Biochemical Characterization and Bioactivity Studies of Natural Products from Marine Sources

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Introduction

The Biochemistry and Nutrition Division of ICAR-Central Institute of Fisheries Technology, Cochin has done extensive work towards development of products, processes and methodologies from marine sources of nutritional and health significance. The concept of wealth from waste has been the focus of research. Several high value compounds/products have been extracted/produced from marine sources which are now in use in the form of tried and tested technologies.

PUFA soft gelatin capsules are an enriched source of heart-healthy omega 3 polyunsaturated fatty acids from sardine oil. Collagen and gelatin, developed from fish processing discards has found successful application in dental and pharmaceutical industries. Collagen and chitosan hydrogel and hand sanitizer developed in combination with wound-healing and antimicrobial constituents respectively, show great promise as commercial products. Nutritional and health benefits of fish and its constituents like shark liver oil, PUFA, taurine, squalene,

chitosan etc. have been well established through rigorous animal experiments.

Collagen peptide, bioactive peptides, functional derivatives of chitins and novel microencapsulated vitamins and marine lipids are the newest developments in our research group. In this chapter biochemical characterization and bioactivity studies of a few marine natural products/ functional products developed by our research group are enumerated.

Collagen peptide from fish skin: Nutraceuticals for treatment of arthritic joints

Collagen peptide is the hydrolysed form of collagen which is easily soluble in cold water. It differs greatly from other proteins as it contains amino acids glycine, proline and hydroxyl proline in a concentration that is around 10-20 times higher than in other proteins. These amino acids play an important role in building fibrous tissues. An insufficient supply of these amino acids results in painful joints, brittle fingernails and hair. Many studies indicate that collagen peptide has a preventive and regenerative effect on bones, cartilage and tendons.

Enzymatic hydrolysis process can produce small fragments of collagen peptides. Furthermore, some of its bioactivity increases and the antigenicity decreases after hydrolysis. We have optimized an enzymatic hydrolysis process using response surface methodology (RSM) for the production of collagen peptide from the skin of Malabar Grouper. RSM can evaluate the influence of all the variables in the multiple factor experiment design. A polynomial regression equation can be framed to predict the optimal condition of variables on the response. Here, we studied four main variables namely pH, temperature, the ratio of enzyme to substrate (E/S) and time to optimize the hydrolysis of fish skin. The Degree of Hydrolysis (DH) was set as response to evaluate the efficiency of hydrolysis. Three-dimensional response surface plots presenting the different combinations of input factors on the DH for protease are given in Figure 1. A three step hydrolysis of Grouper

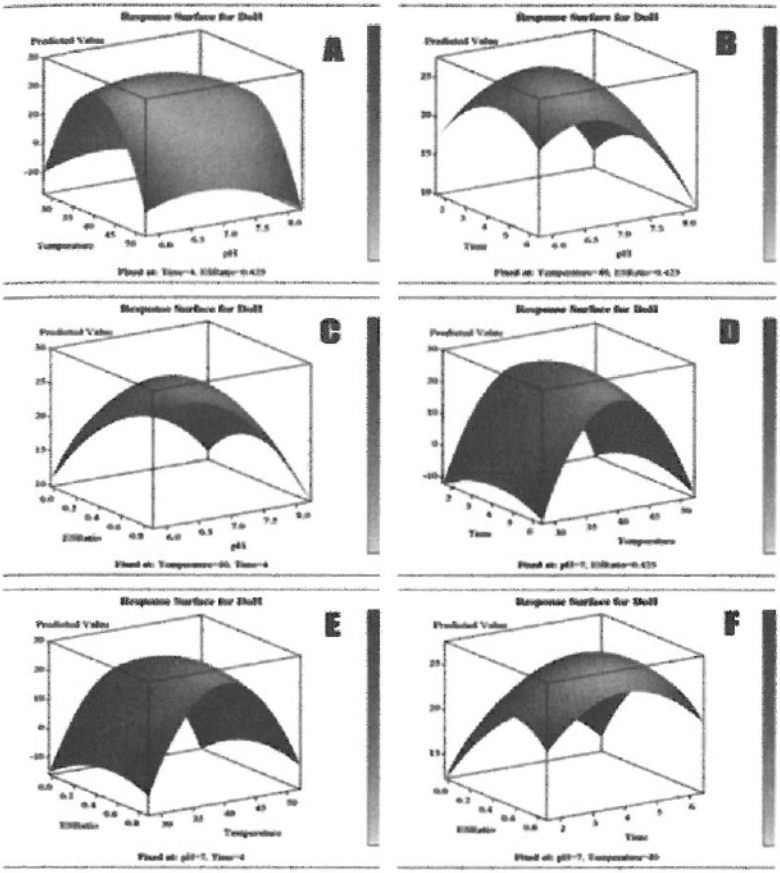

Fig. 1. Protease: Effects of different variables on the degree of hydrolysis presented in response surface (3D) plots

skin collagen was done using the three enzymes consecutively under the optimized conditions. The process resulted in a fine powder of collagen hydrolysate having degree of hydrolysis and nitrogen recovery of 56 ±0.73% and 76.11±1.03%, respectively.

Results from *in vitro* cell line studies clearly indicated that the presence of fish collagen-hydrolysate led to a dose-dependent increase in collagen synthesis by osteoblast cells (Figure 2). However native

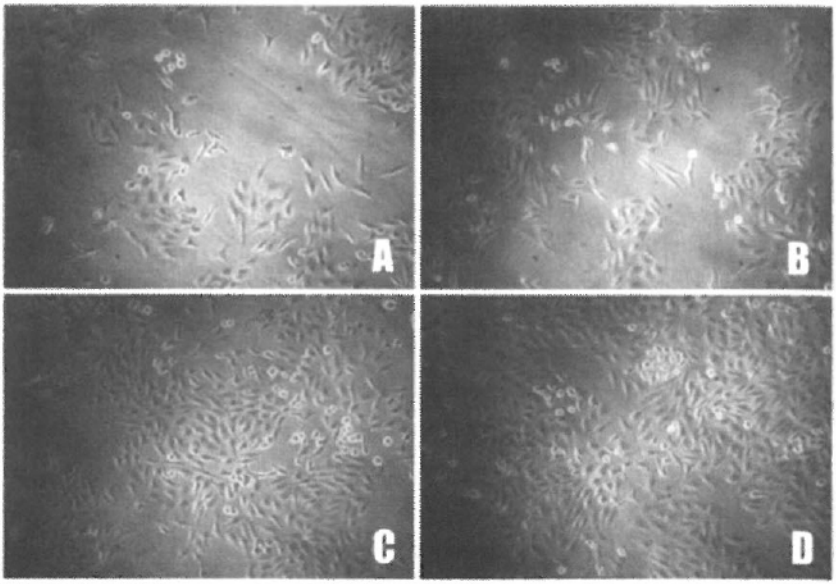

Fig. 2. Human osteoblast (HOS) cells after the different lengths of incubation time (A. HOS cells before FCP treatment, B. 6 hours after FCP treatment, C. 12 hours after FCP treatment, D. 24 hours after FCP treatment)

collagens or collagen free protein hydrolysate failed to stimulate the production of collagen in osteoblast cells. Since marked effects on human osteocytes were observed in our *in vitro* studies it is suggested that FCP from Grouper skin can be used as an effective nutraceutical in the treatment of arthritic joints.

Bioactivity evaluation of chitosan as a potent nutraceutical

Chitin is the second-most abundant carbohydrate biopolymer next to cellulose. It is available in crustacean exoskeleton, insect cuticle and fungi cell wall. However majority of commercial chitin is produced from shrimp shell waste. Chitosan and chito-oligosaccharides are produced by deacetylation and depolymerisation of chitin. In India annually 1,18,652 tonnes of shrimp waste is available for chitin production with a production potential of 3500 tonnes (Sathiadhas and Aswathy, 2004).

Most of the produced chitin and chitosan are exported as raw material to various industries with very little consumption domestically. Hence development of high value products from chitosan domestically will bring higher profitability and growth to domestic industry. ICAR-Central Institute of Fisheries Technology has rich legacy of industry collaboration and has transferred the technology for production of chitin and chitosan in the past. A group of scientists have been working on identifying exiting new properties and application of chitin, chitosan and its novel derivatives and composites. The work done in this aspect is outlined as follows:

Cardio-protection by dietary chitosan supplementation

Despite considerable advances in diagnosis and management over the last three decades, acute myocardial infarction continues to be a major public health problem. It is predicted that ischemic heart diseases will constitute the major disease-burden world-wide in the year 2020. In this study, an attempt has been made to examine the effects of dietary chitosan supplementation on lipid peroxidation and cardiac antioxidant defence system in isoprenaline-induced myocardial infarction in rats, an animal model of myocardial infarction in man.

Reactive oxygen species are designated as potent inducers of cellular injury in myocardial infarction. Peroxidation of endogenous lipid has been recognized as one of the central deteriorative reaction in cellular mechanisms of the cardiovascular pathology. The reaction of isoprenaline-induced hydroxyl radicals (OH•) with polyunsaturated fatty acids present in the cardiac cell membranes eventually leads to the formation of lipid radicals and eventually to short-chain aldehydes and hydroxyalkenals. These events can be followed by the formation of conjugated dienes, malondialdehyde and alkanes.

There was a significant ($p < 0.05$) increase in the level of lipid peroxidation in the heart tissue of Group III isoprenaline-administered rats as compared to controls (Table 1). In the present study, the animals supplemented with chitosan showed a significant ($p < 0.05$) decrease

in the level of lipid peroxidation in the heart tissue, thus indicating the antioxidant nature of chitosan in experimentally induced oxidative

Table 1. Levels of lipid peroxides (LPO), reduced glutathione (GSH), the activities of glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in the heart tissue of normal and experimental groups of rats

Parameters	Group I	Group II	Group III	Group IV
Lipid peroxidation				
LPO	$0.97\pm0.06_{a,c}$	$0.84\pm0.04_{a}$	$2.39\pm0.16_{b}$	$1.08\pm0.07_{c}$
Glutathione-dependent antioxidant system				
GSH	$4.53\pm0.28_{a}$	$5.12\pm0.42_{a}$	$2.15\pm0.15_{b}$	$4.68\pm0.31_{a}$
GPx	$2.55\pm0.19_{a}$	$2.79\pm0.24_{a,b}$	$1.46\pm0.26_{c}$	$3.12\pm0.09_{b}$
GST	$1195\pm72_{a,b}$	$1288\pm95_{b}$	$688\pm46_{c}$	$1078\pm85_{a}$

* Group I and Group III animals, fed standard diet with corn starch at 2% level for 60 days. Group II and Group IV, fed standard diet with added chitosan at 2% level for 60 days. Group III and Group IV animals, intraperitoneally (i.p.) injected with isoprenaline [11 mg (dissolved in physiological saline)/100 g body weight per day for two days] for the induction of myocardial infarction after 60 days feeding. Values expressed: LPO, nmol malondialdehyde released/mg protein; GSH, μmol g^{-1} wet tissue; GPx, nmol GSH oxidized min^{-1} mg^{-1} protein; GST, μmol 1-chloro-2,4-dinitrobenzene conjugate formed min^{-1} mg^{-1} protein. Results are mean ±SD for six animals; one way ANOVA; Duncan's multiple comparison test. Values that have a different superscript letter (a–c) differ significantly ($p < 0.05$) with each other.

stress condition.

Studies by Je *et al.* (2004) have shown that chitosan may eliminate various free radicals by the action of nitrogen on the C-2 position of the chitosan. Investigations by Xie *et al.* (2001) have demonstrated that the scavenging mechanism of chitosan is related to the fact that the free radicals can react with the hydrogen ion from the ammonium ions to form a stable molecule. The present observation also reveals that the supplementation of chitosan is capable of exerting antioxidant property against isoprenaline-induced lipid peroxidation.

There was a significant ($p < 0.05$) decline in the level of reduced glutathione (GSH) in the heart tissue of Group III isoprenaline-administered rats as compared to Group I normal control animals (Table 1). A parallel reduction in the activities of GSH-dependent antioxidant enzymes (GPx and GST) was also observed. Reduction of GSH results in enhanced lipid peroxidation, and disproportionate lipid peroxidation can cause increased GSH consumption, as observed in the present study.

Presence of GSH shields the membrane lipid bilayer from the harmful action of reactive oxygen species. Diminution in the activity of GSH-dependent antioxidant enzymes might be related to the reduced availability of GSH. GPx exerts protection to the myocardial membrane from isoprenaline-mediated oxidative stress by eliminating hydrogen peroxide and lipid peroxide. Reduction in the activity of GPx may lead to the accumulation of reactive oxygen species and makes sensitive myocardial cell membranes easily vulnerable to oxidative deterioration. Reduction in the activity of GST ($p < 0.05$) in isoprenaline-induced myocardial infarction may be due to down regulation of glutathione-S-transferase subunits.

It is possible that aberration in the status of GSH-dependent defense system is, at least in part, related to the pathogenic mechanisms of isoprenaline-induced myocardial dysfunction. In the present study, supplementation of chitosan at 2% level along with feed significantly ($p < 0.05$) prevented the isoprenaline-induced adverse effects on GSH-dependent antioxidant defence and maintained the rats at near normal status. Antioxidant efficiency of chitosan in neutralizing $OH^{\bullet}$ is comparable to that of the endogenous antioxidant GSH. Earlier studies have shown that chitosan potentiates free radical scavenging by a non-enzymatic process of electron donation.

Activities of antiperoxidative enzymes (SOD and catalase) were also significantly ($p < 0.05$) lowered in the heart tissue of isoprenaline-injected rats as compared to controls (Table 2). Our result is in

Table 2. Activities of superoxide dismutase (SOD) and catalase in the heart tissue of normal and experimental groups of rats

Parameters	Group I	Group II	Group III	Group IV
Antiperoxidative enzymes				
SOD	5.31±0.19a	5.82±0.28b	2.54±0.12c	4.58±0.31d
Catalase	6.45±0.28a,b	6.85±0.36b	3.75±0.25c	5.87±0.36a

* Group I and Group III animals, fed standard diet with corn starch at 2% level for 60 days. Group II and Group IV, fed standard diet with added chitosan at 2% level for 60 days. Group III and Group IV animals, intraperitoneally (i.p.) injected with isoprenaline [11 mg (dissolved in physiological saline)/100 g body weight per day for two days] for the induction of myocardial infarction after 60 days feeding. Values expressed: CAT, nmol H_2O_2 decomposed min^{-1} mg^{-1} protein; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation. Results are mean ± SD for six animals; one way ANOVA; Duncan's multiple comparison test. Values that have a different superscript letter (a–d) differ significantly ($p < 0.05$) with each other.

agreement with previously reported study. These two antiperoxidative enzymes, which are responsible for the devastation of reactive oxygen species, have a specific role in protecting myocardial membranes from necrotic damage. Decline in the activities of these antiperoxidative enzymes may lead to the generation of O_2- and H_2O_2, which in turn can form hydroxyl radical (OH•) and bring about a number of reactions harmful to the cellular and subcellular membranes.

Inhibition noted in the activities of these enzymes might be due to the increased formation of highly reactive oxygen radicals such as superoxide and hydrogen peroxide, which cause inactivation of these enzyme activities. SOD contains arginine and histidine residues in its active site. The free radicals formed during isoprenaline-induced myocardial infarction may attack on these highly reactive amino acids, which results in the chemical modification of the amino acids with loss of activity.

In the present study, the oral intake of chitosan significantly ($p < 0.05$) counteracted the isoprenaline-induced aberrations in the

activities of antiperoxidative enzymes and maintained the myocardial antioxidant status in the heart tissue of Group IV rats comparable to that of normal controls. It probably did so by its antioxidant potential against the isoprenaline-induced oxidative stress. Chitosan has been reported to trap free radicals by electron donating and radical resonating mechanisms, thereby blocking the lipid peroxidation chain reaction.

Reports by Jeon *et al.* (2003) have shown that chitosan has strong antioxidative effects, which decrease free radical production and increase antioxidant enzyme activities during CCl_4-induced lipid peroxidation in rats. Chitosan is a potent free radical scavenger and it is capable of acting as a stabilizer of cellular and subcellular membranes. It is possible that stabilization of myocardial membranes by chitosan may prolong the viability of ischemic cardiac muscle from isoprenaline-induced peroxidative damage. The normal rats receiving chitosan alone (Group II) did not show any significant change when compared with normal (Group I) rats, indicating that it does not *per se* have any adverse effects.

Marine nutraceutical squalene: Cardioprotective effect in isoprenaline-induced myocardial infarction in rats

Developing countries like India are struggling to manage the growing burden on the society and health systems caused by non-communicable diseases such as myocardial infarction. In India, myocardial infarction occurs 10 to 15 years earlier than in the west. An increasing number of young Indians are falling prey to myocardial infarction. The major abnormalities noticed in myocardial infarction are lipidemia, peroxidation and loss of plasma membrane integrity.

Natural products have been the starting point for the discovery of many important modern drugs. This fact has led to chemical and pharmacological investigations and general biological screening programmes for natural products all over the world. Squalene is a remarkable bioactive substance present in shark liver oil in higher quantities. It belongs to a class of antioxidants called isoprenoids, which

neutralizes the harmful effects of excessive free radicals produced in the body. Squalene has been reported to possess antilipidemic, antioxidant and membrane stabilizing properties. It has been used to cure cancer, skin disorders, cardiac ailments and liver diseases. Its antiaging, detoxification, cell invigoration and blood purifying properties have already been well studied. Scientific research and clinical trials have shown that squalene is safe as a dietary supplement in food and in capsules and no untoward incidents have been reported in the use of squalene.

An attempt has been made to assess the action of squalene on isoprenaline-induced changes in lipid profile in plasma and heart tissue in male albino rats by virtue of its antioxidant, hypolipidemic and membrane stabilizing properties (Table 3-5).

Table 3. Levels of cholesterol, triglycerides, free fatty acids, phospholipids, LDL-cholesterol and HDL-cholesterol in plasma of normal and experimental groups of rats

Parameter	Group I	Group II	Group III	Group IV
Cholesterol	76.8 ± 6.71	79.1 ± 6.58	118 ± 9.84a,b	87.5 ± 7.9c,d
Triglycerides	38.5 ± 3.18	34.9 ± 2.76	61.4 ± 5.42a,b	44.5 ± 3.47c,d
Free fatty acids	19.7 ± 1.74	18.1 ± 1.41	37.9 ± 2.71a,b	24.1 ± 1.64c,d
Phospholipids	103 ± 8.91	96.5 ± 8.26	134 ± 9.23a,b	112 ± 8.45c,d
LDL-cholesterol	40.1 ± 3.48	36.3 ± 3.14	65.9 ± 5.75a,b	44.9 ± 3.63c,d
HDL-cholesterol	19.4 ± 1.51	24.7 ± 1.76	11.2 ± 0.98a,b	18.0 ± 1.12c,d

*Group I and Group II, normal control, rats received standard diet mixed with coconut oil 2% and squalene 2%, respectively for a period of 45 days; Group III and Group IV, myocardial infarction was induced by intraperitoneal (i.p.) injection of isoprenaline [11mg (dissolved in physiological saline) $100g^{-1}$ body weight day^{-1} for two days] after 45 days of feeding with standard diet mixed with coconut oil 2% and squalene 2% respectively. Results are mean±SD for six animals. Values expressed: mg/dl. $aP<0.001$ significantly different compared with Group I control animals; $bP<0.001$ significantly different compared with Group II squalene administered rats; $cP<0.001$ significantly different compared with Group III isoprenaline-induced myocardial infarcted rats; $dP<0.05$ significantly different compared with Group II animals

Table 4. Levels of cholesterol, triglycerides, free fatty acids and phospholipids in heart tissue (mg/g wet tissue) of normal and experimental groups of rats

Parameter	Group I	Group II	Group III	Group IV
Cholesterol	5.4 ± 0.32	5.2 ± 0.27	10.4 ± 0.96a,b	6.01 ± 0.48c,d
Triglycerides	4.21 ± 0.33	4.03 ± 0.26	7.65 ± 0.63a,b	4.8 ± 0.37c,d
Free fatty acids	0.16 ± 0.02	0.11 ± 0.01	0.28 ± 0.03a,b	0.15 ± 0.01c,d
Phospholipids	23.5 ±1.91	25.4 ± 2.05	14.5 ±1.07a,b	20.4 ± 1.07c,d

*Group I and Group II, normal control, rats received standard diet mixed with coconut oil 2% and squalene 2%, respectively for a period of 45 days; Group III and Group IV, myocardial infarction was induced by intraperitoneal (i.p.) injection of isoprenaline [11mg (dissolved in physiological saline) $100g^{-1}$ body weight day^{-1} for two days] after 45 days of feeding with standard diet mixed with coconut oil 2% and squalene 2% respectively. Results are mean±SD for six animals. Values expressed: mg/dl. aP<0.001 significantly different compared with Group I control animals; bP<0.001 significantly different compared with Group II squalene administered rats; cP<0.001 significantly different compared with Group III isoprenaline-induced myocardial infarcted rats; dP<0.05 significantly different compared with Group II animals

Table 5. Levels of lipid peroxides in plasma and heart tissue of normal and experimental groups of rats

Parameter	Group I	Group II	Group III	Group IV
Plasma	1.78 ± 0.14	1.52 ± 0.12	4.21 ± 0.34a,b	1.95 ± 0.16c,d
Heart	0.94 ± 0.08	0.86 ± 0.08	1.99 ± 0.11a,b	1.03 ± 0.08c,d

* Group I and Group II, normal control, rats received standard diet mixed with coconut oil 2% and squalene 2%, respectively for a period of 45 days; Group III and Group IV, myocardial infarction was induced by intraperitoneal (i.p.) injection of isoprenaline [11mg (dissolved in physiological saline) $100g^{-1}$ body weight day^{-1} for two days] after 45 days of feeding with standard diet mixed with coconut oil 2% and squalene 2% respectively. Results are mean± SD for six animals. Values expressed: nmol MDA/ml in plasma; nmol MDA/mg protein in heart tissue. aP<0.001 significantly different compared with Group I control animals; bP<0.001 significantly different compared with Group II squalene administered rats; cP<0.001 significantly different compared with Group III isoprenaline-induced myocardial infarcted rats; dP<0.05 significantly different compared with Group II animals.

Prior administration of squalene at 2% level along with feed for 45 days significantly prevented the isoprenaline-induced elevation in the levels of cholesterol, triglycerides and free fatty acids in plasma and heart tissue of experimental groups of rats. It exerted an anitlipidemic effect by reducing the level of harmful LDL cholesterol with a parallel rise in the level of HDL cholesterol in plasma of experimental rats. A tendency to prevent the isoprenaline-induced depletion of phospholipids in the myocardium of experimental rats was also observed. In the study, the pre-treatment with squalene significantly counteracted the isoprenaline-induced lipid peroxidation and maintained the rats at near normal status. The results of the study indicate that the overall cardioprotective effect of squalene is probably related to an inhibition of lipid accumulation by its hypolipidemic property and/or to a counteraction of free radicals by its antioxidant nature.

Nutraceutical Oyster Peptide Extract (OPex) from edible oyster (*Crassostrea madrasensis*)

An edible oyster (*Crassostrea madrasensis*) peptide-based nutraceutical, 'OPex' has been developed by ICAR-CIFT. OPex is a 100% natural blend of oyster peptides and oyster protein concentrate that has been scientifically proven in experiments conducted in the Biochemistry and Nutrition Division, to possess several bio-activities. Bio-activities of significant mention are anti-inflammatory, anti-oxidant and anti-bacterial properties.

Oysters are a good source of high quality easily digestible protein and essential amino acids of high amino acid score and hence quite beneficial for human health. Increasing consumer knowledge of the link between diet and health has raised the awareness and demand for functional food ingredients and nutraceuticals. It is well recognized that apart from their basic nutritional role, many food proteins contain encrypted within their primary structures peptide sequences capable of modulation of specific physiological functions.

The antioxidant property of a protein hydrolysate is essentially a function of the inherent amino acid composition. Generally oyster -just as fish- has all the essential amino acids required for adequate human nutrition. Proteins in oyster used in this study occur in their native state and are folded in three-dimensional space in a manner that keep the bioactive amino acids particularly the aromatic and other hydrophobic residues buried in pockets away from exposure to aqueous environment. Enzymatic digestion that specifically cleaves after bulky hydrophobic residues in case of pepsin and basic amino acids in the case of papain, exposes these sites making them amenable to react with various biomolecules giving rise to bio-activities.

The peptide extract characterization has been carried out by sophisticated analytical instruments like high performance liquid chromatography (HPLC), LCMS, electrophoresis etc. This is the first instance in the country that a peptide based nutraceutical has been developed from oyster and it has immense potential that exists in the area of discovering bioactive compounds from marine sources. The product was officially launched by honorable Director General of Indian Council of Agricultural Research Dr. S. Ayyapan in a function held at ICAR-CIFT, Cochin on 5th April, 2012.

The antioxidant peptides present in the oyster protein hydrolysates (OPH) have good relevance in the living organism. Compounds that up-regulate the production of endogenous antioxidants such as glutathione (GSH) and antioxidant enzymes provide novel approaches for the restoration of redox homeostasis.

It is postulated that the active antioxidant peptides preserve the glutathione peroxidase antioxidant system, recognized for its potent antioxidant capabilities, and protect cells from lipid peroxidation and deleterious membrane structure changes. The effect of OPH on the lipid auto peroxidation of linoleic acid was investigated and it was found to inhibit the production of lipid peroxides. This research finding has direct relevance to impart antioxidant protection in humans.

Phenolic acid-grafted chitosan derivatives: Improved grafting ratio and potential application in functional food

Chemically modified biofunctional chitosan derivatives may open up new applications in functional food and nutraceuticals development. Two new conjugates, namely vanillic acid and coumaric acid-grafted chitosan derivatives were developed and comparative evaluation of their antioxidant and antimicrobial activities was carried out with ferulic acid and gallic acid-grafted chitosan.

The synthesized water soluble (1.0-4.5 mg/mL) chitosan-phenolic acid conjugates showed characteristic FTIR-spectroscopy band at around 1644 cm^{-1} and 2933 cm^{-1}. Free phenolic acid was not observed in TLC plate of the chitosan-phenolic acid conjugates and UV-vis spectra showed primary absorption peak of the corresponding phenolic acids confirming the grafting reaction. Total antioxidant activity of the chitosan-phenolic acid derivatives ranged from ~12-29 g ascorbic acid equivalent/100 g of the conjugate. Minute quantity of the derivatives (~57-162 μg and 82-109 μg respectively) could give 50% inhibition of 2, 2'-diphenyl-1-picrylhydrazyl radical and hydroxyl free radical. Broad spectrum antibacterial activity was observed for all four derivatives against an array of food-borne pathogens and spoilage bacteria. Coumaric acid-grafted chitosan showed promise as a food preservative as it inhibited the growth of several food-borne

Table 6. Free radical scavenging activity of chitosan-phenolic acid conjugates

Sample	μg of sample needed for 50 % inhibition	
	DPPH radical	Hydroxyl radical
Chitosan	---	---
Ga-Ch	57±0.25	82±0.57
Fe-Ch	98±0.21	102±0.38
Va-Ch	79±0.56	92±0.37
Co-Ch	162±0.41	109±0.71

pathogens and spoilage bacteria, including *Staphylococcus aureus*. Among all the four derivatives, ferulic acid and gallic acid-grafted chitosan had lowest minimum inhibitory concentration against *Staphylococcus aureus* and *Pseudomonas aeruginosa,* respectively.

Thiamine and pyridoxine loaded ferulic acid-grafted chitosan microspheres for dietary supplementation of water soluble vitamins

Therapeutic potential of water soluble vitamins has been known and recently they are widely supplemented with processed foods. Phenolic acid-grafted chitosan derivatives can serve as an excellent bio-functional encapsulating material for these vitamins. As a proof of concept, thiamine and pyridoxine loaded ferulic acid-grafted chitosan microspheres have been developed. Ferulic acid was successfully grafted on chitosan by a free radical mediated reaction and the structure was confirmed by FTIR and NMR analysis. The grafting ratio of the derivative was 263 mg ferulic acid equivalent/g polymer. Positively charged particles (zeta potential 31 mv) of mean diameter 4.5 and 4.8 μ, corresponding to number distribution and area distribution

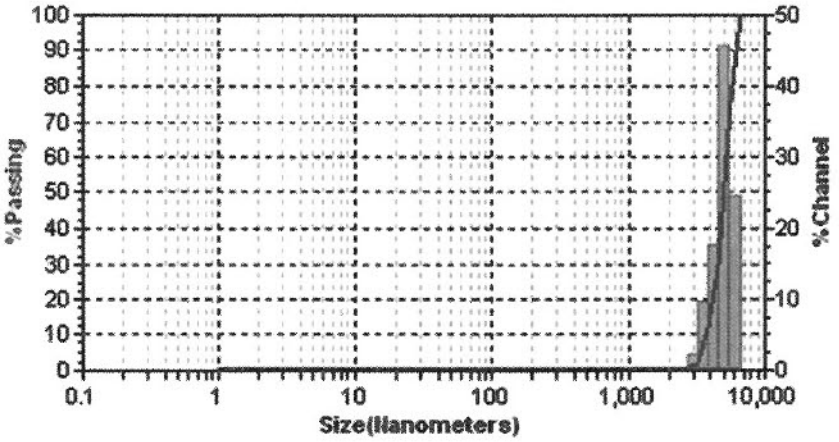

Fig. 5. Particle size distribution of vitamin loaded microspheres

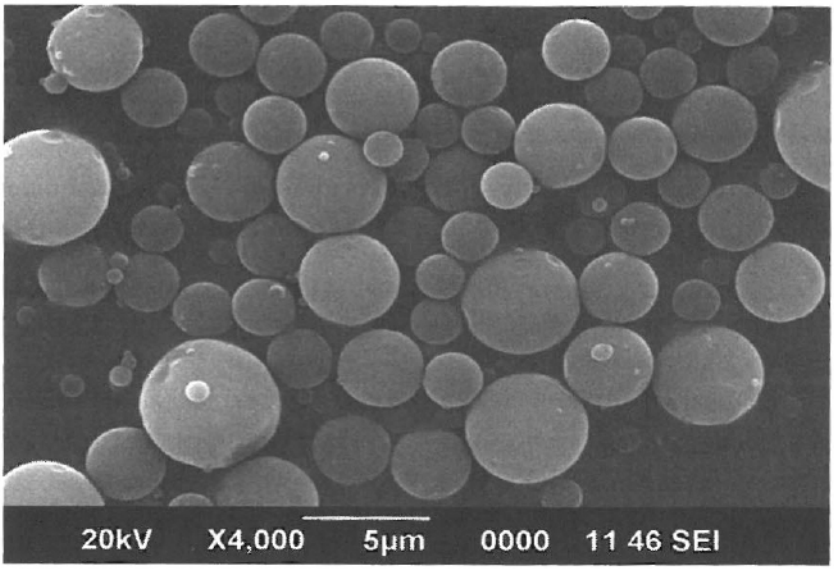

Fig. 6. Scanning electron microscope image of vitamin loaded ferulic acid-grafted chitosan microsphere

respectively were observed. Compact microspheres with smooth surface and no apparent cracks or pores were observed under scanning electron microscope. Efficient microencapsulation was further proved by X-ray diffraction patterns and thermal analysis. Preliminary anti-inflammatory activity of the vitamin loaded microspheres has been demonstrated.

Nutraceuticals from seaweed

Honourable Prime Minister of India, Shri Narendra Modi in his speech during 86th ICAR Foundation Day Celebrations urged the ICAR scientists to take up research work on extraction of valuable phytochemicals from seaweed, to promote commercial utilization of Indian seaweed resources. Despite having rich seaweed resources, the commercial utilization of seaweeds in India is limited to production of agar and alginates. However in western countries and Japan, seaweed is largely considered to be a health food. In China seaweeds form the

part of Chinese traditional medicine. Isolation of unique high value phytochemicals from seaweeds (Fucoxanthin, Phlorotannins, Fucoidan etc.) has drawn popular interest on seaweed globally.

We have developed a novel two-stage enzymatic extraction protocol for better extraction of bioactive compounds from brown seaweed. The extract was totally free from any harmful solvent and all the buffers and enzymes used were of food grade. The extract was further purified with the help of affinity chromatography to selectively isolate the "Fucoidan" and phenolic compounds. The extract showed better antioxidant activity as compared to solvent extract and hot water extract of the brown seaweed. Complete biochemical characterization of the extract involving various spectroscopic techniques were carried out. Based on the enzymatic extract we have developed two ready to serve functional food products, namely, Seaweed Nutridrink and Seaweed extract enriched soup powder (Figure 7 and 8).

The Nutridrink contains rich amount of essential micronutrients, amino acids and water soluble vitamins. The drink contains 38 mg of Fucoidan and 10 mg of gallic acid equivalent of phenolic compounds per mL.

Fig. 7. Seaweed nutraceutical drink

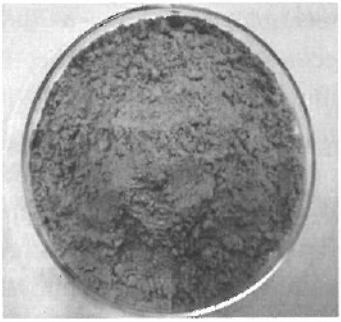

Fig. 8. Seaweed extract incorporated fish soup powder

Microencapsulation of fish oil with novel antioxidant chitosan derivative for enhanced oxidative storage stability

Fish oil has been known for various health benefits and has been commercially exploited in health food industry. However fish oil contains high amount of unsaturated fatty acids such as EPA and DHA. These are prone to oxidation during storage and food processing. Microencapsulation of fish oil with biocompatible biopolymer enhances ease of handling, widens its scope of use in food and provides protection against oxidation during storage and processing.

We have microencapsulated fish oil with vanillic acid-grafted chitosan, which shows excellent anti-oxidant and anti-bacterial property. Storage stability studies showed excellent protection against oxidation as the peroxide values remained within safe level during storage in ambient condition for a period of one month.

Conclusion

Research in the field of Biochemistry is being actively pursued to understand and utilize the potentials of coastal and marine bio-resources. In this respect, several bioactive molecules and processes utilizing the molecules have been developed in the Division. The technologies that were developed in the Division are simple and economically viable. They have been transferred to industry and are in use which is one of the success indicators for viability. Technology for developing collagen-chitosan membranes for dental and wound healing applications has undergone extensive trials in Dental Colleges throughout the country. After transfer and adoption by M/S EUCARE Pvt. Ltd., Chennai, the technology has proven successful. Since the raw material for collagen chitosan membrane preparation is fish processing waste that includes skin, gut and shell of fish and shellfish, the process is also a means of reducing environmental pollution. Fish processing industries generate a substantial amount of processing discards which forms the raw material for the technology and this makes the process sustainable too as the end products are of high value.

PUFA soft gel capsules are a concentrate of heart and brain healthy polyunsaturated fatty acids EPA and DHA. Sedentary lifestyles and unhealthy food choices have left people affected with multiple degenerative diseases like diabetes, heart disease, cancer, Alzheimers etc. The technology uses sardine oil which is simple to extract and enrichment of PUFA is easily accomplished. The venture is profitable to fish processing industries and is safe from the environment point of view. Sardine is abundantly available fish and exploitation of the species can be done in a sustainable way. The technology has been successfully transferred to M/S Strides Arco Lab, Bangalore. We have also successfully demonstrated the technology of enrichment of PUFA in poultry egg and meat. The eggs were found to contain 0.5 to 0.8 g of omega-3 when chicken were fed with PUFA enriched diet. PUFA enriched eggs and chicken meat were commercially produced on a trial basis in collaboration with a private poultry farm in Kerala. A price gain of 40% on eggs and 50% on meat was observed in the market, with the new technology. Commercial level backyard poultry units comprising 100 poultry/ household looked after by the woman at home became a successful model which can be scaled up.

Chitosan, a biopolymer derived from chitin, the second most abundant carbohydrate polymer on earth after cellulose has numerous applications in food and medical industries. A biocompatible and biodegradable composite of chitosan collagen and polyethyleneglycol hydrogel composite developed has wound healing property comparable to commercial wound healing gels. Hand sanitizer incorporating succinyl chitosan has also proved to be as good as commercial hand sanitizers. Trials are currently being carried out by M/S Perma Healthcare and the technologies are waiting to be transferred. Based on our *in vivo* bioactivity studies in several marine biomolecules such as chitosan, squalene, fish oil etc., local industries have come up with innovative products. For example Matsyafed under Kerala State Government is producing Chitone® capsuales (Chitosan and Vitamin C) as health supplement. Squalene is being produced and marketed by M/S Asha

Biochem, Kerala as health supplements.

References

Anandan, R., Chatterjee, N.S., Sivakumar, R., Suseela Mathew, Asha, K.K. and Ganesan, B. (2015) - Dietary chitosan supplementation ameliorates isoproterenol-induced aberrations in membrane bound ATPases and mineral status of rat myocardium. *Biol. Trace Elem. Res.*, **167:** 103-109.

Anandan, R., Ganesan, B., Obulesu, T., Suseela Mathew, Asha, K.K., Lakshmanan, P.T. and Zynudheen, A.A. (2013) - Antiaging effect of dietary chitosan supplementation on glutathione-dependent antioxidant system in young and aged rats. *Cell Stress Chaperones* **18(1):** 121-125.

Anandan, R., Ganesan, B., Obulesu, T., Suseela Mathew, Kumar, R.S., Lakshmanan, P.T. and Zynudheen, A.A. (2012) - Dietary chitosan supplementation attenuates isoprenaline-induced oxidative stress in rat myocardium. *Intl J. Biol. Macromol.* **51(5):** 783-787.

Chatterjee, N.S., Anandan, R., Navitha, M., Asha, K.K., Ashok Kumar, K., Suseela Mathew and Ravishankar, C.N. (2015) - Development of thiamine and pyridoxine loaded ferulic acid-grafted chitosan microspheres for dietary supplementation. *J. Food Sci. & Technol.* pp1-10 (DOI 10.1007/s13197-015-2044-4).

Chatterjee, N.S., Panda, S.K., Navitha, M., Asha, K.K., Anandan, R. and Suseela Mathew (2015) - Vanillic acid and coumaric acid-grafted chitosan derivatives: Improved grafting ratio and potential application in functional food. *J. Food Sci. & Technol.,* **11:** 1-10.

Shiny, K., Hema, G.S., George Ninan, Suseela Mathew, Joshy, C.G. and Lakshmanan, P.T. (2014) - Isolation and characterization of gelatin from skins of Skipjack tuna (*Katsuwonus pelamis*), Dogshark (*Scolidon sorrakowah*) and Rohu (*Labeo rohita*). *J. Food Hydrocolloides* **39:** 68–76.