Valorisation of natural extracts from marine source focused on marine by-products: A review

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A B S T R A C T

The wide chemical and biological diversity observed in the marine environment makes the ocean an extraordinary source of high added value compounds (HAVC) which can be employed in many applications. Minerals, lipids, amino acids, polysaccharides and proteins from marine sources have unique features and, surprisingly, their highest concentration is often found in parts of marine organisms that are commonly discarded. Fish heads, viscera, skin, tails, offal and blood, as well as seafood shells possess several HAVC suitable for human health applications, yet most end up as residues throughout the raw material processing. This review updates information on this issue and conveys critical analysis of the chief methodologies to carry out extraction, purification and eventual transformation, with a focus on their actual and potential applications.

1. Introduction

Fishing is a rather ancient human activity and has played an important role in many human societies. Since the early 1960s, the state of fisheries has been consistently monitored by the Food and Agriculture Organization (FAO) Fisheries Resources Division. According to FAO (2000), the world fish production from marine catches has

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been increasing at an yearly rate of 6%, from 1950 (ca. 19.3 million tons) up to 1970 (ca. around 60 million tons) (Blanco, Sotelo, Chapela, & Pérez-Martín, 2007); however, more recent data point at more than 91 million tons of fish and shellfish caught each year (Rustad, 2003). In the 25 Member States European Union, the annual fish captures (from fishing and aquaculture) added up to 6.9 million ton in 2003 (Fishery Statistic Data, Eurostat, 2006) and the fish processing sector has generated an added value of EUR 3.8 billion (Eurostat, NACE, 2008).

Nowadays, an integrated and sustainable exploitation of fisheries resources is a must as only 50% of the catch is used for actual human consumption. Fisheries worldwide annual discards are estimated to be ca. 20 million tons, which represent 25% of the catch (Rustad, 2003) and include "non-target" species, and processing waste and by-products. In the European Union those discards represent a total of ca. 5.2 million tons per year (AWARENET, 2004; Mahro & Timm, 2007). Fish filleting, salting and smoking generate the major amount of solid waste and by-products (50–75% of the fish) with a total of 3.17 million tons per year; fish canning is regarded as the second source of solid waste and by-products (30–65% of the fish) with an amount of 1.5 million tons per year; crustaceans and mollusk processing also generate significant amounts of solid residues (20–50%), ca. 0.5 million tons per year (AWARENET, 2004). In the UK it is estimated that ca. 313,000 tons of seafood processing residues are produced every year, so only 43% of the catches end up as products for human consumption (Archer, Watson, Garret, & Large, 2005).

Waste and by-products discharged by fisheries are currently rising, driven by both a net increase in fisheries products consumption and the changing consumer trend towards ready-to-use products. The seafood consumption has consistently increased during recent years as seafood has been progressively recognized as an important source of nutrients for human health. Proteins, lipids and polysaccharides but also minerals from seafood possess unique features some of which are a consequence of specific environmental factors prevailing in the marine environment, such as temperature, pressure, salt concentration and oxygen availability (Shahidi, 1997). Seafood by-products and waste constitute at present a serious environmental problem (Arvanitoyannis & Kassaveti, 2008); those by-products and waste require appropriate management, especially because they are highly perishable, owing chiefly to the action of microorganisms that there find an excellent growth medium. Furthermore, more stringent quality and hygienic standards enforced to industries have caused a significant increase in the amounts of waste and wastewater generated from seafood processing in recent years.

Major efforts have been focused on how to deal with the aforementioned by-products and waste in efficient ways, including useful applications; at present, most are taken advantage of as animal feed and plant fertilizers as well as ingredients for adhesive manufacture (AWARENET, 2004). Nutraceutical, functional food formulation or pharmaceutical applications can also be hypothesized as they already exist in such inexpensive marine feedstocks as head, viscera, skin, tails, offal, blood and shells (Kim & Mendis, 2005). Extraction of those high added value compounds (HAVC)—which can be profitable owing to their beneficial role upon human health—joined to the development of new technologies for recovery and purification, will run along with concomitant benefits towards long term sustainability of marine activities.

2. High added value compounds in marine by-products and waste

By-products generated during seafood processing typically range between 20 and 60% of starting raw material. For pelagic fish, such as tuna, cod, mackerel, anchovy and herring, major amounts of residues are represented by offal, head and tail (27% of the fish) collected through eviscerating, cutting and filleting processes. Skin, bones, blood and frames are the second major residues (25% of the fish) collected along skinning and cutting processes (AWARENET, 2004).

At present, the majority of these by-products are sent to fish meal plants, where fish meal and fish oil are produced. Fish meal is by far the most valuable non-edible commodity produced from marine by-products; its global production ranges between 5.5 and 7.5 million tons/year (Hardy & Tacon, 2002). It is a relatively dry product, used either as animal feed or as plant fertilizer, and is composed of protein (70%), minerals (10%), fat (10%) and water (10%), on a weight basis (Blanco et al., 2007). The first stage of fish meal production includes mincing, cooking and pressing of solid fish by-products, from which wastewaters and a solid cake are generated. At a second stage, those wastewaters are dislodged to remove any remaining solid particles, which are then added to the solid cake, and then centrifuged to separate the oil fraction; this crude fish oil is further processed or sold as such. The water is fed back to the process line where the solid cake, along with the material from the wastewater, is dried and grounded to produce fish meal (SEAFISH, 2008). Fish oil can have edible and non-edible applications depending on its composition. Production of soap, glycerol, varnishes, drying and hydraulic oils, fertilizers and substrates for fermentation, are the most common uses, whilst its edible applications are essentially accounted for the production of margarine and shortenings (AWARENET, 2004).

Fish meal can exhibit very diverse specifications, in terms of amino acid profile, digestibility and palatability, depending on the raw material and production process employed (Blanco et al., 2007). Fish meal from pelagic fish is the most widely used, and may generate an income up to 108 Euro/ton depending on its specific composition; currently its average market value is 46 Euro/ton (AWARENET, 2004; SEAFISH, 2008). The main HAVC that can be found in marine by-products are presented in Table 1. The highest concentration of these bioactive compounds is generally found in the parts of the marine organisms that are discarded (AWARENET, 2004). Note that present concentrations can attain up to 80% of the by-product in question—especially in the case of lipids in cod liver. Table 1 also shows the mean market values for selected HAVC that, as expected, are strongly dependent upon purity. It must be highlighted that the least expensive HAVC—gelatin/collagen—hold a value 30-fold higher than fish meal. Finally, for HAVC such as natural free amino acids and hydroxyapatite, a market value has not been established yet, since only synthetic forms have been used until now.

In the following sections, the current and potential uses of the most important types of HAVC will be described in detail together with reference to state-of-the-art technologies for extraction and purification.

2.1. Polyunsaturated fatty acids ω-3

ω-3 fatty acid concentrates remain a topic of general interest for the pharmaceutical and food industries, for the production of drugs with enhanced performance and for the production of nutritional supplements. As known, fat is a source of energy but is also a vital structural component of cellular membranes and is involved in many important cell-signaling pathways (Ma et al., 2004). Since the early studies on long-chain ω-3 polyunsaturated fatty acids (PUFA) carried out by Burr back in 1929, the health benefits of these compounds have been thoroughly investigated, so their market is expected to grow further in the future (Bimbo, 2007). Such HAVC are considered as "essential fatty acids" due to their key role in many metabolic processes and because they cannot be synthesized *de novo* by mammalian cells (Harris, Miller, Tighe, Davidson, & Schaefer, 2007). Dietary ω-3 PUFA ameliorates numerous biological and physiological functions in human body, being particularly valuable in treating some diseases (Ma et al., 2004).

In cell culture, ω-3 and ω-6 PUFA are generated from the precursor α-linolenic acid and linoleic acid, respectively (Tapiero, Ba Nguyen,

Table 1
Content and typical market value of high added value compounds found in marine by-products.

High added value compounds	Marine by-products	Content (% w/w)	Market value (Euro/kg)	References
Polyunsaturated fatty acids (ω-3 and ω-6)	Algae, cod liver, oil of mackerel flesh residues	50–80% in cod liver, 23% are ω-3 PUFA	24 (as purified cod liver oil)	Bimbo (2007), Falch, Rustad, and Aursand (2006), Mondello, Tranchida, Dugo, and Dugo (2006), Ward and Singh (2005), AWARENET (2004)
Free amino acids	Mussels, fresh clams, white fish flesh residues, crustacean shells	0.8–2% of taurine, 2.7% of creatine (on dry matter)	n.a.	Larsen, Stormo, Dragnes, and Elvevoll (2007), Leuchtenberger, Huthmacher, and Drauz (2005), Heu, Kim, and Shahidi (2003)
Chitin and chitosan	Shrimp and crab shells	15–40%	15–750	Abdou, Nagy, and Elsabee (2008), Sini, Santosh, and Mathew (2007), Kurita (2006), AWARENET (2004), Healy, Green, and Healy (2003), Kumar (2000)
Collagen and gelatin	Pelagic fish skin, scales and bones	Up to 80% in skin, up to 50% in scales	9–14	Wang et al. (2008), Kim, Mendis, and Shahidi (2007), Wasswa, Tang, and Gu (2007), AWARENET (2004)
Hydroxyapatite	Pelagic fish scales and bones	60–70% in bones, up to 50% in scales	n.a.	Barakat et al. (2008), Aronov, Karlov, and Rosenman (2007), Haberko et al. (2006)
Antifreeze proteins	Cold water pelagic fish blood and skin	5–35 mg/ml in cold water fish blood	5000	Jin and DeVries (2006), AWARENET (2004), Evans and Fletcher (2004), Crevel, Fedyk, and Spurgeon (2002)
Astaxanthin	Algae, crustacean shells	2.3–33%	3000–12,000	Babu, Chakrabarti, Raja, and Sambasivarao (2008), Yang (2007), López-Cervantes, Sánchez-Machado, Gutiérrez-Coronado, and Ríos-Vázquez (2006), AWARENET (2004)
Enzymes	Algae, pelagic fish viscera	–	14,400 (cod proteases)	SEAFISH (2008); Rasmussen and Morrissey (2007), Shahidi and JanakKamil (2001)

n.a.—not available.

Couvreur, & Tew, 2002); the former leads to eicosapentaenoic acid (EPA, 20:5ω3) and docoesahexaenoic acid (DHA, 22:6ω3), whereas the latter leads to arachidonic acid (AA, 20:4ω6) and other long-chain ω-6 fatty acids (Harris et al., 2007; Tapiero et al., 2002). After absorption in the human body, PUFA are incorporated at cellular level into triglycerides (i.e., 3 fatty acids molecules on a glycerol backbone), phospholipids (i.e., 2 fatty acids molecules on a phosphatidic acid backbone) and cholesterol esters (i.e., 1 fatty acid molecule on free cholesterol). Some studies indicate that ω-3 PUFA are most promptly absorbed from the intestine when taken orally in the form of free fatty acids, moderately absorbed if in the triglyceride form and poorly absorbed if in ethyl esters form. However, ω-3 PUFA in the form of triglycerides are the most stable and desirable for food formulation, unlike as free fatty acids, which are easily oxidized, and as ethyl esters, which continue to be unacceptable for food purposes in terms of safety (Liu, Zhang, Hong, & Ji, 2007). The major reported effects of ω-3 PUFA—EPA and DHA—on human tissues are described in Table 2.

Table 2
Human health effects of ω-3 polyunsaturated fatty acids EPA and DHA.

Claimed function of EPA and DHA	References
Improvement of vision acuity, field of vision and adaptation to light. Alteration of permeability, fluidity, thickness and lipid phase properties of retinal photoreceptor membrane *(DHA only)*	Harris et al. (2007), SanGiovanni and Chew (2005)
Reduction of risk of myocardium lesion and coronary heart disease via lowering blood pressure, triglyceride levels and platelet aggregation, and prevention of arrhythmias	Harris et al. (2007), Moore et al. (2006)
Protective effects against some common cancers (breast and colon) via inhibition of eicosanoids synthesis	Goldberg and Katz (2007), Rose and Connolly (1999)
Increase of level of insulin	Patel et al. (2007)
Beneficial effects in sclerosis treatments via affecting functions of central nervous system	Weinstock-Guttman et al. (2005)
Preventive and curative effects upon non-alcoholic fatty liver disease via lowering liver fat content	Spadaro et al. (2008)
Increase of toleration to noxious therapies (e.g. chemotherapy)	Morisco et al. (2007)
Decrease of heart rate beating	Svensson, Schmidt, Jorgesen, and Christensen (2007)
Improvement of emotional state (e.g. in depression and anxiety conditions)	Raeder, Steen, and Bjelland (2007)
Beneficial effects on autoimmune diseases (e.g. rheumatoid arthritis, psoriasis, systemic lupus, Crohn's disease).	Curtis, Harwood, Dent, and Caterson (2004)
Reduction of joint pain associated with inflammatory conditions, via inhibition of central regulators of inflammation.	Fedacko et al. (2007), Goldberg and Katz (2007)

PUFA can be recovered from a number of marine by-products. Marine protists and microalgae have been referred as the major sources of EPA and DHA (*Schizochytrium* spp. for the specific recovery of DHA; *Phaeodactylum* and *Monodus* spp. for the specific recovery of EPA; *Isochrysis galbana* spp. for the recovery of both EPA and DHA) (Robles Medina, Molina Grima, Giménez Giménez, & Ibañez González, 1998; Ward & Singh, 2005). Antarctic krill (Ju & Harvey, 2004) and by-products from edible fish (e.g. sardine, anchovy, salmon and cod) are the second most important sources of PUFA (Mondello et al., 2006), followed by fish caught especially for fish meal and oil production (Bimbo, 2007).

The most important natural sources of ω-3 PUFA are indeed fish oils of common species such as sardine, mackerel, cod, shark and menhaden with PUFA levels of ca. 30%, which makes them commercially interesting raw materials to prepare ω-3 PUFA concentrates (Chakraborty & Raj, 2007). In particular, cod liver has for long been the most suitable marine by-product for recovery of ω-3 PUFA, for extended use as nutraceutical, having a high content of vitamins A, D and E (Mondello et al., 2006); this oil contains high amounts of lipids (ca. 50–80%), of which 23% is accounted for EPA (Falch et al., 2006; Kolakowska et al., 2002). Anchovy and bluefin possess exceptionally high DHA levels. Salmon head, which represents the main salmon by-product, is also regarded as a good source of PUFA due to a significant content of lipids, ca. 15–18% (Huang & Shativel, 2008). Among fish flesh, mackerel is accordingly the main source of EPA/DHA: it yields ca. 15% (w/w) of oil, with 9% (w/w) of PUFAs and 4% (w/w) of DHA in particular, as triglycerides (Linko & Hayakawa, 1996). Mackerel flesh content of PUFA is in order of 1810 mg per 100 g, followed by salmon with 1800 mg per 100 g, tuna with 1500 mg per 100 g, herring with 1200 mg per 100 g), salmon trout with 1060 mg per 100 g and cod with 240 mg per 100 g (Fedacko et al., 2007).

Microcapsules containing ω-3 PUFA concentrates are a useful form of PUFA supply to the human body, as they convey an extended stability and allow for the release of the active compound in the intestine only (Kantor, Steiner, & Pack, 1990). Microencapsulation also eliminates the well-known unpleasant taste and smell of fish oil, as well as its aftertaste (particularly when ingested in relatively large quantities), and effectively prevents oxidation of PUFA. When microencapsulation is not applied, lipid peroxidation is normally

difficult to avoid and addition of antioxidants to PUFA is required. For preventing oxidation several combinations have been tested: the most successful result has been achieved with a ternary antioxidant system containing α-, γ- and δ-tocopherol concentrates (in the range of 0.2–2.0%), ascorbic acid (or ascorbyl palmitate), soy lecithin, quercetin, morin and catechin (Kamal-Eldin & Yanishlieva, 2002). Other alternative solutions have also been proposed: histidine, for instance, shows a pronounced effect towards preservation of herring oil, whereas catechin, morin and quercetine show strong antioxidative effects on sardine oil up to 60 °C (Kamal-Eldin & Yanishlieva, 2002).

Numerous methods are currently employed for ω-3 PUFA concentration; however, only few are suitable for large-scale production. A single separation/purification method is not often feasible, because the raw material is usually too heterogeneous with relatively high contents of undesirable compounds. Nowadays, PUFA are mainly recovered as free fatty acids, after chemical or enzymatic hydrolysis of marine oil, followed by purification. Depending on the desired yield, available methods include distillation (Chang, Bao, & Pelura, 1989; Shahidi & Wanasundara, 1998), enzymatic splitting (Camacho-Páez, Robles-Medina, Camacho, González-Moreno, & Molina-Grima, 2002; Halldorsson, Kristinsson, & Haraldsson, 2004), low-temperature crystallization (Harris et al., 2007; Shahidi & Wanasundara, 1998), supercritical fluid extraction (Catchpole, Grey, & Noemark, 2000) coupled or not with nanofiltration (Sarrade, Rios, & Carles, 1998), urea complexation (Chakraborty & Raj, 2007; Gámez-Meza et al., 2003), and argentation chromatography (Chakraborty & Raj, 2007; Mondello et al., 2006). Each technique has its own advantages and drawbacks and leads to ω-3 PUFA concentrates in different forms. Chromatography, crystallization and urea complexation are useful techniques for collecting PUFA as free fatty acids (Chakraborty & Raj, 2007; Gámez-Meza et al., 2003) whereas supercritical fluid extraction, distillation and also urea complexation, are suitable techniques for the recovery of PUFA as fatty acids esters (Perretti et al., 2007; Shahidi & Wanasundara, 1998). PUFA as acylglycerols can be obtained by enzymatic methods (Halldorsson et al., 2004; Shimada, Sugihara, & Tominaga, 2001).

Distillation has been mentioned (Chang et al., 1989; Shahidi & Wanasundara, 1998) to produce odorless and flavorless oils, with improved flavor and oxidative stability, which contain only insignificant amounts of undesirable minor constituents, such as thermal and oxidative polymers of unsaturated glycerides, trans-isomers, conjugated dienes and trienes, cholesterols, pesticides and heavy metals. Two distillation methods currently in use are: vacuum steam distillation followed by silica gel treatment, and short-path molecular distillation. The former cannot improve significantly the ω-3 PUFA concentration however the resulting oil is highly pure and can already be subjected to specific techniques such as microencapsulation (Chang et al., 1989). Short-path molecular distillation has been used for partial separation of fatty acid esters; it is a rather old technique, performed at very high temperatures (250 °C) and for very short heating intervals, in the order of seconds. This method leads to increases in EPA and DHA concentrations up to the ranges of 16–28.4% and 9–43% respectively, in the case of menhaden oil (Shahidi & Wanasundara, 1998). However, the ω-3 PUFA alkyl esters form cannot be directly used for food or pharmaceutical purposes, so a further step of conversion to fatty acids or acylglycerols is required (Shahidi & Wanasundara, 1998).

To recover concentrated PUFA as acylglycerols, subcritical and supercritical fluid extraction has been performed with carbon dioxide as solvent and at various conditions depending on the fish oil initial composition. The most interesting result was obtained at 28 °C and 7.8 MPa, with EPA showing the greatest resistance to fractionation (Perretti et al., 2007). Note that in fish oils, EPA and DHA are preferentially located in the middle carbon atom of the glycerol backbone, so it has not been possible to date to achieve very high PUFA concentration if the oil is fractionated as triacylglycerols by physical methods (Corrêa, Peixoto, & Guaraldo Gonçalves, 2008). In this case, as also happened with distillation, the best performance is obtained only if oil is previously subjected to random hydrolysis and esterification (Perretti et al., 2007), where simultaneous hydrolysis and esterification are carried out via an alkaline catalyst (KOH or NaOH) and an alcohol (methanol or ethanol).

Some authors (Gámez-Meza et al., 2003; Halldorsson et al., 2004) observed that chemical methods partially destroy the natural all-*cis* ω-3 PUFA structure, whilst enzymatic hydrolysis provides milder conditions in terms of temperature, pH and pressure, and protects ω-3 PUFA against oxidation, *cis-trans* isomerization and double bond migrations. When an enzymatic process is chosen, it is brought about by lipases, i.e., glycerol ester hydrolases that catalyzes the hydrolysis of triacylglycerols into fatty acids, partial acylglycerols and glycerol. PUFA are firstly enriched in the free fatty acids by hydrolyzing PUFA-containing oil with a microbial lipase (e.g., *Aspergillus niger*, *Candida cylindracea*, *Chromobacterium viscosum* or *Pseudomonas* spp.), and such free fatty acids are then converted to esterified forms with a simple alcohol reaction using a lipase (Shimada et al., 2001). Due to potential benefit of PUFA as acylglycerols, enzymatic esterification of glycerol with individual free fatty acids EPA and DHA recovered by hydrolysis, and purification of PUFA-containing oils is gaining momentum; considerable attention is being given to microbial lipases since EPA and DHA are resistant to hydrolysis by commercial lipases (Gámez-Meza et al., 2003).

2.2. Taurine and creatine

The food and pharmaceutical industries use free amino acids to high levels for the production of food supplements, infant and adult formulae, drugs (European Commission, May, 2003b; Stapleton, Charlses, Redmond, & Bouchier-Hayes, 1997), and nutraceutical energy formulations (Bagchi, 2005). Two amino acids widely used in the free form—taurine and creatine—will be covered by this review paper.

2.2.1. Taurine

Taurine (or 2-aminoethanesulfonic acid, $NH_2–CH_2–CH_2–SO_3H$) is a neutral β-amino acid, which is not utilized in protein synthesis but found mostly in the free form in the human body (Redmond, Stapleton, Neary, & Bouchier-Hayes, 1998); a small amount of taurine is present in small peptides in the brain (Franconi, Di Leo, Bennardini, & Ghirlanda, 2004). Taurine is the dominant free amino acid in many living species; in terms of content, in the human body taurine is second only to glutamic acid. Both the amine and sulphonic groups of taurine can undergo ionization, and their dissociation constant contributes to the biological and physiological activities showed by taurine (Petrosian & Haroutounian, 2000). Osmotic regulation (Chiarla & Giovannini, 2004), cell membrane stabilization (Han, Budreau, & Chesney, 2000), body detoxification (Lourenço & Camilo, 2002), antioxidant protection (Métayer et al., 2008), immune defense enhancement (Redmond et al., 1998) and intracellular calcium homeostasis regulation (Takahashi, Schaffer, & Azuma, 1997) are the biological functions reported for taurine. Central nervous system neuromodulation (Rose, 1996), regulation of renal development and renal function (Han et al., 2000), anti-inflammatory activity (Chiarla & Giovannini, 2004), bile acids conjugation and choleostasis prevention (Lourenço & Camilo, 2002), antiarrhythmic, anti-inotropic and anti-chronotropic effects (McCarty, 2001), endocrine and metabolic effects (Rose, 1996) have been referred as physiological roles of taurine in human body. As reported by Petrosian and Haroutounian (2000), taurine can also convert lipid and lipid soluble compounds into a water-soluble state, a characteristic that makes it an interesting emulsifier in various food and pharmaceutical formulations.

Among water-soluble amino acids, taurine is the most heat-stable and after glycine, is the most water-soluble among all the heat-stable

(Petrosian & Haroutounian, 2000). Although taurine is a conditional essential amino acid for adults (Lourenço & Camilo, 2002), it is essential for newborns (Chiarla & Giovannini, 2004), for whom the availability of taurine is critical to assure the development of their central nervous system and muscles (Stapleton et al., 1997). In newborns, the normal level of taurine is provided by their mother's milk, as it is the predominant free amino acid in human milk. It is particularly important for preterm newborns of gestational age not above 32 weeks, as they possess a limited capacity to convert methionine into cysteine and hence to taurine, due to immaturity of the liver enzymes responsible for taurine synthesis (Rose, 1996). Since taurine is the predominant free amino acid in human milk, it is added in milk-based infant formulae, aimed at substituting mother's milk, at 5 mg/100 ml. On this regard, the European Scientific Committee of Food has proposed that taurine addition to cow's milk and any type of infant formula should not exceed 12 mg/100 ml (European Commission, May, 2003b).

In adult human body, taurine is synthesized from methionine and cysteine, in the presence of vitamin B_6 and it is excreted through urine and bile at daily rates of ca. 0.23 g although varying with age, sex, renal function and clinical condition (Lourenço & Camilo, 2002). Adult individuals are expected to exhibit an average of 1 g/kg of taurine of body weight. However, the synthesis ability varies widely amongst individuals: under stress conditions, such abilities may be impaired, hence making an external supply necessary; for this reason some authors consider taurine as a conditionally essential amino acid for adult population.

High concentrations of taurine have been found in animal sources; conversely, it is essentially undetectable in vegetable matrices. Marine organisms in particular exhibit high levels of taurine, as it is the main organic osmolite in marine species (Omura & Inagaki, 2000). Within seafood, raw mussel is the main source of taurine with typical levels of 655 mg/100 g, followed by fresh clams and raw white fish flesh with 240 mg and 151 mg per 100 g, respectively. Cooking causes no adverse effects on taurine levels in food, due to its thermal stability (Stapleton et al., 1997). In recent years the use of taurine has increased especially in the so called "energy drinks", due to particularly successful marketing strategies (Babu, Church, & Lewander, 2008). In the case of nutraceutical formulations, taurine is added to 400–600 mg/l (European Commission, March, 2003a) often in combination with chromium, D-ribose and withanolides, i.e. steroidal lactones with anti-inflammatory, anti-arthritic and anti-cancer activities (Bagchi, 2005), and also with potassium bicarbonate or ascorbate complexes (Boynton, 2005). Specifically in the case of drug formulation with antihypertensive action, taurine is present as calcium taurate ($NH_2–CH_2–CH_2–SO_3$)–Ca^{2+} in the form of an aqueous suspension (McCarty, 2001).

2.2.2. Creatine

Creatine (or methyl guanidine–acetic acid, $NH_2–C[=NH_2^+]–NH–CH_2–C[=O]–O^-$), is an α-amino acid synthesized from glycine, arginine and methionine (Clark, 1998) and can exist in the free or phoshorylated forms. It is stored in muscle tissues, mainly skeletal, followed by heart and smooth muscles, in which 60–70% thereof exists in the form of phosphocreatine (Clark, 1998). It is also present in the brain, liver, kidney and testes, but in smaller amounts. For a typical 70 kg-man, the total creatine in his body pool amounts to ca. 120 g, of which 95% is stored in the muscle (Greenhaff, 1997). In the human body, the average rate of creatine synthesis is 1–2 g/day (Clark, 1998), which is consumed by the normal physical activity as this amino acid is responsible of skeletal muscle contraction and regeneration (Bishoff & Heitz, 1994). Particularly, it provides energy during intense muscle exercise (Bigard, 1998) and as for taurine, it shows important biological and physiological functions, even in the presence of some diseases. Main claimed biological roles of creatine are neuroprotection against ischemic and oxidative insults, even in Parkinson's disease (Bender, Stamtleben, & Klopstock, 2008; Sullivan, Geiger, Mattson, & Scheff, 2000); slowing down neurodegeneration in Huntington's disease (Bender et al., 2005) and therapeutic effects in mitochondrial encephalomiopathies disease (Komura, Hobbiebrunken, Wilichowsky, & Hanafeld, 2003). Physiological functions of creatine, other than skeletal muscle regeneration and contraction, have been referred as cardiac muscle performance maintenance (Allard, Khursheed, & Sole, 2006; Gordon et al., 1995), energy provision during intense muscular exercise (Bigard, 1998), and fat-free mass promotion of human body (Sullivan et al., 2000).

The amino acid creatine is largely used in various food formulae, drugs and food supplements production. At present, the latter is the most profitable due to a widespread demand in sports at almost every level of athletic performance (Sullivan et al., 2000).

The main marine source of creatine is found in herring with ca. 6.5 g/kg. For comparison beef and pork meat possess levels of 5.5 and 5 g/kg, respectively. Salmon and cod come second and third highest in creatine content with ca. 4.5 and 3 g/kg on dry tissue, respectively (Newsholme & Hardy, 1998). As a food supplement or in drug formulation, creatine has been originally brought onto the market as a water-soluble powder, first in the form of creatine monohydrate, (till now the cheapest and the most widespread form), and later in more expensive versions of creatine phosphate, citrate and malate, even in powder form; tablets, gels, liquids, chewing gums and candies variants are also commercialized but are less used and less efficient as a delivery method for creatine supplementation and are more expensive than powder (Bigard, 1998; Green & Green, 1998). In milk-based infant formula, for instance, creatine is added as creatine monohydrate powder up to 0.8–2 mg/100 ml (European Commission, May, 2003b). In the recent years, a different form of creatine has been introduced into the market: research studies reported in fact that creatine supplements are poorly absorbed by muscle cells when administered in its polar form, leading to the need of larger dosages. Creatine ethyl esters, the esterified lipophilic form of creatine monohydrate, has been reported to increase the bio-availability of creatine since it is able to cross the muscle cells membrane easier, not requiring the presence of creatine transporters in the human body. However, creatine ethyl ester does not show any additional benefit to increase muscle strength or performance than creatine monohydrate; also, this ester form has been demonstrated to undergo an extended hydrolysis in the gastrointestinal tract after ingestion, with a rapid conversion in the derivative product creatinine (Fons et al., 2010; Spillane et al., 2009).

Currently, commercial creatine and taurine are widely produced via chemical synthesis in Europe, Asia and North America, since natural extraction from animal and fish flesh usually incurs in higher processing costs. Taurine is currently produced from monoethanolamine, sulfuric anhydride and sodium sulfate, using a relatively expensive purification process, because taurine is insoluble in most organic solvents commonly used in extraction and purification (Gu, Shi, Yang, & Deng, 2004). Creatine is usually produced in the monohydrate form, starting from sarcosine and cyanamide; however, the final product always contains non-reacted sarcosine, for which there is a low tolerance level by the human body, in the order of ppm, enforced by health authorities agencies, and some other contaminant by-products such as dicyandiamide, dihydrotriazines, creatinine, and several ions, resulting in a formulation with a poor nutrition value.

Extraction from cold water fish protein hydrolysis appears to be a cost-effective technology. The enzymes provided by fish offal and viscera themselves are mixed with fish by-products in water and the resulting enzymatic protein hydrolysate may contain up to 90% of free amino acids, high molecular weight peptides, calcium salt and phosphorus (Pyntikov & Salerno, 2004). The stream can be processed by nanofiltration using inorganic membranes allowing separation of peptides and amino acids based on membrane/solute charge interaction (Martin-Oure, Bouhallab, & Garem, 1997). So far,

commercial developments have been reported encompassing animal sources for free amino acids, but which resort to fermentation processes using a highly performant strain of *Escherichia coli* in the presence of sugar sources such as molasses, sucrose, or glucose (Leuchtenberger et al., 2005). Nevertheless, marine by-products possess great potential, as they are less affected by virus transmissible to humans from higher warm-blooded animals.

2.3. Chitin, chitosan and their oligomers

Chitin and chitosan are ubiquitous marine polysaccharides; over the years they have attracted a great deal of attention in food, pharmaceutical and health applications due to their distinctive biological and physicochemical characteristics (Kurita, 2006; Shahidi, Arachchi, & Jeon, 1999). In fact, the last two decades have witnessed a great amount of work on these biopolymers, which highlighted wide potential uses (Dodane & Vilivalam, 1998). The current trend towards the use of natural products has strongly contributed to the observed increase in demand, which has turned chitin and chitosan production processes into highly profitable ones. The annual production of chitin is estimated to approach that of cellulose (Kumar, 2000), which is ca. 2.6 million tons/year (Davies, 2009).

The name 'chitin' was firstly used by Bradconnot, back in 1811; it is derived from the Greek word 'chiton', that means tunic or 'coat of mail', because it was first found in the exoskeletons of insects and crustaceans. Chitin is an odorless, white to cream-colored solid compound with a structure similar to that of cellulose, and built from *n*-acetyl-glucosamine monomers. It can exhibit various degree of acetylation but pure chitin with full acetylation does not exist in nature (Meyers, Chen, Yu-Min Lin, & Seki, 2008).

Depending on its source, chitin can occur as either of two allomorphs, namely α and β forms (Rinaudo, 2006). The α type is the most abundant and also the preferred form for industrial applications; amongst crustaceans it is found in shrimps and crabs. The relatively rarer β form is found in squid pen and is apparently more reactive than the α form, yet β-chitin cannot be biosynthesized *in vitro*. Both α and β types are insoluble in water and are stable when exposed to acids, bases or organic solvents (Dodane & Vilivalam, 1998; Malinowska & Rozylo, 1997); this water insolubility hampers most applications of chitin (Rinaudo, 2006). Conversely, its deacetylated form named chitosan is soluble in several solvents, depending on its degree of deacetylation.

Chitosan is rather soluble in acidic solutions and slightly soluble in weak alkaline solutions (Dodane & Vilivalam, 1998); the degree of deacetylation is once again the key parameter that dictates solubility of chitosan in aqueous solutions. This polymer is indeed an alkaline or neutral polysaccharide as opposed to other commercial polysaccharides, such as cellulose, dextran, pectin, alginic acid, agar-agar, starch, carrageenan, and heparin, which are all neutral or acidic (Sing & Ray, 2000); this unique feature offers a range of unique applications, as summarized in Table 3. Chitosan possess three types of reactive functional groups: an amino group, and primary and secondary hydroxyl groups at C-2, C-3 and C-6 positions, respectively. Deliberate chemical modifications of these groups have produced several chitosan oligomers (Kurita, 2006; Shahidi et al., 1999) suitable in various fields of applications like normal chitosan, but with tailored features (Table 3).

Chitosan is commercially obtained mainly from chitin isolated from shell waste of crabs, shrimp and krill (Kurita, 2006). In practice, chitin is used almost exclusively as raw material for production of chitosan, oligosaccharides and glucosamine, to levels that amount worldwide to ca. 37,300 tons yearly (Chang, Chen, & Jao, 2007). Shrimp cuticle exhibits the higher amount of chitin, i.e. 30–40%, followed by crab, i.e. 15–30%; in both cases the remaining fraction is made of proteins and minerals (Qin & Agboh, 1997). In extraction process on the industrial level, shells are first treated with dilute hydrochloric acid at room temperature in order to remove metals and salts, primarily calcium carbonate. Decalcified shells are then exposed to an alkaline media to hydrolyse proteins and pigments (Kurita, 2006). Final polishing—via drying—eventually leads to powdered α-chitin. Demineralization and

Table 3
Applications of chitin, chitosan and their oligomers.

Field of application	Claimed applications	References
Nutrition	Dietary fiber Lipid absorption reduction Antigastritis agent Infant feed ingredient Hypocholesterolemic effect	Liao, Shieh, Chang, & Chien, 2007; Muzzarelli et al., 2007
Food Additive	Beverage clarification and deacidification Natural flavour extender Texture controlling agent Emulsifying agent Food mimetic Color stabilizer	Kim & Thomas, 2007; Bautista-Baños et al., 2006
Antimicrobial agent	Bactericidal Fungicidal Mold contamination indicator	Bautista-Baños et al., 2006; Kurita, 2006
Pharmaceutical	Wound healing Drug delivery system Regenerative medicine (bone, skin, liver and cartilage) Hemostatic	Kim et al., 2008; Muzzarelli et al., 2007; Salgado, Coutinho, & Reis, 2004
Edible film	Respiration rate control Antimicrobial substances controlled release Antioxidant controlled release Oxygen partial pressure reduction Temperature control Moisture transfer control	Maher, Elsabee, Abdou, Nagy, & Eweis, 2008; No, Meyers, Prinyawiwatkul, & Xu, 2007
Water purification	Dyes removal Pesticides, phenols and PCB recovery.	Gamage & Shahidi, 2007; Wan Ngah, Kamari, & Fatinathan, 2006
Other	Enzymes immobilization Nutraceutical encapsulation Chromatographic separation Analytical reagent	Grenha, Remuñán-López, Carvalho, & Seijo, 2008; Qiu & Bae, 2006

deproteination of shrimp waste can also be carried out via enzymatic fermentation (Kurita, 2006; Sini et al., 2007); e.g., proteases derived from *Bacillus subtilis* are used for chitin extraction from shrimp shells (Sini et al., 2007), whereas *Pseudomonas maltophilia* seems to be particularly effective in removing proteins from decalcified shrimp cuticle chips (Qin & Agboh, 1997). Based on the final dried form, the yield of this process is of the order of 30–35%, with the higher values observed on crab shells due to the higher content of calcium carbonate in crab cuticle.

Production of chitosan from crustacean is economically feasible, especially if it also includes recovery of pigments such as carotenoids (Abdou et al., 2008). To produce 1 kg of 70% deacetylated chitosan from shrimp shells, 6.3 kg of HCl and 1.8 kg of NaOH are required, in addition to nitrogen and water (Kumar, 2000). Chitosan is largely produced in India, Japan, Poland, Norway and Australia from crustacean shells, and traded under such various forms as powder, paste, film and fiber (Sing & Ray, 2000).

Since chitin and chitosan have peculiar structures and properties that are quite different from synthetic polymers, they have often been considered as promising for development of desirable properties (Kurita, 2006); however, their large molecular weight and high viscosity restricts their *in vivo* use to dietary fiber. Conversely, modified chitin and chitosan have low viscosity and are characterized by low and short-chain length, which make them easily soluble in neutral aqueous solutions, thus readily absorbable *in vivo* (Jeon, Shahidi, & Kim, 2000). Various techniques are suitable to derivatize chitin and chitosan; chemical or enzymatic substitution, chain elongation and depolymerisation are the preferred methods (Senel & McClure, 2004). Chitin and chitosan-based materials find application usually in the form of powders and flakes, but mainly as gels for membrane, coating, capsule, fiber, sponge and scaffold formulations. The methods used for chitosan and chitosan oligomers gel preparation can be tentatively divided into five groups: solvent evaporation, neutralization, crosslinking, ionotropic gelation and freeze-drying (Krajewska, 2004). For all the applications, expect for use as food fibers, chitin and chitosan are accordingly used as their oligomers, owing to the versatility imparted by specific functional groups (Venugopal, 2009).

2.4. Collagen and gelatin

The worldwide industrial demand for collagen and gelatin has undergone an increasing trend and currently accounts to 326,000 tons (Karim & Bath, 2009). Collagen is the major structural protein present in skins and bones of all animals where it accounts for ca. 30% of the total protein content (Wasswa et al., 2007). Collagen is mainly sought for the production of gelatin, a high value functional protein due to its unique gel-forming capacity; of all hydrocolloids in use today, none is as popular as gelatin. Despite its low biological value, gelatin, like collagen, is commonly used in pharmaceutical and medical applications, because of its biodegradability and biocompatibility in the physiological environments, and also for technical applications (Young, Wong, Tabata, & Mikos, 2005). Gelatin is hence utilized, for instance, as ingredient to improve elasticity, consistency and stability of food, as well as for encapsulation and film formation in the pharmaceutical, cosmetic and photographic industries (Gómez-Guillén et al., 2000).

Gelatin is the hydrolyzed form of collagen, and is a heterogeneous mixture of fibrous, denatured, biodegradable and water-soluble protein, with molecular weights typically ranging between 80 and 250 kDa: it contains ca. 88% of protein, as well as 10% of moisture and 1–2% of salt, and is able to retain more than 50 times its weight of water within its gel structure (Ying Liu, Li, & Guo, 2008). Inside gelatin molecule, glycine is the dominant amino acid, constituting ca. 27% of the total amino acid pool (Tabata & Ikada, 1998). Proline and hydroxyproline come second in abundance and play a key role towards thermal stability of collagen. The total amount of those two imino acids is higher in mammalian (20–24%) than in fish (16–20%), which also leads to different rheological properties. Gelatin is amphoteric, so it possesses both acidic and alkaline properties depending on the nature of the solution. It is relatively economical to manufacture gelatin in large amounts since suitable raw materials are easily available (Baziwane & He, 2003). Most gelatin in the market is obtained from mammalian sources, typically bovine and pig skins, which account for 46% of the world gelatin output, followed by bones and hooves, representing 23% and 29% of the total gelatin production, respectively; only the remaining percentage, i.e. 1%, comes from marine sources (Karim & Bath, 2009; Wang et al., 2008). However, a few factors recognized as a serious risk for human health, e.g., the outbreak of bovine spongiform encephalopathy (BSE) and hoof–and–mouth disease (FMD) (*Aphtae epizooticae*), which are transmissible via food, have severely hampered the use of by-products from bovine and porcine origin (Wang et al., 2008). Additionally, the use of land animals as sources of gelatin is restricted by socio-cultural reasons in some countries.

Fish by-products appear then to be a good alternative, and furthermore show peculiar functional properties, which are dependent on the fish environment which is obviously rather different from traditional mammalian sources (Baziwane & He, 2003). Due to similar rheological behaviors, gelatin from warm water fish can be a good alternative to that from pork (Baziwane & He, 2003), which has been considered to be the best among all gelatins due to its higher proline and hydrxyproline contents (Wasswa et al., 2007). Note that collagens derived from species living in cold environments (e.g. cod), have lower contents of proline and hydroxyproline, so they present a lower melting point and inferior thermal stability than those from fish that live in warmer environments (e.g. tuna); 10% of either mammalian or warm water fish gelatins are able to form gel at room temperature, but 10% of cold water fish gelatin only gels at ca. −2 °C (Gildberg, Arnesen, & Carlehog, 2002).

The final characteristics and behavior of gelatin, depends not only on their animal origin, but also on the technology used in the extraction process. Gelatins can be recovered from raw material through acid, basic or enzymatic treatments, all of which are assisted by heat and water. The acid process is preferentially used with fish and pig skins, and is carried out with hydrochloric, sulfuric and phosphoric acids (Ying Liu et al., 2008). In case of high number of cross links, due to the high content of proline and hydroxyproline, lactic acid entertains the best performance upon swelling the collagen (Giménez, Turnay, Lizarbe, Montero, & Gómez-Guillén, 2005). High pressures (i.e., above 200 MPa) facilitate swelling by destabilizing acid-soluble cross links and accelerating hydrolysis of collagen (Gómez-Guillén, Giménez, & Montero, 2005). The acid process takes ca. 3 days and the result is a type A gelatin with a pH between 3.5 and 6, which is the most widely used. Conversely, the alkaline process (which is employed exclusively for bovine bonds) may take up to 20 weeks and generates a type B gelatin which is harder and more viscous than type A, and holds a pH between 5 and 7; its main application is in stabilizing other food hydrocolloids (Wasswa et al., 2007). After either acidic or alkaline extraction, the final gelatin is colorless, transparent, brittle and tasteless, and is thus shapeable onto various forms, such as sheets, flakes or powder. Despite solvent extraction being currently the method of choice, the final gelatin product lacks a few functional properties, so enzymatic hydrolysis has been regarded more and more often as the best way to obtain gelatin with given specifications. In fact, high added value mammalian and warm water fish gelatin can be treated with proteolytic enzymes, to produce gelatin with a very soft color, yet with high gel strength and a wide range of viscosities (Wasswa et al., 2007).

Although collagen and gelatin from fish origin have in general a few drawbacks when compared to their mammalian counterparts, e.g. low stability, fishy odor and dark color, these products are well suited for many industrial applications. Gelatin from warm water fish (e.g.

tuna, sardine and anchovy) is currently exploited as ingredient for food and drugs coating (Pérez-Mateos, Montero, & Gómez-Guillén, 2007), in particular when odor and taste of drugs are unpleasant (Vandervoort & Ludwing, 2004). Gelatin from warm water fish also find application in ophthalmological formulations (Supavititpatana, Wirjantoro, Apichartsrangkoon, & Raviyan, 2008); in production of photographic films (Surch, Decker, & McClements, 2006) and low-set time glues (Wasswa et al., 2007). Cold water fish (e.g. cod, hallock and pollock) gelatin is used as structuring agent in non-gelling formulations (Wasswa et al., 2007) and as active ingredient in cosmetic preparations (Baziwane & He, 2003).

Gelatin possesses a characteristic melt-in-the-mouth property, which make it suitable to a wide range of applications in food and pharmaceutical industries; in particular, fish gelatin releases aroma better and shows a higher digestibility than animal one (Gómez-Guillén et al., 2000). Because of its solubility at room temperature, fish gelatin permits relatively low temperature coacervation, which is not feasible with other gelatins (Gómez-Guillén et al., 2005). Fish collagen is widely used in beauty and cosmetic product formulation (Bae, Osatomi, Yoshida, Osako, Yamaguchi, & Hara, 2008), mainly intended for soft tissues (e.g. skin) as well as cartilage and bone repair (Salgado et al., 2004). In the latter application, and as happens with other biopolymers, e.g. chitosan, starch and hyaluronic acid, natural collagen triggers low immune responses, so it is a good substrate for cell adhesion, with good capacities to interact with host tissues, owing to good chemical versatility and chemotactic properties (Salgado et al., 2004). This biopolymer is nowadays used in the manufacture of biocompatible glues which consist of a mixture of collagen with citric acid as a cross linking agent; particularly powerful in binding tissue–tissue interfaces, yielding less toxicity than such semi-synthetic and synthetic formulations as gelatin-aldehyde and cyanoacrylate-based glues (Taguchi et al., 2006). Its intrinsically high water retention capacity is also taken advantage of in rough skin repairing. Finally, fish and pig collagens exhibit anti-radical action (characterized by $IPOX_{50}$ of 0.18 and 0.45 mg/mol, respectively) and a potential in decreasing blood pressure (characterized by IC_{50} of 0.16 and 0.41 mg/mol, respectively) (Morimura, Nagata, Uemura, Fahimi, Shigematsu, & Kida, 2002).

The intrinsic biological and physicochemical features of collagen and gelatin make them useful in various fields. The current market demand is surging more reliable less expensive sources, and fish by-products clearly fit into this status.

2.5. Hydroxyapatite

Research in the biomedical area has been focusing on the identification of biomaterials with long term physiological compatibility, so current development of advanced materials with biomimetic features is one of the most promising trends in biotechnology. The Institute of Materials (London, UK) estimated a world market of ca. $12,000 million per year, with an average global growth between 7 and 12% per annum (Aronov et al., 2007).

Materials obtained from natural sources obviously show the best biocompatibility with the human body. In many circumstances, synthetic materials are hardly accepted because the organism fights against the invasion by foreign matter. Naturally occurring hydroxyapatite, $Ca_5(PO_4)_3OH$, is a form of bioceramics with the highly desirable physicochemical attributes of stability, inertness and biocompatibility. This mineral is the primary constituent of bones, teeth and calcified cartilage tissues in human and higher animals, as well as fish; it represents ca. 43% of human bone, 65–70% of higher animal bone and 60–70% of fish bone (Aronov et al., 2007; Barakat et al., 2008). It has an elastic modulus that is ca. two orders of magnitude greater than collagen, coupled with a density that is three-fold that of most biological materials, which makes it suitable for several biomedical applications.

Nowadays, polymeric composites involving hydroxyapatite are particularly demanded because of their easy tailorable manufacturing processes; bioinert composites for permanent applications, biodegradable composites for temporary applications and injectable composites include hydroxyapatite among the main compounds, both in pure form or as part of hybrid materials (Mano, Sousa, Boesel, Neves, & Reis, 2004). Bone cement for craniofacial, oral-maxillofacial and orthopedic defect repair, and coating for femoral components are the most common applications of hydroxyapatite so far reported (Mano et al., 2004; Salgado et al., 2004; Woodard et al., 2007). Its beneficial osteoconductivity permits binding to human hard tissues and thus allows implementation of very stable scaffolds in bone as well as in tooth repair and substitution (Deville, Saiz, & Tomsia, 2006; Moshaverinia et al., 2008). Hydroxyapatite has also been reported in combination with other specific materials; for instance, biodegradable metal matrix composites based on magnesium and hydroxyapatite, have been demonstrated to be cytocompatible biomaterials with adjustable mechanical and corrosive properties, which justify their high demand for biodegradable metal implants (Deville et al., 2006; Monkawa et al., 2006; Witte et al., 2007). Hydroxyapatite powder has also been successfully impregnated with cellulose sponges in attempts to obtain porous bodies with adequate porous dimension and distribution that mimic the morphology of a spongy bone and favor osteoconduction (Landi, Celotti, Logroscino, & Tampieri, 2003). Other applications include substrate for adhesion of proteins, peptides, lipids, bacteria and strains, useful for drug delivery (Aronov et al., 2007); cartilage regeneration (Mano et al., 2004; Woodard et al., 2007); wastewater heavy-metal and dyes decontamination (Aronov et al., 2007).

Hydroxyapatite has classically been chemically synthesized to tailored properties using techniques such as the hydrothermal and sol–gel synthesis (Fathi, Hanifi, & Mortazavi, 2007). However, hydroxyapatite obtained from natural source, as fish and animal bones, inherits such structural properties of the original raw material, besides the intended chemical composition, so it is a better alternative for numerous devices based on synthetic hydroxyapatite (Haberkom et al., 2006; Kim & Mendis, 2005). Hydrothermal alkaline hydrolysis is the most common route for hydroxyapatite extraction from fish and animal bones (Barakat et al., 2008). A method recently proposed for hydroxyapatite extraction from natural sources consists of subcritical water extraction, which has the further advantage of producing carbonated hydroxyapatite, which is highly required in biomedical applications. The former and more usual process also keeps the carbonate ions of the natural hydroxyapatite source, although to a lower extent than the subcritical water one. On the other hand, alkaline hydrothermal process produces relatively better nanoparticles (Barakat et al., 2008), which is highly desirable to human bone applications (Li, Chen, Yin, Yao, & Yao, 2007).

Therefore, there is a continuing interest in exploring features offered by hydroxyapatite composites, so commercial applications are expected to rely on more accessible and less expensive raw materials as is typically the case of fish by-products.

2.6. Antifreeze proteins

Antifreeze proteins, also known as thermal hysteresis proteins, are ice-structuring proteins able to influence the growth of ice crystals and inhibit ice recrystallization. Those unique molecules were first identified by de Vries back in 1969 in the blood of fish living in frozen sea areas (Crevel et al., 2002); they apparently serve to lower fish blood freezing point below seawater freezing point, thus avoiding the increase in plasma osmotic pressure and without altering the blood melting point (Barrett, 2001). Proteins with similar features have meanwhile been found, although to lesser levels, in plants and insects that survive at very low temperatures (Cheng, 1998).

There are two major types of antifreeze proteins: glycoproteins and non-glycoproteins, based on presence or absence of carbohydrate, respectively (Harding, Anderberg, & Haymet, 2003). However, both types can lower the freezing point of an aqueous solution via a non-colligative mechanism, by binding to the ice crystal surfaces and inhibiting further crystal growth (Evans & Fletcher, 2004). Since antifreeze proteins prevent fish from freezing, a significant percent is present only in species adapted to very cold sea water; the greatest amount has been found in the blood of Antarctic fish, particularly in cod (Harding et al., 2003). Their antifreeze proteins are mainly glycoproteins, the blood concentration of which varies from 5 to 35 mg/ml (Jin & DeVries, 2006). In both glycol- and non-glycoproteins, alanine makes up two-thirds of the total number of amino acid residues, with the remaining third being accounted for threonine and cysteine.

Antifreeze proteins find large application in frozen food technology, low-fat content food manufacture, transplanted organs cryopreservation, cryosurgery and aquaculture (Feeney & Yeh, 1998; Venketesh & Dayananda, 2008). Two applications arise as particularly important in terms of frozen foods: ice-cream manufacturing and frozen meat technology. Antifreeze proteins may improve quality of frozen foods, hence allowing for the maintenance of their natural texture, reduction of cellular damage and loss of nutrients, all of which contributes to preserve their nutritional value (Li & Sun, 2002). It is illustrative that meat (bovine and ovine muscle) soaked in solutions of up to 1 mg/ml of antifreeze glycoproteins prior to freezing at −20 °C showed evidence of reduced ice crystal size, and conveyed a better nutritional value after thawing. Furthermore, antifreeze proteins have been used in ice-cream manufacturing to reduce the overall fat content down to 1%, conferring a good texture thanks to their nanostructuring ability (Feeney & Yeh, 1998). A few companies have recently invested in this feature and consequently earned substantial economic benefits, by designing and launching a new range of low-fat products with pleasing texture, which are becoming even more popular among consumers around the world due to the low caloric content (Sigman-Grant, Warland, & Hsieh, 2003).

Although yeasts and some other types of microorganisms are a suitable source of antifreeze proteins, the most promising one is fish. For the recovery of such unique proteins, fish skin or blood are usually frozen in nitrogen, powered and eventually homogenized in an alkaline medium. After centrifugation, the lyophilized supernatant is dissolved in the same alkaline medium, and finally is subjected to preparative chromatography, using a specific silica gel column (Evans & Fletcher, 2004). An alternative isolation/purification method is adsorption on ice, using a cold finger apparatus; depending on the intended final purity, a further chromatographic step may also be required (Kuiper, Lankin, Gauthier, Walker, & Davies, 2003).

More performing and less expensive purification technologies are now urged, as the final cost of 1 g of antifreeze proteins is very high, with reported values of ca. $50 (Feeney & Yeh, 1998) to ca. $10.000 (A/F Proteins, Walthma, USA), currently accounting for the purification steps mainly. If that constraint is successfully addressed, a wide diversification of the novel food portfolio employing those specialty proteins is likely.

2.7. Enzymes

Nowadays, enzyme-based technology represents an important contribution (and, in some cases, essential one) for many industrial applications (Rasmussen & Morrissey, 2007). The boom in life sciences has led worldwide industries to a sustained rate of annual growth of ca. 7.5% for enzymes, with an estimated volume of up to US$ 7000 million in 2013 (Market Research Report, 2009). This is so because enzymes possess a high selectivity, and a very high activity even at very low concentrations and mild conditions of pH and temperature, which leads to fewer unwanted side-effects and by-products (Shahidi & JanakKamil, 2001).

Aquatic invertebrates at large, as well as the internal organs of fish and the shells of crustaceans, constitute natural sources of enzymes with huge interest. Due to the prevailing environmental conditions, marine enzymes can effectively operate at low temperatures, below 4 °C, and within a neutral to alkaline pH values (SEAFISH, 2008). As emphasized elsewhere (Shahidi & JanakKamil, 2001), most enzymes from fish and aquatic invertebrates also occur in higher animals, yet they possess unique characteristics, in terms of molecular weight, amino acid composition, pH and temperature stability, inhibitory behavior and kinetic properties.

Proteases constitute at present the dominant group of marine enzymes with a commercial expression; recall that proteases bring about hydrolysis of peptide bonds, and have been termed as proteinases or peptidases, depending on whether they act on proteins or polypeptides, respectively. Gastric, intestinal and hepatopancreas proteinases have been scrutinized more thoroughly (Beynon & Bond, 2001); pepsin from polar cod is one of the most extracted gastric proteases, followed by collagenase, elastate, trypsin and chymotrypsin, as well as non proteolytic enzymes, such as transglutaminase, lipases and chitinolytic enzymes to lower extents (Rasmussen & Morrissey, 2007). The main enzymes of fish, aquatic invertebrates and marine mammals that may be potentially recovered for practical uses are presented in Table 4.

Even though no reliable market data are presently available, recovery of marine enzymes is potentially profitable; well established commercial applications already exist (Table 5) but there are major opportunities for developing new niche markets.

There is an outstanding profitable market for cod transglutaminase in surimi manufacture (Shahidi & JanakKamil, 2001) as well as for lipases in the detergent industry (Esposito et al., 2009). A recent addition to this list is the use of lipases in polyester production (Schmid et al., 2001). A great number of research studies are currently

Table 4
Sources of enzymes from fish, aquatic invertebrates, marine mammals and their by-products (adapted from Shahidi & JanakKamil, 2001).

Group	Enzyme	Source
Gastric proteases (aspartic protease family)	Pepsin	Atlantic cod, Greenland cod, salmon, mackerel, sardine, capelin, American smelt, tuna (bluefin), orange roughy
	Pepsinogen	Shark, tuna (bluefin), rainbow trout
	Chymosin	Harp seal
	Gastricsin	Atlantic cod, hake
Intestinal proteinases (serine protease family)	Trypsin	Atlantic cod, Greenland cod, sardine, capelin, cunner, chum salmon, Atlantic salmon, Coho salmon, anchovy, carp, Atlantic white croaker, palometa, hybrid tilapia, krill, crayfihs, oyster
	Chymotrypsin	Capelin, herring, Atlantic cod, spiny dogfish, rainbow trout, scallop, abalone, white shrimp, grass carp
	Collagenases	Fiddler crab, freshwater prawn, crayfish, Atlantic cod, king crab
	Elastases	Carp, catfish, Atlantic cod
Chitinolytic enzymes	Chitinases	Crustaceans, squid, octopus,
	Lysozymes	Arctic scallop
Transglutaminases		Red sea bream, rainbow trout, Atka mackerel, walleye pollok liver, scallop muscle, Botan shrimp, Squid
Lipases		Shark, Atlantic cod

Table 5
Current and potential applications of enzymes obtained from marine products and by-products (adapted from Esposito et al., 2009; Shahidi & JanakKamil, 2001).

Field of application	Claimed applications
Fish curing and fermentation	Fish sauce and silage production
Hydrolysed products production	Fish protein hydrolysate
	Flavour compound
Pigment extraction	Pigments recovery from shellfish waste
Protein coagulation	Rennet substitute in cheese manufacturing
	Removal in milk oxidized flavor
Selective tissue degradation	Fish and aquatic invertebrates deskining
	Fish roe purification
	Cod liver membrane removal
	Shellfish exoskeleton removal
	Salted cod swim bladder production
Wastewater treatment	Viscosity reduction of sticky water
Detergents industry	Laundry detergent production
Other potential applications	Meat tenderizing
	Fish oil enzymatic extraction from raw material
	ω-3 fatty acids concentrates production
	Antibacterial enzymes
	Antioxidant enzymes
	Gene cloning technology

focusing on novel purification methods and immobilization methodologies that can improve both stability and enantioselectivity of the enzymes and thus render those processes more competitive (Mohapatra, Bapuji, & Sree, 2003).

2.8. Astaxanthin

Astaxanthin (3,3-dihydroxy-β,β-carotene-4,4-dione) is a ketocarotenoid oxidized from β-carotene, which plays biological roles and possesses a number of desired features for food applications, such as natural origin, nil toxicity, high versatility, and both hydro and liposolubility (Delgado-Vargas & Paredes-López, 2003). Its attractive pink color, its biological functions as vitamin A precursor and antioxidant, make astaxanthin widely sought for food and medical applications (López-Cervantes et al., 2006). Its superior antioxidant power has even coined his label of "superior vitamin E"; in fact, it possesses an antioxidant effect greater than β-carotene, as well as vitamins C and E (Stepnowski, Olafsson, Helgason, & Jastorff, 2004), especially in quenching singlet oxygen and scavenging free radicals. Moreover, astaxanthin is active in protecting against chemically-induced cancers and age-related macular degeneration, and also in enhancing the immune system and in preventing damages arising from ultraviolet radiation (Yang, 2007).

This carotenoid occurs naturally in a wide variety of marine and aquatic organisms (López-Cervantes et al., 2006) with algal cells and crustacean shells being the major sources, followed by plants, bacteria and egg yolk. Astaxanthin represents between 74 and 98% of the total pigments in crustacean shells, which contains from 2.3 to 33.1 g/100 g of carotenoids (Shahidi, Metusalach, & Brown, 1998). Due to these high contents, crustacean shells are used not only for recovery of chitin but also for recovery of carotenoids.

The methods currently available for the extraction of astaxanthin from shell matrices employ different elements such as edible oils, hydrochloric acid and organic solvents, besides proteolytic enzymes (Babu, Chakrabarti, et al., 2008). Such plant oils as sunflower oil, groundnut oil, ginger oil, mustard oil, soybean oil, coconut oil, rice bran oil and palm oil have accordingly been used to extract astaxanthin from crustaceans and fish by-products (Handayani, Sutrisno, Indraswati, & Ismadji, 2008). Some countries, such as India, use proteolytic enzymes, such as trypsin, papain and pepsin, dissolved in citrate phosphate, to precipitate carotenoids from the shells. At present, a feasible technique for partial concentration of astaxanthin from crustacean shells is via lactic acid fermentation, which also has the advantage of protecting the biomass from bacterial decomposition. The silage formed contains insoluble chitin, a protein-rich fraction, and a lipid-rich fraction composed of astaxanthin, sterols, and vitamins A and E (Babu, Chakrabarti, et al., 2008).

Owing to its useful properties, astaxanthin from natural sources is increasingly being marketed as a functional food ingredient in many countries (Yang, 2007) with prices that range between 3000 and 12,000 US$/kg (AWARENET, 2004) and fish by-products are thus expected to become increasingly important as industrial feedstock.

3. Conclusions

The dual problem arising from the surplus of several commodities, which bear low economic returns for the farmers, while entertaining especially in the view of increasingly tighter environmental regulations, has been promoting the commercial exploitation of many products that were so far discarded, and eventually leading to better manufacturing practices and more sophisticated processing technologies. Valorisation of by-products arising from fish catching and processing is a must order, since it constitutes a central issue for the long-term sustainability of the fish industry at large. Moreover, a few high value added compounds recovered from such type of by-products are economically more attractive than the target products themselves. Development of novel and clean technologies aimed at more efficient recovery of bioactive nutraceutical compounds from marine by-products will lead to the development of more profitable processes, thus giving rise to many great opportunities to the marine industry.

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