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Director's Message



ICAR-CIFT is completing its 58 years of service to the nation, on 29th April 2015. With its Head Quarters at Cochin and Research Centres at Veraval (Gujarat), Mumbai (Maharashtra) and Visakhapatnam (Andhra Pradesh), the Institute is unique in bringing out technologies suitable for the betterment of fish harvest and post harvest sectors. Know-how and solutions relating to the fisheries sector are made out of basic and applied research organized under seven scientific Divisions of the Institute. As an Institute dedicated to overall development of fisheries sector, it is committed to extend its research findings to the community. On this occasion, we are very pleased to bring out a new publication, 'FishTech Reporter' which proposes to cover articles and reports on current and immediate research findings of the Institute. This definitely is a major step forward in filling the gap between the contributions of the researcher, on one hand, and the requirements of the end-user on the other. Hope this initiative will be appreciated by fishers, academicians, processors and policy makers.

Dr. Ravishankar C.N. Director

From the Editorial Board

Fisheries, around the world, as also in India, is prone to several undesirable practices. Such practices range from wrong harvesting practices, to inefficient use of resources at the post harvesting stage. The research community, definitely, has a responsibility towards addressing these issues and finding out solutions. Technology is the key word for bringing out change. However, success of any technology depends on its adoption by the end-user. In order to ensure adoption by the end-user, stakeholder participation in dissemination is crucial. `FishTech Reporter', the ICAR-CIFT publication is placed against this background.

It gives us immense pleasure to come out with this inaugural issue of the `FishTech Reporter'. It brings to you a range of technologies having direct social impact. Articles on β -carotene fortified fish powder; incorporation of glucosamine hydrochloride as food supplement, and shrimp protein powder; and hydrolysate incorporated snacks depict some simple, but health and nutrition-oriented technologies. Another focal theme is green technologies. Articles on plant extracts and vibriocin for fish preservation highlight on the greening opportunities in fisheries. This issue also has, in its scope, a new approach for safe sea-foods: Detection of fish spoilage and adulteration using polyaniline composites comes with such a new approach. 'Combination fishing', the optimizing use of different seasons and resources, forms another key theme of this issue. A study on design standardization of a multipurpose fishing vessel for commercial applications has been included under this theme.

Encapsulated β-carotene and its use in nutrient formulations against malnutrition

According to the WHO, 2013, India is one of the most malnourished countries in the world. The prevalence of under-weight children in India is among the highest in the world with dire consequences for mobility, mortality, productivity and economic growth. In India 44% of children under the age of five are underweight. Figure 1 shows the state-wise percentage of children under five year of age who are under-weight. The 2011 Global Hunger Index (GHI) Report ranked India 15th, amongst countries with hunger situation.

According to the National Family Health Survey India, in Kerala 56.1% children between 6-35 months and 32.7% ever-married women aged between 15-49 are anemic. Theeradesha Samrakashna Samithi, a grassroot organization working among coastal people, and RIGHTS, an NGO, have submitted a detailed report high-lighting the prevalence of hunger and malnutrition particularly among children belonging to the Adivasi, Dalit and fishing communities.

Fish as a great source of nutrients

Hundreds of millions of children and adolescents in India take nutrient deficient diets. Current approaches to address malnutrition have serious limitations. The fortified cereals presently being supplied through food aid do not meet minimal standard requirements. They need access to energy-dense, nutrient-rich foods. Interestingly, fish is probably the most affordable food to provide up to 40 essential nutrients.

Fortified fish-based foods for intervention with an aim to reduce malnutrition

A project, supported by the International Fund for Agricultural Development, aimed to improve nutrition, especially in women and children in Bangladesh, through increased intake of nutrientrich small fish. In a study on Ghanaian infants, fortification of a product Weani-mix with fish



Fig. 1. State-wise percentage of children under five years of age in India who are under-weight. Source: National Family Health Survey, India, 2005-06

powder, vitamins and minerals improved iron stores and vitamin A status. In Gambia an intervention study in women used millet pap ogi, enriched with peanut paste and dried fish; alternative additional ingredients were bean flour, butter, with the result that mil feeding also increased. A nutritional intervention in Indonesia used locally made Nasi tim bayi, rice enriched with fish. In a study in Philippines to improve infant and young mothers' health, interventions were made by supplementing rice with flaked fish and vegetables.

Vitamin A deficiency

One out of three people in developing countries is affected by vitamin and mineral deficiencies, according to the World Health Organization (Fig. 2). Vitamin A deficiency has serious health consequences for poor people in



Fig. 2. Clinical and subclinical vitamin A deficiency prevalent in most developing countries. Source: WHO, 2010

developing countries. It reduces the body's resistance to disease and can also cause blindness and growth retardation. In a study undertaken in rural Thiruvananthapuram in 2001, vitamin A intake of adolescent girls was found to be less than 50% of recommended dietary allowance.

Most common cause of vitamin A deficiency is insufficient intake of vitamin A which is available from animal sources of food, especially in lowincome food deficit countries where predominantly vegetarian-based diets are consumed. Although considerable progress has been made in controlling vitamin A deficiency worldwide, there is still a need for additional prevention efforts in the form of dietary diversification, fortification, and supplementation. With this background, Biochemistry and Nutrition Division of ICAR-CIFT is currently conducting research on fortification of fish soup powder with β -carotene. It was hypothesised that β -carotene fortified fish soup powder could provide/fulfil a part of the daily requirement of vitamin A in severely vitamin A deficient malnourished populations. However, the utilization of supplemented β -carotene is limited due to its instability. In order to render protection to β -carotene to be fortified, owing to its highly unsaturated nature and its easy susceptibility to rancid degradation, β -carotene was microencapsulated. The bioavailability of β -carotene from specific foods as well as from microencapsulated particles is not well understood. Before we continued with fortification of fish soup powder (Fig. 3), we presumed that it is essential to assess whether microencapsulated β -carotene can positively contribute to β -carotene content



Fig. 3. Fish soup powder

of the supplemented rats.

In this study, the rate at which free β -carotene (FBC) and microencapsulated β -carotene (EBC) appeared in plasma, liver and urine post-dosing which would reflect the bioavailability of β -carotene were compared. In view of the anti-oxidant nature and health benefits demonstrated by β -carotene, the effect of β -carotene supplementation (free and encapsulated) on the content in plasma and liver of glutathione (GSH), an intracellular anti-oxidant molecule and thiobarbituric acid reactive substances (TBARS), an index of oxidative damage were measured. The outcome of the study is expected to support the eventual purpose of β -carotene fortification as a strategy to address vitamin A deficiency.

Thirty six rats divided into three groups of 12 animals each were taken for the study. The groups were as follows: Group I - control was given placebo; Group II - free β -carotene fed at the level of 20 mg/kg body weight and Group III encapsulated β -carotene at the level of 20 mg/kg body weight (prior to feeding of Group III rats, weight of β -carotene was corrected for encapsulation efficiency). Microencapsulated β carotene and free β -carotene dissolved in oil phase were administered by gavage and the study lasted for a period of 14 days. After the study period, rats were sacrificed following chloroform anaesthesia. Blood, liver and urine were collected and stored at -20°C until analysis for β carotene-reduced glutathione and TBARS. After the last dosing on Day 14, rats were fasted overnight and sacrificed following chloroform

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anaesthesia. Blood and liver samples were used for biochemical analyses.

Microencapsulation of β -carotene was successfully accomplished as evidenced by SEM images (Fig. 4) and FTIR (Fig. 5) data. β -carotene was not detected in plasma, liver and urine of control rats (Fig. 6). Plasma and liver content of β -carotene was significantly high in EBC administered group when compared with FBC. This may be explained by the possibility that encapsulation of β -carotene allowed better intestinal absorption due to enhanced solubility. Urinary levels were significantly high in group FBC rats when compared to EBC suggesting that the loss of



Fig. 4. Scanning Electron Micrograph of encapsulated β-carotene



Fig 5. FTIR of vannilic acid grafted chitosan and encapsulated β -carotene



Fig. 6. Levels of β -carotene in plasma, liver and urine of control and experimental groups of rats. FBC- free β -carotene, EBC- encapsulated β -carotene

free β -carotene *via*. urinary excretion was much more when compared to encapsulated β -carotene.

TBARS assayed in liver and plasma of the experimental rats were essentially unchanged across all groups (Fig. 7). This is a significant outcome of the study as it establishes that at the dose of β -carotene employed in the study, it is not pro-oxidant in contrary to some reference reports that purport β -carotene's pro-oxidant nature at high doses.



Fig. 7. Levels of reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) in plasma of rats

From our study it is evident that microencapsulation of β -carotene had positive effect on the levels of β -carotene in liver, plasma and urine; and it increased the levels of glutathione in plasma and liver. Administration of microencapsulated β -carotene also did not induce any change in the content of TBARS, an indicator of peroxidative effects.

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Detection of ammonia and amines using polyaniline composites

Conducing polymers are very much sought in recent years due to their low cost, ease of synthesis, processing and its ability to sense in the room temperature. Polyaniline, polypyroll, polythiophene and polyacetylene are some of the important conducting polymers exploited extensively for a variety of applications. Among these, polyaniline (PANI) is the most preferred one since it has the ability to switch between the insulating and conducting phase through an acid/ base doping or dedoping process (Mac Diarmid, 2001). The aim of the study was to synthesize a polyaniline composite by introducing different amine responsive molecules. Polyanilinecurcumin-copper-cobalt (PC3) was synthesized and the composite was characterized using FTIR (Fig. 1). The FTIR evaluation exhibited the formation of the composite by showing the characteristic peaks of curcumin, copper and cobalt. The wave numbers at 488 and 543 cm⁻¹ highlight the presence of copper and cobalt, whereas 945, 1249,1011, 1510, 1650 cm⁻¹ are due to the presence of curcumin. The characteristic peaks of polyaniline was 660, 829, 1180, 1290 and 1560 cm⁻¹. There was clear shift in the peaks of designated individual molecules which showed the formation of composite. The composite was tested for the detection of ammonia, methyl, dimethyl



Fig. 1. FTIR spectra of polyaniline-curcumin-coppercobalt composite

and trimethyl amines (TVBN). TVBN detection was done through cyclic voltammetry, electrochemical impedance and visual methods. The composite responded to TVBN from 1 ppm to 200 ppm by exhibiting gradation of bluish green colours with increasing concentration (Fig. 2). The nitrogen and amine molecules responded comparatively on similar pattern. The colouring intensity was increased with increasing concentrations (Fig. 3).



Fig. 2. Response of polyaniline composite with different concentrations (1 to 200 ppm) of ammonia, methylamine, dimethylamine and trimethylamine

Detection of very low concentration of TVBN can be carried out by cyclic voltammetric and impedance techniques. The composite was embedded over polished glassy carbon electrode and then dried. The ammonia and the amines were added into 1:1 solutions of potassium ferro and ferricyanide electrolyte and the analyte was detected electrochemically by using Pt as counter, glassy carbon electrode as working electrode and



Fig 3. Electrochemical impedance spectral response of different concentrations of ammonia. The graph shows the impedance of real (Z') and imaginary (Z") with different concentrations, which followed linear relationships

Ag/AgCl electrode as reference electrode. Figure 4 shows the cyclic voltammetric pattern of methylamine in different concentrations and other amines also exhibited similar patterns. Ammonia did not respond with cyclic voltammetry since it does not undergo any oxidation and reduction reaction.

The results show that the synthesized composite is a potential candidate for the detection of ammonia, methyl, dimethyl and trimethyl amines



Fig. 4. Cyclic voltammogram of methylamine in different concentrations and the bottom is the peak current response at different concentrations. Upper line shows the oxidation response and the bottom due to the reduction

and the method can be utilized for the quantitative detection of fish spoilage and adulteration. The response with electrochemical technique highlights that it can be utilized for detection through electronic devices.

Reference

Mac Diarmid, A.G. (2001) - "Synthetic metals" - A novel role for organic polymers (Nobel lecture), Angew. Chem. Int. Ed. 40: 2581.

Muhamed Ashraf P.

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Design standardization of a commercial combination fishing vessel

There is a lack of standard designs for commercial combination fishing vessels in India. ICAR-CIFT has developed several designs of fishing vessels, fishery research vessels, mother ships and training vessels for state governments and union territories of India. The lack of standard designs for commercial purposes has been a long term negative impact on policy making in fisheries at national and state levels. Hence, design of a 19.75 m vessel was developed to meet this requirement of all the maritime sates of India to conduct multipurpose fishing commercially. The design was

developed and standardized under the project "Green Fishing Systems for Tropical Seas" funded by the National Agricultural Science Fund, ICAR for developing the design, model testing and construction of the prototype at Goa Shipyard Limited. A national survey was conducted in all the maritime states of India and designs of existing commercial vessels were collected. Thirty five most popular designs were short listed and were analyzed for fuel economy, performance and stability. Design of a 19.75 m L_{OA} vessel with 6.5 m breadth and 2.8 m depth was developed from this data. This vessel is equipped for trawling, long lining and gillnetting with onboard hydraulic fishing equipment. Indigenously developed Refrigerated Sea Water (RSW) tank is another specialty of this design.

From the computer generated lines plan of the vessel developed at ICAR-CIFT, a 3-D model was prepared and the same was exported to stability analysis software, Maxsurf for further analysis. The simulation studies of this hull were conducted in Computer Fluid Dynamics (CFD)



Fig. 1. Model of the hull generated using CFD



Fig. 2. Model of the 19.75 m multipurpose fishing vessel used for testing at IIT Chennai

software for arriving at the resistance of the vessel. Figure 1 shows the model of the hull generated using CFD.

The 1:10 model of the 19.75 m multipurpose fishing vessel developed at CIFT was prepared (Fig. 2) by IIT Chennai and was tested in the towing tank for various speed range up to 10 knots to confirm the resistance values obtained from CFD analysis (Fig. 3).



Fig. 3. Model test progressing in the towing tank at IIT Chennai

After establishing the preliminary stability, the general arrangement drawing was prepared with subsequent stability verification. This vessel is designed and constructed under the IRS classification. The approval of the initial drawings is already done by IRS and the proto-type construction is underway at Goa Shipyard Limited, Goa.

Baiju M.V.

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Effectiveness of proper hand washing on sanitation in seafood processing microenterprise units

Global Hand Washing Day is an annual global advocacy day dedicated to increasing awareness and understanding of hand washing with soap. The first Global Hand Washing Day was celebrated on October 15, 2008, as per recommendation of UN General Assembly in 2008, as International Year of Sanitation.

Sanitation is the first line of defense in any effective food safety programme. Food handlers' correct knowledge of problems, conditions of handling and good manufacturing practices are some of the most important conditions contributing to good hygienic quality in food preparation and/or quality deterioration. There are more than 250 different food-borne diseases and millions of people fall ill every year and many die as a result of eating unsafe food. Often hands act as vectors that carry disease-causing pathogens from person to person through direct contact or indirectly through contact surfaces and foods. Hand washing with soap is among the most effective and inexpensive way to interrupt the transmission of microorganisms in a food processing environment. Although people around the world wash their hands with water, many often forget washing their hands with soap at critical moments, particularly after going to the toilet and before handling or eating food. The challenge, therefore, is to transform simple hand washing to hand washing understanding scientific principles. Food handlers have specific responsibilities in food safety related to their health and hygiene. Proper and adequate training on personal hygiene practices are absolutely essential for food handlers for safe handling of food and thus to prevent food-borne diseases.

Effective hand washing is an important strategy to prevent the risk of infection and it will take nearly one minute which involves the application of a soap or any antimicrobial agent onto wet hands; then rubbing of both hands to form a lather and effective rubbing on back of the palm, between the fingers, back of the fingers, finger tips, under finger nails, thumbs, wrists etc., rinsing with clean water; and drying hands with clean towel or dryer (Fig. 1).

In connection with the 'Global Hand Washing Day' an awareness campaign on "Importance of effective hand washing in seafood processing and food-borne illnesses" was conducted at Moothakunnam, Ernakulam on 15th October, 2014. The campaign was organized as part of the DST project entitled "Food safety interventions for women in fishery based micro enterprises in

Fig. 1. Demonstration of hand washing procedure as per WHO guidelines



1st Step (Wet your hands with clean water & Apply enough soap)



2nd Step (Rub hands palm to palm)



3rd Step (Rub the back of both hands with interlaced fingers)



5th Step (Back of fingers to opposing arms)



7th Step (Rotational rubbing of fingertips on palms)



(Dry hands thoroughly with a clean towel)





4th Step (Palm to palm with fingers interlaced)



6th Step (Rotational rubbing of thumbs)



8th Step (Rinse hands thoroughly with clean water)



10th Step (Use towel to turn off faucet)

11th Step (Clean hands = Safe hands) coastal Kerala". To assess the impact of training, stakeholders from micro enterprise units were divided into two groups. The group to which training was imparted was designated as Group A and the other group as Group B. Eleven steps of proper hand washing as per WHO guidelines on hand hygiene was demonstrated during the training session (Fig. 1). The level of hand hygiene before and after the training was assessed by conventional microbiological swabs. Swabs were collected from the critical areas of hands or the parts often missed during hand washing and brought to the laboratory in aseptic condition. Swabs were taken before and after hand wash to compare the variations. Analysis was performed as per standard protocol. The efficiency of training was evaluated by comparing the two groups with respect to time taken for hand washing, persons wearing jewelry, uncut nails, open wounds and other hand hygiene practices etc.

All food handlers washed their hands with tap water. In Group B about half of them (43.75%) used soap, rubbing of the hand areas (between fingers, finger tips, around wrist, thumbs etc.) was either not practiced at all (78.12%) or partially done (37.50%). Initial mesophilic bacterial count was 1910 \pm 154 cfu/cm² in Group A and 1829 \pm 153 cfu/cm² in Group B which reduced by 90.55% in Group A and 63.74% in Group B after hand washing (Fig. 2). In Group B, 84.38% of stakeholders took less than 20 sec. for hand washing whereas food handlers in Group A, who acquired training took 40-55 seconds. Prior to washing, a variation in mesophilic count of about 154 cfu/cm² was noticed



Fig. 2. Impact of training on hand hygiene

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between both hands of the food handlers in Group A while the second group showed a variation of 153 cfu/cm² which indicates that initially there was no significant difference (P < 0.05) between the two groups. The variation in mesophilic counts between two hands of first group of people after hand washing was 11.7 cfu/cm² but the second group exhibited a significant variation of about 131 cfu/cm² between two hands even after washing. This probably indicates that most of them may have omitted the critical steps of friction and rubbing during hand washing. Escherichia coli and Staphylococcus aureus count in Group A people was significantly reduced after hand washing being less than 10 cfu/cm² in all cases. Even though the hand washing method followed by the food handlers in Group B significantly decreased both E. coli and S. aureus counts, they were reduced only to 66.67% and 76.48% respectively, indicating the necessity of an intervention.

The skin underneath rings had more microorganisms than control sites. Training was found to be useful that 91% and 92% of food handlers in Group A followed the system of removing rings and bangles prior to hand wash but only 14.29% and 18% of Group B removed rings and bangles prior to washing indicating the need of an intervention. Almost all of them were aware about the unhygienic aspects of long nails before the training itself but many forgot to cut their nails, may be due to laziness or time pressure. Open wounds are also a source for cross contamination and it was found that 6.25% of food handlers had open wounds.

Microorganisms thrive well in a damp environment. So hand drying is an important step to prevent cross contamination. Most of the food handlers (87.5%) in the present study followed wiping their hands on towels after hand wash but they rarely noticed the cleanliness of the wiping material. It was found that the entire food handlers from Group A wiped their hands on clean towels after training. Taps should not be touched again with freshly washed hands. Group which acquired training turned off the faucets using the towels whereas Group B used washed hands to turn off the faucets. Foot operating taps are more recommended in food processing units. Petri plates with microbial growth were exhibited to make them aware about the bacteria in dirty hands and to emphasize the importance of proper hand washing. Posters were also displayed on foodborne diseases, treatment, preventive measures and personal hygiene practices.

Most of the food-borne disease outbreaks are attributable to poor personal hygiene practices and improper handling of food. The spectrum of food diseases are widening with emerging pathogens. Judicial washing of hands is a simple step in personal hygiene which can significantly reduce the risk of food-borne illness. Present study revealed that besides inadequate hygiene facilities, lack of proper training plays a vital role in unhygienic practices. Significant reduction (P <0.05) in bacterial counts indicated the impact of intervention. Training was found to be effective in this study but long-lasting improvement in hand hygiene practice needs a multifaceted approach involving both behavioural change and continuous monitoring facility.

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Perception of fishers on fisheries co-operative services

Fisheries Co-operatives Societies plays significant roles in fisheries resource management, managing fishing effort, fish marketing etc. in various countries. The services offered by a particular Co-operative Society are uniform. But, the perception and utilization of the services may vary. A study was carried out at Narakkal -Navarambalam Fishermen Development Welfare Co-operative Society at Narakkal panchayat, Vypeen Island, Ernakulum, Kerala to assess the perception of fishers on the services offered by the Society. The three-tier structure viz., primary co-operatives at village level, secondary cooperatives at district level and apex co-operatives at state level with definite roles and responsibilities was first brought into effect in 1917. The primary activities of credit/production have diversified into various business and welfare activities. Fish selling through fishermen cooperatives is one such activity which is a way of ensuring fair return to the fishermen and keeps the Societies functional through profits generated. A sample of 75 fishers was contacted for the study. The fishermen perception on co-operative services was estimated using a five point Likert Scale.

From the results, it was found that 94 per cent of respondents agreed or strongly agreed that

they were earning profit by selling fish through co-operatives (Table 1).

More than 80 per cent accepted that cooperatives were the best credit source, ensured unity among fishermen and improved the standard of living. More than 50 per cent felt that cooperatives had no role in fisheries management.

Table 1. Fishers' perception on fisheries co-operative services (in %)

Particulars	SA*	A*	N*	D*	SD*
Potential and attra-	2	86	4	8	0
ctive credit source					
Ensure fishermen	4	78	6	12	0
unity					
Provide training on	0	18	24	54	4
fishing activities					
Satisfying the	2	52	20	26	0
needs of fishermen					
Fish selling is	4	90	4	2	0
profitable					
Improves standard	2	86	2	6	0
of living of fishermen					

*SA - Strongly Agree, A - Agree, N - Neither agree nor disagree, SD - Strongly Disagree, D - Disagree

Particulars	SA*	Α*	N*	D*	SD*
Client-friendliness	6.25	85.42	4.17	4.17	0.00
Timely loan availability	4.17	29.17	16.67	50.00	0.00
Profitability	4.17	83.33	4.17	6.25	2.08
Technical assistance	0.00	22.92	25.00	52.08	0.00
Input supply	2.08	43.75	31.25	16.67	6.25
Hassle-free procedures	2.08	87.50	4.17	2.08	4.17
Repayment flexibility	6.25	43.75	12.50	33.33	4.17

Table 2. Determinants of fishers' perception on co-operative services (in %)

*SA - Strongly Agree, A - Agree, N - Neither agree nor disagree, SD - Strongly Disagree, D - Disagree The major determinants of the fishers' perception were the role of co-operatives in input supply (94%), client friendliness (92%) and hassle-free procedures for approval and repayment of loans (89%) (Table 2).

The co-operatives were preferred least due to the lack of timely loans (33%) and non-provision of technical assistance (29%). For societies to better their performance, assessment of stakeholders' perception and satisfaction on cooperative services can play an important role.

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Proximate composition and biochemical quality of smoked fishes from the markets of Manipur

Smoked fish is important in the diet of the people of North-east India. Fishes caught from the water bodies are preserved through traditional smoking and such fishes are used as essential ingredients in vegetable curry preparation and also as replacement for fresh fish. The smoking of fish in Manipur is unique in nature as no salting is involved in the entire smoking process. Biochemical quality is an important consideration in food processing which will help processors to define the optimum processing and storage condition. Only limited information is available on the quality of traditionally smoked fish of Manipur markets. The present report is a study of the proximate composition and biochemical guality of the most commonly available smoked fish species in the markets of the state of Manipur, India.

Smoked fish species were collected from markets of different districts (Imphal East, Imphal West, Thoubal and Bishnupur) in Manipur. Proximate composition and biochemical analysis were assessed with standard procedures and the results are depicted in Table 1 and 2, respectively. Moisture content of the smoked fish was in the range of 9.15 to 61.64% and the highest and lowest moisture content was recorded in *Cirrhinus mrigala* and *Pseudoambassis ranga* respectively. The relatively higher mean percentage (31.25) of moisture content could be attributed to the fact that the fishes were not smoked optimally or the storage conditions were not proper which resulted in absorption of moisture from the surroundings. Protein level in the analyzed smoked fishes varied from 23.50 to 65.65% and the highest level was found in *Channa striatus*. Crude fat content ranged from 3.60 to 24.08% with the highest level in *Puntius titto*. The ash content varied between 5.20 and 27.48% in all fishes.

High values of TVBN were found in all the samples. It may be due to the release of volatile compounds during the smoking of fish. TVBN values obtained for *Clarius batrachus* and *Notopterus notopterus* were very high indicating sub standard quality of the product. It could be due to usage of spoiled fishes for smoking. Generally TVBN is an index of decomposition and consists of

Samples	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)
Puntius titto	24.12	41.09	24.08	10.72
Clarius batrachus	43.46	41.61	10.10	5.20
Channa striatus	27.57	65.65	1.27	6.47
Noptopterus notopterus	43.00	42.68	6.12	8.16
Glossogobius guiri	34.35	42.56	3.60	19.34
Colisa fasiata	20.36	36.44	15.90	27.48
Anabas testudineus	27.63	44.98	17.69	9.40
Hypophthalmichthys molitrix	36.19	45.73	9.78	9.36
Pseudoambassis ranga	9.15	55.68	19.61	11.12
Monopterus albus	49.27	40.43	3.23	6.63
Puntius sarrana	14.36	59.57	7.05	18.83
Cirrhinus mrigala	61.64	23.50	7.14	7.59
Amblypharyngodao mola	12.72	57.53	15.48	14.35
Puntius sophore	33.76	52.82	4.66	11.91

Table 1. Proximate composition of smoked fishes from Manipur

Table 2. Biochemical quality of smoked fishes from Manipur

Samples	TVBN	PV (meqO ₂ /	FFA (% as	TBA (mg
	(mg N%)	kg of fat)	oleic acid)	MDA/kg)
Puntius titto	280.23	1.90	69.50	0.40
Clarius batrachus	1044.98	2.81	68.64	0.30
Channa striatus	144.01	16.23	4.58	1.75
Noptopterus notopterus	522.48	8.60	2.43	0.63
Glossogobius guiri	98.13	12.10	17.51	0.27
Colisa fasiata	18.37	17.57	4.95	0.48
Anabas testudineus	146.04	ND	80.79	3.38
Hypophthalmichthys molitrix	42.00	18.43	6.77	20.67
Pseudoambassis ranga	70.00	4.87	6.43	7.25
Monopterus albus	98.00	10.73	17.13	16.32
Puntius sarrana	70.00	3.22	8.18	2.57
Cirrhinus mrigala	56.00	4.81	3.39	5.15
Amblypharyngodao mola	56.00	3.63	6.40	3.51
Puntius sophore	42.00	4.47	6.31	4.64

ND: Analysis not done

ammonia liberated due to deamination of amino acids by microbial or enzymatic degradation. Peroxides value (PV) which is a primary indicator of oxidation of fat was in the range of 1.90 to $18.43 \text{ meqO}_2/\text{kg}$ fat. The peroxide values corresponding to spoilage are usually in the order of 20-40 meqO₂/kg fat. This indicated that the collected samples generally were of good quality and also the phenolic content in the smoke gave protection against oxidation. The free fatty acid (FFA), which is an indicator of hydrolytic rancidity ranges from 2.43 to 69.50%. *Puntius titto* had the highest FFA corresponding to its high fat content. *Hypophthalmichthys molitrix* had the highest TBA value of 20.67 mg MDA/kg.

The present study showed that the smoked fishes were rich source of protein and minerals. However, difference was observed in the proximate composition and biochemical indices of different species. In the market, smoked fishes were sold in open environment which will easily accelerate biochemical changes in the product. Hence, by adopting good packaging bags to display and store the products and by using good quality raw materials, the quality of the smoked fishes can be improved.

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Functional tilapia sausage incorporated with Glucosamine hydrochloride

Functional foods provide benefits beyond basic nutrition and play an important role in minimizing the risk of certain diseases. The global demand for functional food is estimated to be ~ US \$ 120 billion by 2015. In many countries, the aged population is growing rapidly which is expected to reach $\sim 25\%$ of population by 2036. This changing population distribution may result in increase / accelerate the occurrence of chronic diseases such as cancer, cardio-vascular diseases. Type 2 diabetes and osteoarthritis. Many of these chronic diseases are also associated with diet and physical activity of an individual which increases the per capita health expenditure resulting in huge economic burden. This places impetus on developing functional foods as a preventive strategy to decrease the risk of these chronic diseases. Osteoarthritis is one such painful chronic disease characterized by a progressive degeneration and loss of cartilage and related tissues. Osteoarthritis has no cure and hence becomes a life-long process. Although a variety of medicines are available for treating osteoarthritis, health supplements in the form of food is gaining popularity due to the prevailing stomach-related rheumatic diseases associated with these medicines. Osteoarthritis can be managed by proper nutritional supplement containing glucosamine, chrondroitin sulphate and unsaponifiables from avocado, soybean etc. The market value of health supplements containing glucosamine and/or chondroitin sulphate was over US \$425 million during 2011 and are expected to reach US \$ 510 million by 2018. Glucosamine is a water soluble amino mono-saccharide available as glucosamine hydrochloride, glucosamine

sulfate, and N-acetyl-D-glucosamine. Both the sulfate and hydrochloride forms generate glucosamine-free base at gastric pH. Glucosamine is a very popular dietary supplement marketed in many countries mainly to reduce joint pain and to improve mobility in persons with osteoarthritis. Although many clinical trials support its usefulness in decreasing osteoarthritis, there is a conflict of interest on the effectiveness of glucosamine when consumed as drug. Very limited information is available on the studies concerned with the incorporation of glucosamine hydrochloride as food supplement.

With this background, a study was carried out to develop a functional fish sausage from tilapia incorporating glucosamine hydrochloride (GAH) and to assess the effect of GAH on the quality of the sausage. Quality attributes were compared between control and glucosamine hydrochloride (1 and 2%)-incorporated tilapia sausage. Addition of GAH did not affect the protein and fat content, whereas it resulted in an increase of total carbohydrate content significantly (p<0.05). pH value of control tilapia sausage decreased significantly (p<0.05) with the addition of glucosamine hydrochloride. Total volatile base nitrogen showed a significant increase (p<0.05)from an initial value of 11.2 to 19.6 and 22.4 mg N₂ 100g⁻¹ for 1 and 2% glucosamine hydrochlorideincorporated tilapia sausage, respectively (Table 1). A slight increase in the thiobarbutiric acid value and decrease in conjugated dienes formation was observed with the incorporation of glucosamine hydrochloride in tilapia sausage. Although incor-

	5			5		
Sausage samples	TVB-N (mg%)	рН	Peroxide Value (millieq kg ^{.1})	Free Fatty Acid value (as % oleic acid)	TBA value (mg malon- aldehyde kg ⁻¹)	Conjugated Diene
CONTROL 1% GAH 2% GAH	11.20±0.95 19.60±0.75 22.40±0.84	7.37±0.01 6.93±0.04 6.77±0.03	2.17±0.11 2.56±0.14 2.67±0.21	1.01±0.09 1.26±0.11 1.31±0.15	0.09±0.01 0.48±0.03 0.16±0.01	13.86±0.98 8.63±0.88 7.32±0.79

Table 1. Changes in biochemical quality of tilapia sausage incorporated with GAH

Table 2. Changes in physical and sensory quality of tilapia sausage incorporated with GAH

Sausage	Hardness	Springiness	Chewiness	Gel strength	Folding	Overall
samples	1 (N)	(mm)	(Nmm)	(gf /cm)	test	acceptability
CONTROL	57.91±0.36	9.79±0.40	266.20±7.66	1158.15±18.65	2	7.62±0.32
1% GAH	53.63±0.92	9.21±0.40	237.36±5.45	1031.08±22.34	1	7.06±0.57
2% GAH	45.84±3.22	9.60±0.37	218.75±4.33	1040.21±23.44	1	6.62±0.43

poration of GAH resulted in an increase in volatile bases and lipid oxidation products, it did not exceed the acceptable levels. No significant (p<0.05) variation was observed for instrumental L*, a* and b* values. Gel strength, hardness and chewiness was better for control tilapia sausage followed by 1% glucosamine hydrochlorideincorporated sausage (Table 2). The scores for folding test ranged from 1 to 2 for different products indicating that the samples retained gel strength even after addition of GAH. A significant (P<0.05) decrease in the hardness and chewiness was observed for tilapia sausage incorporated with 2% GAH compared to control samples. Sensorily, tilapia sausage incorporated with 1% glucosamine hydrochloride was found acceptable, whereas sausage with 2% glucosamine hydrochloride was not found suitable. The results indicated that glucosamine hydrochloride can be incorporated at a level of 1% in tilapia sausage without affecting its quality adversely. Regular consumption of glucosamine-incorporated food helps in augmenting the osteoarthritis related problems as it improves bone health.

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Study on water exudation and salt uptake during salting of Ribbonfish steaks

F ish preservation by salting and drying is a traditional method. Salt exerts bacteriostatic effect by osmosis and coagulation of metabolic enzymes. Drying in combination with salting achieves a greater reduction in water activity. Thus, the salting and drying practices extend the shelf life of fish. In addition to the preservative effect, this process leads to formation of unique aroma and flavor to the product. The salt uptake

and moisture loss is a simultaneous process which determines the quality and shelf life of the product. Salt uptake may vary with the species, size, thickness, weight, composition (lipid content and distribution), physiological state, salting method, brine concentration, duration of salting step, and fish-to-salt ratio. The present study was aimed at elucidating the mechanism of salt uptake and water exudation by Ribbonfish steaks during

Table	1.	Proximate	composition	of	Ribbonfish
(Leptu	irad	canthus save	ala)		

Parameter	Average value
	(% wet weight basis)
Moisture	76.17
Crude Protein	20.05
Crude Fat	3.18
Ash	0.80

brine salting.

Freshly landed Ribbonfish (*Lepturacanthus savala*) was used for the experiment. The proximate composition of fish was analyzed by the methods of Association of Official Analytical Chemists (AOAC, 1984). Steaks were prepared and brine salted using saturated brine (26.5% (w/v) sodium chloride in distilled water) at room temperature for 6 h. Samples were drawn at regular interval of 30 min. for analysis. Salt content was estimated by Mohr's Method.

The proximate composition of fresh Ribbonfish indicated that the moisture content of the fish was 76.17%. The protein content and crude fat content of fresh Ribbonfish was 20.05% and 3.18%, respectively.

The changes in the salt uptake and water exudation are given in Fig. 1. The Initial salt content of fish was 0.41% (dry weight basis) and



Fig. 1. Changes in the moisture loss and salt uptake of muscle



Fig. 2. Changes in the salt content of muscle



Fig. 3. Correlation coefficient between salt uptake and moisture loss

the final salt content was 17.6% (dry weight basis). Increase in salting time, increased the salt uptake and water exudation. A rapid rise in the salt content of muscle was observed in the first few hours of the process (Fig. 2). The impregnation level directly affected the moisture content. There exists a correlation coefficient between the salt uptake and moisture loss (Fig. 3). During the later stage the rate of salt uptake was reduced. The gradient between the salt content of the fish and brining system was the driving force responsible for the faster salt gain at the first few hours of the process. Over the salting time, the gradient was reduced as the salt concentration in the muscle was increased. The salt content layer that is formed on the fillet surface acts as a barrier against further salt uptake. The study clearly shows that the salt content of 17.6% could be achieved within 6 hr of brine salting process in Ribbonfish steaks.

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Modified icing system using plant extracts for fish preservation

Shelf life of ice stored fish, especially fatty fish is often limited due to post-mortem autolysis, microbial growth and lipid oxidation. Even though a number of synthetic antioxidants/anti-microbials were traditionally being used for the control of fish spoilage, currently, the use of synthetic additives are under strict regulation due to their potential health hazard in the promotion of carcinogenesis as well as general rejection of synthetic preservatives by consumers. Plant extracts, which are called bio-preservatives or green chemicals are now being viewed as potential alternatives to chemical preservatives. The use of plant extracts to extend the shelf life of chilled fish product is a promising technology since extracts from many plant sources have been established to control microbial growth and lipid oxidation in chilled fish. Recent studies have shown significant inhibitory effects of ice incorporated with natural antimicrobials and antioxidants on the microbiological and biochemical mechanisms involved in fish spoilage, as compared to conventional icing. Hence, a study has been undertaken to evaluate the efficacy of ice incorporated with extracts from mint (Mentha arvensis) leaf and citrus (Citrus aurantium) peel against that of conventional icing system on improving the quality and shelf life of whole Indian mackerel during storage.

Extracts were prepared from mint leaves and citrus peel using ethanol as solvent. Mint extract solution (700 mg/l) and citrus extract solution (1000 mg/l) were prepared in 2 ppm chlorinated water. Two litres of each solution were packed in a polyethylene bag and frozen to 0 °C in a commercial plate freezer. Conventional ice was prepared from 2 ppm chlorinated water. The whole mackerel was washed thoroughly in potable water and divided into three lots. First lot was stored in conventional ice (C ice), the second was stored in mint extract ice (ME ice) and the third lot was stored in citrus extract ice (CE ice) in individual thermocole boxes in 1:1 fish to ice ratio. The boxes

were further stored in a vertical chiller maintained at 0-2 °C. The ice was renewed during storage as and when required. Three fishes from each box were withdrawn at regular intervals and analyzed for its biochemical, microbiological and sensory qualities.

Icing with plant extract has significantly controlled the biochemical, microbiological and sensory quality changes during chilled storage. The development of Total Volatile Base-Nitrogen (TVB-N) was significantly higher in conventional iced groups than that in citrus extract and mint extract iced groups (Fig. 1). Similarly, presence of plant extracts in ice, particularly mint extract significantly (p<0.05) reduced the generation of Trimethylamine nitrogen (TMA-N) and free fatty acids (FFA) during storage. Moreover, a marked inhibition of lipid oxidation could be detected in fishes stored in ices with extracts as determined by the Peroxide Value (PV), (Fig. 2) and Thio Barbituric Acid Reactive Substances (TBARS). Additionally, the extract icing system also led to substantially lower counts of total viable bacteria (Fig. 3), Enterobacteriaceae and Pseudomonas spp., as compared with the fishes stored under conventional ice. The shelf life of fishes stored in traditional ice was determined as 13 days and that of extract iced fishes as 15 and 17 days for citrus



Fig. 1. Effect of plant extract icing system on the TVB-N of Indian mackerel





Fig. 2. Effect of plant extract icing system on the PV of Indian mackerel

extract and mint extract respectively. It can be concluded that natural plant extracts can be used by the fish processing industry to extent the shelf life of fish since they exhibited promising antiFig. 3. Effect of plant extract icing system on the Aerobic Plate Count (APC) of Indian mackerel

oxidant and antimicrobial effects when incorporated into the chilling medium.

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Medicinal role of shark liver oil against human ailments

Shark liver oil has been used for centuries as a folk medicine in India and abroad. Deep sea sharks are considered as good source of liver oil, since the liver of these species can account for up to 20% of their total weight. Shark liver oil is a rich source of squalene, squalamine, mono unsaturated fatty acid (MUFA), poly unsaturated fatty acid (PUFA), vitamin A, vitamin E, and alkyl glycerols, many of which are known for their immunological properties.

Bramble shark (*Echinorhinus brucus*) (Fig. 1) belongs to the family Echinorhinidae and typically seen at depths of 400 - 900 m of ocean. Bramble shark is known for its rich content of liver oil and this species is not included in the list of endangered fish species according to Zoological Survey of India, Ministry of Environment and Forests, Government of India.

Nutritional parameters such as proximate composition, amino acid and fatty acid profiles



Fig. 1. Bramble shark (Echinorhinus brucus)

of Bramble shark were analyzed in the Bio-chemistry and Nutrition Division of ICAR-CIFT, Cochin. Experiments on antiulcer and anti-inflammatory properties of liver oil from Bramble shark have been carried out in animal models revealing its biological activities against the said ailments.

Chloroform-methanol extraction of shark oil

Oil was extracted from Bramble shark liver (60 g) following the method of Folch *et al.* (1957) and Sankar *et al.* (2010). The oil was purified and

used to evaluate various bio-activities.

Animal study

Anti-inflammatory and anti-ulcer effects of Bramble shark were evaluated in experimental models of male albino rats.

Anti-inflammatory effect

Anti-inflammatory effect of liver oil extracted from *E. brucus* was determined by formalininduced rat paw edema test. Significant reduction of paw edema was observed in oil-treated rats and the activity was comparable to standard drug. Oil-treated subjects also showed significant reduction of paw size compared to untreated rats (Fig. 2). The result showed good anti-inflammatory potential of shark liver oil (Mathew *et al.*, 2008).



Fig. 2. Anti-inflammatory effect of shark liver oil as shown by reduction in rat paw edema

Anti-ulcer effect

Anti-ulcer effect of the liver oil was evaluated in animal model using albino rats. Hydrochloric acid (0.6% v/v) was used as ulcerogenic agent which was administered orally at the rate of 2.0 ml/kg body weight (Ganesan *et al.*, 2010). Significant anti-ulcer effect as seen in stomach lesion photographs was observed in oil treated animals, whereas vehicle (DMSO) showed no preventive effect (Fig. 3).

Conclusion

Bramble shark is nutritionally rich and the liver oil is proved to contain anti-inflammatory and anti-ulcer properties. Shark liver oil has been used as folk medicine by humans since ancient



Fig. 3. Stomach lesion showing anti-ulcer effect of shark liver oil

D. DMSO treated

times. Through the study, anti-inflammatory and anti-ulcer activities of oil derived from Bramble shark (*E. brucus*) were observed. Shark liver oil is rich in micronutrients like n-3, n-6 fatty acids, alkyl glycerols, vitamins and sterol derivatives which may have contributed to the anti-inflammatory and anti-ulcer properties.

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C. Oil+DMSO treated

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Development of extruded snacks by incorporating shrimp protein powder and hydrolysate

Shrimp processing industries generate large guantities of waste in the form of head waste and shell which guite often becomes a considerable waste disposal problem. The shrimp head waste, which represents about 33% of total weight is presently either converted in to manure or discarded. These wastes are rich in useful biomolecules and nutrients such as chitinous polymers, carotenoid pigment and protein. Protein hydrolysates have many uses in developing specialty foods such as non-allergic infant formula, sports drinks, diet supplement etc. Hydrolysates can be prepared from shrimp head waste by enzymatic hydrolysis and can also be used for food flavouring, colouring and protein enrichment. In modern days there is an ever increasing awareness about health foods. Fish and shellfish are gaining acceptance due to its nutritional and functional properties. A wide range of fish based products are available in the markets. Extrusion cooking is a food processing technique which is used worldwide to transform various ingredients in to ready-to-eat snack products. Different types of cereal flours are mainly used for extrusion processing.

In the present study, shrimp hydrolysate (SH) was prepared from shrimp head waste and shrimp powder was prepared from peeled and cooked shrimp. Further, it was incorporated with cereal flour (rice flour and corn flour) and extruded using twin screw extruder. Extruder conditions maintained were screw speed 350 rpm; barrel temperature 120 °C and the die diameter 2.5 mm. Shrimp hydrolysate and shrimp powder was added to cereal flour at the concentration of 5-10%. Fifteen formulations were made by using mixture response surface methodology.

Proximate composition of shrimp hydrolysate was 4% moisture, 70% protein, 10.97% fat and 11.44% ash on dry weight basis and that of shrimp powder was 7.3% moisture, 77.87% protein, 4.85% fat and 4.58% ash on dry weight basis. The average moisture content of all the extruded products varied between 4.20% and 4.72% and the average protein content of the extruded products varied between 9.19% and 15.75%. The effect of addition of shrimp hydrolysate and shrimp powder on the colour of extruded snacks revealed that L* values decreased with increase in addition of SH. Further, an increase in a* values was observed with addition of SH. Sensory evaluation showed that among the 15 formulations, the four formulations (Table 1 & Fig.1) showed more acceptability by the panelist. Table 1. Ingredient composition of extruded snacks preferred by sensory panel

-	-			
Sample	Rice	Corn	Shrimp	Shrimp
	flour	flour	powder	hydroly
	(%)	(%)	(%)	sate (%)
A	90	-	10	-
В	90	-	5	5
С	22.5	65	7.5	5
D	65	22.5	7.5	7.5



Fig. 1. Extruded snacks preferred by sensory panel

Further, B formulation had higher score than others. It was also observed that addition of shrimp hydrolysate in excess of 5% level reduced the expansion ratio and increased the crispiness. Results suggested that shrimp hydrolysate and shrimp powder can be used at 5-10% level for

developing protein-rich extruded products without affecting sensory characteristics.

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Isolation and characterization of Vibriocin from marine environment

In recent years regulation authorities are more stringent about the use of synthetic preservatives in the food industry. On the other side the emergence of new type of pathogens continues in food leading to higher inciden-ces of food poisoning outbreaks. Hence, pre-venting the growth of pathogenic microorganisms is essential for food quality and safety. Most of the decontamination technologies such as cooking, pulsed light, high pressure, ozone, ultrasound processing etc. are not efficient to destroy the pathogenic bacteria and are not compatible with the delicate texture and flavour of seafood. Hence, a new technology that is gaining widespread attention is the bio-preservation technology. Biopreservation implies inoculating the food with microorganisms, or their metabolites, which have potent antibacterial properties. One such biopreservative approach is the use of bacteriocins. Bacteriocins are bacterial substances having an essential biological protein moiety and a bactericidal mode of action centred against other bacteria. For example, Nisin has already been given the status of a preservative by USFDA and is being used commercially in food industries. Hence, an attempt has been made in Mumbai Research Centre of ICAR-CIFT for the isolation and purification of Vibriocin, a bacteriocin from Vibrio species. Further, its efficacy in inhibiting the growth of major seafood spoilage and pathogenic organisms has been evaluated.

A total of 40 Vibrio bacteria isolated from clam samples were tested for antimicrobial activity (Fig. 1). The activity was tested against nine different pathogens such as Aeromonas hydrophila, Bacillus cereus, B. subtilis, Escherichia coli, Listeria monocytogenes, Salmonella, Staphylococcus aureus, Vibrio cholerae and V. parahaemolyticus. Only two strains of Vibrio (Isolate 7 and 8) were shown to produce a bacteriocin-like substance (Fig. 1). Isolate 7 showed potent antimicrobial activity against Staphylococcus aureus (Fig. 2), whereas Isolate 8 showed potent antimicrobial activity against S. aureus and B. subtilis (Fig. 3).





Fig. 1. Bio-screening of the isolates against *Bacillus subtilis* and the zone of clearance around isolate Number 8

Fig. 2. Isolate 7 showing maximum activity against *S. aureus* after dialysis

The bacteriocin producing culture was centrifuged at 10,000 rpm for 10 minutes and the cell-free supernatant (CFS) was filter sterilized through 0.45µm syringe filter and tested for its activity under varying conditions such as pH, temperature and activity after proteolytic enzymes treatments. The bacteriocins retained their activity over a wide range of pH (4 to 8); however, maximum activity was observed at



Fig. 3. Isolate 8 showing maximum activity against *B. subtilis* after dialysis



Fig. 4. Enhanced effect of Vibriocin after treatment with SDS at different pH (Well No. 3, 4, 7, 8, 10); untreated control (Well No. 12)

neutral pH. Bacteriocin possessed the maximum activity at a temperature of 40 °C. Even though, the activity was observed at 60 °C, it was lost on heating beyond 30 min. at 60 °C, which indicates that the bacteriocins are well adapted to the environment. The bacteriocins showed complete sensitivity to the proteolytic enzymes such as lysozyme, papain, proteases and proteinase K, which suggests that the Vibriocin is a protein and will be destroyed by the intestinal enzymes. Hence, it is very safe for the consumers. It was also observed that, bacteriocins showed an enhanced zone of inhibition while treating with surfactant *viz.*, Sodium dodecyl sulfate (SDS). But, no clear zone was formed on treatment with urea in comparison to the un-treated control (Fig. 4). It could be assumed that the bacteriocin compound contains a disulphide bond which was cleaved by urea. Since, some of the bacteriocin gene is plasmid associated, an attempt has been made to isolate the plasmid by alkaline lysis method. However, no plasmid could be detected, this would suggest that the Vibriocin isolated in this study is not associated with plasmid.

The cell-free supernatant/crude bacteriocin was further subjected to concentration by lyophilisation technique, and then purified by salt precipitation technique with the use of 60% ammonium sulphate and dialysed with molecular weight cut off of 12000-14000 Da. It has been observed that the antimicrobial activity of the bacteriocin against *S.aureus* and *B. subtilis* was enhanced after concentration and purification.

The purpose of characterisation of bacteriocin was to utilize the antibacterial substance as a bio-preservative in foods. Strong inhibition activity of this potent bacteriocin against *Staphylococcus aureus* could be used as a natural preservative to enhance the shelf life of different processed food products. In future, the conventional and harsh chemical methods can be replaced with a safer and environment friendly bacteriocin.

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