscuits ranges from 47.62-48.33 mm and 4.11-4.65 mm, respectively. Fucoidan supplementation reduced the breaking strength of the biscuit from 43.25 to 19.55 N. Water activity-the measure of free water in the product-of the biscuits varied from 0.25 to 0. 33. The different descriptive sensorial attributes viz. color, appearance, flavour, odour, texture, taste and overall acceptability of both the biscuits varied from “Like moderately (7)” to “Like

*Fig.2. Fucoidan enriched biscuit (left); control biscuit (right)*

extremely (9)" on the 9-point hedonic scale.

Thus, crude fucoidan was successfully extracted and supplemented in biscuits without adversely affecting the sensorial attributes of the biscuits. Biscuits may serve as the carrier to deliver the bioactive compound to the human nutrition as biscuit is widely consumption throughout the world.

# Influence of hydrocolloids on oil absorption of fried tuna kebab

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The recent trend in adopting healthier lifestyle has led to the development of low-fat products by reducing the fat content in fried foods using batter formulations with specific ingredients. Hydrocolloids, which have been widely using in food products, are known for reducing the oil uptake of fried foods. In the present study, hydrocolloids, guar gum (GG) and tragacanth gum (TG) were selected for adding into the batter to act as oil barriers and an attempt was done to prepare a low fat tuna kebab. The oil uptake and other quality characteristics of kebab were further evaluated. Kebab is a Middle Eastern dish, originally based on grilled meat and the traditionally used meat is mutton. But, depending on local taste and other preferences, kebab has been modified and now different varieties are available with other meats such as beef, goat, chicken, fish/seafood, or more rarely, pork. Mostly, kebabs are grilled on skewers or cooked in tandoor after marinating in special spices and it is even fried, especially in many places in India.

For preparing tuna kebab, ground tuna meat was mixed with minced onion, ginger-garlic paste, green chilly, coriander powder, chilly powder, turmeric powder, cumin powder, garam masala powder and salt. The final mixture was divided into two batches. Minced beetroot was added to one batch and the second batch was without beetroot. Further, it was formed into the shape of a kebab with the beetroot added mixture inside and the first batch of mixture outside. Thus, when the kebab is cut open, the cross-section will reveal two different colours. In this study, after battering and breading, the tuna meat based kebab was deep fried in oil instead of being grilled. Both GG and TG were added separately at 1% into the simple egg batter. The control tuna kebab was coated with only egg white. Values of the colour parameters, L* (lightness), a* (redness) b* (yellowness) of cross-section of kebab were 34.07±0.32, 26.10±0.10, and 18.19±0.16 for control and 34.17±0.52, 25.56±0.15, and 19.42±0.48 for TG sample. Fat content of control sample was

**Table 1. Moisture and fat content of control and treated tuna kebabs**

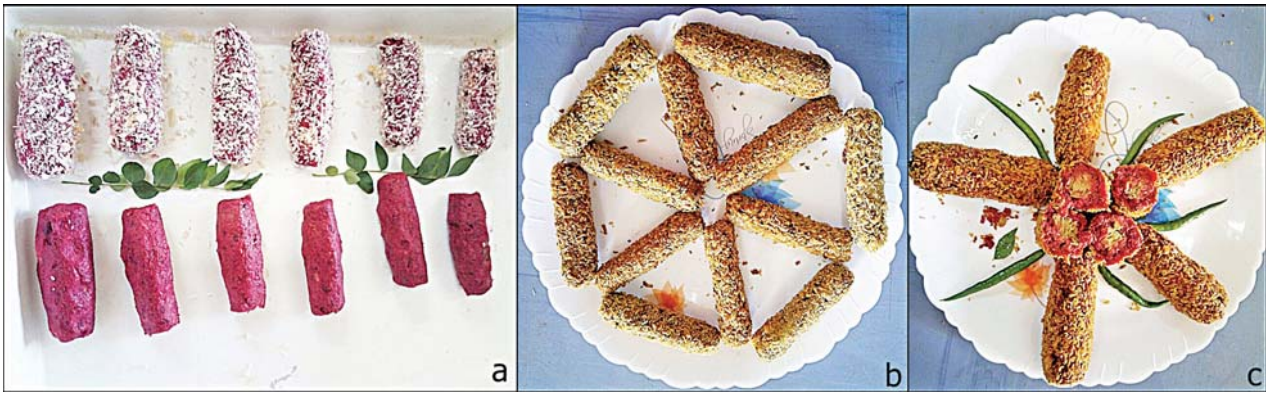
| Parameter | Control | | GG | | TG | |
|---|---|---|---|---|---|---|
| | Crust | Core | Crust | Core | Crust | Core |
| Moisture (%) | 32.89±0.42 | 52.64±1.12 | 36.1±0.75 | 58.81±0.44 | 38.24±0.65 | 64.14±0.92 |
| Fat (%) | 18.5±0.14 | 15.12±0.20 | 14.41±0.34 | 10.62±0.11 | 12.64±0.55 | 8.6±0.82 |

Values are expressed as Mean ± Standard Deviation

**Table 2. Colour of control and treated tuna kebabs**

| Parameter | Control | | GG | | TG | |
|---|---|---|---|---|---|---|
| | Surface | Cross-section | Surface | Cross-section | Surface | Cross-section |
| L* (lightness) | 35.64±0.50 | 34.07±0.32 | 37.12±0.49 | 35.1±0.47 | 36.15±0.72 | 34.17±0.52 |
| a* (redness) | 28.02±0.33 | 26.10±0.10 | 26.04±0.40 | 24.1±0.33 | 27.14±0.44 | 25.56±0.15 |
| b* (yellowness) | 22.84±0.61 | 18.19±0.16 | 22.12±0.34 | 17.75±0.50 | 21.16±0.40 | 19.42±0.48 |

Values are expressed as Mean ± Standard Deviation



*a. Tuna kebab before frying* *b. Tuna kebab after frying* *c. Cross-section of the tuna kebab*

18.5±0.14 % for crust and 15.12±0.20 % for core while the samples treated with batter containing gums had significantly lower fat content. Among the treated samples, TG tuna kebab had highest moisture content and lowest oil content. GG and TG tuna kebab had fat content of 14.41±0.34 % and 12.64±0.55 % for crust and 10.62±0.11 % and 8.6±0.82 % for core, respectively. Moisture content of crust of control and TG samples were 32.89±0.42 % and 38.24±0.65 % and core contained 52.64±1.12 % and 64.14±0.92 % moisture. The film forming ability of hydrocolloids such as GG and TG reportedly helps to reduce oil absorption while enabling to retain the natural moisture in the food. There were no significant differences in the sensory scores for overall acceptability of control and treated tuna kebabs. The results of the present work showed that use of hydrocolloids, guar gum and tragacanth gum, in the batter, significantly reduced oil absorption in tuna kebab without negatively influencing its sensory properties and fat reduction was highest in TG sample.

# Chitosan coated activated carbon for removal of iron content from water

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One of the most important natural resource present in the earth is water. But it is often contaminated by natural and manmade sources. Iron is the second most abundant metal in earth and it exist in two forms in water. One is soluble ferrous iron which is colourless in nature. The other one is insoluble ferric iron which is reddish brown in colour. Iron is mostly present in the nature in the form of hydrocarbons, sulphates, chlorides, in combination with humus or phosphates. When it will come in contact with water, iron is precipitated in the form of dark deposits changing the color of water to turbid, dark brown. In India high iron content in drinking water is a major problem in many geographic areas. Presence of iron in ground water at higher level can impart the water unusable due to its metallic taste, discoloration, odor, turbidity and staining of laundry. Overload of iron in drinking water can result vomiting, bleeding and circulatory disorders.

The different methods used for the removal of iron from water include methods such as precipitation, coagulation-flocculation, ultra filtration, reverse osmosis, electrochemical treatment etc. Adsorption is considered as one of the promising techniques for the removal of contaminants. Activated carbon is often used in water filtration devices for purification. Chitosan coated activated carbon (CCAC) was prepared by a modification to the method reported by Okoya et al. (2016) and evaluated its efficiency in iron removal by batch sorption studies in comparison to activated carbon (AC). Activated carbon was given a coating of chitosan having a degree of deacetylation 90.2. The effect of adsorbent dosage, contact time of treatment and initial iron concentration on iron removal efficiency was evaluated.

Stirring treatment of 10 mg/Kg iron standard solution for a period of 30 minutes using CCAC at 0.1, 0.5, 1, 1.5 and 2 % levels in comparison to AC was carried out. CCAC was found to be highly efficient in iron removal in comparison to simple activated carbon and showed maximum iron removal efficiency at 1.5% level (99.28%). But in the case of simple AC at 1.5% level, iron removal efficiency was only 63.7 %. In case of 10 mg/Kg initial iron concentration at varying contact time of stirring from 15, 30, 60 & 120 min, maximum removal efficiency was found at a timing of 30 min (98.31%). While changing the initial concentration from 5, 10, 15 and 20 mg/Kg, the maximum removal efficiency was found in the range of 10 to 20 mg/Kg (97.48 – 99.71%). Hence chitosan coated activated carbon can be used as an alternative to activated carbon in water filtration devices for augmenting the removal efficiency of iron content more effectively.

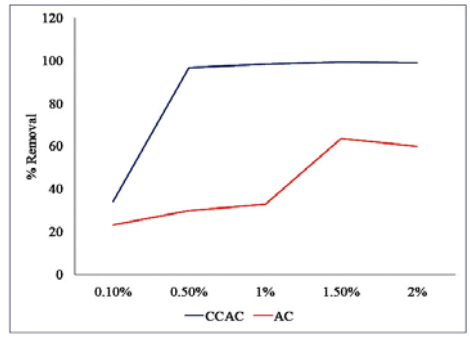


*Fig 1. Effect of adsorbent dosage on iron removal efficiency*

**Reference**

Aderonke A. Okoya, Abimbola Bankole Akinyele, Omotayo Sarafadeen Amuda and Ifeanyi Emmanuel Ofoezie1. Am. Chem. Sci. J. 11(3) (2016) 1-14.

# Drying characteristics of nandan (*Chanda nama*) fish in portable household electrical dryer

**Aniesrani Delfiya D.S., Murali S., Alfiya P.V. and Manoj P. Samuel**

ICAR-Central Institute of Fisheries Technology, Cochin - 29

Fresh fish is a highly perishable food product since it contains up to 80% of water. It has a very short span of shelf life (Bala and Mondol, 2001). Fish spoilage occurs as a result of the action of enzymes and microorganisms present in fish and also chemical oxidation of fat which causes rancidity. Fish become putrid within a few hours of capture unless it is preserved or processed in some way to reduce this microbial and autolytic activity. Salting and drying are traditional preservation technique and salted dried fish products are popular in many areas.

Drying preserves fish by inactivating enzymes and removing the moisture necessary for bacterial and mould growth (Duan et al., 2004). Traditionally, drying of fish is carried out under open sun with the natural flow of air. Open air sun dying is a low cost processing technique to preserve fish. However it has some limitations like no control over drying process and parameter, weather uncertainties, high labour costs, requirement of large drying area, insect infestation, mixing with dust and other foreign materials and so on. To overcome those limitations, it is necessary to dry fish in simple closed chamber made up of low cost materials. A low cost, multipurpose, portable electrical dryer for fish and other agricultural commodities is designed and developed at Engineering division, ICAR-Central Institute of Fisheries Technology, Cochin. It can be used in small scale fish processing units, laboratories and household environments for in-house drying of fishes throughout the year. In this study nandan (Elongate glassy perchlet, *Chanda nama*) fish was dried in portable household electrical dryer in order to evaluate the performance of dryer and drying characteristics of fish during drying.

Portable household electrical dryer (Fig. 1) comprises of a base frame, fan housing, two heating coils of 1.5 kW each, drying chamber with ten stainless steel trays and an exit for exhaust air. Base frame of dryer is fabricated using angle iron bar with dimensions (865×498×770 mm).

*Fig. 1 Portable household electrical dryer*

The frame is welded to shape and provides support for other components of the dryer. The rectangular shaped drying chamber is made up of marine plywood which can withstand humid and wet conditions. The dimensions of the drying chamber were 560×610×1650 mm. Inside of the chamber is coated with Aluminium foil for insulation. Air inlet to the chamber is located at the bottom end and the moisture laden air escapes through the exhaust at the top end. The ambient air is forced into the dryer by a fan located at the bottom which in turn gets heated by two electrical coils of 1500 W each arranged parallel to each other for heating the incoming air. Then, the resulting hot air passes through the drying chamber across the material to be dried. The chamber consists of ten trays which are made up of Aluminium frames and Stainless Steel mesh. The trays are stacked one over the other at a spacing of 10 mm. Dimensions of each tray is 560×500×250 mm accounting for a total tray area of 2.8 $m^2$. Also the chamber has provisions for a hinged door which permits easy access for loading and unloading of fishes. Capacity of dryer is 10 kg of fish per batch. The drying time required is around 6-7 hours for fishes and the maximum temperature attainable in the drying chamber is 50ºC.

Nandan (*Chanda nama*) fish for this drying study was purchased from the fishing harbour kalamukku, Cochin. Fish was cleaned using water and salted in the ratio of 1:8 (Salt: Fish) for 24 h. Next day excess salt was rinsed and then fish was placed in household electrical dryer for drying. Moisture content of fish was determined by using the following formula (AOAC, 1990):

$$\text{Moisture content } (M_{wb}), \% = \frac{\text{Weight of water removed (g)}}{\text{Weight of sample taken (g)}} \times 100 \quad .....(1)$$

$$\text{Moisture content } (M_{db}), \% = \frac{\text{Weight of water removed (g)}}{\text{Weight of dry matter in sample (g)}} \times 100 \quad .....(2)$$

Drying rate of fish sample during drying period was determined as follows:

$$k = \frac{Ww}{T} \quad .....(3)$$

where, k - drying rate, kg/h; $W_w$ - quantity of moisture evaporated, kg; T - time taken (for drying) to remove water $W_w$ g of moisture, h

The moisture ratio of fish was calculated as

$$MR = \frac{M}{Mo} \quad .....(4)$$

where, MR - moisture ratio, dimensionless value; M - moisture content at any time t (%db); Mo - initial moisture content (%db)

Five kilograms of nandan (*Chanda nama*) fish was placed inside the dryer. Initial moisture content of fish was found to be 67.71±1.68 (% wb) and the time required for complete drying of nandan (*Chanda nama*) fish was observed as 6 hours. Drying behaviour of nandan (*Chanda nama*) fish in portable household electrical dryer has been studied and the variation of moisture content and drying rate with respect to time at specified drying temperature of 50±2ºC was also observed (Fig. 2 and 3).

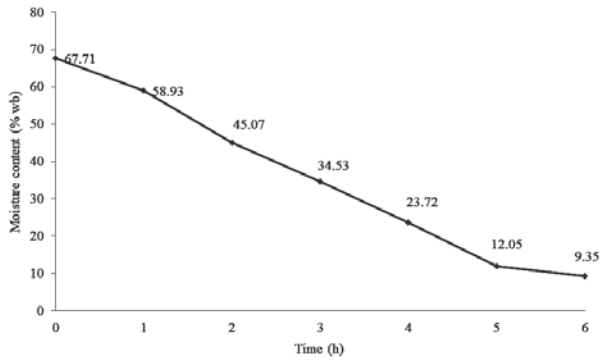


*Fig. 2 Changes in moisture content of nandan (Chanda nama) fish during drying*

Moisture content of fish samples decreased as drying time progresses. Moisture content of fish was reduced from 67.71 (% wb) to 9.35 (% wb) in 6 hours of drying (Fig. 2). This moisture reduction with increased drying time might be due to the reduction of water content of fish. Drying rate of fish was observed to be 1.305 kg/h during the first hour of drying and then drying rate

was gradually decreased as drying proceeded (Fig.3). Generally drying rate reduces with increased drying time. As the drying process

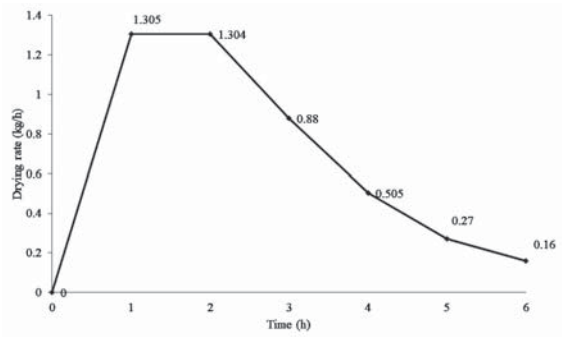


*Fig. 3 Drying rate curve of nandan fish during drying in portable electrical dryer*

continues, less free water is available on fish surface and then drying rate could be dominated by the moisture diffusion from inside to the surface of fishes. Therefore, drying rate starts to decrease as drying time increase. The entire drying process occurred under falling rate drying period (Fig. 3).

This study showed that nandan (*Chanda nama*) fish drying using portable household electrical dryer is a suitable method for quality dry fish production within 6 hours.

## References

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CIFTFISHPRO

# CIFTFISHPRO – A mobile APP for android systems on CIFT Value Added Fish Products

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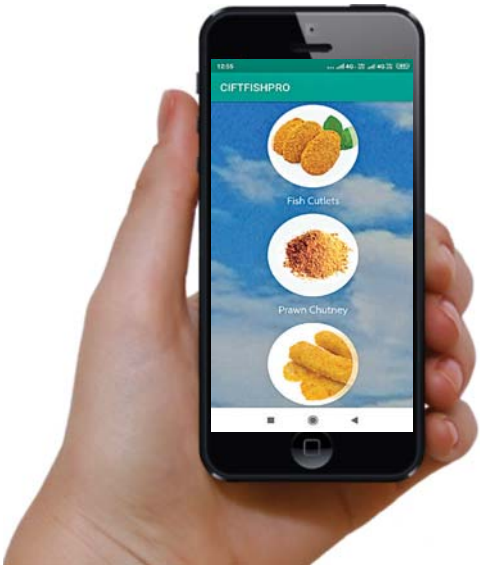
ICAR-CIFT has developed an interactive mobile application on CIFT Value Added Fish Products (CIFTFISHPRO). CIFTFISHPRO is a web based mobile APP for android systems. This system contains information about series of value added fish products including description about the product, active ingredients of the product and method of preparation. The series of fish products includes coated fish products like fish cutlets, fish fingers, fish burger, fish balls etc.; marinated products like fish and prawn pickles; extruded products like fish kure and noodles; wrapped fish products like fish momos, fish kebabs, fish samosa and fish rolls; cured products



like dried fish and prawn; other products like fish sausage and prawn chutney powder. This system will also help the users to automatically quantify the amount of ingredients required when the product is taken up for up-scaling and the user can give input cost for different ingredients to get an idea about approximate cost required for up-scaling. Another important feature of the APP is that users can get quick response to their queries placed through the APP.

The important features of CIFTFISHPRO are

1. Product description
2. Active ingredients of the product
3. Method of preparation of product
4. Ingredients requirement for Up-scaling of the product
5. Approximate cost for Up-scaling
6. Instant query making system

The mobile app is available in the Google play store and it is available in the link

https://play.google.com/store/apps/details?id=com.icar_ciftfishpro&hl=en.

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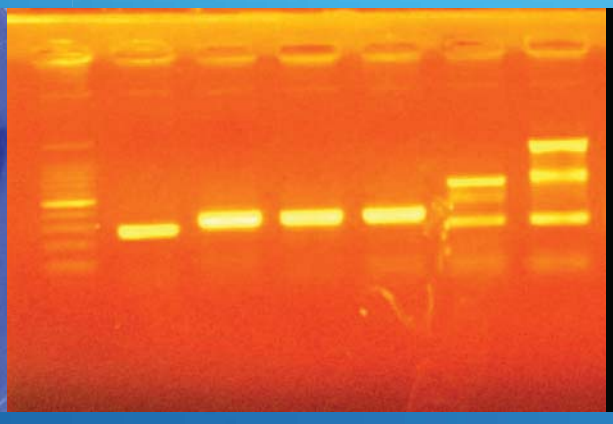

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