Fishaceuticals – A Detailed Investigation

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Introduction

In the last two decades, consumer's preference towards food/ food supplements has shifted to look beyond the nutritional balance particularly for performance enhancement and disease prevention. This attitude of consumers has led to the emergence of a new class of products called nutraceuticals. It combines "nutrition" and "pharmaceutical" to indicate a food that has both health beneficial and nutritional properties. Approximately 20 years ago, the word nutraceutical didn't exist in the text. Nutraceutical ingredients are natural chemical entities possessing health promoting, disease preventing properties. In fact, the term is so broad that functional foods and beverages, dietary supplements, and any other type of food with health benefits fit into the nutraceutical category.

Fishaceuticals

Fishaceutical is a linguistic blend of words 'fish' and 'nutraceuticals' refering to the nutraceuticals produced/derived/isolated/extracted from fish. The term fish refers to the vertebrates with gills that live in water. In the present context the term 'fishaceutical' refers to neutraceuticals from finfishes, mollusks and crustaceans. Fish as such could be considered as functional food as it offers health promoting substances other than the major nutrients. Fishaceuticals can be classified into the following headings (Venugopal and Lele, 2015):

- Protein-based fishaceuticals (Proteins, bioactive peptides and protein hydrolysates, free amino acids, taurine etc.)
- Lipid-based fishaceuticals (Omega-3 fatty acids)
- Polysaccharide-based fishaceuticals (Chitin, chitosan, glucosamine, glucosaminoglycans etc.)
- Mineral-based fishaceuticals

Fish muscle protein

Seafood proteins are rich source of essential amino acids and are easily digestible. Upon consumption, fish proteins undergo digestion in the gastro-intestinal tract and release peptides of varying sizes. The size and sequence of peptides produced during the digestion are affected by various factors including the sequence of parent proteins, conditions prevailing in the gastro-intestinal tract, the way the fish is processed before consumption and the physiological state of individuals. The peptides produced may exert various health beneficial properties upon absorption other than the nutritional benefit. Thus, the fish proteins as a whole could also be considered as fishaceuticals. Fish protein powder is a dried fish product, meant for human consumption, in which the protein is more concentrated than in the original fish flesh. Different methods for the separation of meat from fish are employed, such as washing meat with water for two to three cycles and concentrating, solubilization of muscle by pH adjustment and iso-electric precipitation, solvent extraction method to remove fat, cooking and drying, enzyme/acid hydrolysis and a combination of various methods.

Earlier studies conducted on rat have shown that fish proteins have greater cholesterol lowering ability (Ammu *et al.*, 1989) and can protect the animal against lipid peroxidation. Fish protein reduces serum cholesterol, triglycerides and free fatty acids and increases the proportion of HDL cholesterol. In general, protein supplements claims to help weight loss and muscle building. Fish protein supplements have shown beneficial effects on blood levels of glucose and LDL-cholesterol as well as glucose tolerance and nutritional composition of body in overweight adults (Vikoren *et al.*, 2013). In another study, dietary scallop protein completely prevented high-fat, high-sucrose-induced obesity whilst maintaining content of lean body mass and improving the lipid profile of plasma in male C57BL/6J mice (Tastesan *et al.*, 2014).

Collagen and gelatin

Collagen is a structural protein found mainly in the skin and bones of all animals. Collagen is the most abundant protein originating from animal source, comprising approximately 30% of total animal protein. It is composed of three α-chains which are inter-twined to form a triple-helix. It is present in the connective tissue matrix that makes the framework of skin, bones and joints, cornea, blood ducts, and the placenta. There are many types of collagens, but 90% of our body's collagen protein is Type-I collagen. It is found to be rich in amino acids such as glycine, valine, alanine, proline and hydroxyproline (Burghagen, 1999). Glycine constitutes one third of the total amino acid content of collagen followed by hydroxyproline and proline, which account for another one-third. Owing to this structural uniqueness of collagen molecule, there is increasing interest for the direct consumption of collagen in the form of their easily digestible derivatives. World-wide, this interest has been taken-up by the nutraceutical industry, especially in developing countries.

Currently, collagen is used in many pharmaceutical and cosmetic products, due to its structural role and better compatibility with human body. It is commonly used in the cosmetic industry for the production of skin lotions as it forms a superior protective film to soothen and hydrate the skin. Such potential of collagen has tremendous bearing on anti-aging treatment. Apart from that, collagen has a wide range of applications in the field of cosmetic and burn surgery, especially as dermal fillers in the reconstruction of skin and bone. Collagen gels have potential clinical importance in the preparation of 'artificial skin' used in treating major wounds. Injectable collagen hydrogels have been successfully used for soft-tissue augmentation, drug delivery carriers and hard-tissue augmentation. Microfibrous collagen sheets are used as promising drug carriers for the treatment of cancer. It is also an essential component in diverse orthopedic and dental treatments. Further, collagen is recently projected as a joint mobility supplement.

Gelatin

Gelatin is a soluble polypeptide obtained by denaturing the insoluble collagen. Procedures to derive gelatin involve the breakdown of cross-linkages existing between the polypeptide chains of collagen along with some amount of breakage of intra-polypeptide chain bonds. Tissues that contain collagen are subjected to mild degradative processes, i.e., treatment using alkali or acid followed or accompanied by heating in the presence of water, the systematic fibrous structure of collagen is broken down irreversibly and gelatin is obtained. It is the only protein-based food material that gels and melts reversibly below the human body temperature (37 °C). Gelatin possesses unique and outstanding functional properties and can be obtained at reasonable cost, making it one of the most widely used food and pharmaceutical ingredient.

Fish skins and bones can be utilized to produce gelatin, thus contributing to solve the problems of waste disposal with the advantage of value addition. The main drawback of the fish gelatins are that the gels based on them tend to be less stable and have inferior rheological properties compared to mammalian gelatins. It may be noted that fish gelatin has its own unique properties like better release of the product's aroma and flavor with less inherent off-flavor and off-odor than a commercial pork gelatin, which offer new opportunities to product developers.

Fish protein hydrolysates (Bioactive peptides)

Apart from being highly nutritious, fish muscle proteins can be made use for preparing fish protein hydrolysates (FPH) which comprises of bioactive peptides with valuable nutraceutical and pharmaceutical potentials. Fish protein hydrolysates are the mixture of amino acids and peptides obtained by digesting proteins from fish meat or fish processing waste with proteases. The size of these peptides may range from 2 to 20 amino acid residues with the molecular masses of <6000 Da and are highly bioactive (Meisel and Fitzgerald, 2003). The food derived peptides can be used as functional food ingredients or as nutraceuticals to benefit human health and prevent disease. In this context, large pharmaceutical companies are more interested to invest in bioactive peptide research to open therapeutic prospects.

Collagen peptide

Collagen peptide is alternatively known as 'collagen hydrolysate', 'gelatin hydrolysate' and 'hydrolysed collagen'. Since collagen and gelatin are high molecular weight proteins of approximately 300 kDa, it is difficult to digest and hence becomes unavailable to human body for their biological functions. Consequently, in recent years, much attention has been paid to the development of small molecular weight peptides from the native collagen with improved biological activities. This can be achieved by the process of hydrolysis in which the native collagen/ gelatin molecules are cleaved to small fragments. The hydrolysis process leads to the formation of fragmenting from the collagen of about 300 kDa to small peptides having an average molecular weight of less than 5 kDa. The visible consequence of this hydrolytic transformation is the complete dissolution of resultant peptide mixture in cold water, which further widens the application prospects of collagen peptide.

Small peptides are desirable for nutraceutical and pharmaceutical applications, whereas large peptides are desirable for the functional modification of food products. Standardization of collagen production technology is a stepping stone in the nutraceutical and health food industry. From a nutritional perspective, peptides are more bioavailable than proteins or free amino acids and at the same time, less allergenic than their native proteins (Otani *et al.*, 1990). Apart from this, collagen peptides are shown to promote the absorption of vitamins and minerals. Hence, recently combined formulations of collagen peptide with minerals and vitamins are coming up in the market. Apart from their nutritional benefits, bioactive collagen peptides possess a wide range of physiological functions including antihypertensive, antioxidative, anticancer, immunomodulatory, antimicrobial, mineral binding, antithrombotic and hypocholesterolemic effects (Gomez-Guillen *et al.*, 2011). Enzymatically hydrolyzed collagen have shown better biological activities compared to the peptides derived from fish muscle protein with antioxidants and antihypertensive agents.

Type-1 collagen is the major type of collagen found in the scales, skin, bones and fins of various fish species. This indicates that there are ample opportunities for a fish filleting or surimi manufacturing factory to convert the so called 'waste' into biologically active molecules. In fact, this will be much lucrative than the food industry itself. ICAR-Central Institute of Fisheries Technology has developed a nutritional mix based on collagen peptide derived from fish scale containing 78% protein content (Fish Technology Newsletter, 2013). The product can be consumed along with milk as a health drink, can be a component of chappathi, or can be sprinkled over salads. The health-mix also contains natural mango pulp and oat fibres as the major ingredients, which effectively masks the blank taste of collagen peptide as well as modify the viscosity of the product (in drink). The product is mainly intended for middle aged and old people, ladies and sports-persons who need regular supply of collagen for healthy joints and bones. It may also be beneficial for patients suffering from osteoporosis and long-term-nursing home residents where there is a possibility of development of pressure ulcers.

Health beneficial claims of FPH

The peptides present in the FPH exhibit various biological activities such as:

- Antioxidant activity
- Antihypertensive activity
- Antiproliferative activity
- Anticoagulant activity
- Calcium-binding activity
- Antiobesity or antidiabetic activities
- Anti HIV properties

Among the bioactive properties of fish protein hydrolysates studied, antihypertensive activity and antioxidant activity has received wide attention over the years.

Taurine

Fish is a good source of taurine. Taurine is conditionally an essential amino acid that has been shown to involve in certain aspects of mammalian development. This molecule contains a sulfonic-acid group, rather than the carboxylic acid moiety, that is not incorporated into proteins. Taurine is one of the most abundant free amino acids in many tissues, including skeletal muscle, cardiac muscle and the brain tissue. Taurine is present in mackerel, albacare tuna, ray, shark, whiting fish and several other species. A wide range in taurine content from from 41-1040 mg/100 g has been reported in different fish species (Zhao *et al.*, 1998).

Health benefits of taurine

- Reduces blood pressure
- Improves the performance of cardiac muscle
- Reduces the level of blood cholesterol

Polysaccharide-based fishaceuticals (Chitin, chitosan, glucosamine and amino glycans)

Chitin

Chitin is a nitrogenous polysaccharide (poly N-acetyl Amino D-glucose) found in the outer skeleton of insects, exoskeleton of crabs and shrimps and internal structure of lobsters and other invertebrates. It is the most abundant organic compound next to cellulose in earth. Chitin represents 14-27% and 13-15% of the dry weight of shrimp and crab processing waste, respectively (Ashford *et al.*, 1977). Chitin is present as chitin protein complex along with minerals, mainly Calcium carbonate. So the process of chitin production consists of deproteinisation with dilute alkali and demineralization with dilute acids. Chitin on deacetylation gives chitosan and on hydrolysis with concentrated HCl gives glucosamine hydrochloride. ICAR-CIFT has developed a technology for production of chitin, chitosan and glucosamine hydrochloride from prawn shell waste.

Carboxy methyl chitin

Carboxy methyl chitin is another value added derivative of chitin. The conversion of chitin into carboxy methyl chitin came in to practice by carboxymethylation. It has successfully proved its use in the field of cosmetics as moisturizer, skin smoothener and a cleaner for face skin conditioning and it is used for the preparation of food products also.

Chitosan

Chitosan is prepared by deacetylation of chitin. Chitosan is almost colourless, light in weight and soluble in dilute organic acids. It's uses are hindered due to its insoluble nature in water, alkali and organic solvents. It gives viscous solution when dissolved in dilute organic acids such as formic acid, acetic acid, citric acid etc. Chitosan finds extensive applications in pharmaceutical applications, food industries and chemical industries. Chitosan is used in dental and surgical appliances as a haemostatic agent, wound healing, biodegradable films as a replacement for artificial skins for removing toxic heavy metals, wine clarification, industrial effluent treatment, agriculture, photography, cosmetic applications and textiles and as an immobilizing agent for enzymes.

Advantages of chitosan

Chitosan is soluble at acidic pH and becomes insoluble at pH above 6.5. Chitosan is a versatile polymer which has a variety of useful forms that are commercially available or can be made available. Since chitosan is non-toxic and can be administered orally, it has been mainly studied as an oral delivery system in the form of tablets or particles (microspheres, beads, etc.). Its muco-adhesive and permeation enhancement properties have been used for transdermal and sustained gastro-intestinal drug delivery. Its biodegradability and tissue compatibility make it a suitable compound for implantable delivery devices.

(a) Cholesterol and overweight lowering

Oral administration of chitin is generally recognized as safe (Harrison, 2002). Sugano *et al.* (1978) were the first to report the cholesterol lowering effect of chitosan and the report suggests that a diet containing 5% chitosan reduced liver cholesterol to half or more in cholesterol-fed rats. In similar studies reduction of both liver and serum cholesterol in rats fed on diets containing 1% chitosan and 0.1% bile salts was observed. Chitin has also shown to reduce plasma cholesterol in cholesterol-fed boiler chicken with diet containing 1.5 to 3% chitin. Thus the ability of reducing cholesterol in animals is well established (Razdan and Peterson, 1996).

Adult males fed on chitosan containing biscuits for two weeks (3g/day for Week 1, 6g/day for Week 2) experienced a significant decrease of 6% in total cholesterol. The reports of animal studies and human trials provide convincing evidence that chitosan is effective in lowering total and LDL cholesterol. Chitosan is also effective in lowering serum cholesterol and hypertension in humans with restricted diet. Chitosan is also being used as food supplement in persons suffering from obesity.

(b) Osteoarthritis prevention and treatment

A variety of pharmaceuticals such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), cyclo-oxygenase 2-inhibiters, intra-articular steroids, viscos supplements, vitamins etc. are effective in pain relief; but do not address the basic problem. Glucosamine and N-acetyl-D-glucosamine are involved in building up connective tissue in joints; e.g. glycosamionoglycans (GAG), chondroitin and hyauronic acid. Glucosamine serves not only as a substrate for the synthesis of GAGs but also trigger their synthesis and prevent degradation. In addition, these compounds provide protection against oxidative damage (Deal and Moskowitz, 1999). Different combinations of glucosamine are now in use for treatment of arthritis and the annual global consumption of glucosamine now exceeds 6000 tonnes.

Chitosan derivatives and their applications

Chitosan is not soluble in water but is soluble in dilute acid solutions like 1% acetic acid. This has limited its applications in water soluble environments like human health and plant protection. Hence, the free amino and hydroxyl groups can be derivatized with new molecules to improve the functional properties of chitosan.

Advantages of chitosan derivatives

1. They are biodegradable and biocompatible
2. They are non-toxic and water soluble
3. They can be modified to impart special properties

Important derivatives

1. **N-Trimethylene Chloride Chitosan:** N-Trimethylene chloride chitosan (TMC) is a quaternary derivative of chitosan and it has a superior aqueous solubility, intestinal permeability as well as higher absorption over a wide pH range.
2. **Chitosan Esters:** Esters of chitosan with glutamate, succinate and phthalate have a differential solubility profile. These esteric

forms are insoluble in acidic condition and provide sustained release of drugs in basic condition.

3. **Chitosan Conjugates:** Chitosan can be conjugated with a bioactive excepients for delivery of active ingredients such as Calcitonin. Chitosan conjugates such as 5-methyl pyrrolidinone chitosan, chitosan-4-thiobutyl amidine conjugate have exhibited enhanced absorption as well as muco-adhesives properties.

4. **Carboxymethyl Chitosan (CM-Chitosan):** CM-Chitosan is prepared by reacting mono chloroacetic acid with chitosan. The derivative is soluble in water and gives viscous solutions. An unique property of CMC is that it is more thermo-stable compared to the similar structural polymer carboxymethyl cellulose.

Major Applications

1. Controlled release and drug delivery
2. Scaffolds for biomedical applications like stents, organs etc.
3. Tissue engineering, wound healing and regenerative medicine
4. Food supplements and natural preservatives
5. Anti-viral and anti-tumor applications
6. Bio-composite materials with functional properties

Glucosamine hydrochloride

Glucosamine is chemically glucose in which a hydroxyl group on the second carbon atom is substituted with an amino group. It crystalizes as glucosamine hydrochloride during purification under acidic conditions. It is one of the amino sugars used by biological systems for bringing modification to the functions of proteins (Ronda and Zynudheen, 2014).

Health claims of Glucosamine

Although glucosamine was discovered long back, the interest in neutraceutical use received great attention since last two decades.

- **To treat joint pain**

  The rationale in using glucosamine for arthritis is that it is absorbed by the body and distributed to all organs. In the joint and synovial fluid this glucosamine will stimulate the synthesis of proteoglycans that help in repair of damaged cartilage. Although many clinical trials have shown benefits, the evidence is not equivocal. There are more than 100 generic preparations of glucosamine alone or in combination in the market.

- **As stomach antacid**

  Research in mice has shown that glucosamine is having good acid neutralization and peptic ulcer healing properties. Peptic ulcers is a major problem affecting adult human beings and oral administration of glucosamine will relieve pain associated with it and also help in synthesis of gastric mucosa to repair ulcer.

- **Anti-aging property**

  Latest research has claimed that glucosamine supplementation mimics low calorie diet in rats and increase the life span compared to control animals. Calorie restriction was proven in animals to improve the life span in laboratory studies. Although the mechanism of action of glucosamine is not clear in this case, it was shown to reduce the amount of glucose metabolized through the glycolytic pathway, thus mimicing low calorie diet.

- **Wound healing**

  Hyaluronic acid is highly hydrated and provides strength and elasticity to the skin. By binding and retaining some moisture in a wound re-epithelialization can proceed more quickly. This water-binding effect is also important for cosmetic uses of glucosamine. It can increase the skin's content of hyaluronic acid to increase moisturization, leading to enhanced skin barrier properties and reduced dryness.

- **Cosmetics**

Glucosamine has also been reported to have potential to inhibit skin melanin production. Glucosamine has been shown to inhibit glycosylation, the addition of polysaccharide units to proteins in *in vitro* melanocyte cell culture. Glycosylation is a required step in the conversion of certain inactive pro-enzymes to their active forms. Active tyrosinase, a key enzyme in the pathway for melanin production, is glycosylated. Thus, glucosamine inhibits the production of melanin in melanocytes.

Glycosaminoglycans (GAGs)

Glycosaminoglycans (GAGs) are heteropolysaccharides consisting of repeated units of disachcharides and no branched chains. Out of two monosaccharides, one is always an amino sugar (N-acetylgalactosamine or N-acetylglucosamine) and the other one is an uronic acid (Ronca *et al.*, 1998; Kogan *et al.*, 2007). They are present in the extracellular matrix of all animal cell surfaces and their function is to regulate different proteins such as growth factors, enzymes or cytokines. They found applications in food, cosmetic and clinical areas. The identified potential sources of glycoconjugates include mainly the marine organisms like sponges, sea cucumbers, squids or mollusks. The matrix of collagen associated with proteoglycans, macromolecules having a core protein to which the GAGs (chondroitin sulfate, keratan sulfate, dermatan sulfate and heparan sulfate) are covalently bonded by means of a tri-saccharide linked to a serine residue and form the cartilage tissue (Seno and Meyer, 1963). Hyalurunic acid (HA) is the only non-sulfated GAGs and is not covalently bound to the protein in any tissue. Chondroitin sulphate (CS) and HA are the most valued GAGs in market because of its abundance in mammalian tissues and notable physiological functions and high activity (Imberty *et al.*, 2007).

Chondroitin sulphate

Chondroitin sulphate (CS) consist of repeated disaccharide units of glucuronic acid (GlcA) and N-acetylgalactosamine (GalNAc) linked by β-(1→3) glycosidic bonds and sulfated in different carbon positions (CS non-sulfated is CS-O). The classification and type of CS is dependent on sulfate group placing: carbon 4 (CS-A), 6 (CS-C, more common), both 4 and 6 (CS-E), positions 6 of GalNAc and 2 of GlcA (CS-D) and 4 of GalNAc/2 of GlcA (CS-B). The function of the organism and tissue influence the composition (shark, CS-D; dogfish, CS-A and CS-D; squid and salmon, CS-A and CS-E; ray, CS-A and CS-C) and concentration (e.g., 9% in shark fin and 14% in chicken keel) of CS. Thus, CS from marine sources differs in chain length and over-sulfatation (Vázquez *et al.*, 2013).

Chondroitin sulfate is involved in various biological processes such as the function and elasticity of the articular cartilage, hemostasis, inflammation, cell development, cell adhesion, proliferation and differentiation by being an essential element of extracellular matrix of connective tissues. Chondroitin sulfate with less than 20 kDa is orally administered in nutraceutical formulations indented to treat and prevent osteoarthritis.

Hyalurunic acid (HA)

In marine organisms, the eyeball of fish species is the only clear source of HA which is present in the vitreous humor (VH). VH volume and HA concentration varies with the fish species, for instance, HA is obtained from eyeballs of shark and swordfish at 0.3 g/L from 18 mL of VH and 0.055 g/L from 70 mL, respectively (Imberty *et al.*, 2007). HA is also present in the cartilage matrix as structural element and of the most researched benefits of hyaluronic acid is its ability to alleviate pain in joints. Its effectiveness in this area is not surprising since hyaluronic acid is especially concentrated in the knees, hips, and other moving joints. It is a major component of both cartilage and the synovial fluid that bathes these joints, binding to water to create a thick, gelatinous substance that lubricates and protects the cartilage. In addition to playing a vital role in joint health, another major benefit of hyaluronic acid is its effectiveness of maintaining a healthy, youthful skin. The reason is because hyaluronic acid is an essential component of the skin. The suggested oral dose of hyaluronic acid is 100–200 mg per day.

Lipid-based fishaceuticals

Omega-3 fatty acids

The health benefits of seafood have mainly been attributed to the marine lipids. The beneficial effects of seafood have traditionally been ascribed to the presence of omega-3 polyunsaturated fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The amounts of fatty acids most frequently found in seafood range between 8 and 12% EPA and between 10 to 20% DHA. These fatty acids accumulate in the fish muscle *via.* the food chain from plankton. This arises from the fact that marine phytoplankton has a high ratio of EPA and DHA, and thus these fatty acids are accumulated in the food chain. These fatty acids have pleiotropic effects and influence the *in vivo* production of inflammatory components, blood theology, and membrane functionality. Due to their potential effects in promoting health, recommendations for daily intakes of n-3 PUFAs have been published by several international scientific authorities and several organizations (Jeyakumari, 2014).

Health benefits claims of ω-3 polyunsaturated fatty acids

- Improvement of vision acuity, field of vision and adaptation to light. Alteration of permeability, fluidity, thickness and lipid phase properties of retinal photoreceptor membrane (DHA only)
- Reduction of risk of myocardium lesion and coronary heart disease *via.* lowering blood pressure, triglyceride levels and platelet aggregation, and prevention of arrhythmias
- Protective effects against some common cancers (breast and colon) *via.* inhibition of eicosanoids synthesis and increase of tolerence to noxious therapies (e.g. chemotherapy)

- Increase of level of insulin and decrease of heart beating rate
- Beneficial effects in sclerosis treatments *via* affecting functions of central nervous system
- Preventive and curative effects upon non-alcoholic fatty liver disease *via.* lowering liver fat content; Improvement of emotional state (e.g. in depression and anxiety conditions)
- Beneficial effects on auto-immune diseases (e.g. rheumatoid arthritis, psoriasis, systemic lupus, Crohn's disease) and reduction of joint pain associated with inflammatory conditions, *via.* inhibition of central regulators of inflammation.

Squalene

One of the most interesting compounds available from fish is squalene, which is found in deep sea shark's liver oils. Squalene is most abundant in the liver oils of deep sea sharks, commonly called spiny dogfish. The liver oil of these species contains about 40 to 85% squalene by weight of oil. Squalene is a polyunsaturated hydrocarbon, better known as Isoprenoid or Isoprene. By virtue of its double bond structure attached to six methyl [- CH_3] groups, the isoprenoid has an exceptionally strong antioxidative effect in cell metabolic activities (Sreedharan, 2014).

Health beneficial claims

- Involved in eventual formation of cholesterol and its steroid metabolites
- Squalene protects the cellular and cytoplasmic organoids and bio-membranes from oxidative stress
- Reduce the LDL cholesterol (bad cholesterol)
- Maintain and protect the youthfulness of skin by increasing collagen synthesis
- Squalene protects normal cells and vital organs from the highly

toxin-free radicals generated by secondary metabolites of chemotherapy drugs by free radical scavenging action

- Protects bone marrow, thereby enhances the production of RBC cells during chemotherapy drug administration
- Squalene can reduce the inflammatory effect of radiation and DNA damage to normal organs during radiation therapy
- Squalene improves immunity and can be used in immunity-compromised diseases and conditions
- Squalene is found effective in anti-inflammatory effects on psoriasis vulgaris and atopic dermatitis
- Various epidemiological and laboratory data suggest that squalene help prevent cancer at the outset and can also fight established tumors

Mineral-based fishaceuticals

Fish bone calcium - Calcitone

It is well documented that consumption of whole small fish is nutritionally beneficial providing with a rich source of calcium. Calcium and phosphorus comprise about 2% (20 g/kg dry weight) of the whole fish. Small fish are often eaten whole, including bones. However, in the case of large fishes like tuna, bone is a major component of processing waste. Thus, the filleting wastes of tuna and other bigger fishes are very good sources for calcium when the quantity of calcium is concerned. Calcium derived from the filleting waste can be used for pharmaceutical purposes, as it is mainly in the form of calcium phosphate with ideal calcium-phosphorus ratio. ICAR-CIFT, Cochin has optimized the process to extract calcium from fish bone which is mainly treated as processing discard during filleting operation of larger fishes, *viz.* tuna, carps etc. Before packing, the calcium powder was supplemented with vitamin D which is known to enhance absorption and bio-availability of calcium in the body. *In vivo* studies conducted at ICAR-CIFT in albino rats have shown that fish calcium powder supplemented with vitamin D has improved the absorption and bio-availability. The product is packed in 400 mg capsules containing 200 mg calcium per capsule.

Carotenoid pigments in shrimp

Studies on efficient utilization of shrimp industry byproducts have concentrated mainly on recovery of chitin and chitosan from waste. In recent years, much attention is being paid for extracting valuable constituents from shrimp waste like natural carotenoid pigments. The most prevalent carotenoid found in shrimp is astaxanthin, 3,3'-dihydroxy-i,i-carotene-4,4'-dione representing about 65-98% of the total carotenoids present and consisting of three sterioisomers (3S,3'S; 3S,3'R; 3R,3'R). Three different forms of this particular pigment are recognized in crustaceans, namely diesters, monoesters and the free form. Normally, wild caught shrimps will have more pigmentation compared to their cultured counterparts. Highest carotenoid content was reported in the head of deep-sea shrimp *A. alcocki* (180-185 μg/g) and marine shrimp *P. stylifera* (150-155 μg/g) followed by *P. monodon* (120-135 μg/g) and *P. vannamei* (120-130 μg/g). High levels of carotenoids were also reported in carapace of *A. alcocki* (115-120 μg/g), *S. indica* (117-120.0 μg/g) and *P. stylifera* (100-105 μg/g). Relatively low levels of carotenoids were reported in shrimp *P. indicus*, freshwater prawn *M. rosenbergii* and crabs. The major carotenoids in shrimps, freshwater prawn and marine crab was reported as astaxanthin and its esters. β-carotene and zeaxanthin were reported at low levels in these species. Most of the carotenoids in shrimps are distributed in the shell (nearly 84-87%) and only 12-15% is present in flesh. The recovered carotenoids can be used as colourants in food products, used as antioxidant and as pigment source in diets of ornamental fishes, farmed salmon, trouts etc. Carotenoids from shrimp waste can be used in prevention of cancer, enhancement of immune responses and improvement of visual function. Currently the major application of carotenoids include nutritional supplements, pharmaceuticals, food colourants, and animal feed additives (Mohan *et al.*, 2014).

Nutraceuticals in the global market

Global scenario

The global nutraceutical market comprising of functional foods and beverages and dietary supplements, was valued at around $250 billion in 2014. Consumer demand for nutraceuticals is rapidly increasing with the market and is expected to reach around $385 billion by 2020. Nutraceutical products have a highly competitive market and price compared with conventional therapies; in particular their market share is increasing in many regions including the United States of America (USA), Europe, Japan, Asia Pacific, Middle East and Latin America. The analysis of the global industry offers a detailed report of the industry structure, market drives, trends, issues and competition. The global market is dominated by the USA, Europe and Japan who contribute more than 85%. It is predicted that these three regional markets will remain at the forefront of the nutraceutical industry both as producers and consumers due to greater product awareness, higher income levels and a preference for nutraceutical products, preventive medicine and self-treatment. It is estimated that the Asia Pacific region, particularly China and India, will experience significant growth in the long term, because of increasing awareness, income and wealth (Source: Frost and Sullivan; www.frost.com).

In the regions where nutraceutical product consumption is high, a number of dietary supplements, medical foods, food complements, and functional foods have invaded the food and health market with the aim of maintaining health and preventing disease. Within these regions, consumption rates of these products vary with the country, age and sex of the population. It has also been found that intake of nutraceutical products varies with lifestyle. Thus individuals with healthy lifestyles are more commonly the users of nutraceutical products. The intake of these types of products is also of special relevance for aging populations. Elderly people are more susceptible to micronutrient deficiencies due to a variety of factors including social, physical, economic and emotional obstacles to eating. Marine nutraceutical products represent a large portion of the global market and are derived from a diverse range of sources that provide a myriad of bioactive molecules (Source: Frost and Sullivan; www.frost.com).

Indian Scenario

India's nutraceuticals market is projected to cross $6.1 billion by 2020 from the current level of $2.8 billion growing at compound annual growth rate (CAGR) of about 17 per cent (Hindu News, August 20, 2015). The Indian nutraceuticals market is at a nascent stage but is expected to grow at fast pace in the coming years. Currently, India accounts for around 1.5 per cent of the global market. At present, India does not have any kind of regulatory guidelines for the approval or monitoring of the products under this segment.

These products are regulated under the guidelines of FSSAI, 2006 amended in 2011 for registration, licensing, approval, labelling and packaging, import, marketing and distribution, laboratory testing like conventional food products. It is highly demanding that FSSAI should come up with proper guidelines related to manufacturing, storage, packaging, labelling, distribution, sales, claims and imports. This will bring clarity to the industry stakeholders and they can invest in the industry with no fear of counterfeiting. In the absence of regulations, the products take much longer time to reach the market. For industry growth, it is utmost necessary to give faster approvals for eligible nutraceuticals. For faster growth of the domestic market, both private players and government should create awareness about the health benefits of nutraceuticals among masses through campaigns, social media and television.

Fishaceuticals - Today and tomorrow

Fish nutraceuticals take a small share of the $22 billion global nutraceuticals market projected in 2013. The marine nutraceuticals market will grow steadily as the global nutraceuticals and functional foods markets continue to expand. Developers and marketers of fishaceuticals and bio-ingredients should focus on identifying products for domestic or global markets; exploring indigenous bio-resources for domestic markets, building on the existing infrastructure, and expanding to overseas markets; securing sustainable resources through aquaculture and establishing high-throughput screening tests (Lee *et al.*, 2009). Development of fishaceutical products and ingredients is highly market-driven and requires good marketing strategies. Special attention should be given to types of products (i.e., fishaceutical ingredients or functional foods) and to the most suitable form of product in terms of compatibility, stability, consumer acceptability, and regional preference. To succeed in the marketplace, products require good science-based clinical evidence.

References

Ammu, K., Jose Stephen and Devadasan, K. (1989) - Influence of dietary proteins on cholesterol levels in albino rats. *Fish. Technol.* **26(2):** 125-130.

Ashford, N.A., Hattis, D. and Murray, A. E. (1977) - Industrial prospects for chitin and chitosan from prawn shell waste. MIT, Sea Grant Report. MITSG 77-3, MIT, Cambridge.

Burghagen (1999) – Collagen, In: Food Chemistry (2nd Ed.), Belitz, H.D. and Grosch, W. (Eds.), Springer, Berlin, pp. 540–547.

Deal, C.L. and Moskowitz, R.W. (1999) - Nutraceuticals as therapeutic agents in osteoarthritis. The role of glucosamine, chondroitin sulfate, and collagen hydrolysate. *Rheum Dis. Clin. North Am.* **25(2):** 379–395.

Gòmez-Guillèn, M.C., Turnay, J., Fernández-Diaz, M.D., Ulmo, N., Lizarbe, M.A. and Montero, P. (2011) - Structural and physical properties of gelatin extracted from different marine species: A comparative study. *Food Hydrocolloids* **16:** 25–34.

Hindu News, August, 20, 2015. http://www.thehindu.com/business/Industry/nutraceuticals-global-market-to-touch-2629-b-by-2020/article7562397.ece.

Imberty, A., Lortat-Jacobb, H. and Pérez, S. (2007) - Structural view of glycosaminoglycan–protein interactions. *Carbohydr. Res.* **342:** 430–439.

Jeyakumari, A. (2014) - Health and functional foods from fish. In: Development of nutraceuticals, health foods and fish feed from fish and shellfish processing discards, Zynudheen, A.A., Bindu, J., George Ninan, Mohan, C.O. and Ronda, V. (Eds.). CIFT Cochin, pp-228-232.

Kogan, G., Soltés, L., Stern, R. and Gemeiner, P. (2007) - Hyaluronic acid: A natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnol. Lett.* **29:** 17–25.

Lee, C.M., Barrow, C.J., Kim, S.K., Miyashita, K. and Shahidi, F. (2009) - Global R&D Trends in Marine Nutraceuticals. IFT Annual Meeting. http://static1.squarespace.com/static/50529c7ce4b020ebebdf5cfe/t/528103e9e4b0c24b160fc5f1/1384186857791/Food+Tech+Mar+Nutra+1-16-12.+pdf.pdf.

Meisel, H. and Fitzgerald, R.J. (2003) - Biofunctional peptides from milk proteins: Mineral binding and cytomodulatory effects. *Curr. Pharm. Des.*, **9:** 1289-1295.

Otani, H., Dong, X.Y. and Hosono, A. (1990) - Preparation of low immunogenic peptide fragments from milk casein. *Milchwissenschaft*, **45:** 217-220.

Razdan, A. and Peterson, D. (1996) - Hypolipidaemic, gastrointestinal and related responses of broiler chickens to chitosans of different viscosity. *British J. Nutrition*, **76:** 387-397.

Ronca, F., Palmieri, L., Panicucci, P. and Ronca, G. (1998) - Anti-inflammatory activity of chondroitin sulfate. *Osteoarthr. Cartil.* **6 (Suppl. A),** 14–21.

Ronda, V. and Zynudheen, A.A. (2014) - Glucosamine hydrochloride and its applications. In: Development of nutraceuticals, health foods and fish feed from fish and shellfish processing discards. Zynudheen, A.A., Bindu, J., George Ninan, Mohan, C.O. and Ronda, V. (Eds.). CIFT, Cochin, pp-228-232.

Seno, N. and Meyer, K. (1963) - Comparative biochemistry of skin mucopolysaccharides of shark skin. *Biochem. Biophys. Acta* **78:** 258–264.

Sreedharan, M. (2014) - Therapeutic application of squalene – A component of fish oil. In: Development of nutraceuticals, health foods and fish feed from fish and shellfish processing discards, Zynudheen, A.A., Bindu, J., George Ninan, Mohan, C.O. and Ronda, V. (Eds.). CIFT, Cochin, pp-228-232.

Tastesen, H.S., Keenan, A.H., Madsen, L., Kristiansen, K and Liaset, B. (2014) - Scallop protein with endogenous high taurine and glycine content prevents high-fat, high-sucrose-induced obesity and improves plasma lipid profile in male C57BL/6J mice. *Amino Acids* **46(7):** 1659–1671.

Vázquez, J.A., Rodríguez-Amado, I., Montemayor, M.I., Fraguas, J., González, M.P. and Murado, M.A. (2013) - Chondroitin sulfate, hyaluronic acid and chitin/chitosan production using marine waste sources: Characteristics, applications and eco-friendly processes: A review. *Mar. Drugs* **11:** 747-774.

Venugopal V.M. and Lele, S.S. (2015) - Nutraceuticals and Bioactive compounds from seafood processing waste. Kim, S.K. (Ed.), Springer Hand Book of Marine Biotechnology. Springer Dordrecht Heidelberg, London, pp, 1406-1426.

Vikøren, L.A., Nygard, O.K., Lied, E., Rostrup, E. and Gudbrandsen, O.A. (2013) - A randomised study on the effects of fish protein supplement on glucose tolerance, lipids and body composition in overweight adults. *British J. Nutrition* **109:** 648–657.

Zhao, X.U., Jia, J. and Lin, Y. (1998) - Taurine content in Chinese food and daily taurine intake of Chinese men. In: Advances in Experimental Biology and Medicine, Lombardini, J.B., Schaffer, S.W. and Azuma, J. (Eds). Plenum Press, New York, USA, pp. 501-505.