MARINE BIOTECHNOLOGY FOR PRODUCTION OF FOOD INGREDIENTS

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The marine world represents a largely untapped reservoir of bioactive ingredients that can be applied to numerous aspects of food processing, storage, and fortification. Due to the wide range of environments they survive in, marine organisms have developed unique properties and bioactive compounds that, in some cases, are unparalleled by their terrestrial counterparts. Enzymes extracted from fish and marine microorganisms can provide numerous advantages over traditional enzymes used in food processing due to their ability to function at extremes of temperature and pH. Fish proteins such as collagens and their gelatin derivatives operate at relatively low temperatures and can be used in heat-sensitive processes such as gelling and clarifying. Polysaccharides derived from algae, including algins, carrageenans, and agar, are widely used for their ability to form gels and act as thickeners and stabilizers in a variety of foods. Besides applications in food processing, a number of marine-derived

ADVANCES IN FOOD AND NUTRITION RESEARCH VOL 52 ISSN: 1043-4526

DOI: 10.1016/S1043-4526(06)52005-4

compounds, such as omega-3 polyunsaturated fatty acids and photosynthetic pigments, are important to the nutraceutical industry. These bioactive ingredients provide a myriad of health benefits, including reduction of coronary heart disease, anticarcinogenic and anti-inflammatory activity. Despite the vast possibilities for the use of marine organisms in the food industry, tools of biotechnology are required for successful cultivation and isolation of these unique bioactive compounds. In this chapter, recent developments and upcoming areas of research that utilize advances in biotechnology in the production of food ingredients from marine sources are introduced and discussed.

I. INTRODUCTION

The term biotechnology is associated with a number of meanings. In a broad sense, it can be defined as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use" (FAO, 2000). However, to some, biotechnology fits into a narrower definition restricted to "the commercial application of living organisms or their products, which involves the deliberate manipulation of their DNA molecules" (USDA, 1994). While this chapter will include some discussion of genetic research, it will be largely focused on the broader meaning of biotechnology, exploring new advances in the controlled manipulation and utilization of marine organisms for the production of food ingredients.

Although the marine world represents nearly three-fourths of the Earth's surface, it is one of the most underutilized biological resources, containing a vast array of organisms with unique biological systems and characteristics. Marine organisms such as macro- and microalgae, sponges, fish, and bacteria have all developed diverse and unique characteristics that allow them to survive under conditions with varying degrees of salinity, pressure, temperature, and illumination. Thanks to the tools of marine biotechnology, molecules that promote survival in marine environments have begun to be identified and methods of extraction are being developed and improved upon. In 2003 alone, over 650 new marine compounds were isolated from marine microorganisms and phytoplankton, green algae, brown algae, red algae, sponges, coelenterates, bryozoans, mollusks, tunicates, and echinoderms (Blunt *et al.*, 2005), and the majority of marine organisms, mostly microorganisms, remain unidentified (Colwell, 2002; USDA, 1995). Biomolecules derived from marine organisms are useful to the food industry in a number of applications, including efficient food production under unique conditions such as low temperature or high pressure; providing added nutritional benefits to foods; and/or using "natural" pigments, preservatives, or flavors. As shown in Table I, some major categories of marine-derived food

TABLE I

MARINE-DERIVED FOOD INGREDIENTS

Category	Food ingredient	Application	Major marine sources	Health benefits and other advantages	References
Photosynthetic pigments	Carotenoids: β-carotene, astaxanthin, and lutein	Natural food colorings, nutraceutical agents, farmed salmon pigmentation	Microalgae: *D. salina, S. maxima, C. protothecoides, C. vulgaris*, and *H. pluvialis*	Vitamin A precursors, antioxidants, anticarcinogenic, anti-inflammatory, natural pigments	Ben-Amotz, 1993; Gregory, 1996; Guerin *et al.*, 2003; Maeda *et al.*, 2005; Ramirez and Morrissey, 2003; Shi and Chen, 2001; von Elbe and Schwartz, 1996
	Carotenoids: fucoxanthin	Stimulation of UCP1, resulting in metabolic thermogenesis	Seaweed: *U. pinnatifida*	Possible antiobesity effect and reduced risk of type II diabetes	Maeda *et al.*, 2005
	Phycobilins: phycoerythrin phycocyanin	Natural food colorings	Red and blue-green algae: e.g., *P. cruentum*	Natural source of pigmentation	Borowitzka, 1993; Roman *et al.*, 2002
	Chlorophylls	Natural food and beverage colorants	Aquatic plants and bacteria: e.g., *S. platensis* and *A. flos-aquae*	Anticancer activity, natural source of pigmentation	Bhattacharya and Shivaprakash, 2005; Chernomorsky *et al.*, 1999; de Oliveira Rangel-Yagui *et al.*, 2004; Donaldson, 2004; Egner *et al.*, 2001; Kay, 1991; Sarkar *et al.*, 1994
Lipids	Omega-3 fatty acids: SDA, EPA, DHA	Nutraceuticals, fish oil capsules, fortification of livestock, feed and infant formula	Fish (e.g., salmon, sardine, tuna, herring), microalgae (*Navicula* spp., *N. frustulum, B. sinensis, P. tricornutum, C. cohnii, A. carteri, G. simplex, G. cohnii, C. minutissima, P. cruentum, Thraustochytrium, Schizochytrium*), fungi (phycomycetes), extremophiles, macroalgae (Rhodophyceae, Bryophytes), krill (*E. superba*), transgenic terrestrial plants	Numerous health benefits (e.g. visual and neurodevelopment, reduce risk of cardiovascular problems, ameliorate diseases such as arthritis and hypertension)	Borowitzka, 1993; Cohen *et al.*, 1988; Horrocks and Yeo, 1999; Jiang *et al.*, 2001; Kendrick and Ratledge, 1992; Nettleton, 1995; Radwan, 1991; Sijtsma and de Swaaf, 2004; Ursin, 2003; Venugopal and Shahidi, 1995; Yap and Chen, 2001; Yongmanitchai and Ward, 1989
	Sterols	Aquaculture feed	Microalgae (thraustochytrids)		Borowitzka, 1993; Lewis *et al.*, 1999

(*continued*)

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Category	Food ingredient	Application	Major marine sources	Health benefits and other advantages	References
Polysaccharides	Phycocolloids: algins	Thickener, stabilizer, and emulsifier in foods such as salad dressings, ice cream, jam, and mayonnaise	Brown seaweed (*S. confusum, L. japonica, E. maxima, L. pallida, M. angustifolia*)	Water soluble, stable at high temperatures, high viscosity	BeMiller and Whistler, 1996; FAO, 2004; Ohshima, 1998; Sutherland, 1996; Tseng, 2001
	Phycocolloids: carrageenans	Gel formation and coatings in the meat and dairy industry	Red algae (*K. alvarezii, E. denticulatum, B. gelatinum*)	Anti-HIV activity and anticoagulant properties, water soluble, high viscosity, stable over wide pH ranges	BeMiller and Whistler, 1996; FAO, 2004; Ohshima, 1998; Renn, 1993; Tseng, 2001; Vlieghe *et al.*, 2002
	Phycocolloids: agar	Gel formation and food gums	Red algae (*Gelidium, Grateloupia, Gracilaria, Hypnea, Gigartina*)	Water soluble, ability to gel aqueous solutions at low concentrations	BeMiller and Whistler, 1996; FAO, 2004; Freile-Pelegrin and Murano, 2005; Marinho-Soriano and Bourret, 2005; Ohshima, 1998; Renn, 1993
	Fucans/fucanoids	Potential use as nutraceuticals	Cell walls of brown algae, sea urchin eggs, sea cucumbers	Anticoagulant, antithrombotic, anti-inflammatory, antiviral, cellular antiproliferative, and adhesive activities	Berteau and Mulloy, 2003; Kuznetsova *et al.*, 2003; Mourao, 2004
	Exopolysaccharides from cyanobacteria	Emulsion stabilizers, bioflocculants	Cyanobacteria (*C. capsulata, Nostoc, Cyanotheca*)	Unique and unusual properties	de Philippis *et al.*, 2001
	Exopolysaccharides from extremophiles	Unique thickening, gelling, stabilizing, suspending, coagulating, film-forming, and water retention properties	Extremophiles (*Pseudoalteromonas, Alteromonas, Vibrio, H. mediterranei*)	Unique properties: unusual gelling, high metal-binding and thickening capacities, resistance to high salinities, temperatures, and pH	Guezennec, 2002; Herbert, 1992

	Chitin, chitosan, and their derivatives	Gelling agents, antimicrobial activity, edible protective films, clarification and deacidification of fruit juices, emulsifying agents, nutraceuticals, water purifiers, and others	Crustaceans (shrimp, crab, lobster, prawn, krill), lactic acid bacteria (*L. plantarum*)	Increase dietary fiber, reduce lipid absorption, antitumor, bactericidal and fungicidal activities	Haard *et al.*, 1994; Rao and Stevens, 2005; Shahidi and Abuzaytoun, 2005; Shahidi *et al.*, 1999; Synowiecki and Al-Khateeb, 2003
Proteins	Collagen	Edible casings in the meat industry (e.g., sausages)	Fish (albacore tuna, silver-line grunt, bigeye snapper, brown-backed toadfish, hake, trout, lingcod, catfish, rainbow trout, yellow sea bream, common horse mackerel, tiger puffer, and others)	Can be extracted from processing by-products	Jongjareonrak *et al.*, 2005; Noitup *et al.*, 2005; Senaratne *et al.*, 2006
	Gelatin	Stabilizer, texturizer, or thickener in ice cream, jam, yogurt, cream cheese, margarine, confectionaries, and low-fat foods; clarifiers (e.g., isinglass) in beverages such as wine, beer, cider, and vinegar; film- and foam-forming agents	Fish, especially cold-water (pollock, cod, haddock, hake, cusk)	Forms gels at low temperatures, isinglass has been shown to prevent and treat chronic atrophic gastritis	Choi and Regenstein, 2000; Djagny *et al.*, 2001; Gomez-Guillen *et al.*, 2002; Haard *et al.*, 1994; Norland, 1990; Xu *et al.*, 2004
	Albumin	Replacement for egg albumin as a whipping, suspending, or stabilizing agent	Mollusks, crustaceans, low-fat fish	High flexibility and strength, health benefits (e.g., anticoagulant, antioxidant)	Haard *et al.*, 1994; Nicholson *et al.*, 2000; Ockerman and Hansen, 1988
	Protamine	Antibacterial agent, preservative in fruits, rice, and confectionaries	Fish spermatozoa (e.g., herring and salmon milt)	Alters the cell structure of some bacteria, does not coagulate under heat, natural preservative	Islam *et al.*, 1986; Ohshima, 1998; Potter *et al.*, 2005
	Protein powders	Mariculture and animal feed, use as functional ingredients and nutritional supplements	Extremophile (*Dunaliella*), fish processing by-products (e.g., arrowtooth flounder and herring)	High productivity, favorable nutritional and functional properties	Herbert, 1992; Sathivel *et al.*, 2004

(*continued*)

TABLE I (*continued*)

Category	Food ingredient	Application	Major marine sources	Health benefits and other advantages	References
Enzymes: Digestive proteases	Gastric proteases (e.g., pepsins, gastricsins, chymosins)	Cold renneting milk, fish feed digestion aid	Fish viscera (Atlantic cod, carp, harp seals, American smelt, sardine, capelin, salmon, mackerel, orange roughy, palometa, tuna)	Demonstrate catalytic activity at lower temperatures, thus minimizing unwanted chemical reactions and bacterial growth	Shahidi and Janak Kamil, 2001; Simpson, 2000
	Serine and cysteine proteases (e.g., trypsins, chymotrypsins, collagenases, elastases, cathepsin B)	PPO inactivation (preventing unwanted color changes in food products such as shrimp and fruit), food processing (low-temperature protein digestion, meat tenderizing, curing of Herring, squid fermentation)	Pyloric ceca, pancreatic tissues, intestines, hepatopancreas (stomachless bone fish, sardine, capelin, cod, cunner, salmon, anchovy, palometa, Atlantic white croaker, carp, hybrid tilapia, herring, spiny dogfish, rainbow trout, crustaceans, mollusks, short-finned squid)	Demonstrate catalytic activity at lower temperatures, thus minimizing unwanted chemical reactions and bacterial growth	Eichler, 2001; Gudmundsdottir and Palsdottir, 2005; Haard and Simpson, 2000; Haard *et al.*, 1994; Raksakulthai and Haard, 2001; Shahidi and Janak Kamil, 2001; Simpson, 2000
Enzymes	Lipases	Numerous uses in the fats and oils industry (e.g., production of omega-3-enriched triglycerides)	Atlantic cod, seal, salmon, sardine, Indian mackerel, red sea bream, and others	Higher specificity for omega-3 fatty acids	Shahidi and Janak Kamil, 2001; Shahidi and Wanasundara, 1998
	Polyphenol oxidases (e.g., tyrosinase, polyphenolase, phenolase, catechol oxidase, cresolase, catecholase)	Processing and fermentation of tea, coffee, raisins, and prunes	Crustaceans, microorganisms	Have higher activity at lower temperatures, as compared with terrestrial counterparts	Haard *et al.*, 1994; Whitaker, 1996
	Chitinolytic enzymes	Replace HCl for converting chitin into oligomeric units	Digestive tracts of fish, shellfish and shellfish waste, squid liver; octopus saliva	Less harsh than HCl and results in more consistent products	Shahidi and Janak Kamil, 2001
	Transglutaminase	Creates protein cross-links to improve rheological properties of gels, i.e., surimi, gelatin	Red sea bream, rainbow trout, atka mackerel, walleye, pollock liver, scallop muscles, botan shrimp, squid	Strengthens gels with protein cross-linkages	Ashie and Lanier, 2000; Chen *et al.*, 2003; Ohshima, 1998; Shahidi and Janak Kamil, 2001

	Red algae enzymes in the starch degradation pathway (e.g., α-1,4-glucan lyase and others)	Production of the natural sugar 1,5-anhydro-D-fructose and the antifungal compound microthecin	Red algae (genera *Gracilariales*)	Production of compounds that exhibit antioxidant, antimicrobial, anti-blood-clotting, and/or antitumor properties	Yu, 2005
Extremophilic enzymes	Thermophilic enzymes (e.g., heat-adapted α-amylase, glucoamylase, cellulase, chitinase, pectinase, β-galactosidase, xylose isomerase, pullulanase, neutral proteases, lipases, and serine and acid proteases)	Baking and brewing, food processing, production of natural sweeteners, use in transesterification and oligosaccharide, peptide, and phospholipid syntheses	Thermophiles	Active at moderate to high temperatures (45–100°C or more)	Eichler, 2001; Gomes and Steiner, 2004; Herbert, 1992
	Psychrophilic enzymes (e.g., cold-adapted alcohol dehydrogenase, α-amylase, β-galactosidase, lipase, aspartate chitinase, pectinase, transcarbamylase, Ca^{2+}–Zn^{2+} protease, citrate synthase, β-lactamase, malate dehydrogenase, subtilisin, triose phosphate isomerase, and xylanase)	Beer, wine, and dough fermentation, cheese production, milk and fruit juice processing	Psychrophiles (e.g., Antarctic and Arctic microorganisms)	High activity at low to moderate temperatures (−5 to +20°C), can replace mesophilic counterparts, reduce residual heat coagulation that commonly occurs during cheese production	Cavicchioli *et al.*, 2002; Gerday *et al.*, 2000; Gomes and Steiner, 2004; Herbert, 1992
	Alkaliphilic enzymes (e.g., cyclomaltodextrin glucanotransferase, extracellular β-mannanases, pectinolytic enzymes, xylanases)	Low-cost production of cyclodextrins from starch, guar gum hydrolysis, treatment of pectin-containing effluent, production of wheat and rice straw	Alkaliphiles (e.g., *Bacillus* sp.)	Active under moderate to high alkalinities	Gomes and Steiner, 2004; Herbert, 1992

ingredients used commercially are photosynthetic pigments, polyunsaturated fatty acids (PUFAs), sterols, polysaccharides, proteins, and enzymes.

Many marine-based food ingredients fall under the category of nutraceuticals, which are bioactive substances with medicinal characteristics or added health benefits such as anticancer or anti-inflammatory activity. Fortification of foods with nutraceuticals has become an increasingly popular method for providing nutritional food products to health-conscious consumers. Marine-based nutraceuticals are already an active industry in Japan and Europe, and the US market has experienced significant growth over the past decade. According to Ohr (2005), consumer awareness of marine-based nutraceuticals has been growing due to reports on their extensive health benefits such as enhanced antioxidant activity and immunity. Some examples of marine nutraceuticals currently marketed in the United States include products such as fish and algal oils rich in omega-3 fatty acids, chitin and chitosan, fish and shark liver oil, marine enzymes and chondroitin from shark cartilage, sea cucumbers, and mussels. Omega-3 fatty acids are well known for their wide range of health benefits, including reduced risk of cardiovascular disease and enhanced brain development in infants, while chondroitin has been shown to have anti-inflammatory and anticancer properties.

Marine-based food ingredients and nutraceuticals can be derived from a vast array of sources, including marine plants, microorganisms, and sponges, all of which contain their own sets of unique biomolecules that allow them to thrive in their respective habitats. Another growing source for marine-based food ingredients has been fish and seafood by-products resulting from post-harvest processing. This chapter will cover current applications of marine biotechnology in the production of food ingredients from marine plants, animals, microorganisms, and processing by-products. In Section II, the major sources of marine-based food ingredients will be introduced and in Section III, specific biomolecules that have applications in the food industry will be discussed.

II. SOURCES OF MARINE-DERIVED FOOD INGREDIENTS

A. MACRO- AND MICROALGAE

The term alga refers to a plant or plantlike organism from one of several phyla of mostly aquatic, chlorophyll-containing nonvascular organisms. Algae are divided into two general categories—macroalgae, such as red, yellow-green, green, and brown algae, and microalgae, such as blue-green algae. According to Chen and Jiang (2001), there may be over 50,000 species of algae worldwide. Humans utilize algae as a source of health food, food ingredients, and

high-value chemicals and pharmaceuticals. Recently, there has been a renewed interest in developing high-quality products from algae, with algae showing great potential as a source of bioactive substances. Some cultivated marine microalgae, such as *Spirulina, Dunaliella, Chlorella*, and *Crypthecodinium cohnii*, have exhibited promising nutraceutical properties, including the presence of β-carotene, omega-3 fatty acids, and antioxidants (Molyneaux and Lee, 1998). The algal biotechnology industry is growing, with an aquaculture sector that produces large amounts of seaweeds, such as *Laminaria, Porphyra*, and *Gracilaria*, and microalgae, including *Dunaliella* and *Spirulina*. Additionally, the utilization of algae-derived colloids (phycocolloids) such as algin, agar, and carrageenan has developed into an important industry (Tseng, 2001). Global production of aquatic plants for 2002 was 11.6 million tons, generating US$6.2 billion, with the highest production coming from Japanese kelp (*Laminaria japonica*) (4.7 million tons) followed by Nori (*Porphyra tenera*) with 1.3 million tons (FAO, 2004).

Despite the growing promise of algae as a source of food ingredients, the industry has developed with only varying amounts of success and its biotechnological potential remains to be fully exploited (Chen and Jiang, 2001; Ramirez and Morrissey, 2003). A major setback is in achieving efficient production methods, as the traditional means of cultivating algae using open systems brings with it many limitations, and new closed-system cultivation technologies (photobioreactors) are not yet fully developed. Additionally, extraction of food ingredients from cultivated algae in a profitable manner can be quite challenging. Recent advances in transgenic work with algae offer interesting and promising alternatives to traditional production methods. In this section, biotechnologically important species of macro- and microalgae as a source of food ingredients will be reviewed, along with major developments and challenges facing the industry.

1. Macroalgae

Macroalgae, or seaweeds, provide a wide range of food and food ingredients, with an estimated total annual production value of US$5 billion. Wild and farmed seaweed combined amount to an annual usage of 7.5–8 million tons (wet seaweed), with increasing algae cultivation in response to growing demands. Cultivation of macroalgae now contributes to over 90% of the global seaweed demand, with the remainder being naturally harvested (FAO, 2004).

There are four major classes of macroalgae: Rhodophyta (red algae), Phaeophyta (brown algae), Chlorophyta (green algae), and Cyanophyta (blue-green algae) (Renn, 1993). Some specific commercially important cultivated seaweeds and seaweed products include the brown seaweed

L. japonica, wakame from the brown seaweed *Undaria pinnatifida*, Hizikia from *Hizikia fusiforme*, and the high-value product Nori, worth about US$16,000/dry ton, from the red seaweed *Porphyra* sp. (FAO, 2004). The *Laminaria* cultivation industry is one of the largest producers worldwide of the hydrocolloid algin, providing 13,000 tons per year (Tseng, 2001). Biotechnological advances regarding macroalgae cultivation include establishment of cell and tissue cultures that can biologically synthesize desired compounds, such as eicosanoids, on a large scale under a controlled environment (Rorrer *et al.*, 1998). For example, by feeding *Laminaria saccharina*, a specific diet rich in linoleic and γ-linolenic acids, production of three desired bioactive hydroxy fatty acids [15-hydroxy-5,8,11,13-eicosatetranenoic acid from arachidonic acid; 13-hydroxy-9,11-octadecatetraenoic acid from stearidonic acid (SDA); and 13-hydroxy-9,11-octadecadienoic acid from linoleic acid] was increased up to 400% (Rorrer *et al.*, 1998). According to Rorrer *et al.* (1998), results of their studies with macroalgae demonstrate that biotechnology for controlled production of bioactive substances from cell and tissue cultures of marine seaweeds utilizing bioreactor systems holds great promise.

2. *Microalgae*

Microalgae are the most primitive and simply organized members of the plant kingdom, with the majority existing as small cells of about 3–20 μm, and a few species organized into simple colonies (Ramirez and Morrissey, 2003). This group of microorganisms is extremely diverse and a rich source of potential bioactive ingredients such as vitamins, pigments, fatty acids, sterols, and polysaccharides (Borowitzka, 1993; Grobbelaar, 2004; Kay, 1991; Yap and Chen, 2001). There is great potential for use of microalgae in production of food ingredients, as they are photoautrophic microorganisms that can grow on a very simple culture medium containing seawater, nitrate, phosphate, trace amounts of certain metals, and carbon dioxide (Luiten *et al.*, 2003). Thanks to development of new production and environmental technologies, biotechnology of microalgae has grown in importance (Volkman, 2003), with well-established microalgae production plants in Taiwan (*Chlorella*), Thailand (*Spirulina*), the United States (*Spirulina, Dunaliella*), Australia (*Dunaliella, Chlorella*), Israel (*Dunaliella*), Czechoslovakia (*Scenedesmus*), and Mexico (*Spirulina*), and smaller operations in Cuba, Iran, India, China, Vietnam, Chile, France, Spain, and South Africa (Ben-Amotz, 2004; Borowitzka, 1993). Cultivation of microalgae offers several advantages over use of conventional higher plants, including high growth rates, high uptake and release rates promoted by a large surface to volume ratio, strains that can tolerate extreme conditions, no need to obtain high-quality agriculture soils, possibility for high-density growth using

closed photobioreactors with semicontrolled parameters, and highly valued end-products (Grobbelaar, 2004). Some products obtained from microalgae include β-carotene, astaxanthin, vitamins C, A, E, H, B_1, B_2, B_6, and B_{12}, supplements in health foods, PUFAs, and viscosifier gums from polysaccharides (Luiten *et al.*, 2003). Commercially produced microalgal oils have been incorporated into infant milk formulations and used as dietary supplements and food additives (Volkman, 2003).

Despite the great potential for production of food ingredients from microalgae, only a few species are being cultivated on a large scale. Some noteworthy strains include *Aphanizomenon, Nostoc, Spirulina* (*Arthrospira*), *Chlorella, Dunaliella salina*, and *Haematococcus pluvialis* (Grobbelaar, 2004; Kay, 1991; Pulz, 2001; Richmond, 2004). The cyanobacteria *Aphanizomenon* is a relatively new source of microalgae for human consumption that is harvested in temperate lakes of the United States and sold as a health food and dietary supplement (Kay, 1991; Pulz, 2001). Large-scale commercial production of *Aphanizomenon flos-aquae* began in the early 1980s in Oregon. This *Aphanizomenon* strain is desirable as a health food source because it is high in protein and rich in vitamins, minerals, carotenoids, and phycobiliproteins (Hu, 2004). *A. flos-aquae* has also been reported to promote digestion and to have immune-enhancing effects along with anti-inflammatory activity (Jensen *et al.*, 2001). Despite the economic advantages to natural cultivation of *A. flos-aquae*, there exist numerous inconsistencies along with the possibility of contamination. Therefore, future use of photobioreactors may provide a safer, more controlled environment for harvesting this biomass (Hu, 2004).

Nostoc is tolerant to extreme environments and is found throughout the world in places such as hot springs, the polar region, and deserts. *Nostoc*, which has been a part of the Chinese diet for 2000 years, is recognized as a healthy food owing to its low fat content and high levels of protein and natural pigments (Danxiang *et al.*, 2004). The two *Nostoc* species of the most economic value are *N. flagelliforme* and *N. commune*, which are harvested in several Asian and South American countries.

Spirulina is a photoautotrophic blue-green alga that commonly flourishes in brackish, saline lakes with extremely high pH (Mao *et al.*, 2005). This cyanobacterium appears to be one of the most important microalgal species utilized by humans: it has been used as food for thousands of years by the Aztecs and Mayans (Wang *et al.*, 2005) and for centuries by African populations near the alkaline lakes of Chad and Niger (Grobbelaar, 2004). In the later part of the 1970s, the first commercial production plant for *Spirulina* was established, and *Spirulina* is now cultivated around the world using open raceway ponds, with major facilities in the United States (Hawaii and California), China, Taiwan, and Japan (Hu, 2004; Pulz, 2001). *Spirulina* is

widely produced and generally recognized as safe (GRAS) approved *Spirulina* is presently available for use in foods and supplements (Mao *et al.*, 2005; Ohr, 2005). *Spirulina* has great potential as a nutrient source, as it is high in protein (60–70% depending on strain) (Mao *et al.*, 2005) and contains significant levels of vitamins, minerals, essential fatty acids, and antioxidants such as carotenoids (especially chlorophyll) and phycobiliproteins (de Oliveira Rangel-Yagui *et al.*, 2004; Hu, 2004; Liu and Cao, 2001). *Spirulina* has also been reported to have numerous health benefits. Chronic or subchronic treatment with *Spirulina* has been suggested to reduce lipid peroxidation, increase antioxidant levels, reduce reactive nitrogen species toxicity, and reduce cholesterol levels (Wang *et al.*, 2005). *Spirulina* reduces potential brain damage from strokes and other neurological disorders (Wang *et al.*, 2005), and daily 2-g supplements of *Spirulina* reduced allergy symptoms in allergic rhinitis patients (Mao *et al.*, 2005). According to Mao *et al.* (2005), *Spirulina* exhibits anti-inflammatory activity thanks to the compound c-phycocyanin, which is a pigment commonly found in blue-green algae that also has antioxidant activity. Two of the most well-known species are *Spirulina (Arthrospira) platensis* and *Spirulina (Arthrospira) maxima*.

Chlorella is a freshwater, unicellular green microalga that is widely used as a food supplement in Japan and around the world. Mass commercial cultivation of *Chlorella* for use as a health food supplement has taken place for over 35 years, with a more recent application in mariculture feed (Iwamoto, 2004). Many strains of *Chlorella* can be grown heterotrophically, allowing for production of a high-quality powder without contamination. *Chlorella* supplements are taken in the form of tablets, capsules, liquid, or as food additives. Claims for health benefits of *Chlorella* include improved immune function and improved control of hypertension, fibromyalgia, and ulcerative colitis (Halperin *et al.*, 2003).

Dunaliella is a motile green alga with a cell volume ranging from 50 to 1000 μm^3. This ovoid biflagellate predominates in aquatic systems with salinity contents of 10% or higher and may be the most halotolerant eukaryotic organism known, as it can survive at salinities ranging from 0.2% to 35% (Ben-Amotz, 1993). There is great biotechnological potential for use of *Dunaliella* in the production of antioxidants such as β-carotene, ascorbic acid, and tocopherol. When stressful culture conditions (i.e., nitrogen deficiency, high light intensity, and high NaCl) are induced, the production of these antioxidants has been reported to reach 13.1%, 2.5%, and 1.2%, respectively (El Baz *et al.*, 2002). The halotolerance of *Dunaliella* allows for outdoor cultivation under high salt conditions with minimal contamination from other bacteria, while its high β-carotene content helps to protect it from solar irradiation (Ben-Amotz, 1993). Carotenoids are generally the main products from cultivation of *Dunaliella* and they are sold in three

major forms: *Dunaliella* powder, β-carotene extract, and dried *Dunaliella* for use in feeds (Ben-Amotz, 2004). *Dunaliella* cultivation takes place in Australia using large unstirred shallow ponds, in the Ukraine with natural ponds, and in the United States and Israel using raceway ponds (Borowitzka, 1993; Pulz, 2001). Numerous smaller facilities are located around the world in Mexico, Cuba, Chile, India, Iran, Taiwan, and Japan.

H. pluvialis is a freshwater, green, unicellular alga that is used extensively for production of the orange-red pigment astaxanthin in both open and closed culture systems (Grobbelaar, 2004; Orosa *et al.*, 2005; Pulz, 2001). In order to produce astaxanthin from *Haematococcus* using a large-scale outdoor system, the algae are first grown under conditions that promote rapid growth. Next, environmental stressors, such as nitrogen starvation, introduction of compounds that prevent cell division and high light intensity, are used to induce carotogenesis (Guerin *et al.*, 2003; Orosa *et al.*, 2005). Then the cells are harvested by settling and centrifuging, the cell biomass is cracked to improve the bioavailability of astaxanthin, and then the extract is dried and encapsulated or the astaxanthin is further extracted from the biomass (Guerin *et al.*, 2003). It has been reported that use of malonate as a carbon source can increase carotenoid yields by up to 13-fold (Orosa *et al.*, 2005). Haematococcus-derived astaxanthin has value as a nutraceutical and as a source of pigment in aquaculture feed, and *Haematococcus* has been approved by the FDA as a dietary supplement ingredient (Cysewski and Todd Lorenz, 2004).

3. *Algae cultivation*

Although microalgae have numerous potential biotechnological applications for use in the food, cosmetics, and pharmacy industries, several setbacks remain for achieving efficient production rates (Pulz, 2001). The main limitation for microalgae and microalgal products to reach their economic potential is the need for closed culture systems in the form of closed photobioreactors, which are costly to run because of high light requirements and slow growth rate of some organisms (Ramirez and Morrissey, 2003). Open systems have traditionally been the dominating method for mass production of cultured microalgae; however, these systems are in contact with the air and have significant drawbacks, such as evaporative losses, diffusion of CO_2 to the atmosphere, an ongoing possibility of contamination and pollution, and light limitation in the high layer thickness (Pulz, 2001). Open systems are also dependent on weather and climate and therefore quality of products can be highly variable (Sijtsma and de Swaaf, 2004). Inefficient use of light energy and limited growth rates can have undesirable consequences such as slow cell growth rates and production of secondary metabolites

(Hejazi and Wijffels, 2004). Therefore, in order to create a profitable industry, it seems necessary to improve production methods for microalgae. A hopeful solution to open systems is the use of closed systems (closed photobioreactors) in which all the important culture conditions are controlled and regulated. Closed systems present the opportunity to reduce the risk of outside contamination, reduce CO_2 loss, and provide reproducible cultivation conditions such as regulated water and temperature parameters (Pulz, 2001). However, as already mentioned, closed systems are costly to run due to light requirements and the often slow cell growth of microalgae. One biotechnological advancement is the concept of "milking" microalgae for their secondary metabolites. This method allows for reuse of the same culture and continuous removal of desired compounds, and would be a way to compensate for the low productivity often experienced with algal cultures (Hejazi and Wijffels, 2004).

One of the main cultivation systems utilized on a large scale is the shallow open raceway pond, which is used for production of *D. salina* at high salinity, *S. platensis* at high alkalinity, and *Chlorella* sp. (Luiten *et al.*, 2003). Additionally, some strains, such as *Chlorella, Nitzschia, Cyclotella*, and *Tetraselmis*, can be grown heterotrophically in fermenters. Heterotrophic growth makes use of glucose as a source of both carbon and energy, and it is generally less costly and easier to regulate as compared to photoautotrophic cultivation. *Crypthecodinium* and *Chlorella* are both grown on a large-scale using fermentative technology (Apt and Behrens, 1999).

4. *Algal transgenics*

Although still in its beginning stages, algal transgenic biotechnology shows great potential for more efficient and specialized production of food ingredients. The possibility of using transgenic algae as "cell factories" is an appealing new technology that could be utilized for the production of compounds such as carotenoids, PUFAs, and enzymes (Leon-Bañares *et al.*, 2004). Genetic work with algae thus far has resulted in the establishment of molecular tools and sequence information that can be used to integrate transgenes into select strains of algae, especially in the case of the green algae *Chlamydomonas reinhardtii* and *Volvox carteri* and the diatom *Phaeodactylum tricornutum* (Walker *et al.*, 2005). However, the term "algae" refers to a wide range of organisms with varying biological systems, and the methods of transformation developed for one type of algae may not function in another. Therefore, more work is necessary to standardize methods of transgenic introduction and expression in different types of algae. For a thorough review of the genomic information available for select algae, see Grossman (2005).

Transgenes can be integrated into algae via nuclear or chloroplastic transformation. Both methods have several advantages and disadvantages. For example, chloroplastic transformation allows for high levels of expression without foreign gene silencing, while nuclear transformation provides a greater range of possibilities in the areas of algal metabolism and protein expression (Leon-Bañares *et al.*, 2004). Nuclear transformation is a routine, standardized procedure in several species of microalgae, with reports of stable transformations in diatoms, dinoflagellates, and chlorophytes, while strains of red, green, and euglenoid algae have all been successfully transformed using chloroplastic techniques (Dunahay, 1996; Walker *et al.*, 2005). In the following sections, specific genetic advances among groups of micro- and macroalgae will be highlighted along with their potential applications in the food industry.

a. Diatoms. Diatoms are an abundant and diverse group of unicellular heterokont (flagellated) algae that dominate the phytoplankton of cold, nutrient-rich waters. Diatoms are known to contain the antioxidants fucoxanthin and chlorophyll along with a silica-based cell wall (Graham and Wilcox, 2000). The metabolic pathways involved in the generation of the silica cell wall are largely unknown and show potential for the discovery of novel enzymes and proteins that may prove to be commercially useful (Leon-Bañares *et al.*, 2004). Over the last decade there has been significant progress in the genetic study of diatoms, with developments in genetic techniques and increasing numbers of species with genomes that have been sequenced and transformed with foreign genes (Montsant *et al.*, 2005; Walker *et al.*, 2005).

Although transgenic diatoms have not yet been used in commercial applications, there are several promising areas of research with the potential for development into large-scale industries. One example is in the manipulation of algal lipid metabolism pathways to produce specific oils such as the production of biodiesel from transgenic diatoms (Dunahay, 1996). This type of transgenic lipid manipulation could also be valuable in the production of PUFAs from microalgae. Another interesting development has been the conversion of the obligate photoautotroph *P. tricornutum* into an organism capable of heterotrophic growth (Zaslavskaia *et al.*, 2001). This was achieved by transformation of the diatom with a human glucose transporter gene. The ability to convert photoautotrophs to heterotrophs could be valuable to algal biotechnology because the cultivation of algae dependent on light can be expensive and inefficient.

b. Dinoflagellates. After diatoms, the next most important eukaryotic primary producers in marine coastal waters are the dinoflagellates, a group of unicellular algae that contain chlorophyll, carotenoids including β-carotene,

and PUFAs (Graham and Wilcox, 2000; Walker *et al.*, 2005). Although there is potential for use of dinoflagellates in algal transgenics, these organisms have very large genomes that make gene sequencing impractical. Despite setbacks, gene transformation has been successfully carried out in two species of dinoflagellates, *Amphidinium* and *Symbiodinium*, and expressed sequence tag (EST) operations are underway (Lohuis and Miller, 1998; Walker *et al.*, 2005).

c. Green algae. Chlorophytes that have experienced significant progress in the field of transgenics include *Chlorella* sp., *C. reinhardtii, Haematococcus* sp., and *Dunaliella* sp. Some of these species have been considered for use as cell factories in the production of valuable food ingredients or pharmaceuticals such as proteins. Plant-derived proteins are GRAS approved, as they show low risk of contamination due to viruses, prions, or bacterial endotoxins (Franklin and Mayfield, 2004). The green alga *Chlorella* has experienced major advancements in nuclear transformation, with promising applications in the production of valuable proteins and peptides. Some examples include production of antimicrobial and bioinsecticide peptides and human and fish growth hormones for use in aquaculture (Walker *et al.*, 2005).

C. reinhardtii is a freshwater, eukaryotic alga whose genome is now publicly available (along with only two other eukaryotic algal genomes) (Montsant *et al.*, 2005). *C. reinhardtti* has numerous favorable attributes that make it an interesting candidate for use as a cell factory in the production of valuable proteins: it can be grown either heterotrophically or phototrophically, it can be cultivated on a large scale, and stable transgenic lines can be created relatively quickly (Franklin and Mayfield, 2004). Major biotechnological advances have taken place in the nuclear transformation of *C. reinhardtii*, with potential applications in the food and pharmaceutical industries. For example, *C. reinhardtii* produces an antigenic protein of the pathogenic bacteria *Renibacterium salmoninarum*, which causes kidney disease in salmonids. When this antigenic protein was fed to trout and rabbits, it promoted generation of antibodies against the bacterium (discussed in Leon-Bañares *et al.*, 2004). Although *C. reinhardtii* shows promise in the field of transgenic algal protein production, several challenges remain, including reducing the silencing of foreign gene expression and enhancing translation in chloroplast-based expression (Walker *et al.*, 2005).

In addition to transgenic advances in *Chlorella* sp. and *C. reinhardtii*, there has been great effort to develop the biotechnological tools required to research and exploit the commercially important green microalgae *H. pluvialis* and *D. salina*. These microalgae are the world's major suppliers of natural astaxanthin and β-carotene, respectively, and successful DNA

transformation has been carried out in both species. Research into the metabolic pathways and regulatory mechanisms involved in the production of these antioxidants could result in transgenic technologies designed to develop commercial strains with increased antioxidant productivity. Although gene transformation in these species has been met with limited success, in part due to the high salt environment required by *Dunaliella* sp., progress involving transformation with foreign genes coding for hygromycin resistance and the human hexose/H^+ symporter has shown promising results (Walker *et al.*, 2005). In a recent example of biotechnological applications for microalgae, the *Dunaliella* species *D. bardawil* was mutated and strains rich in the carotenoids phytoene and phytofluene were selected for growth in small outdoor ponds in Israel. These strains were gradually brought to large-scale growth in open raceways for commercial carotenoid production (Ben-Amotz, 2004; Werman *et al.*, 2002).

d. Macroalgae. Although macroalgae are commercially important producers of food and phycocolloids such as alginates, agars, and carrageenans, transgenic research in this field remains far behind that for land plants and microalgae. However, the first macroalgae genome project was announced in the brown alga *Ectocarpus siliculosus* (Peters *et al.*, 2004) and genetic work has been carried out with *Laminaria* and *Porphyra*. Recent advances in the field of plant transgenics include genetic engineering of the chloroplast genome, which allows for numerous advantages over nuclear transformation, including increased expression levels of transgenes, use of operons, and more precise and predictable loci (Walker *et al.*, 2005). Further research into chloroplast transgenics could lead to more efficient production of desired compounds, such as polysaccharides, from macroalgae cultivation.

B. EXTREMOPHILES

Extreme aquatic environments are oftentimes inhabited by microorganisms that have developed unique biological properties in order to survive and thrive under conditions outside the tolerance levels of most living things. This diverse group of organisms, referred to as extremophiles, includes bacteria, cyanobacteria, algae, and yeasts that exhibit high tolerances for certain conditions such as extreme salinities, temperatures, pressures, radiation levels, and heavy metal concentrations (Herbert, 1992).

Biomolecules such as enzymes isolated from extremophiles can be highly useful in the food industry due to their unique activities under abnormal conditions, and it has been widely accepted that extremophiles have strong potential to be valuable resources for use in biotechnology (Fujiwara, 2002; Guezennec, 2002; Herbert, 1992). Additionally, the discovery of deep-sea

hydrothermal vents has revealed an increasing number of new bacterial species with incredible diversity and the ability to produce novel biomolecules such as enzymes, polysaccharides, and other bioactive molecules with potential commercial importance in the food industry (Guezennec, 2002). For more extensive reviews on extremophiles and their biotechnological potential, please see Gomes and Steiner (2004), Guezennec (2002), Fujiwara (2002), Cavicchioli *et al.* (2002), and Eichler (2001).

C. MARINE SPONGES

Marine sponges are lower invertebrate animals belonging to the phylum Porifera. They have porous skeletons composed of double-walled cell colonies that become permanently attached to underwater surfaces. From a biotechnological perspective, marine sponges represent a rich source of new, undiscovered bioactive natural products. The Porifera phylum contains approximately 15,000 different species with diverse growth forms (Belarbi *et al.*, 2003). Lower invertebrates, such as sponges, possess a far greater diversity of certain lipid components, such as fatty acids, sterols, and other unsaponifiable compounds, as compared with higher-up animals. Biotechnological interest in sponges began in the early 1950s with the discovery of unknown nucleosides such as spongothymidine and spongouridine in the marine sponge *Cryptotethya crypta*. These nucleosides were found to be the basis for synthesis of cytosine arabinoside (Ara-C), the first marine-derived anticancer agent (Luiten *et al.*, 2003). Since then, more than 5300 different products (about one-third of all marine natural products) have been isolated from sponges, with hundreds more being discovered every year (Blunt *et al.*, 2005; Luiten *et al.*, 2003; Wang *et al.*, 2003). Most of the bioactive compounds from sponges have antibiotic, antitumor, anti-inflammatory, antiviral, immune suppressive, antifouling, or antimalarial properties. Besides nucleosides, sponges have also been found to be a source of compounds such as bioactive terpenes, sterols, cyclic peptides, alkaloids, fatty acids, peroxides, and often-halogenated amino acid derivatives (Luiten *et al.*, 2003). As explained by Osinga *et al.* (1999) and Belarbi *et al.* (2003), a number of these metabolites are actually produced by endosymbiotic microorganisms that live in the sponge tissue rather than by the sponges themselves.

Although sponge mariculture was attempted in the late nineteenth and early twentieth centuries, it has not yet proven to be very lucrative, as little is known about how to replicate the sponge's natural environment and life cycle (Luiten *et al.*, 2003). Additionally, the bioactive compounds of interest are often only produced in trace amounts by sponges. However, recent developments using a primmorph system may facilitate production of bioactive compounds from sponges. Primmorphs are very densely packed,

spherical sponge-cell aggregates (~1-mm diameter) that are produced by gently agitating a dissociated cell suspension. *In vitro* cell culture of certain sponges, such as *Geodia cydonium, Dysidea avara*, and *Suberites domuncula*, for use in producing bioactive compounds is a developing area of research, and use of the primmorph system has allowed for the discovery of basic mechanisms involved in cell proliferation and programed cell death (Le Pennec *et al.*, 2003). According to Le Pennec *et al.* (2003), sponge cell culture using the primmorph system is available for biotechnological applications. Additionally, the possibility of ''milking'' primmorphs (as discussed previously with microalgae) has been suggested as a potential technology to increase metabolite production (Muller *et al.*, 2000).

Two additional recent biotechnological methods for production of metabolites from sponges include *ex situ* culture and sponge-cell culture. In *ex situ* culture, functional sponges are grown outside of the sea and they are fed powdered substrates, guaranteeing a product of consistent quality; however, growth rates remain low compared to functional sponge growth in the sea. Optimization of growth rates may be achieved with increasing knowledge of the biology of Poriphera. Sponge-cell culture has shown promising growth rates and corresponding metabolite production, but a major setback with this method has been large amounts of contamination. Current work has been focused on developing methods to discriminate between sponge cells in culture and contaminants, with promising results. In a comparison of metabolite production using various methods, including chemical synthesis, wild harvest, mariculture, primmorphs, *ex situ* culture, sponge-cell culture, and genetic modification, it was determined that optimal techniques vary depending on the compound of interest. Mariculture proved to be the most economically feasible production method, while *ex situ* culture shows promise for the future production of valuable compounds that require close monitoring of growth parameters. Wild harvest was determined to be less feasible due to the logistics and environmental issues of removing massive amounts of sponges from their natural marine habitats. Due to the often small concentrations of metabolites present in sponges, cell culture was also not a highly recommended production technique. Use of primmorphs did not appear feasible either, as the amount of biomass required to form the primmorphs was larger than the biomass necessary for direct extraction of the desired metabolites (Sipkema *et al.*, 2005).

The use of genetic modification for production of specific metabolites is an interesting alternative to more traditional methods such as mariculture, cell culture, and chemical synthesis. Transfer of genetic material from a sponge host into bacteria that are easier to grow could allow for increased production of desired metabolites at a much lower cost. However, the majority of the metabolites of interest are not simply proteins, but molecules that are

formed as a result of complex biochemical pathways involving numerous intermediate compounds and enzymes. It has been predicted that future production methods will likely involve transfer of genetic material into bacteria, followed by bacterial fermentation to produce metabolite precursors and then chemical synthesis of the final product (Sipkema *et al.*, 2005).

D. FISH AND SEAFOOD BY-PRODUCTS

Production of food ingredients from fish and seafood by-products is a growing area of interest, as it is a way to reduce processing waste and more efficiently utilize raw materials. Along with increased commercial production of fish and shellfish worldwide, there has been an increase in the amount of fish processing discards, at times amounting to up to 70–85% of the total weight of the catch (Shahidi, 1994). For example, in Quebec it has been estimated that 90% of all shrimp landings are processed and 70–75% of the raw material ends up as processing waste (Goldsmith *et al.*, 2003). Fish processing discards have traditionally been dumped inland, incinerated, or hauled to the ocean (Shahidi, 1994; Suresh and Chandrasekaran, 1998). However, thanks to technological advances, a higher proportion of fish and seafood by-products are being utilized to meet growing demands for fish meal. Also, new product development and extraction of commercially important biomolecules from underutilized bycatch and processing discards is an important area of research (Okada and Morrissey, 2007; Shahidi and Janak Kamil, 2001). Numerous countries have expressed heightened interest in this area of work because it could help to reduce waste, thereby catering to ethical and environmental concerns over processing discards, and it could result in the development of valuable, natural marine-derived products (Gudmundsdottir and Palsdottir, 2005; Ohshima, 1998; Okada and Morrissey, 2007). For example, in Japan by-products have been used for the production of a number of commercially successful biomolecules, such as chitin and chitosan, PUFAs, growth hormones for aquaculture, protamines for use as antibacterial agents in food, and some pharmaceutical compounds (Ohshima, 1998). Some additional food ingredients obtained from fish and seafood include proteins in the form of albumin and gelatin, and a number of valuable enzymes with unique properties.

One approach to extracting valuable compounds from shellfish processing waste is through the use of marine microorganisms in a procedure referred to as solid state (substrate) fermentation, or SSF. SSF may prove to be an economically advantageous tool for the production of certain compounds from marine waste. For example, the marine fungus *Beauveria bassiana* can be used to produce chitinase from chitinous prawn waste. Without the fungus, this conversion step normally accounts for 12% of the

total production cost of chitinase; however, use of SSF may result in a more economically favorable method for obtaining chitinase, with a maximum yield of 248 units/g initial dry substrate reported after 5 days of incubation (Suresh and Chandrasekaran, 1998).

III. MARINE-DERIVED FOOD INGREDIENTS

A. PHOTOSYNTHETIC PIGMENTS

Photosynthetic pigments are bioactive compounds used by autotrophs, such as plants, algae, and cyanobacteria, to capture solar energy for photosynthesis. Due to the fact that each pigment captures light only over certain wavelength ranges, autotrophs use multiple pigments in order to absorb more of the sun's energy. These photosynthetic pigments fall into three major categories: carotenoids, phycobilins, and chlorophylls (Table I).

1. Carotenoids

Carotenoids, which are present in all plants and many photosynthetic bacteria, represent photosynthetic pigments in the red, orange, or yellow wavelengths. Nature's most widespread pigments, carotenoids are linear polyenes that function both as light energy harvesters and as antioxidants that inactivate reactive oxygen species formed by exposure to light and air (von Elbe and Schwartz, 1996). Of the approximately 600 known carotenoids, about 50 have been shown to exhibit some provitamin A activity, which is their primary beneficial role in the diet of humans and animals (Gregory, 1996; von Elbe and Schwartz, 1996). As potent antioxidants and vitamin A precursors, carotenoids have been suggested to have protective activity against cancer, aging, ulcers, heart attack, and coronary artery disease (Li and Chen, 2001). Carotenoids are commonly used in food products as food-coloring or nutraceutical agents, and they can either be produced synthetically or derived from natural sources. Microalgal production of carotenoids such as β-carotene and astaxanthin is an attractive area of research, as they are valuable bioactive ingredients and can be present at relatively high concentrations in some algal cells. Strains of algae that are currently being investigated for use as natural producers of commercial carotenoids include *D. salina, S. maxima, Chlorella protothecoides, Chlorella vulgaris*, and *H. pluvialis* (Ramirez and Morrissey, 2003).

a. β-Carotene. The carotenoid that is most commonly found in plant tissues and has been reported to exhibit the most provitamin A activity is β-carotene (Gregory, 1996; von Elbe and Schwartz, 1996). High accumulations

of β-carotene among marine plants and algae are species specific and involve the combination of several stress factors such as high light intensity, limited nitrates, and high salt concentrations. Cultivated algae can therefore be induced to produce more β-carotene by controlling certain environmental growth conditions. A major marine producer of β-carotene is the extremely halophilic microalgae *D. salina*, which is the most β-carotene-enriched eukaryotic organism known (Ben-Amotz, 1993; El Baz *et al.*, 2002). Although producers of microalgal β-carotene may find it hard to compete economically with synthetic production plants, natural β-carotene (a mixture of *cis* and *trans* isomers) has been reported to be more biologically active than the synthetically produced, fat-insoluble, crystallizable, all-*trans*-β-carotene (Ben-Amotz, 1993). Additionally, microalgal-derived β-carotene can be marketed as a "natural" food additive for products catered toward consumers interested in buying organic and natural foods. β-Carotene derived from *Dunaliella* has been marketed commercially in several forms, such as an extract in edible oils (containing 1.5–30% β-carotene) and dried *Dunaliella* powder in capsules or tablets (containing ~5% β-carotene). These β-carotene-rich powders can be used in the health food and pharmaceutical industries to prepare naturally colored products (e.g., margarine) and to provide antioxidant activity for cancer prevention (Borowitzka, 1993). Additionally, β-carotene can be used in the aquaculture industry as a natural pigment in fish tissues.

Despite promising advances in algal production of β-carotene, numerous disadvantages remain. For one, harvesting the algae is a complicated procedure, as the biomass growth is rarely higher than 1 g/liter and the density of the algal cells is nearly identical to that of the growth medium. Also, most of the world relies on synthetically produced β-carotene, which is easily produced in large amounts, making it quite challenging for cultivators of microalgae to compete (Borowitzka, 1993).

b. Astaxanthin. Another important carotenoid that can be derived from marine sources is the orange-red pigment astaxanthin. Astaxanthin is the major carotenoid pigment present in aquatic animals and the primary pigment responsible for the pink color of salmon flesh and the reddish color of shrimp and lobster exoskeletons following heating (astaxanthin is blue when complexed with proteins prior to heating) (von Elbe and Schwartz, 1996). Astaxanthin cannot be synthesized by animals and is obtained through consumption of carotenoid-containing plants and algae (Guerin *et al.*, 2003). With an antioxidant activity up to 10 times stronger than other carotenoids (including β-carotene, canthaxanthin, and lutein) (Miki, 1991), astaxanthin provides protective activity against cancer, inflammation, and UV light (Guerin *et al.*, 2003). The health benefits of astaxanthin along

with its strong coloring properties make it an important potential ingredient for use in the nutraceutical, cosmetics, food, and feed industries (Guerin *et al.*, 2003; Miki, 1991). Microalgae-derived astaxanthin supplements have been available to the public since about 2000 (Guerin *et al.*, 2003), and astaxanthin in commercial form (*Zanthin*) was just approved by the FDA in June of 2005 as a dietary supplement with a patent for use in slowing development and ameliorating the effects of diseases of the eye and/or central nervous system (Ohr, 2005). Many astaxanthin supplements, including *Zanthin*, are derived from the green microalgae *H. pluvialis*, which is cultivated at an industrial scale and is the richest known source of natural astaxanthin, producing more than 30 g astaxanthin per kilogram of dry biomass in commercial operations (Guerin *et al.*, 2003). In addition to its use in dietary supplements, *H. pluvialis* can be added to aquaculture feed for enhanced pigmentation of salmon flesh (Molyneaux and Lee, 1998). However, farmed salmon-fed carotenoid pigments are labeled as "color added," which can cause confusion among consumers.

Naturally derived astaxanthin is a highly valued bioactive compound and has experienced rapid market growth. However, there are several inherent disadvantages to microalgal production of astaxanthin. For example, *H. pluvialis* is a freshwater alga, making open-air cultivation difficult due to high risk of contamination from undesirable organisms (Borowitzka, 1993). Additionally, optimal conditions for astaxanthin production change throughout the growth cycle of *Haematococcus*. Although current producers of natural astaxanthin have a hard time competing with synthetically produced astaxanthin, there are hopes that with increased cultivation and extraction technologies and rises in public demands for natural foods (e.g., naturally pigmented farmed salmon), microalgal production of astaxanthin will become more economically feasible and profitable. A promising new extraction technology that could increase the commercial success of natural astaxanthin is use of the milking process with a two-phase bioreactor for continuous removal of astaxanthin from *Haematococcus* (Hejazi and Wijffels, 2004; Pulz, 2001).

c. Other carotenoids. Naturally derived carotenoids other than β-carotene and astaxanthin do not appear to be a very promising field of algae cultivation thus far. However, work with the microalgae *C. protothecoides* resulted in the development of a three-step cultivation process for high yield of the carotenoid lutein (Shi and Chen, 2001). The cultivation procedure involves a fed-batch system, in which nutrients are supplemented to achieve high algal growth, followed by a nitrogen-limiting stage and then maintenance of the culture at elevated temperatures. The latter two steps in the process increase environmental stress, thus promoting carotogenesis (i.e., lutein synthesis).

Another interesting carotenoid with potential commercial value is fucoxanthin, from the edible seaweed *U. pinnatifida* (Maeda *et al.*, 2005). Fucoxanthin has been reported to be of potential use in treating obesity and reducing the risk of certain diseases, such as type II diabetes, due to its ability to promote expression of the uncoupling protein UCP1. UCP1 stimulates metabolic thermogenesis, a process by which metabolism leads to production of heat rather than adenosine triphosphate (ATP) production and excess fat accumulation. Although UCP1 is primarily found in brown adipose tissue and humans store most of their fat as white adipose tissue, there is hope that fucoxanthin may promote expression of UCP1 in the white adipose tissue as well. Rats and mice that were fed a diet rich in fucoxanthin were reported to have significantly increased expression of UCP1 and weight reductions in the abdominal white adipose tissue (Maeda *et al.*, 2005).

2. *Phycobiliproteins*

Phycobiliproteins are protein-pigment complexes, such as phycoerythrobilin (red) and phycocyanobilin (purple to deep blue), which can be easily isolated from eukaryotic algae (e.g., red algae, glaucophytes, and cryptomonads) or cyanobacteria (Apt and Behrens, 1999; Borowitzka, 1993). Aquatic autotrophic organisms contain additional photosynthetic pigments such as phycobiliproteins because more than 10 m below the water surface light wavelengths for some colors are almost completely absorbed (Voet *et al.*, 1999). Phycobiliproteins, which generally make up 1–10% of the dry weight of algal biomass, have a high market value but a small market size (Skulberg, 2004). They possess a number of qualities with potentially valuable commercial applications. For example, they are able to form stable conjugates with numerous compounds, such as biotin and antibodies, they are fully water soluble, and they can emit fluorescence (Apt and Behrens, 1999). Phycobiliproteins are utilized as natural food colorings in products such as chewing gums, dairy products, jellies, and ice sherbets in Japan, Thailand, and China (Borowitzka, 1993; Roman *et al.*, 2002).

3. *Chlorophylls*

Chlorophylls are green pigments that can be found in any plant, alga, or cyanobacterium that carries out photosynthesis. Chlorophylls are primarily used in the food industry as natural colorants in foods and beverages (de Oliveira Rangel-Yagui *et al.*, 2004). Additionally, chlorophylls and their derivatives exhibit anticancer activity in their ability to bind carcinogenic hydrophobic compounds such as polycyclic aromatic hydrocarbons, heterocyclic amines, and aflatoxin. The resulting complexes are less bioavailable and

are therefore excreted from the body rather than absorbed (Chernomorsky *et al.*, 1999; Donaldson, 2004; Egner *et al.*, 2001; Sarkar *et al.*, 1994). Although the majority of industrial chlorophylls are extracted from vegetable sources, there is a growing interest in developing the biotechnological tools necessary for production of chlorophylls from microalgae. Microalgal chlorophyll production can be carried out using fermentation processes, which allow for several advantages over traditional methods, including the potential for continuous cultivation as well as rapid multiplication and growth of microorganisms (de Oliveira Rangel-Yagui *et al.*, 2004). Some possible microalgal sources of chlorophylls include *Spirulina* sp., such as *S. platensis*, and *A. flos-aquae* (Bhattacharya and Shivaprakash, 2005; de Oliveira Rangel-Yagui *et al.*, 2004; Kay, 1991).

B. LIPIDS

1. Marine-based long-chain PUFAs (LC-PUFAs)

PUFAs are essential structural components of cell and organelle membranes, contributing to regulation of membrane properties such as fluidity, structure, phase transitions, and permeability (Yap and Chen, 2001). Marine-based LC-PUFAs have 20 or more carbons with 2 or more double carbon bonds, and they are classified by the position of the first double bond from the methyl (omega) terminus. Of particular interest are the omega-3 LC-PUFAs (n-3 LC-PUFAs) in which the first double bond is located at the third carbon from the methyl terminus. n-3 LC-PUFAs can contain up to six double bonds and cannot be synthesized by animals. The most well-studied marine n-3 LC-PUFAs are eicosapentaenoic acid (EPA), with 20 carbons and 5 double bonds, and docosahexaenoic acid (DHA), with 22 carbons and 6 double bonds (Sijtsma and de Swaaf, 2004). These two bioactive ingredients have been of increasing interest lately due to research revealing their beneficial effects on many aspects of human health such as reducing risk factors associated with cardiovascular problems, assisting visual and neurodevelopment, and ameliorating diseases such as arthritis and hypertension (Bao *et al.*, 1998; Grimm *et al.*, 2002; Horrocks and Yeo, 1999; Hu *et al.*, 2002; Leaf *et al.*, 2003; Nettleton, 1995). Although a precursor to EPA and DHA, called α-linolenic acid (ALA, 18:3), can be obtained from terrestrial plant sources, it is converted to EPA and DHA very inefficiently by the human body (Pawlosky *et al.*, 2001; Salem *et al.*, 2003).

The market for omega-3 fatty acid nutraceuticals in Europe and Japan has long been established, and the US market is experiencing rapid growth. Although most Western diets do not include enough n-3 LC-PUFAs, advances in marine biotechnology are helping to incorporate these by use

of fish oil capsules, fortification of livestock and aquaculture feed to produce omega-3-enriched farmed fish, eggs, and milk, and addition of DHA to infant formula (Sijtsma and de Swaaf, 2004). Marine-based oils used in the food industry as omega-3 dietary supplements and in functional foods include algal oil, cod liver oil, sardine oil, tuna oil, and salmon oil (Ohr, 2005). Many of these marine oils, along with fish oil marketed as omega-3 concentrate, have been GRAS approved by FDA, provided that the combined intake of DHA and EPA does not exceed 3 g/person/day. Although fish oil supplements have long been popular in Europe and Japan, a more attractive option for many in the food industry is to enrich everyday foods with n-3 LC-PUFAs (Garcia, 1998). Some examples of foods that have been marketed as omega-3-enriched products are bread and margarine (Europe); cakes, pasta, and dog food (United Kingdom); and infant formula (Japan) (Garcia, 1998). Major applications, sources, and benefits of n-3 LC-PUFAs are summarized in Table I.

a. Microalgae as a source of n-3 LC-PUFAs. Algae are believed to be the primary manufacturers of n-3 LC-PUFAs in the marine food chain and the only plant source available for EPA and DHA (Ackman *et al.*, 1964; Ohr, 2005). Although the current contribution of n-3 LC-PUFAs derived from microorganisms to the market is very low, EPA and DHA have been found at high levels in various species of marine micro- and macroalgae with relatively high oxidative stability compared to fish oils (Bajpai and Bajpai, 1993; Sijtsma and de Swaaf, 2004). Some examples can be found in several species of diatoms, dinoflagellates, and thraustochytrids. Diatoms generally contain fairly high levels of EPA (15–30% of total fatty acids) and no DHA. Some examples of diatoms rich in EPA are *Navicula pelliculosa* (freshwater) and the marine diatoms *Nitzschia frustulum, Navicula incerta*, and *Biddulphia sinensis* (reviewed in Yap and Chen, 2001). The diatom *P. tricornutum* has been reported to contain more than 35% of its total fatty acids as EPA (Borowitzka, 1993; Yongmanitchai and Ward, 1989). Alternatively, dinoflagellates, such as *C. cohnii* (nonphotosynthetic), *Amphidinium carteri, Gymnodinium simplex*, and *Gyrodinium cohnii*, have high potential for use in commercial production of DHA, which ranges from 12% to 51% of the total fatty acids in these organisms (Yap and Chen, 2001).

Microalgal n-3 LC-PUFA production technologies. Although the basic production of bioactive compounds from marine microorganisms was already discussed in Section II.A.3, advances in cultivation of microalgae specifically for production of n-3 LC-PUFAs will be explored here. To begin with, oil obtained from bioreactor cultivation of select microorganisms is referred to as single-cell oil (SCO). The main application of SCO biotechnology, in which there have been several successes and increasing industrial

interest, is in producing high-value products such as n-3 LC-PUFAs (Sijtsma and de Swaaf, 2004). However, numerous challenges remain, including optimization of algae culturing methods, bioreactor operations, and omega-3 extraction and isolation procedures.

Maximum omega-3 production can be induced by altering growth conditions: lipids found in actively growing and dividing algae contain high percentages of n-3 PUFAs (Yap and Chen, 2001). For example, under optimal culture conditions, the microalga *Chlorella minutissima* can produce an EPA content of up to 45% of its total fatty acids and is therefore a promising source for commercial production of EPA (Seto *et al.*, 1984). Large-scale production of EPA was reported using the red alga *Porphyridium cruentum* with controlled cell concentrations and temperatures (Cohen *et al.*, 1988). For a thorough review of EPA production from microorganisms, see Bajpai and Bajpai (1993).

One interesting alternative to use of photobioreactors is the possibility of growing heterotrophic organisms on organic substrates, which eliminates the need for light, a limiting factor in mass production of algae, and allows for higher cell densities (Sijtsma and de Swaaf, 2004). However, there are several setbacks that need to be overcome, such as the limited number of heterotrophic species available, the risk of contamination due to the rich media required, the slow growth rates of microorganisms, and the need to reduce production costs to well below the market price of omega-3 fatty acids (Sijtsma and de Swaaf, 2004). Some promising heterotrophic marine organisms are the thraustochytrids, *C. cohnii*, and *P. tricornutum* (following genetic transformation) (Domergue *et al.*, 2002; Sijtsma and de Swaaf, 2004; Volkman, 2003).

Thraustochytrids, which are taxonomically aligned with heterokont algae, have promising applications in DHA production because they can be grown on an industrial scale using fermentation technology and they yield high levels of DHA under dense biomass concentrations (Barclay, 2006; Barclay *et al.*, 1994; Lewis *et al.*, 1999). Some examples include *Thraustochytrium* and *Schizochytrium*, which have been reported to contain between 25% and 60% of total fatty acids as DHA, predominantly as triglycerides or oils (Kendrick and Ratledge, 1992). *Schizochytrium* can contain over 70% of its body weight as lipids and is currently used for commercial production of concentrated DHA oil and dried microalgae, some of which are used as a poultry feed additive to produce omega-3-enriched eggs and for enriching rotifers and brine shrimp prior to feeding them to cultured finfish larvae or shrimp (Barclay and Zeller, 1996; Lewis *et al.*, 1999; Sijtsma and de Swaaf, 2004; Volkman, 2003). Additionally, it has been suggested that thraustochytrid oils, which contain mostly DHA, offer an advantage over many fish-derived oils used in the feed industry

because many aquaculture species require proportionally more DHA than EPA (Lewis *et al.*, 1999). Accompanying the ongoing research developments involving thraustochytrids have been a number of approved US patents, with the most recent being a procedure outlining fermentative growth of *Schizochytrium* and *Thraustochytrium* for the production of high levels of n-3 PUFAs to be used in food products or aquaculture feed (Barclay, 2006).

C. cohnii is known to accumulate especially high amounts of DHA (30–50% of total fatty acids) with other PUFAs only present at trace amounts (Jiang *et al.*, 2001). With the addition of vitamin B_{12} and tryptone as a nitrogen source, *C. cohnii* produced up to 64% DHA and had a higher specific growth rate and biomass concentration (Jiang *et al.*, 2001). *C. cohnii* is being cultivated in large-scale bioreactors with well-controlled parameters [pH, temperature, air flow, pressure, dissolved oxygen (DO), agitation] for use as a commercial source of DHA (Sijtsma and de Swaaf, 2004). However, a complicating factor is the production of excess polysaccharides by *C. cohnii*, leading to an increased viscosity and a strong decrease in oxygen transfer, thereby impeding the development of high-density cell biomass for DHA production (de Swaaf *et al.*, 2001). One hopeful solution is use of a commercial polysaccharide hydrolase, which could be used to lower the viscosity of the culture and decrease the amount of stirring required (de Swaaf *et al.*, 2003). Also, although the extracellular polysaccharides produced are by-products in DHA production, they could potentially be used in the food industry for functional food products (Sijtsma and de Swaaf, 2004). Another possibility for more efficient production of DHA from *C. cohnii* is the use of milking technology (discussed in Section II.A.3), in which the cells would first be grown according to normal growth conditions and then they would be stressed in order to produce higher concentrations of DHA, which would be continuously removed from the system (Hejazi and Wijffels, 2004).

Similar to "milking" is the idea of semicontinuous culturing in which microorganisms are held at steady state with renewal every 24 hours. Semicontinuous culturing under nitrogen-limiting conditions was reported to increase the percentage of EPA in total fatty acids among the marine microorganisms *P. cruentum, P. tricornutum*, and *Isochrysis galbana*, with different culture strategies being recommended for the different species (Otero *et al.*, 1997). Further work with a strain of *I. galbana* has revealed important information regarding optimal lighting conditions (i.e., photon flux density and photoperiods) for maximum biomass production (Tzovenis *et al.*, 2003).

b. Fish as a source of n-3 LC-PUFAs. Although uni- and multicellular marine plants such as phytoplankton and algae are the primary sources of n-3 LC-PUFAs, high levels can be found in the tissue of many marine fish

as a result of transfer through the aquatic food chain. The flexible, unsaturated properties of n-3 LC-PUFAs help fish to maintain membrane fluidity in cold environments (Shahidi and Wanasundara, 1998). A major source of omega-3 fatty acids for human consumption has traditionally been fish such as salmon, sardine, mackerel, menhaden, anchovy, tuna, and herring (Sijtsma and de Swaaf, 2004; Yap and Chen, 2001).

Although incorporation of n-3 LC-PUFAs into foods and beverages is a growing area of interest, a major challenge remains in the high oxidative susceptibility of these unsaturated fatty acids, which can result in strong fishy odors and flavors (Garcia, 1998). Some additional challenges to the industry are related to oil quality and purification. Marine fish oil can vary in quality depending on factors such as fish species, season, and catch location, and it is a complex mixture of fatty acids that has to undergo purification steps in order to isolate DHA and/or EPA (Sijtsma and de Swaaf, 2004). Liver oil from cod and halibut carries with it the risk of vitamins A and D overdose, which can cause toxic effects, and the risk of increased cholesterol and saturated fatty acid intake (Shahidi and Wanasundara, 1998). In attempts to avoid the possible negative side effects of capsule consumption, concentrated forms of n-3 LC-PUFAs are being developed. These are preferred over crude marine oils for use in pharmaceuticals and food enrichment, as they allow for increased intake of DHA and EPA while keeping the intake of other lipids minimal (Shahidi and Wanasundara, 1998).

An important area of research is development of technologies that allow for efficient, cost-effective, high-quality extraction of omega-3 oils. Numerous methods have been established for concentration of n-3 LC-PUFAs, including adsorption chromatography, fractional or molecular distillation, enzymatic splitting, low-temperature crystallization, supercritical fluid extraction, and urea complexation; however, each has its own drawbacks and thus far only a few are suitable for mass production (Shahidi and Wanasundara, 1998). According to Shahidi and Wanasundara (1998), there is growing industrial interest in use of enzymatic methods due to the potential benefits of obtaining the concentrated oils in acylglycerol form. A promising new n-3 LC-PUFA extraction technique called the pH-shift method may prove beneficial in the industry because no heat is required, thereby limiting decomposition reactions and oxidative damage that normally occur during extraction (Morrissey and Okada, 2005).

Due to a growing interest in the bioactive properties of n-3 LC-PUFAs, there is an increasing demand for purified concentrates for use as dietary supplements and in functional foods; however, it is believed that traditional sources will be insufficient in meeting this demand (Bajpai and Bajpai, 1993; Lewis *et al.*, 1999). In addition to development of improved methods of omega-3 extraction from fish oils, a possible solution is the use of alternative,

sustainable sources of omega-3 fatty acids such as marine microalgae, fungi, extremophiles, macroalgae, and krill (Sijtsma and de Swaaf, 2004; Yap and Chen, 2001).

c. Fungi as a source of n-3 LC-PUFAs. Another promising source of n-3 LC-PUFAs is the class of algae-like fungi called phycomycetes. These lower marine fungi, especially those from the *Mortierella* genus (e.g., *M. alpina, M. elongata, M. ramanniana*, and *M. isabellina*), are known to produce high levels of either γ-linolenic acid, arachidonic acid, or EPA (Yap and Chen, 2001). Another type of phycomycete (*Phythium* sp.) produces EPA at high yields, while other species, such as *Entomophthora obscura* and *Phytophthora infestans*, have been reported to produce significant levels of DHA.

d. Transgenic organisms as a source of n-3 LC-PUFAs. An alternative biotechnological approach to maximizing production of n-3 LC-PUFAs is the use of genetic enhancement. For example, it was shown that *P. tricornutum* could be converted into a heterotrophic organism through the insertion of a glucose transporter gene (discussed in Section II.A.4) (Zaslavskaia *et al.*, 2001). Use of this type of fermentation technology could prove to be economically beneficial over photoautotrophic growth because production of n-3 LC-PUFAs would no longer be dependent on light, but rather on glucose supply.

Work in plant genomics has led to the development of terrestrial plants capable of producing significant amounts of SDA, an n-3 PUFA that is a precursor to EPA (Ursin, 2003). Traditionally, terrestrial plants have been unable to synthesize marine omega-3 fatty acids such as EPA and DHA, but rather they are known for their ability to produce the n-3 PUFA ALA. As discussed previously, ALA is converted to EPA and DHA very inefficiently by the human body, with the rate-limiting step being a reaction involving n-6 fatty acid desaturase. In research led by the food biotechnology company Monsanto, the genes for n-6 and n-12 desaturases isolated from the marine fungi *M. alpina* were introduced into canola seed, along with the n-15 fatty acid desaturase from canola (*Brassica napus*). The resulting transgenic canola lines accumulated SDA at up to 23% of total lipids and more than 55% of total lipids was in the form of omega-3 fatty acids (ALA + SDA). As a metabolic intermediate between ALA and EPA, SDA can be converted to EPA with much greater efficiency than ALA. Clinical trials demonstrated that SDA could increase plasma EPA levels at a rate three to four times greater than ALA and one-third as effectively as dietary EPA (James *et al.*, 2003). Use of transgenic land-based plants in the commercial production of n-3 LC-PUFAs is a novel technology that could greatly increase worldwide consumption of omega-3 fatty acids. However, challenges remain in the consumer acceptability of genetically modified products and in the reduced

effectiveness of dietary SDA compared to dietary EPA in raising plasma EPA levels.

In another gene transfer study, three enzymes necessary for EPA production were cloned from the genomes of *P. tricornutum* and *Physcomitrella patens* and then expressed in the yeast *Saccharomyces cerevisiae* (Domergue *et al.*, 2002). Although the biosynthetic pathways of arachidonic acid and EPA were reconstructed in the yeast, a high number of side products were reported. The ability of *P. tricornutum* to accumulate high levels of EPA with few side products is therefore thought to be indicative of a highly effective regulatory system that is not present in *S. cerevisiae*. These results show the importance of understanding not only biosynthetic pathways but also regulatory mechanisms in order to successfully use gene transfer technology for the production of n-3 LC-PUFAs.

The genes that code for the two enzymes necessary for the conversion of EPA into DHA have been identified and cloned from the genomes of the marine microalgae *Pavlova* and *Isochrysis* (Pereira *et al.*, 2004). These genes were expressed in yeast and conversion of EPA to DHA was successfully carried out. This study marks the final step in the elucidation of the biosynthetic pathway and isolation of enzymes necessary for production of DHA. Future work in this area may lead to commercial production of DHA using genetically enhanced microorganisms.

e. Extremophiles as a source of n-3 LC-PUFAs. Some deep-sea bacteria have been found to contain large amounts of EPA and DHA, presumably to allow their membranes to be fluid and adaptive to extreme temperatures and pressures (Yap and Chen, 2001). For example, psychrophiles, including some species of yeast, fungi, and microalgae, can accumulate up to 80% of their biomass as lipid, mostly in the form of triglycerides (Herbert, 1992). Additionally, psychrophilic PUFA synthesis is carried out under low temperatures using conventional fermentation technology, resulting in faster production rates and more consistent product quality and yields as compared with use of mesophilic phototrophs. For these reasons, psychrophilic PUFA production is considered by some to be a potentially lucrative field of work. Some promising organisms are *Mortierella* sp. such as *M. alpina*, which can produce EPA as 15% of total extractable fatty acid at 12°C (Bajpai and Bajpai, 1993), and the microalgae *P. tricornutum* and *C. minutissima*, which produce significant amounts of EPA at low temperatures (Herbert, 1992).

f. Macroalgae and mosses as a source of n-3 LC-PUFAs. Some macroalgae and mosses are known to produce fairly high yields of PUFAs; however, efficient culturing systems remain to be established. For example,

macroalgae in the class Rhodophyceae contain relatively high amounts of C20 PUFAs such as arachidonic acid and EPA; however, owing to their size they are almost impossible to culture under controlled conditions (Yongmanitchai and Ward, 1989). Another potential source of PUFAs is Bryophytes, which are lower plants such as *Mnium, Polytrichum, Marchantia, Matteuccia, Bryum, Sphagnum, Ctenidium*, and *Pogonatum* spp. (Radwan, 1991). One species of moss, *Pogonatum urnigerum,* can yield more than 70% of its total fatty acids as EPA; however, culturing of this moss is not profitable as a result of low cell proliferation (Yap and Chen, 2001).

g. Krill as a source of n-3 LC-PUFAs. Krill are small, shrimp-like crustaceans, the most abundant being the Antarctic krill, *Euphausia superba* (Venugopal and Shahidi, 1995). This zooplankton is rich in EPA and DHA, and contains potent antioxidants such as astaxanthin, vitamins A and E, and a novel flavonoid (Bunea *et al.*, 2004). It has been suggested that the combination of n-3 LC-PUFAs with phospholipids in krill oil facilitates passage through the intestinal wall, thus improving the bioavailability of the omega-3 fatty acids (Sampalis *et al.*, 2003). Krill oil has been shown to alleviate/reduce some of the symptoms of premenstrual syndrome and help manage hyperlipidemia by reducing levels of glucose, total cholesterol, triglycerides, low-density lipoprotein (LDL), and HDL as compared to both fish oil and a placebo (Bunea *et al.*, 2004; Sampalis *et al.*, 2003). Krill oil is sold as a dietary supplement to benefit the cardiovascular system (Ohr, 2005).

2. *Sterols*

Sterols are membrane lipids that are essential to all eukaryotic organisms and are synthesized by microeukaryotes and some bacteria (Lewis *et al.*, 2001; Volkman, 2003). Although microalgae are a rich source of commercially important bioactive molecules from the sterol biosynthetic pathway, significantly more research is necessary in order to develop the biotechnological tools for their exploitation within the food industry. Thus far, the only commercial use of sterol-containing microalgae has been as feed for aquaculture stocks, such as crustaceans and mollusks, which lack the mechanism to synthesize sterols (Volkman, 2003). A major limitation for the use of microalgae-derived sterols in the food industry is that algal cells typically have a low sterol content, amounting to less than 0.1% of the dry weight (Borowitzka, 1993; Volkman, 2003). Therefore, in order for microalgal production of sterols to be economically beneficial, the bioactive molecules would need to command a high market price. Numerous unusual and unidentified sterols have been reported in microalgae that could have potentially valuable uses in the food industry (Borowitzka, 1993; Lewis *et al.*, 2001).

A DHA-producing strain of thraustochytrids was found to produce 20 sterols, some of which were the same as those found in common food plants and 7 of which were unknown (Lewis *et al.*, 2001). The study also showed that environmental conditions, such as temperature and DO, and culture age can influence the sterol content and profiles of the thraustochytrid strain analyzed. A maximum dry weight percentage of sterols of 0.32% was reported 2 days prior to reaching peak biomass concentration in cultures grown at 20°C with high DO (Lewis *et al.*, 2001).

C. POLYSACCHARIDES

Polysaccharides are polymers of simple sugars (monosaccharides) linked together by glycosidic bonds. They have numerous commercial applications in products such as adhesives, paper, paints, foods, and beverages. Algae are a well-established source of hydrocolloids, which are used extensively in the food industry as thickeners, stabilizers, and emulsifiers (FAO, 2004; Tseng, 2001). Although red and brown algae are the most prominent sources of marine-derived polysaccharides, a major limiting factor in the commercial production of algal polysaccharides has been the high costs involved in cultivating algal biomass (Borowitzka, 1993). Alternative sources that are being investigated include cyanobacteria and extremophiles living in deep-sea vents. An additional marine-derived polysaccharide that will be discussed is chitin, which is found abundantly in the exoskeletons of crustaceans and has numerous potential uses in the food industry. For a summary of marine polysaccharides discussed in this section, along with their applications, sources, and benefits, see Table I.

1. *Polysaccharides from algae*

a. Hydrocolloids. Hydrocolloids are carbohydrates that dissolve in water to form a viscous solution. Phycocolloids (hydrocolloids extracted from algae) are a growing industry, with about 1 million tons of seaweed harvested annually for hydrocolloid extraction (FAO, 2004). The three major phycocolloids used as commercial food ingredients are algins, carrageenans, and agar. These are used for applications such as thickening aqueous solutions, forming gels, forming water-soluble films, and stabilizing products such as ice cream (FAO, 2004; Tseng, 2001).

Algins are extracted from brown seaweed and are available in both acid and salt form. The acid form is referred to as alginic acid and it is a linear polyuronic acid. The salt form, alginate, is an important cell wall component in all brown algae, constituting up to 40% of the dry weight of algal biomass (Graham and Wilcox, 2000). Algins are extracted commercially from several

different species such as *Sargassum confusum* and the cultivated plant *L. japonica* (Ohshima, 1998; Tseng, 2001). Despite a limited number of successes, brown seaweed has proven too expensive to cultivate and most algins are extracted from wild algal strains, such as those found abundantly off the coast of South Africa and commercially gathered for algin extraction and exportation (*Ecklonia maxima, Laminaria pallida, L. pallida* var. *schinzii*, and *Macrocystis angustifolia*) (FAO, 2004; Stirk, 2004).

The high numbers of carboxyl groups that make up alginic acid allow it to combine easily with cations and, once converted into its salt form, it can be used to provide high viscosity at low concentrations (BeMiller and Whistler, 1996; Ohshima, 1998). The high viscosity of sodium alginate or propylene glycol alginate solutions and the fact that they remain stable during pasteurization and cooking make them valuable natural food ingredients that can serve as thickeners, emulsifiers, and stabilizers in foods including salad dressings, ice cream, jam, and mayonnaise (BeMiller and Whistler, 1996; Ohshima, 1998).

Carrageenans are a group of biomolecules composed of linear polysaccharide chains with sulfate half-esters attached to the sugar units, giving them an overall negative charge and preventing precipitation at low pH. These properties allow carrageenans to dissolve in water, form highly viscous solutions, and remain stable over a wide pH range. There are three general forms of commercially available carrageenans (kappa, lambda, and iota), each with their own gel-forming abilities (Renn, 1993). They are extracted from red algae using dilute alkaline solutions to produce the sodium salt of carrageenan. In the past, carrageenan was extracted from wild seaweeds such as *Chondrus crispus* (Irish moss); however, advances in biotechnology have resulted in the successful cultivation of several carrageenan-containing species, especially *Kappaphycus alvarezii* and *Eucheuma denticulatum* from the Philippines and *Betaphycus gelatinum* from Hainan (FAO, 2004; Tseng, 2001).

The primary applications for carrageenans in the food industry are in dairy and processed meat products (Ohshima, 1998). Carrageenans can form gels with milk and water and can be used to coat meats to help retain moisture, seasonings, and flavors and to serve as a protective barrier (BeMiller and Whistler, 1996). From a human health perspective, it has been reported that carrageenans have antihuman immunodeficiency virus (HIV)-1 activity and some anticoagulant properties (Vlieghe *et al.*, 2002).

A third marine-derived hydrocolloid of importance to the food industry is agar, which is a mixture of polysaccharides with similar structural and functional properties as carrageenan (BeMiller and Whistler, 1996). Agar, which is composed of 70% agarose and 30% agaropectin, is extracted from red algae such as *Gelidium, Grateloupia, Gracilaria, Hypnea*, and *Gigartina*

(FAO, 2004; Ohshima, 1998). Agars are commercially important in the food industry due to their capacity to gel aqueous solutions at low concentrations and for their use as food gums (BeMiller and Whistler, 1996; Renn, 1993). Although it is the oldest phycocolloid produced in China, agar is currently the lowest in production compared with algin and carrageenan (Tseng, 2001). Methods to produce agar from cultivated seaweeds using tanks and ponds have been developed, but so far they have proven to be economically unsuccessful (FAO, 2004). However, *Gracilaria dura* from the Mediterranean Sea was reported to be a commercially viable source of agar, yielding 32–35% agar with high gel strength that can be further improved with alkali treatment (Marinho-Soriano and Bourret, 2005). Another strain of *Gracilaria* growing off the coast of the Yucatan Peninsula, *G. crassissima*, was also reported to produce a good quality agar and to be a possibly exploitable source of commercial grade agar (Freile-Pelegrin and Murano, 2005).

b. Fucans/fucanoids and other polysaccharides. Fucans are a group of polysaccharides primarily composed of sulfated L-fucose, with less than 10% other monosaccharides. They are found widely in the cell walls of brown algae (Phaeophyceae), but not in green, red, golden, or freshwater algae or in terrestrial plants. Besides brown algae, the only known reported sources of fucans are the egg jelly coats of sea urchins and the body walls of sea cucumbers. For an excellent review of the sources, structures, functions, and biological properties of fucans, the reader is referred to Berteau and Mulloy (2003).

Although the major physiological purposes of fucans are not thoroughly understood, they are known to possess numerous biological properties with potential human health applications. Fucoidans, or fucans from brown algae, have been reported to exhibit anticoagulant, antithrombotic, antiviral, and cellular antiproliferative and antiadhesive activities, as well as having an effect on the inflammatory and immune systems. These properties make fucanoids an attractive alternative to the mammalian-derived anticoagulant, heparin, which is more likely to carry with it infectious agents such as prions or viruses (Berteau and Mulloy, 2003; Kuznetsova *et al.*, 2003; Mourao, 2004). Additionally, fucanoids can make up more than 40% dry weight of isolated algal cell walls and can easily be extracted using either hot water or an acid solution (Berteau and Mulloy, 2003). The toxicity of fucanoids from *L. japonica* was tested in rats and no adverse effects were reported at levels of 300 mg/g body weight per day; however, significantly prolonged blood-clotting times were observed at three times that level and higher (Li *et al.*, 2005). Although fucanoids have yet to be exploited in the food industry, the fact that they are easy to isolate and have numerous health benefits gives them the potential to serve as valuable bioactive ingredients in natural health foods.

Another group of sulfated polysaccharides with potential use as nutraceutical agents have recently been isolated from the Chlorophyta *Ulva pertusa*. These polysaccharides were reported to affect levels of low- and high-density cholesterols and triglycerides in the plasma and serum of mice, with the resulting conclusion that they have great potential for use in preventing ischemic cardiovascular and cerebrovascular diseases (Pengzhan *et al.*, 2003a,b).

2. *Exopolysaccharides from cyanobacteria*

Cyanobacteria are considered by some to be a promising source of exocellular polysaccharides (de Philippis *et al.*, 2001). Certain strains of cyanobacteria are known to contain large amounts of released exocellular polysaccharides, which consist of a relatively large number of monosaccharides and display unique, unusual properties (de Philippis *et al.*, 2001). Cyanobacterial polysaccharides have demonstrated the potential to be used for the stabilization of emulsions or as bioflocculants; however, further research is necessary for commercial development. Polysaccharides produced by *Cyanospira capsulata*, one *Nostoc* strain, and two *Cyanothece* strains were reported to have viscosity values comparable to or above those for aqueous solutions of xanthan gum under similar concentrations (de Philippis *et al.*, 2001).

3. *Exopolysaccharides from extremophiles*

Extremophiles such as deep-sea bacteria contain polysaccharides with a wide variety of chemical and physical properties that are oftentimes not present in or are variations of the more traditional, terrestrial plant-derived polysaccharides (e.g., thickening, gelling, stabilizing, suspending, coagulating, film-forming, and water retention) (Guezennec, 2002). Exopolysaccharides (EPSs) secreted by deep-sea hydrothermal microorganisms have been identified in *Pseudoalteromonas, Alteromonas*, and *Vibrio*. One strain of *Alteromonas* was found to produce an anionic EPS with potential use as a thickening agent, while other *Alteromonas* strains produced polymers with qualities such as unusual gelling properties, significant thickening ability, and high metal-binding capacity (reviewed in Guezennec, 2002). Additionally, halophiles such as *Halobacterium mediterranei* have been reported to contain EPSs with highly favorable rheological properties and resistance to high salinities, temperatures, and pH (Herbert, 1992). Although EPSs from extremophiles exhibit unique and potentially valuable properties as food ingredients, their commercial application in biotechnology and industry will ultimately be dependent on factors such as yield, price, and markets (Guezennec, 2002).

4. *Chitin and chitosan*

Chitin is a homopolymer of *N*-acetyl-D-glucosamine residues and is a major structural component in the exoskeletons of crustaceans, mollusks, arthropods, and the cell walls of numerous fungi and algae. Owing to its widespread presence in both terrestrial and aquatic organisms, chitin is second only to cellulose as the most abundant biopolymer on the Earth (Shahidi and Abuzaytoun, 2005). On a dry weight basis, shrimp, crab, lobster, prawn, and crayfish have been reported to contain between 14% and 35% chitin, while deproteinized dry shell waste of Antarctic krill contains approximately 40% crude chitin (Haard *et al.*, 1994). Crustaceans are the primary sources of chitin used in industry. Chitin can be extracted from shellfish and crustacean waste by mixing with a dilute acid to induce demineralization, followed by a deproteinization step in a hot alkaline solution (Synowiecki and Al-Khateeb, 2003).

Once isolated, chitin can be deacetylated to create chitosan, a large cationic polymer with numerous commercial applications in the food, pharmaceutical, and waste treatment industries. Chitosan can be used in meat preservation, as it inhibits growth of spoilage bacteria in foods (Darmadji and Izumimoto, 1994). Chitosan has been sold as a weight loss supplement due to the belief that it absorbs and binds fat, inhibits LDL cholesterol, and boosts HDL cholesterol; however, studies on its effectiveness in this area have been inconsistent (Ohr, 2005; Shahidi and Abuzaytoun, 2005). The majority of the chitosan produced in Japan is used to treat wastewater in the food industry, as it can remove water-soluble proteins with high biochemical oxygen demands (Ohshima, 1998). Additionally, chitin derivatives have numerous properties that could be further utilized commercially, such as ability to form gels, high capacity for adsorption, polyelectrolyte properties, reactive functional groups, biodegradability, and antitumor, bactericidal, and fungicidal activities (Synowiecki and Al-Khateeb, 2003). Although chitosan is used in wound healing (i.e., sutures and poultices), there exist a variety of food applications for chitin, chitosan, and their derivatives, including use as antimicrobial agents, edible films, additives (e.g., for clarification and deacidification of fruit juices or emulsification), nutraceuticals (e.g., increasing dietary fiber, reducing lipid absorption), and water purifiers (Shahidi and Abuzaytoun, 2005; Shahidi *et al.*, 1999).

Despite the various potential applications of chitosan, there are several drawbacks. For example, the costs of production often outweigh the economic benefits of its application. Extraction yields for chitosan from waste are generally very low (~3–5% of raw material) and production is limited by seasonal variations in crustacean harvesting. Also, additional waste streams are created during the alkali deproteinization of chitin during isolation (Ludlow, 2001; Synowiecki and Al-Khateeb, 2003). One interesting

new technology for extraction of chitin that offers an alternative to the more harsh chemical methods traditionally utilized is the use of the lactic acid bacteria *Lactobacillus plantarum*. Fermentation of shrimp waste with *Lactobacillus* results in production of a solid portion of chitin and a liquor containing shrimp amino acids, minerals, and pigments (Rao and Stevens, 2005).

D. PROTEINS

Proteins from marine sources show promise as functional ingredients in foods because they possess numerous important and unique properties such as film and foaming capacity, gel-forming ability, and antimicrobial activity (Table I). Some of the most prevalent marine proteins used in foods are collagen, gelatin, and albumin, all of which can be extracted from fish and seafood by-products. The protein protamine has also shown promise for use in the food industry as a natural antibacterial preservative.

Collagen is a connective tissue protein found in skin, bones, cartilage, and ligaments. It has been reported to contribute to up to 30% of the total proteins in animals and it can be isolated from both terrestrial and marine sources (Senaratne *et al.*, 2006). Collagen is used widely in the food, cosmetic, and pharmaceutical industries. A major food application is in the meat-processing industry as edible casings for products such as sausages. The most widely used form of collagen (type I) was extracted from the skins of two different fish species, albacore tuna (*Thunnus alalunga*) and silver-line grunt (*Pomadasys kaakan*), and compared to bovine type I collagen (Noitup *et al.*, 2005). Interestingly, the fish skin type I collagens were less stable in that they had lower denaturation temperatures and lower levels of hydroxyproline, a cross-link promoter. In another study, collagen isolated from the skin of brown backed toadfish (*Lagocephalus gloveri*) was reported to have a significantly lower denaturation temperature compared to porcine collagen (Senaratne *et al.*, 2006). These differences between land-based and marine collagens are presumably due to the differences in habitats of the organisms. The distinct qualities of marine collagens are useful in the food industry in products that require formation of gels or casings at low temperatures. Also, collagen can be extracted from fish processing by-products in an effort to reduce and utilize marine discards. Some additional marine sources of collagens include bigeye snapper, hake, trout, lingcod, catfish, rainbow trout, yellow sea bream, common horse mackerel, and tiger puffer, among others (Jongjareonrak *et al.*, 2005; Noitup *et al.*, 2005).

Gelatin is a protein product formed by the partial hydrolysis of collagen. It has a unique gel-forming ability and is used in the food industry as a stabilizer, texturizer, thickener, or foaming agent in ice cream, jam, yogurt,

cream cheese, margarine, marshmallows, bakery products, and low-fat foods. Traditionally, gelatin has been derived from beef or pork; however, gelatin can also be extracted from marine sources for commercial use in foods (Choi and Regenstein, 2000). Marine gelatin can be derived from the skins of flatfish, such as sole and megrim; cold-water fish species including pollock, cod, haddock, hake, and cusk; or alternative sources such as squid and octopus, by either acid or alkaline treatment methods (Djagny *et al.*, 2001; Gomez-Guillen *et al.*, 2002; Norland, 1990). Although many properties of marine and land-based gelatins are similar, marine gelatins tend to have lower melting points and form weaker gels at relatively low temperatures (Choi and Regenstein, 2000; Haard *et al.*, 1994; Leuenberger, 1991). These differences are likely due to the lower levels of proline and hydroxylproline found in marine gelatins compared to land-based sources (Gomez-Guillen *et al.*, 2002). Marine gelatins are excellent emulsion stabilizers, crystal growth inhibitors, and foam and film-forming agents, and can serve as edible protective coating materials or clarifiers (Djagny *et al.*, 2001; Haard *et al.*, 1994; Norland, 1990). A particularly important application is isinglass, a high-grade gelatin made from the swim bladders of fish. Isinglass is widely used as a commercial clarifier in beverages such as wine, beer, cider, and vinegar due to its ability to induce aggregation of yeast and other insoluble particles (Hickman *et al.*, 2000). In addition to its clarifying properties, isinglass was reported to prevent and treat symptoms of chronic atrophic gastritis in rats (Xu *et al.*, 2004).

Albumin is a blood plasma protein synthesized in the liver. It is a flexible protein that will readily change shape as a result of ligand binding or changes in environmental conditions; however, the presence of disulfide bridges provides strength and allows albumin to easily regain its original structure. Albumin has exhibited several properties that make it beneficial to human health, such as antioxidant and anticoagulatory activities and ability to maintain microvascular integrity (Nicholson *et al.*, 2000). Although it is typically derived from egg whites, albumin can also be isolated from mollusks, crustaceans, and low-fat fish (Ockerman and Hansen, 1988). In order to extract albumin from marine sources, minced flesh is cooked in acid and the resulting digested protein is pressed, ground, extracted, digested with sodium hydroxide, and neutralized with lactic acid to produce a mixture of mostly polypeptides that is spray-dried for use in industry. Marine-derived albumin can be used as a replacement for egg albumin as a whipping, suspending, or stabilizing agent (Haard *et al.*, 1994).

Also of interest is protamine, a simple, cationic protein consisting largely of arginine residues. Protamine associates with DNA in the place of histones in spermatozoa and can be extracted from the spermatic cells of fish, birds, and mammals (Ohshima, 1998; Potter *et al.*, 2005). Commercial marine

sources include salmon and herring milt. The major application of protamine in the food industry thus far has been as a preservative in products such as fruits, rice, and confectionaries. Protamine is a promising antibacterial agent in foods because it does not coagulate under heat and can kill or significantly inhibit the growth of some bacteria, disrupting the cell envelopes of both Gram-negative and Gram-positive bacteria (Islam *et al.*, 1986; Johansen *et al.*, 1997). The antibacterial effect of protamine is slightly reduced in food matrices due to interferences such as nonspecific binding to negatively charged food particles and the presence of divalent cations (Ca^{2+} and Mg^{2+}) (Pink *et al.*, 2003; Potter *et al.*, 2005). Fortunately, these inhibitory effects have been shown to be reduced by altering the electrostatic properties of protamine (Potter *et al.*, 2005).

A protein mixture similar in composition to soy meal can be commercially extracted from the extremophile *Dunaliella* for use in mariculture and animal feed. The industrial-scale growth of *Dunaliella* can turn out protein extract at about 100-times greater productivity than that reported in agriculture and 50-fold greater than in fish farming (Herbert, 1992). Protein powders can also be extracted from the processing discards of fish such as arrowtooth flounder (*Atheresthes stomias*) and herring (*Clupea harengus*). These freeze-dried powders have been reported to possess favorable nutritional and functional properties, exhibiting desirable mineral levels and amino acid profiles along with high fat adsorption and emulsifying capacities (Sathivel *et al.*, 2004).

E. ENZYMES

1. *Marine enzymes: Introduction and sources*

Enzymes are bioactive compounds with the ability to transform other molecules, making them valuable biotechnological tools for use in the food and feed industries. As ingredients in food, enzymes can influence factors such as processing, storage, spoilage, and safety. Thanks to advances in biotechnology, the use of marine-derived enzymes in food applications has grown into a promising field of research (Table I) (Diaz-Lopez and Garcia-Carreno, 2000; Venugopal and Shahidi, 1995). Excellent reviews with in-depth discussions of the use of marine-derived enzymes available include Okada and Morrissey (2007), Shahidi and Janak Kamil (2001), and Haard and Simpson (2000).

Owing to its vast diversity of organisms and habitats, the marine world is a rich source of unique and valuable enzymes with potential applications in the food industry. Many marine-derived enzymes have physical, chemical, and/or catalytic properties unparalleled by their terrestrial counterparts. For example, most marine enzymes have cold-adapted properties that are useful in food and

feed processing, such as their ability to have high catalytic activity at low temperatures and to be inactivated at moderate temperatures (Diaz-Lopez and Garcia-Carreno, 2000). Additionally, marine-based enzymes are valuable as food ingredients and in food processing due to their specificity, diverse properties, salt tolerance, and high activity at mild pH (Okada and Morrissey, 2007; Shahidi and Janak Kamil, 2001). Taken together, these properties allow for production of food without the undesired side effects and by-products resulting from use of enzymes that operate under more extreme conditions such as higher temperatures. Use of enzymes in the food industry also has benefits over chemical or mechanical methods, which are oftentimes harsher and more damaging to a product (Shahidi and Janak Kamil, 2001). The main challenges for use of marine-derived enzymes are limited availability depending on harvest; instability of raw material; and potentially poor economic advantages depending on technologies for extraction, potential markets, and quality of by-products (Haard *et al.*, 1994). A potential biotechnological approach to these challenges is in the transfection and overexpression of fish enzyme genes in select marine microorganisms (Simpson, 2000).

Major sources of marine enzymes are by-products produced as a result of fish and shellfish processing such as the viscera, heads, skin, bones, exoskeletons, and shells. Specific sources for different marine enzymes are listed in Table I. Some novel sources of enzymes with unique properties include extremophiles and red algae.

2. *Marine enzymes: Applications*

Traditionally, marine-based enzymes have been used in a limited number of products, that is, fish sauce, cured herring, or fish protein hydrolysate; however, more recent uses include the accelerated production of other products such as PUFAs and improving processing techniques such as the removal of skin, scales, and membranes from fish; purification and cleaning of roe for caviar production; extraction of carotenoproteins from shellfish processing waste; substituting rennet during cheese manufacturing; removal of the oxidized flavor from milk; ripening and fermentation of fish product (for fish sauce); and preparation of fish protein hydrolysates and concentrates (Diaz-Lopez and Garcia-Carreno, 2000; Shahidi and Janak Kamil, 2001; Venugopal and Shahidi, 1995).

3. *Marine enzymes available for use in the food industry*

There are numerous marine-derived enzymes available for use in the food industry (Table I). Although an attempt will be made to discuss the major categories of important marine enzymes, a large number of enzymes are

available and the reader is referred to the reviews mentioned in the beginning of the Section III.E.1 for further information. Enzymes that have been isolated from the marine world for use in the food industry include digestive proteolytic enzymes such as gastric, serine, and cysteine or thiol proteases; lipases; polyphenol oxidases (PPOs); chitinolytic enzymes; muscle proteases; transglutaminase; extremophilic enzymes; and a novel red algae enzyme.

a. Digestive proteases. Proteases are enzymes that cleave the peptide bonds in proteins. Although they are considered to be the most important and widely used group of enzymes in the food industry, proteases extracted from marine organisms are used in limited amounts, in part due to a lack of basic knowledge regarding these specific enzymes and also due to consumer attitudes toward their source—fish and seafood discards (Simpson, 2000). However, digestive proteases from marine sources have received growing interest from researchers and food processors owing to their high enzymatic activities at low temperatures and an increasing availability of raw materials such as viscera (Okada and Morrissey, 2007). Fish viscera are a rich source of digestive enzymes, such as pepsin, trypsin, chymotrypsin, and gastricsin, and numerous researchers have been developing methods for their recovery on a large scale (Haard *et al.*, 1994; Reece, 1988). Work is being carried out around the world in countries such as Japan, Great Britain, and Denmark, with specific examples being the Icelandic Fisheries Laboratory, which has developed a way to recover trypsin-like enzymes from cod viscera (Stefansson and Steingrimsdottir, 1990) and Marine Biochemicals (Tromso, Norway), which has developed industrial-scale methods for the recovery of trypsin, pepsin, chymotrypsin, alkaline phosphatase, and hyaluronidase from fish viscera (Almas, 1990). An alternative new technology is the utilization of microorganisms to produce enzymes from marine sources, for example cold-adapted proteases from marine invertebrates have been successfully expressed in yeast (Kristjansdottir and Gudmundsdottir, 2000).

Due to their ability to hydrolyze proteins, proteases can significantly alter the texture of food products. One of the most economically important applications of proteases is in the tenderizing of meat after rigor mortis (Whitaker, 1996). They can also be used to enhance the texture of cereals and baked products, remove membranes from organs and egg sacks, thereby improving drying and quality of egg products, ripen cheeses, remove skin from fish and squid, and recover bone proteins (Haard *et al.*, 1994; Simpson, 2000). In shrimp, proteases can be used to loosen shells from the meat, recover flavor compounds for use in surimi-based and cereal-based extrusion products, and recover carotenoprotein (up to 80% of the protein and 90% of the astaxanthin in shell waste) (Haard *et al.*, 1994). Stomachless marine organisms, such as crayfish, cunner, and puffer, also contain digestive

proteases that can be used to inactivate PPO and/or pectin-esterases in fruit juices (Shahidi and Janak Kamil, 2001). Three of the most widespread types of digestive proteases found in the marine world are acid/aspartyl (gastric) proteases, serine proteases, and cysteine or thiol proteases.

Acid/aspartyl proteases are found in the stomachs of animals and are therefore active under acidic conditions and inactive in alkaline environments (Simpson, 2000). Three main types of gastric proteases are pepsins, gastricsins, and chymosins. Pepsins are aspartic endopeptidases that have been found in the gastric fluid of numerous marine and freshwater species (Shahidi and Janak Kamil, 2001). Cold-adapted pepsins from Atlantic cod (*Gadus morhua*) are being recovered in Norway, with commercial applications in the cheese industry for cold renneting milk and in the fish feed industry to assist in digestion (Simpson, 2000). Gastricsin is a gastric protease that has been identified in marine organisms and has similar properties as pepsin. A third type of acid protease of interest is the rennin chymosin, which is typically present in the digestive compartment of young ruminant stomachs. Chymosin has been identified in the stomachs of marine organisms such as carp and harp seals (Shahidi and Janak Kamil, 2001). Cheddar cheese prepared with seal chymosins was reported to have higher sensory scores compared to cheese made with calf rennet (Simpson, 2000).

Digestive serine proteases are present in the pyloric ceca, the pancreatic tissues, and the intestines of animals and have been reported in numerous species of Archaea (Eichler, 2001). Serine proteases are inactive at acidic pH and have high activity under neutral to slightly alkaline conditions (Simpson, 2000). Although fish serine proteases are quite similar to their mammalian counterparts, they have been reported to be more active under alkaline rather than neutral conditions (Shahidi and Janak Kamil, 2001). Some of the most well-known serine proteases from marine sources include trypsins, chymotrypsins, collagenases, and elastases.

Trypsin-like enzymes can be found in both cold- and warm-water marine organisms such as stomachless bone fish (*Carassius auratus gibelio*), sardines, and others (Shahidi and Janak Kamil, 2001). They can inactivate enzymes such as PPO, giving them potential use in the food industry for preventing undesired color changes in PPO-containing products such as shrimp and fruit (Haard *et al.*, 1994). Trypsins and other digestive proteases have also been isolated from crustaceans and mollusks in an organ called the hepatopancreas, which is a combination of the mammalian liver and pancreas (Shahidi and Janak Kamil, 2001). A popular marine source for trypsins is the Atlantic cod, which contains cold-adapted enzymes that have catalytic activity between 4 and 55°C and are sensitive to inactivation by autolysis, low pH, and/or moderate heat (65°C or above). Research conducted over the past two decades has helped to develop industrial methods for extraction

of cold-adapted trypsins from fish processing by-products such as the pyloric cecum of cod. These trypsins have promising applications in areas of food processing that require protein digestion at low temperatures in order to avoid undesirable chemical reactions and bacterial contamination (Gudmundsdottir and Palsdottir, 2005). For example, cod trypsins have been used for low-temperature curing of herring (Matjes) and in squid fermentation, thereby accelerating the ripening process which traditionally takes about 1 year (Haard *et al.*, 1994; Simpson, 2000).

Another type of serine protease, elastase, is produced by the pancreas and is meant for the digestion of proteins such as elastin, a fibrous protein found in connective tissues. Elastase is an intestinal protease that operates at alkaline pH and has been isolated from marine animals such as carp, catfish, and Atlantic cod (Shahidi and Janak Kamil, 2001; Simpson, 2000). A fourth category of marine serine proteases is the digestive collagenases, which have been isolated from the digestive organs of many fish and from the hepatopancreas of marine invertebrates such as crab, prawn, and lobster (Haard *et al.*, 1994; Shahidi and Janak Kamil, 2001). Collagenases are thought to be one of the principal compounds responsible for flesh mushiness observed in the seafood industry following handling and storage; therefore, these enzymes have potential applications as meat tenderizers in the manufacturing of high-quality meat and meat products (Haard *et al.*, 1994).

Like the gastric proteases, digestive cysteine or thiol proteases are active at acidic pH and inactive at basic pH. They are important components of the hepatopancreas of many marine crustaceans, and are responsible for over 90% of the protease activity in the hepatopancreas in short-finned squid (*Illex illecebrosus*) (Raksakulthai and Haard, 2001). Cathepsin B is one example of a marine-derived digestive thiol protease. Only a few marine sources have been identified for cathepsin B, including surf clam (*Spisula solidissima*), horse clam (*Tresus capax*), and mussel (*Perna perna* L.) (Simpson, 2000).

b. Lipases. Lipases are mainly produced in the pancreas and catalyze the hydrolysis of triglycerides into free fatty acids, mono and/or diglycerides, and glycerol at the lipid-water interface. Marine sources of lipases include Atlantic cod, seal, salmon, sardines, Indian mackerel, red sea bream, and others. Lipases have valuable applications in the food industry because they have distinct specificities and they are able to catalyze processes such as esterification, hydrolysis, and exchange of fatty acids in esters (Shahidi and Wanasundara, 1998). These characteristics provide numerous opportunities for use of marine lipases in the fats and oils industry such as the production of triglycerides enriched with n-3 LC-PUFAs. Production of these triglycerides using commercially available lipases from nonmarine sources is

generally difficult because nonmarine lipases are either less specific than marine lipases or they have different specificities than desired (Shahidi and Janak Kamil, 2001). A marine lipase isolated from Atlantic cod was shown to preferentially hydrolyze LC-PUFAs over shorter-chain fatty acids (Lie and Lambertsen, 1985), and lipase-assisted hydrolysis of seal blubber oil and menhaden oil has been used for enrichment of acylglycerols with n-3 LC-PUFAs (Shahidi and Wanasundara, 1998).

c. Polyphenol oxidases. PPOs, including tyrosinase, polyphenolase, phenolase, catechol oxidase, cresolase, and catecholase, are found in plants, animals, and a number of microorganisms. They are responsible for the postharvest brown discolorations that appear in certain crustaceans, fruits, and vegetables, and for the brown and black colors of products such as tea, coffee, raisins, and prunes (Whitaker, 1996). These dark pigments are a result of the PPO-catalyzed oxidation of diphenols to form quinones, which then undergo further oxidation and polymerization. Traditionally, plant-derived PPOs have been used in tea fermentation; however, marine PPOs are actually better suited for this process because they are cold-adapted and therefore have higher activity at low/moderate temperatures compared with their terrestrial counterparts. In addition to extraction of PPOs from crustacean by-products, use of genetic engineering to produce PPOs from marine microorganisms has also been suggested (Haard *et al.*, 1994).

d. Chitinolytic enzymes. In nature, chitinolytic enzymes are utilized for degradation of chitin during molting of insects and crustaceans and as digestive aids, as they are able to disrupt the exoskeleton of prey, allowing access to the soft inner tissues. The genes for these chitinolytic enzymes have been cloned from several different organisms (Shahidi and Abuzaytoun, 2005). Chitinases have been identified in the digestive tracts of numerous fish, in shellfish and shellfish waste, and in squid liver and octopus saliva. Chitin degradation enzymes have also been identified in the hyperthermophilic archaea *Thermococcus chitonophagus* (Andronopoulou and Vorgias, 2004) and in the marine bacterium *Bacillus* sp. LJ-25 (Lee *et al.*, 2000). Chitinases have a wide range of potential applications in the food industry; for example, they can replace hydrochloric acid in the conversion of chitin into commercially available oligomeric units, resulting in products with more consistent characteristics (Shahidi and Janak Kamil, 2001).

e. Transglutaminase. While most enzymes utilized in the food industry are responsible for the breakdown of specific compounds, transglutaminase is unique in that it is able to modify protein functions by promoting cross-links (Ashie and Lanier, 2000). There exist numerous marine sources for

transglutaminase, including red sea bream, rainbow trout, atka mackerel, walleye, pollock liver, muscles of scallop, botan shrimp, and squid (Shahidi and Janak Kamil, 2001). A major commercial application of transglutaminase is in cross-linking proteins for the production of surimi, thereby improving the rheological properties of the protein gel (Ohshima, 1998; Shahidi and Janak Kamil, 2001). Recently, a microbial transglutaminase was reported to catalyze gel formation from gelatin. Unlike typical gelatin-based gels, transglutaminase-catalyzed gels were thermally irreversible, presumably due to the strong cross-linking ability of this enzyme. Addition of chitosan was reported to promote gel strength and results in more rapid formation of gels (Chen *et al.*, 2003).

f. Extremophilic enzymes. Enzymes produced by extremophiles can have valuable applications in the food industry owing to their ability to function under a diverse range of extreme conditions. For example, thermophilic enzymes can operate within a temperature range of 45–100 °C or more. These enzymes have current use in the production of natural sweeteners and also have potential applications in reactions involving transesterification and synthesis of oligosaccharides, peptides, and phospholipids. Specific examples include: enzymes that interact with carbohydrates, such as α-amylase, glucoamylase, cellulase, pectinase, β-galactosidase, xylose isomerase, and pullulanase; neutral (fungal) proteases for baking and brewing; and lipases and acid proteases for use in food processing (Herbert, 1992).

Psychrophilic enzymes, which operate within temperatures ranging from −5 to +20 °C, also have numerous advantages for use in the food industry: they have high activity at low to moderate temperatures, they are easily inactivated with heat, and bacterial contamination and competing reactions are reduced at low temperatures (Gerday *et al.*, 2000; Herbert, 1992). Specific cold-adapted enzymes that have been isolated from Antarctic and Arctic microorganisms include alcohol dehydrogenase, α-amylase, aspartate transcarbamylase, Ca^{2+}–Zn^{2+} protease, citrate synthase, β-lactamase, malate dehydrogenase, subtilisin, triose phosphate isomerase, and xylanase (Gerday *et al.*, 2000). Psychrophilic enzymes can be useful as replacements for their mesophilic counterparts in processes such as beer and wine fermentation and cheese production. Cold-adapted amylases, proteases, and xylanases are useful to the dough industry for reducing fermentation time and improving textural properties. Psychrophilic enzymes can replace calf rennet in manufacturing cheese, thereby reducing residual heat coagulation. Some examples of commercially available extremophilic rennet agents are: Marzyme II® and Modilase®. The cold-adapted form of β-galactosidase is useful to the milk industry because it allows for the breakdown of lactose

into glucose and galactose at lower temperatures, thereby reducing bacterial activity and speeding productivity (Herbert, 1992).

Another potential commercial application of extremophiles is in the use of the alkaliphilic form of the enzyme cyclomaltodextrin glucanotransferase for production of cyclodextrins (CDs) from starch. CDs are widely used in the food industry as emulsifying, foaming, and stabilizing agents, and the possibility for their low-cost commercial production using alkaliphilic *Bacillus* sp. has been demonstrated. *Bacillus* sp. alkaliphiles also have been reported to contain extracellular β-mannanases, which have potential in the food industry because they can hydrolyse products that contain mannan, such as guar gum. Another product of *Bacillus* sp. alkaliphiles is pectinolytic enzymes, which have been used to treat pectin-containing effluent at an orange-canning operation. Alkaliphiles are also known to synthesize xylanases, which could be used to produce valuable products from plant residues such as wheat and rice straw (Herbert, 1992).

g. Enzymes from red algae. Recent research has revealed a type of red marine algae (genera *Gracilariales*) that produces enzymes important in the starch degradation pathway, including α-1,4-glucan lyase, which catalyzes the formation of the natural sugar 1,5-anhydro-D-fructose (AF). AF, which is also present in seaweeds and edible mushrooms, is a versatile molecule that is a precursor to compounds with antioxidant, antimicrobial, and/or anti-blood-clotting and antitumor properties (Yu, 2005). Researchers also isolated an enzyme in the starch degradation pathway that is known to convert AF into the antifungal compound microthecin.

IV. CONCLUSIONS

Marine biotechnology for the production of food ingredients has experienced rapid growth and shows great potential for the future. The number of food ingredients that can be derived from marine sources is ever-increasing thanks to advances in the biotechnological tools utilized for their identification and extraction. These resulting components can be used in a variety of applications, such as fortification/nutraceuticals, natural pigments, stabilization, antimicrobial food coatings, and in the development of more efficient and natural food processing techniques.

Despite the number of opportunities that the tools of marine biotechnology provide for production of food ingredients, numerous challenges remain. In order to maximize profits in the production of marine-derived compounds, cultivation techniques for select marine organisms must be

refined and made more efficient. Also, in many cases, there lies the challenge of finding niche markets for marine-derived ingredients in which they can economically compete with or surpass their synthetic counterparts. Although recent developments in genetics show promising results, aquatic plant transgenic research remains far behind that for terrestrial plants. Overall, the field of marine biotechnology for production of food ingredients is still in its developing stages, with many challenges to overcome; however, there is great potential and what seem to be unlimited opportunities for growth and progress.

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