

History, Production Development and Industry of Seafood in Japan

Toshiaki Ohshima

Tokyo University

The utilisation of natural products from marine resources for the production of nutraceuticals and functional foods is now common. For example, formula milk products enriched in docosahexaenoic acid by adding purified fish oils are available in Japan and other countries. There are now more than 200 food products which contain added fish oil. Topics on other natural compounds from marine resources will be introduced. These include milt proteins which can be isolated from fish gonad and decrease the growth rate and heat resistance of certain spoilage bacteria and therefore used in food preservation as an antibacterial agent. Transglutaminase and ϵ -polylysine improve the elastic properties of heat-induced gel (*kamaboko*) produced from low grade surimi. Many advanced scientific studies on isolation and utilisation of bioactive natural products for pharmaceutical applications, including anti-microbial anti-mould, anti-virus and anti-tumour compounds, have been carried out. Certain bioactive compounds have already been applied to pharmaceutical uses. Eicosapentaenoic acid ethyl ester encapsel products with over 92% of purity are used for pharmaceutical for arteriosclerosis and for hyperlipidemia. Chitosan sheets are used as an artificial protector against skin damage such as cut and burns in surgical operations. This chapter summarises the use of marine nutraceuticals and functional foods in Japan

1. TRADITIONAL JAPANESE SEAFOOD

There have been certain marine food products that are considered to be rather specialties of Japan from ancient time. That is canned products, dried products, including boiled-dried products, salted-dried products, dried fish sticks, frozen-dried products, seasoned-dried fish as well as smoked fish and shellfish, Japanese style fish meat pastes, fish sausage, fish oils and meals. Among these products, using traditional processes from ancient resume has produced

fermented fish products.

Fermentation means the phenomenon that organic components in raw materials are hydrolysed by microorganisms. Alcoholic beverages, dairy products and seasonings are well known agricultural fermented products. Fermented marine products include salted preserves "*shiokara*", liquid fish sauce, matured sushi "*nare-zushi*" as well as pickled products "*su-zuke*".

Each fermented marine food is produced by hydrolysing the materials with the autolysis and the action of microorganisms, suppressing the decomposition in the presence of sodium chloride. Consequently, the fermented marine products have *umami* taste due to formation of glutamic acid and inosinic acid as well as characteristic flavor.

1) Fermented seafood

There are many varieties of foods prepared by the fermentation of fish meat, roe, milt or visceral mass as well as shellfish. These may be summarized as follows:

- a) Fermented squid meat "*ika-shiokara*" : Squid (*Ommastrephes sloani pacificus*) mantle meat and the visceral mass (liver) are fermented together, spoilage being prevented by the addition of salt (about 15~20%).
- b) Fermented bonito visceral mass "*shu-to*" : The pyloric appendage of bonito (*Katsuwonus Pelamis*) is fermented with sufficient salt to prevent spoilage.
- c) Fermented sea-urchin "*uni-no-shiokara*": The roe or milt of the sea-urchin are fermented with salt to prevent spoilage. When the fermented sea-urchin is drained and kneaded, the commodity is called "neri-uni" (kneaded sea-urchin).
- d) Fermented "*ayu*" : There are several kinds in the products, including "*uruka*" (fermented meat of "*ayu*"), "*ko-uruka*" (fermented roe), "*shiro-uruka*" (fermented milt), "*niga-uruka*" (fermented visceral mass excluding roe and milt), "*kirikomi-uruka*" (fermented mixture of muscle meat and visceral mass).
- e) Fermented visceral mass of the sea-cucumber
- f) Fermented roe of crabs
- g) Fermented visceral mass of abalone
- h) Fermented *Calanus blumchrus* "*ami-shiokara*" There are also many other products made by fermenting mollusc meat (shellfish meat), roe or visceral mass of other fishes.

2) Cured fermented seafood

Various kinds of cured fermented seafood are marketed as follows

- a) Cured fermented in boiled rice "*I-sushi*"

The products of fermented-"sushi" are the old style Japanese-"sushi" of today ('nigiri-

sushi). The product is prepared from "ayu" (*Plecogrossus altivelis*), "funa" (prussian carp; *Carracius auratus*), "tai" (sea bream; *Pagrosomus major*), sand fish (*Aretoscopus japonicus*), "bora" mullet; (*Viugil cephalus*), salmon, etc. The procedure is different according to the place of production and kind of fish meat employed. Generally speaking, fish bodies from which visceral mass has been removed are cured with 20~30% of their weight of salt. In particular the ventral cavity is filled with salt. The cured fish bodies are stored under a stone weight for one to two months. After the cured fish is re-salted, the bodies are drained. Boiled rice and *koji* are sprinkled the bottom of a barrel, and then the desalted fish bodies are placed the layers of boiled rice and "*koji*"; the ventral cavity is also filled with boiled rice and "*koji*". The amount of boiled rice added is about 50% of the fish material, and that of "*koji*" is a half that of the boiled rice. The filled barrel is pressed with a weight. The fermentation will be completed after 10 days. It has the best taste after two months of fermentation.

b) Cured fermented in "*Koji*" which is prepared by inoculating *Aspergillus oryzae* on steamed rice and grow it "*Koji-zuke*"

Cured products in "*koji*" are prepared from various kinds of fish, mollusca and their roe. For example, "*ayu*", sea-bream, mackerel, anglerfish, herring, octopus, squid, mackerel roe, etc. The common method for the preparation of the cured products in "*koji*" is as follows. Fish or roe is first cured with salt. After removal of the liberated liquid "*koji*" is added to the salted fish or roe and the material is fermented for some period. Protease in "*koji*" ferments the meat or protein during the storage giving good flavor and taste.

c) Cured-fermented in rice-bran "*Nuka-zuke*"

Fish meal or roe is employed after salt-curing or drying. The amount of salt added is sufficient to preserve the fish meat or roe and rice bran itself from spoilage. It is about 30~35% by weight of the salt-cured or dried fish meat or roe. The amount of rice bran is 30~70% the weight of the fish meat. During the fermentation of fish meat or roe and rice bran, the mixture must be thrown away.

d) Fermented in "*kasu*" which is "*sake*"-lees "*kasu-zuke*"

After fish meat or roe have been salted they are cured in "*sake*" - lees, or sweet "*sake*"-lees. The flavor and taste of the lees are transferred to the fish meat or roe and their taste becomes delicious. On the other hand, the fish meat or roe and autolyzed somewhat, becoming soft. "*Ayu*", salmon, cod or abalone meat are employed as raw materials.

e) Vinegar-pickled "*Suzuke*"

Vinegar-pickled products are prepared at home. In Japan octopus and squid are soaked in vinegar or pickled as commercial products. The products include sardine, herring, or mackerel meat in vinegar, Alaska pollack meat in vinegar, as well as octopus or squid meat in vinegar.

3) Fermented fish sauce

This product is prepared by continuing the fermentation of the fermented fish meat for longer than one year. "*Sbottsuru*" which is prepared from sand fish, *Arctoscopus japonicus* "*hata-hata*" is the most famous product. Products from squid visceral mass, sardine, anchovy or other mollusca meat are also prepared, though the amounts of production are small.

2. MODERN SEAFOOD INDUSTRIES

According to the up-to-date annual report on Japanese fisheries (MAFF, 2000), Japanese fishery products output in 1998 amounted to 6.7 million metric tons valued at 2.0 trillion yen, and has gradually decreased for the last decade. Annual imported fishery products in that year amounted to 5.2 million metric tons. A Japanese self-supplying rate for fishery products excepting animal feed and fertilizer was about 57% in 1998, 23 point down from 1988.

Under these severe conditions surrounding the fisheries of Japan, maximum recovery from marine resources is very important because recent under-utilized marine resources in Japanese coastal area are not so rich. In this chapter, some topics on value-added utilization of marine resources carried out in Japan will be introduced.

1) Fish oils with omega-3 polyunsaturated fatty acids (3-PUFA)

Fish oils are produced from the whole body or guts of various fish species. Their main component is triacylglycerol which is rich in PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Epidemiological research of Dyerberg *et al.* (1975) on comparison between the Inuit inhabitants in Greenland and the inhabitants in Denmark itself revealed that; 1) in both cases 40% of the total calorie intake came from fat-based high-calorie foods, and 2) death originating in coronary heart diseases (CHD) was lower among the Inuit people living in Greenland. Based on more detailed comparative studies on food consumption and the lipid compositions of the serum, they found that; 1) differences in dietary PUFA were accurately reflected in the fatty acid compositions of plasma lipids, and 2) the occurrence of CHD was not solely genetic but affected by EPA consumed from fish

and fur seals, the diets of the Intuits. At that time, however, relationship between the frequency of CHD and EPA intake remained still unknown. After highly purified EPA became available, the researches have accumulated a large volume of data on physiological functions of EPA.

Much of EPA consumed from diet is -oxidised in liver cells and synthesised *in vivo*, but some EPA is incorporated into cell membrane by the action of phospholipase A₂. Since the melting point of EPA is low at -54.4°C, the EPA incorporated in glycerophospholipids of biomembrane in this way causes physical properties of the membrane, including biomembrane fluidity. Indeed, dietary ingestion of sardine oils, which are rich in EPA (Otsuji *et al.*, 1984) or EPA (Makrides *et al.*, 1993), increases the fluidity of human erythrocyte membrane. A wide range of physiological functions of eicosanoids is summarised in Table 1.

Table 1. Physiological actions of eicosanoids

(Hwang, 1992)

Eicosanoids	Effect
PGE ₁	Inhibits platelet aggregation
PGE ₂	Vasodilation, increases cAMP levels, decreases gastric acid secretion, suppresses immune response, luteotropic action
PGI ₂	Relaxes smooth muscle, vasodilation, inhibits platelet aggregation, raises cAMP levels
TXA ₂	Contracts smooth muscle, causes platelet aggregation, bronchoconstriction
PGD ₂	Inhibits platelet aggregation, raises cAMP levels, causes peripheral vasodilation
LTB ₄	Neutrophil and eosinophil chemotaxis, leakage in microcirculation, raises cAMP levels, causes neutrophil aggregation
LTC ₄ -LTD ₄	Contracts smooth muscle, constricts peripheral airways, leakage in microcirculation, decrease camp levels
12-HETE- 12-HPETE	Neutrophil chemotaxis, stimulates glucose-induced insulin secretion
15-HETE	Inhibits 5- and 12-lipoxygenase
Lipoxin A	Superoxide anion generation, chemotaxis, activates protein cell activity

Dietary supplement of fish oil or EPA over a period of time has the effect of reducing blood triglycerides in healthy subjects, hyperlipidemia sufferers, laboratory animals, and hyperlipidemia model animals alike. The detailed mechanism of this physiological action of EPA remains obscure. When the lipoprotein composition of plasma and their constituent lipid classes were compared after dietary ingestion of fish oils to hypertriglyceridemia subjects for a few weeks, a marked reduction of very low density lipoprotein (VLDL) that bear the role of transporting triacylglycerols. This was caused by a decrease in triacylglycerols (Inagaki and Harris 1990).

Dietary ingestion of EPA has the recognised action of inhibiting platelet aggregation. Decrease

in the platelet membrane arachidonic acid (AA) with substitution by EPA leads to the decrease in production of thromboxane A₂ (TXA₂) which is effective in promoting platelet aggregation. At the same time, cyclooxygenase produces TXA₃ which is not effective to antiplatelet action from EPA incorporated into membrane phospholipids. In addition to this, changes in membrane physical properties due to the incorporation of EPA causes affinity of phospholipase A₂ located in the surface of membrane and the separation of AA is suppressed (Akahane *et al.*, 1995; Surette *et al.*, 1995). If the membrane supply of AA decrease with an increase in the ingestion amount of EPA, production of prostaglandin I₂ (PGI₂), which is produced from AA and has the effect of expanding arteries, also decreases (Mann *et al.*, 1994). In this way, EPA is important as a factor in anti-arteriosclerosis action.

Epadel[®] (Mochida Pharmaceuticals, Co., Tokyo, Japan), which contains more than 92% EPA ethyl ester, received approval for commercial production as a pharmaceutical for arteriosclerosis in 1990 and for hyperlipidemia in 1994. It is produced from sardine oils in which the original level of EPA is 15~16%. Sardine oils are generally ethyl-esterified and purified by an ultra-vacuum rectification system consisting of four fractionating towers lined in series (Fig. 1). After this distillation process, the level of EPA ethyl ester increases to about 82%. The partially purified EPA ethyl ester is then treated by a urea-adduction process and silica gel treatment to increase the level of EPA ethyl ester to more than 92% (Hata and Makuta, 1990; Hata *et al.*, 1992a, b). It is then encapsulated in a gelatin capsule, and α -tocopherol is added to increase its stability against autoxidation. The final product is stable for 6 months at 50° C or 3 years at room temperature, and its acid value remains about 0.1 mg KOH/g lipid after 3 years under nitrogen atmosphere (Hata, 1993).

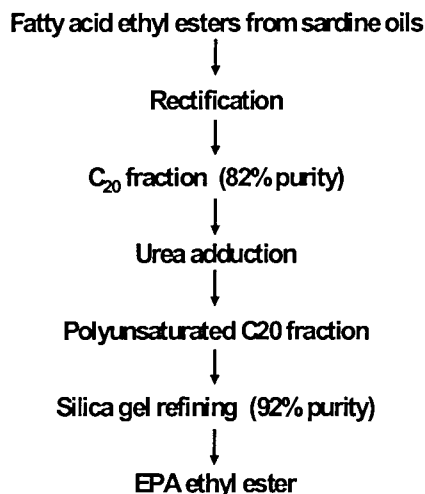


Fig. 1. Purification of EPA ethyl ester.

The partially hydrogenated fish oils have a higher viscosity and are used as a raw material for the production of margarine, shortening and detergents. Uauy *et al.* (1992) found that the retinal functions are more or less complete in infants after 57 weeks of conception (4 months after birth) and revealed that the component ratio of PUFA with 20 and 22 carbon numbers in phosphatidylethanolamine in erythrocyte phospholipids was significantly high in the infant group fed with formula milk containing fish oil (Fig. 2). When the retinal potential of infants was measured in the 36th week after conception (8~13 weeks after the start of test feeding), electrical activity in the retina was found to be in proportion to the ω 3 fatty acid content (Uauy *et al.*, 1992). Thus, while the effect of DHA on retinal functions has been under some scrutiny in recent years, this shows that DHA is essential to the development of retinal functions, particularly in premature infants (Gibson *et al.*, 1994; Harris *et al.*, 1998). Thus, formula milk products enriched with DHA by the addition of purified fish or algal oils are now commercially available in Japan and the USA. The number of other fish oil-added food products has now reached more than 200, including pudding, candy, juice, and others. Their market value is expected to be more than 25 billion yen.

Fish oils rich in DHA (50~65%) with enzymatic modification and chemical esterification of acyl groups are commercially available. However, DHA is quite susceptible to autoxidation under ambient conditions because it has six ethylenic double bonds. Therefore, stabilisation of DHA against oxidation is necessary for commercial application in food products. Encapsulated micro-powders (20~30 mm in diameter) containing 50% or 30% DHA-rich oil were developed to prevent off-flavour development due to the oxidation of DHA during processing. They are

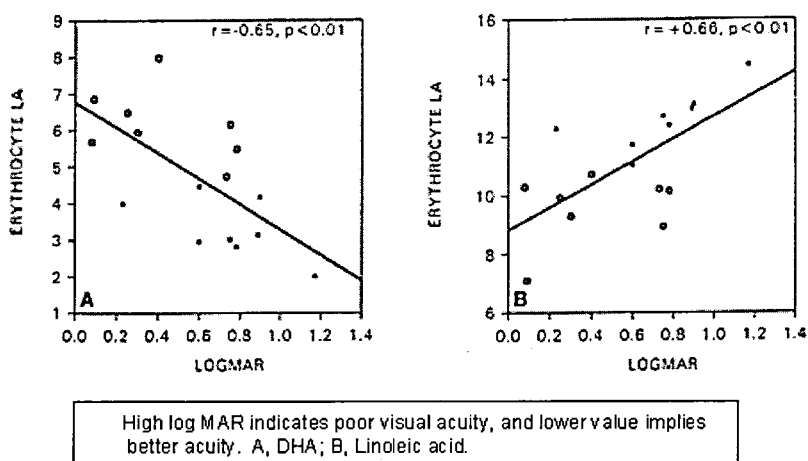


Fig. 2. Relationship between erythrocyte phospholipids DHA and visual acuity of infants.

○, infants exclusively fed breast milk; ■, infants predominantly fed infant formula. (Adapted from Makrides *et al.*, 1993).

produced as follows: Cornstarch is treated with a starch-degrading enzyme to provide elasticity (Monma *et al.*, 1990). A DHA-rich oil is encapsulated in the enzyme-treated starch, followed by coating with an alcohol-soluble protein obtained from corn. The oxygen permeability of the protein coat is almost 0 mL/m²/24 hr/atm., so the DHA covered with the protein becomes stable against autoxidation (Suzuki, 1995).

2) Effects of marine lipids on the taste

According to Japanese traditional concepts, the best season for consuming sardine and scallop is generally between July and August, for the Pacific saury is between September and November, and for the Pacific oyster and yellowtail is between December and February. Generally, fish accumulate lipids gradually in their tissues with sexual maturation, and their lipid contents reach maximum just before the spawning season. This season is cited as "shun" in Japanese, and most consumers in this country know that fish become tasty in this particular season. Addition to this, many Japanese consumers prefer a part of fish meat with high fat content compared to lean part. For example, many Japanese consumers believe that fatty tuna meat "Oh-toro" is tasty compared to lean part "Akami" of the same fish meat, though the pure extracted oils themselves do not have any taste. In our own sensory perception tests in humans, we obtained results showing that triacylglycerols extracted from southern bluefin tuna enhanced Umami taste of glutamic acid and inosinic acid in emulsion. Fig. 3 shows the effect of DHA-rich triacylglycerols in enhancing "umami" taste. Triacylglycerols enriched in 22:6 ω 3 showed a similar function to the tuna triacylglycerols (Ohshima, 2000; Ushio *et al.*, 2001). Similar function of marine oils in the enhancing of Umami taste was reported in the case of emulsions with bigeye tuna oils (Koriyama *et al.*, 2000a, 2000b). On the other hand, emulsions consisting of soybean oils and lard and the fish extract did not reveal the "Umami" enhancing function. Detailed mechanisms for the enhancement of "Umami" taste by marine lipids are still obscure. Gilbertson *et al.* (1997)

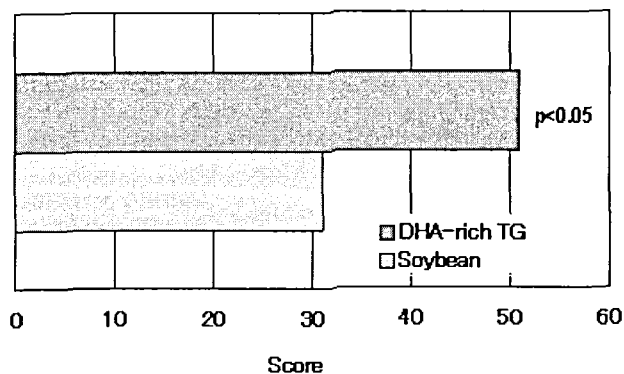


Fig. 3. Effect of DHA-rich triacyl-glycerols in enhancing 'umami' taste.

reported physiological evidence for the gustatory cue for fat in which the patch-clamp recordings of isolated rat taste receptor cells revealed that cis-PUFA, including 18:3 ω 3 and 20:4 ω 6 inhibited delayed rectifying K^+ channels. When mouse tongue was treated by EPA or DHA, the bitter taste response of chorda timpani against quinine hydrochloride or denatonium was inhibited (Nakajima and Ninomiya, 2001). These results from neurophysiological studies suggest that PUFA can play a role in terms of controlling "Umami" taste of seafood.

3. APPLICATION OF MARINE NUTRACEUTICALS AND FUNCTIONAL FOODS

1) Chitin and Chitosan

Chitin is one of the prominent components of arthropoda and mollusca tissues. The exoskeleton of crustaceans is one of the suitable raw materials for production of chitin. Demineralisation is carried out by washing the exoskeleton with dilute sulphuric acid. Proteins are removed by washing with concentrated alkaline solution, then rinsing with water. The crude chitin thus prepared is then treated with concentrated hydrochloric acid, and purified chitin is obtained as the precipitate (Fig. 4). Chitin is a smooth, flaky powder, and white when pure. It is insoluble in water and requires certain solvents to obtain chitin solution. Water-soluble chitosan is obtained by removing acetyl groups from chitin with an alkaline solution (Inui, 1997). Annual production of chitin and chitosan is about 700 metric tonnes, and its marketability is estimated to be around

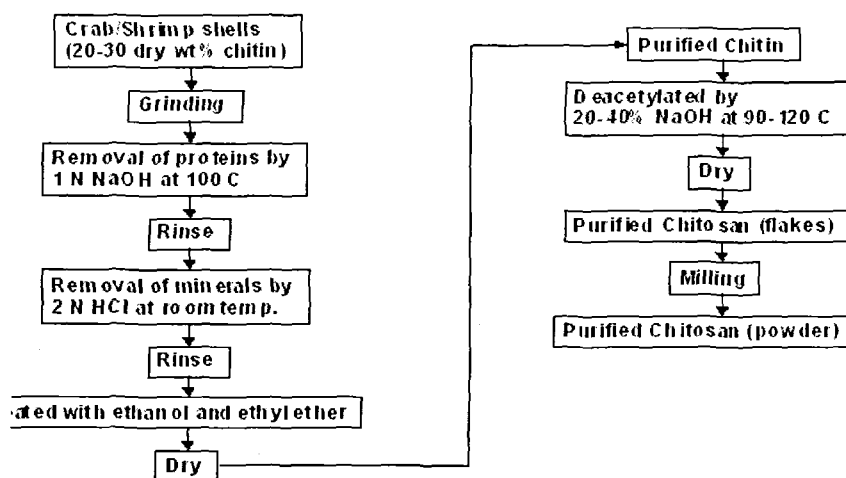


Fig 4. Manufacturing process of chitin and chitosan from crab and shrimp outer shells. (Adapted from Inui, 1997).

5 billion yen. About 85% of chitosan produced in Japan is used for wastewater treatment in the food industry. It removes high-BOD water-soluble proteins through flocculation.

Another notable function of chitosan is inhibition of spoilage bacterial growth in foods. Addition of 0.01% chitosan prolonged the reproductive induction period by two days compared to the control group, while the addition of 0.02% or more inhibited the growth of *E. coli* altogether (Table 2)(Uchida, 1988). Added chitosan in liquid media prevented the growth of spoilage bacteria during incubation at 30°C for 24 hr (Darmadji and Izumimoto, 1994). Its application for meat preservation is expected in the near future. Chitosan is macromolecular, even if it reacts with the cell wall of bacteria it would not be expected to pass through the cell membrane and react with the nucleic acid and proteins inside the cell wall. Consequently the antibacterial nature of chitosan is closely linked to the molecular weight of chitosan; lower the molecular weight of chitosan, the greater its antibacterial effect (Uchida, 1988).

Table 2. Effect of chitosan derivatives on the growth of *E. coli* (Uchida, 1988)

Chitosan derivatives (0.004%)		Degree of decomposition (Total reducing sugar produced mg/g)	Microbial account ($\times 10^2$ /ml)
Control		-	140 ++
Derivative	A	540	44 ++
	B	260	29 +
	C	40	5 -
	D	8	15 -
Chitosan		4	13 -

Shaken culture at 30°C, 24 hr.

-, No growth; +, Growth; ++, Marked growth.

Chitin and chitosan are processed into a film for medical use as an artificial protector against skin damages such as cuts and burns (Oshima *et al.*, 1986). Burnt skin tissues wrapped with a chitosan film regenerated faster and more favourably than those treated without the film. Artificial skin prepared from crab chitin (Beschitin[®]-W distributed by Unichika, Co., Ltd. Osaka, Japan) is commercially available. This processed film of chitin is of 0.08~0.5 mm thick. However, the availability of this preparation is limited by their high prices (Feofilova *et al.*, 1999).

Rats fed on a diet containing 7% tallow, 1% cholesterol and 5% chitin had reduced levels of liver triacylglycerols and cholesterol and similar levels of serum and fecal cholesterol compared to rats fed on the diet without chitin, suggesting that chitin may be effective in controlling lipid absorption from intestine (Zacour *et al.*, 1992). In hamsters the inclusion of feeding chitin and chitosan in the diet is associated with a reduced food intake and the cholesterol-lowering effect

seems mainly related to an increase in the fecal excretion of neutral sterols (Tautwein *et al.*, 1997). Ormrod *et al.* (1998) showed a direct correlation between lowering of serum cholesterol with chitosan and inhibition of atherogenesis, and suggested that the chitosan could be used to inhibit the development of atherosclerosis in individuals with hypercholesterolaemia. Thus, orally administered chitosan binds fat in the intestine, blocking absorption, and lower blood cholesterol in animals and humans.

Chitosan has immunomodulatory activity. Phagocytosis of leucocyte in rainbow trout was significantly activated after injection with 100 mg/kg of chitosan in peritoneum, compared to a control injected with bovine serum albumin (Sakai *et al.*, 1992).

Other applications of chitosan include improving the physical properties of heat-induced gel of surimi, kamaboko (Benjakul *et al.*, 2001), use as a matrix for bioreactor, strengthener for pulp sheet, and moisturiser in cosmetics (Table 3). More information about food application of chitinous materials can be obtained in Chapter 15.

2) Chondroitin sulphate

Chondroitin sulphate is a typical mucopolysaccharide sulphate. There are three isomers differing in the position of the sulphuric acid groups. Chondroitin polysulphate, with two sulphuric acid residues in a molecule, exists in the cartilage of sharks. It is mostly used as a base for cosmetics such as hand cream.

Chondroitin sulphate dose-dependently inhibited the pancreatic lipase activity in an assay system using triolein emulsified with phosphatidylcholine. In addition, it inhibited the palmitic acid uptake into the brush border membrane vesicles of the rat jejunum. Chondroitin sulphate caused the reduction of body weight and parametrial adipose tissue weight and prevention of fatty liver and hyperlipidemia in mice fed a high-fat diet (Han *et al.*, 2001).

3) Milt proteins as food preservatives

Milt protein (protamine) is a basic peptide containing over 80% arginine. Protamine changes the cell morphology of certain bacteria and releases certain soluble constituents from the cells (Islam *et al.*, 1987). Its unique properties include thermo-stability and the ability to prevent the growth of *Bacillus* spores (Table 4). For this reason, protamine is being used as an antibacterial agent in food processing and preservation (Islam *et al.*, 1986). The preservative effects of protamine product after added to a wide range of food, including cooked rice, omelet, cooked vegetables, salad, sweet potato, custard cream, kneaded bean jam, cooked waxy rice flower, steamed bread and white sauce were investigated (Nozaki 1999). Fig. 5 shows the effect of salmin (milt protein from salmon) on the shelflife of boiled rice.

Gel by forming ϵ -(γ -Glu)-Lys cross-links in the gels. The enzyme preparation is not of marine

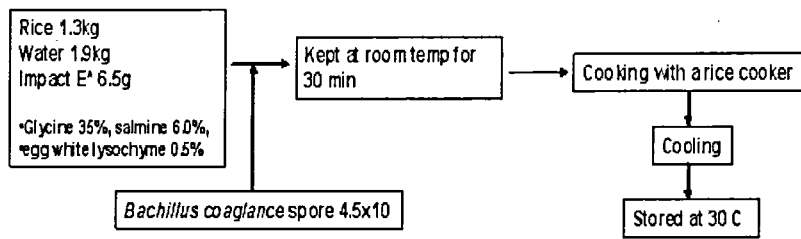
origin, but is purified from the culture broth of a variant of *Streptoverticillium mobaraence* (Sakamoto *et al.*, 1995).

Table 3. Applications of chitin, chitosan, and their oligomers (Jeon *et al.*, 2000)

Fields	Chitin and chitosan	Oligomers
Food	Antimicrobial agent Edible film	Preservative agent
Pharmaceuticals	Protective effect on bacterial infection Antitumor agent Immunopotentiating agent Carrier for drug derivaty system	Protective effect on bacterial Infection Antitumor agent Immunopotentiating agent
Medical	Accelerator for wound healing Artificial skin Absorbable sutures	
Nutritional	Dietary fiber Hypocholesterolemic agent Antihypersensitive agent <i>in vitro</i>	Hypocholesterolemic agent Calcium absorption accelerator
Biotechnological	Carrier for immobilized enzyme and cell Porous beads for bioreactor Resin for chromatography Membrane material	
Agricultural	Seed coating preparation Activator of plant cell	Activator of plant cell
Others	Coagulant for waatewater Protein recovery preparation in food processing Removal of heavy metal from Wastewater Cosmetic materials	

Table 4. Effect of milt protein from salmon (salmine) on growth of bacteria (Nozaki, 1999)

Bacteria	Concentration (ppm)						
	0	200	300	400	500	700	1000
<i>B. subtilis</i>	+	+	+	-	-	-	-
<i>E. coli</i>	+	+	+	+	-	-	-
<i>Sal. typhirium</i>	+	+	+	+	+	+	-
<i>Pichia farinosa</i>	+	+	+	+	-	-	-
<i>Strept. lactis</i>	+	+	-	-	-	-	-



		Storage, days			
		0	24	48	72
Control	CFU/g	10	2.2×10^4	4.0×10^7	**
	pH	6.7	6.7	6.3	**
Impact E added	CFU/g	10	5.3×10^3	2.5×10^3	4.5×10^6
	pH	6.5	6.6	6.4	6.7

Fig. 5. Effect of salmin (milt protein from salmon) on the shelflife of boiled rice. (Adapted from Nozaki, 1999).

4. CONCLUSIONS

The structure of Japanese fisheries has changed for last decade; the productions from the distance water and offshore fisheries have drastically decreased. On the other hand, the imports of fishery products in terms of quantity and value have increased. Thus, the conditions of Japanese fisheries face to hard in these days. The Oceans are, however, rich in marine organisms, including fish, mammals and microorganisms which all have potentials as the resources for value-added utilization for not only human consumption but also medical applications (Ohshima, 1996). A cooperative research and development between industries and researchers will have a chance to open the difficulties around the Japanese fisheries.

REFERENCES

1. Akahane N, Ohba S, Suzuki J, Wakabayashi T, Nakahara T, Yanagi K, Ohshima N. Antithrombotic activity of a symmetrical triglyceride with eicosapentaenoic acid and gamma-linolenic acid in guinea pig mesenteric microvasculature. *Thrombosis Research* 1995; 78:441-450.
2. Benjakul S, Visessanguan W, Tanaka M, Ishizaki S, Suthidham R, Sungpech O. Effect of chitin and chitosan on gelling properties of surimi from barred garfish. *Journal of the Science*

- of Food and Agriculture* 2001; 81:102-108.
3. Darmadji P, Izumimoto T. Effect of chitosan in meat preservation. *Meat Science* 1994; 38:243-254.
 4. Dyerberg J, Bang HO, Hjörne N. Fatty acid composition of the plasma lipids in Greenland Eskimos. *Journal of Clinical Nutrition* 1975; 28:958-966.
 5. Feofilova EP, Tereshina VM, Memorskaya AS, Alekseev AA, Evtushenkov VP, Ivanovskii AG. A new field of biotechnology: polyaminosaccharides-based wound-healing preparations. *Microbiology* 1999; 68:735-738.
 6. Gibson RA, Makrides M, Neumann MA, Simmer K, Mantzioris E, James MJ. Ratios of linoleic acid to α -linolenic acid in formulas for term infants. *Journal of Pediatrics* 1994; 125:S48-S55.
 7. Gilbertson TA, Fontenot DT, Liu L, Zhang H, Monroe WT. Fatty acid modulation of K⁺ channels in taste receptor cells. *American Journal of Physiology* 1997; 272:C1203-1210.
 8. Han LK, Sumiyoshi M, Takeda T, Chihara H, Nishikiori T, Tsujita T, Kimura Y, Okuda H. Inhibitory effects of chondroitin sulfate prepared from salmon nasal cartilage on fat storage in mice fed a high-fat diet. *International Journal of Obesity* 2000; 24:1131-1138.
 9. Harris MA, Reece MS, McGregor JA, Manchego JM, Allen KGD. Possible roles of maternal and perinatal long-chain fatty acids in preterm birth. In: Huang YH Sinclair AJ (eds). *Lipids in Infant Nutrition*. Champaign, USA: AOCS Press, pp 1-18, 1998.
 10. Hata K, Makuta M. Method for continuous urea adduction and its apparatus. Japan patent H2-180996, 1990.
 11. Hata K, Noda H, Makuta M. Production method of eicosapentaenoic acid or its ester with high concentration. Japan patent H4-128250, 1992.
 12. Hata K, Noda H, Makuta M. Production method of eicosapentaenoic acid and its ester. Japan patent H4-41457, 1992.
 13. Hata, K. Application of eicosapentaenoic acid to medicine. In: Fujimoto K (ed.) *Properties of Physiological Activities of Fish Oils*. Tokyo: Koseisha-Koseikaku, pp 101-110, 1993.
 14. Hirata Y, Uemura D. Halichondrins-antitumor polyether macrolides from a marine sponge. *Pure and Applied Chemistry* 1986; 58:301-310.
 15. Hwang D. Dietary fatty acids and eicosanoids. In: Chow CK (ed). *Fatty Acids in Foods and Their Health Implications*. New York: Marcel Dekker, pp 545-557, 1992.
 16. Inagaki M, Harris WS. Changes in lipoprotein composition in hypertriglyceridemic patients taking cholesterol-free fish oil supplements. *Atherosclerosis* 1990; 82:237-246.
 17. Inui H. Biochemical function of chitin and chitosan in mammals and higher plants, and possibilities in applications in medical and agricultural fields. *Applied Biological Science* 1997; 3:55-65.
 18. Ishibashi M, Ohizumi Y, Yamashita M, Nakamura H, Hirata Y, Sasaki T, Kobayashi J. Amphidinolide B. A novel macrolide with potent antineoplastic activity from the marine

- dinoflagellate *Amphidinium* sp. *Journal of the Chemical Society - Chemical Communications* 1987; 16:1127-1129.
19. Islam NMD, Motohiro T, Itakura T. Combined effect of heat treatment and protamine on the growth and heat resistance of *Bacillus* spores. *Bulletin Japanese Society of Scientific Fisheries* 1986; 52:919-922.
 20. Islam NMD, Oda H, Motohiro T. Changes in the cell morphology and the release of soluble constituents from the washed cells of *Bacillus subtilis* by the action of protamine. *Nippon Suisan Gakkaishi* 1987; 53:297-303.
 21. Jeong YJ, Shahidi F, Kim SK. Preparation of chitin and chitosan oligomers and their applications in physiological functional foods. *Food Reviews International* 2000; 16:159-176.
 22. Kamiya H. Utilization of high molecular-bioactive substances from marine organisms. In: Reijiro H (ed). *Frontier Researches in Fisheries Science*. Tokyo: Kouseisha-Kouseikaku, pp 131-154, 1994.
 23. Koriyama T, Kohata T, Watanabe K, Abe H. Chemical components of bigeye tuna muscle and the effects of lipid on the taste. *Nippon Suisan Gakkaishi* 2000; 66:462-468.
 24. Koriyama T, Kohata T, Watanabe K, Abe H. The effects of oils on the taste of tuna extract. *Nippon Suisan Gakkaishi* 2000; 66:876-881.
 25. Lavelle F, Zerial A, Fizames C, Rabault B, Craudeau A. Antitumor activity and mechanism of action of the marine compound girodazole. *Investigational New Drugs* 1991; 9:233-244.
 26. Makrides M, Simmer K, Goggin M, Gibson RA. Erythrocyte docosahexaenoic acid correlates with the visual response of healthy, term infants. *Pediatric Research* 1993; 33:425-427.
 27. Mann N J, Warrick GE, O'Dea K, Knapp HR, Sinclair AJ. The effect of linoleic, arachidonic and eicosapentaenoic acid supplementation on prostacyclin production in rats. *Lipids* 1994; 29:157-162.
 28. Matsunaga S, Fusetani N, Hashimoto K, Koseki K, Noguchi H, Noma M, Sankawa U. Bioactive marine metabolites, Part 25. Further kabiramides and halichondramides cytotoxic peptides from *Hexabranhus* egg masses. *Journal of Organic Chemistry* 1989; 54:1360-1362.
 29. Matsunaga S, Fusetani N, Hashimoto K, Koseki K, Noma M. Bioactive marine metabolites, Part 13. Kabiramide C, a novel antifungal macrolide from nudibranch egg masses. *Journal of the American Chemical Society* 1986; 108:847-849.
 30. Ministry of Agriculture, Forestry and Fisheries, Government of Japan. *Fisheries Statistics of Japan 1998*. Tokyo: Association of Agriculture-Forestry Statistics, pp. 2-14, 2000.
 31. Monma M, Tanamoto Y, Kainuma K. Ultrastructure of corn starch-granules digested by *Chalara paradoxa* glucosamylase. *Denpun Kagaku* 1990; 37:13-19.
 32. Moore RE, Patterson GML, Mynderse JS, Barchi J Jr, Norton T, Furusawa E, Furusawa S. Toxins from cyanophytes belonging to the acytonemataceae. *Pure and Applied Chemistry* 1986; 58:263-271.
 33. Murakami S, Takemoto T, Zensho T, Daigo K. Effective principles of *Digenea simplex*. VIII.

- Structure of kainic acid. *Journal of Pharmaceutical Society of Japan* 1955; 75:866-869.
34. Nagai H, Murata M, Torigoe K, Satake M, Yasumoto T. Gambieric acids, new potent antifungal substances with unprecedented polyether structures from a marine dinoflagellate *Gambierdiscus toxicus*. *Journal of Organic Chemistry* 1992; 57:5448-5453.
 35. Nakajima S, Ninomiya U. Umami of seafood and taste. In: Yamasawa M (ed). *Investigation and Utilization of Sea Food Components to Enhanced Human Health*. 2001. (in press).
 36. Norton TR, Kashiwagi M, Shibata S. Drugs and food from the sea: myth or reality? In: (PN Kaul and CJ Sindermann (ed). Norman; The University of Oklahoma, pp. 37-50, 1978.
 37. Nozaki K. Preservation measures for food products. The effect of milt protein on food preservation and its application. *Gekkan Fudo Kemikaru* 1999; 15:50-57.
 38. Ohshima T. By-products and seafood production in Japan. *Journal of Aquatic Food Product Technology* 1996; 54:27-42.
 39. Ohshima T. Preference for food lipids. *Nippon Suisan Gakkaishi* 2000; 66:133-134.
 40. Oshima Y, Nishino K, Yonekura Y. Utilization of chitin film as a covered sheet for a wound part after skin surgery. *Nishinihonhifuka* 1986; 48:1119-1122.
 41. Ormrod DJ, Holmes CC, Miller TE. Dietary chitosan inhibits hypercholesterolaemia and atherogenesis in the apolipoprotein E-deficient mouse model of atherosclerosis. *Atherosclerosis* 1998; 138:329-334.
 42. Otsuji S, Kamada T, Yamashita T, Soejima Y, Setoyama S, Hashiguchi J, Chuman Y. Effect of dietary sardine oil (eicosapentaenoic acid) on the erythrocyte membrane fluidity in diabetic patients. *Rinsho Byori* 1984; 32:764-771.
 43. Perry NB, Blunt JW, Munro MHG, Thompson AM. Antiviral and antitumor agents from a New Zealand sponge, *Mycale* sp. 2, structures and solution conformations of mycalamides A and B. *Journal of Organic Chemistry* 1989; 55:223-227.
 44. Pettit GR, Herald CL, Doubek DL, Herald DL, Arnold E, Clardy J. Isolation and structure of bryostatin 1. *Journal of the American Chemical Society* 1982; 104:6846-6848.
 45. Sakai M, Kamiya H, Ishii S, Atsuta S, Kabayashi M. The immunostimulating effects of chitin in rainbow trout, *Oncorhynchus mykiss*. In: Shariff M, Subasinghe RP, Arthur JR. (eds). *Diseases in Asian Aquaculture I*. Manila, Philippines: Asian Fisheries Society, pp 413, 1992.
 46. Sakamoto H, Kumazawa Y, Toigichi S, Seguro K, Soeda T, Motoki M. Gel strength enhancement by addition of microbial transglutaminase during onshore surimi manufacture. *Journal of Food Science* 1995; 60:300-304.
 47. Sakemi S, Ichiba T, Kohmoto S, Saucy G, Higa T. Isolation and structure elucidation of onnamide A, a new bioactive metabolite of a marine sponge, *Theonella* sp. *Journal of the American Chemical Society* 1988; 110:4851-4853.
 48. Shibata S, Norton TR, Izumi T, Matsuo T, Katsuki S. A polypeptide (AP-A) from sea anemone (*Antiopeleuda xanthogrammica*) with potent positive inotropic action. *Journal of*

- Pharmacological Experimental Therapy*. 1976; 199: 298-309.
49. Shimada S. Antifungal steroid glycoside from sea cucumber. *Science* 1969; 163: 1462.
 50. Surette ME, Whelan J, Lu G, Hardard'ottir I, Kinsella JE, Dietary n-3 polyunsaturated fatty acids modify Syrian hamster platelet and macrophage phospholipid fatty acyl composition and eicosanoid synthesis: a controlled study. *Biochimica Biophysica Acta* 1995; 1255:185-191.
 51. Suzuki K. Utilization and properties of a microcapsule DHA. *Shokuhin To Kagaku* 1995; 37:1-7.
 52. Trautwein EA, Jürgensen U, Erbersdobler HF. Cholesterol-lowering and gallstone-preventing action of chitosans with different degrees of deacetylation in hamsters fed cholesterol-rich diets. *Nutrition Research* 1997; 17:1053-1065.
 53. Uauy R, Birch E, Peirano P: Visual and brain function measurements in studies of n-3 fatty acid requirements of infants: *Journal of Pediatrics* 1992; 120:S168-180.
 54. Uchida Y. Antibacterial properties of chitin and chitosan. *Food Chemical* 1988; 34:22-34.
 55. Ushio H, Ohshima T, Koizumi C. Functionality and preference of marine lipids. In: Yamasawa M (ed). *Investigation and Utilization of Sea Food Components to Enhanced Human Health*. 2001. (in press).
 56. Zacour AC, Silva ME, Cecon PR, Bambirra EA, Vieira EC. Effect of dietary chitin on cholesterol absorption and metabolism in rats. *Journal of Nutritional Science and Vitaminology* 1992; 38:609-613.