In: Bioactive Compounds Extraction from Marine Resources and Wastes.
Interdisciplinary Biotechnological Advances (IBA), Springer, Singapore, pp. 49-67
DOI:10.1007/978-981-96-1253-6_3

Chapter 3. Biotechnology strategies for bioactive compound extraction

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Abstract

The marine environment is a relatively unexplored source of bioactive compounds that can be used in several industries in a wide range of ways. Some of these organisms live in harsh environmental conditions, resulting in complex compounds with unique biological properties. One of the main challenges for their application into the industry is their extraction, which is one of the most crucial stages in the development of products containing bioactive compounds from marine sources. Soxhlet extraction is the most common method applied at lab and pilot scales to obtain compounds from any natural source, however, this method is based on the use of solvents that can be harmful to the environment, also it can affect the biological properties of the compounds. For this, biotechnological extraction techniques such as fermentation, enzyme-assisted extraction, and molecular tools have emerged as alternatives to traditional techniques enabling the extraction of bioactive compounds of high quality without the use of harmful solvents. Besides, these technologies have shown great potential to be applied at the industrial scale once the optimization of the process has been carried out. This chapter focuses on the potential use of biotechnological tools for the extraction considering the factors that affect the yields and downstream process of marine-derived bioactive compounds for further applications in the industry.

Keywords: Solid-state fermentation; Submerged fermentation; Enzyme-assisted extraction; Purification; Marine bioactive compounds

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The final published version (version of record) is available online at DOI:10.1007/978-981-96-1253-6_3.
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1. Introduction

Nowadays, more people are aware and more interested in developing healthier habits to improve their quality of life. Through diet, people can take care of and improve their health with an adequate nutritional balance and the incorporation of food or food products with high-value bioactive compounds (HVBCs). HVBCs are defined as natural compounds that can improve health beyond basic nutritional value by being able to modulate some metabolic processes (Arshad et al. 2021). Bioactive compounds are present in a wide variety of sources in the terrestrial environment the most common include fruits, vegetables, and grains but are also present and able for exploitation in food, agro-industrial by-products, such as peels, seeds, pomaces, straws, bagasse, mead, and molasses (Inamuddin 2021). These HVBCs also can be found in marine ecosystems that cover over 70% of the Earth's surface, harboring an extraordinary diversity of life forms from microscopic algae to marine mammals, many of which remain untapped reservoirs of HVBCs. Marine bioactive compounds exhibit remarkable biological activities, ranging from antimicrobial and antiviral properties to antioxidant and anti-inflammatory effects. For this reason, in recent years, the exploration of marine sources for bioactive compounds has become a focal point in the field of biotechnology, owing to their potential to revolutionize industries ranging from pharmaceuticals to nutraceuticals (Furniturewalla and Barve 2022; Kim et al. 2022).

HVBCs from marine sources have shown promise in the development of novel pharmaceuticals, functional foods, and cosmeceuticals. Moreover, their applications extend to industrial sectors, where their unique properties can be harnessed for diverse purposes, such as bioremediation, bioenergy production, and materials science (Furniturewalla and Barve 2022; Kim et al. 2022). The potential of HVBCs from marine sources is vast, but their extraction presents several

challenges. The harsh and dynamic marine environment, coupled with the often-limited availability of certain organisms, demands innovative and sustainable extraction strategies. Biotechnology offers a transformative approach to harnessing the potential of marine bioresources, applying enzyme-assisted extraction (EAE), fermentation processes, and molecular modifications of the organisms to increase the extraction of HVBCs from marine organisms minimizing the environmental impact and ensure the cost-effectiveness of the process (Furniturewalla and Barve 2022; Kim et al. 2022).

The synthesis of HVBCs is affected by several factors, including culture media composition, nutrient supplementation, light, temperature, pH, and so on (Ekpe et al. 2018). However, bioactive compounds from marine sources have a high economic value in the market. For example, astaxanthin has an estimated value in the market of US $3.4 billion of this, only ~1% is obtained from algal sources (Lim et al. 2018; Grand View Research 2023). The obtention cost of 1 kg of synthetic astaxanthin is estimated at US $1000, although it sells for more than US $2000 on the market. Instead, its production by using *Xanthophyllomyces dendrorhous* and *Haematococcus pluvialis* has an estimated production and sell costs of US $2500 and US$7000 per kilogram, respectively (Panis and Carreon 2016). This compound has great importance owing to its antioxidant activities up to 800, 6,000, and 550 times stronger than coenzyme Q, vitamin C, and green tea catechins (Ekpe et al. 2018). Therefore, the implementation and optimization of biotechnological tools to produce HVBCs from marine sources is imperative since they promote the sustainability of products with a high economic value. This chapter introduces the biotechnological strategies employed for the extraction of bioactive compounds from marine sources, reviewing their applications, limitations, and potential advancements.

2. Biotechnology strategies for the extraction of bioactive compounds from marine sources

Depending on their structure, HVBCs can display different activities such as antioxidant activity, prebiotic activity, anti-inflammatory, and immunomodulatory properties, among others (Furniturewalla and Barve 2022; Kim et al. 2022). Pigments such as carotenoids, carbohydrates like fructooligosaccharides, fatty acids, amino acids, peptides, flavonoids, and polyphenols also are considered HVBCs (Zhang et al. 2014; Maltsev and Maltseva 2021). Their specific activity can depend on their source, concentration, extraction technique, and their stability. Marine sources such as micro- and macroalgae, shrimp and crab shells, and fish by-products are rich in lipids, carbohydrates, pigments, and peptides which can be extracted to generate value-added products (Saldarriaga-Hernandez et al. 2021; Zhang et al. 2024). Conventional extraction techniques involve high temperatures and a high quantity of solvent utilization with the potential degradation of some thermolabile valuable compounds and compromising the application of the extracted products (Inamuddin 2021; Muñoz-Miranda and Iñiguez-Moreno 2023). Hence to promote their production and extraction of HVBCs from marine sources alternatives such as fermentation and EAE have been implemented.

2.1 Fermentation

Nowadays, extensive efforts have been made to improve extraction techniques that allow improved efficiency, more cost-effective, and more environmentally friendly (Inamuddin 2021; Taveira et al. 2021). In response to all these needs, assisted extraction methods involving the adaptation of new technologies or strategies to the well-known extractive methods have been implemented. Among the assisted-extractive methods, fermentation for assisted-extraction has emerged as a

valuable alternative strategy for the release and bioconversion of targeted bioactive compounds (Saldarriaga-Hernandez et al. 2021; Taveira et al. 2021).

Fermentation is an ancestral biotechnological tool utilized by cultures around the world to modify chemical substances in certain substrates through the action of microorganisms (Taveira et al. 2021). Microorganisms involved in fermentation include bacteria, yeasts, and fungi strains used for liquid and solid-state fermentation. These microorganisms can produce valuable sets of enzymes for bioactive compound-assisted extraction, such as cellulases, chitinases, lipases, proteases, and oxidases, among others (Liu et al. 2020; Tan et al. 2020).

2.1.1. Liquid fermentation

Among marine sources, shellfish wastes are generated in high quantities, causing economic and environmental problems because of inadequate disposal or the lack of an effective strategy to valorize these residues (Doan et al. 2019; Tan et al. 2020; Furniturewalla and Barve 2022). These kinds of wastes like shrimp and crab shells are rich in valuable bioactive compounds such as chitin, chitosan, carotenoids, and protein hydrolysates (Tan et al. 2020).

Fermentation of crustacean wastes has been reported as an alternative for its valorization for the generation of bioactive compounds. *Brevibacillus parabrevis* TKU046 strain was reported as a producer of an extracellular protease which was used in fermentation for deproteinization and subsequent recuperation of chitin from shrimp waste, in the said article up to 95% of deproteinization was achieved in 4 days of fermentation for cooked tiger shrimp shell (Doan et al. 2019). Extraction of chitin has also been reported applying successive two-step fermentation in which *Lactobacillus rhamnoides* and *Bacillus amyloliquefaciens* (BA01) were utilized for demineralization and deproteinization in which 96.8% of deproteinization and 19.6% of chitin yield was achieved after 84 h of fermentation (Liu et al. 2020). Another study reports the use of

lactic acid bacteria for fermentation of shrimp wastes to produce chitin. In the aforementioned studies, different lactic acid bacteria strains were evaluated selecting the strain *Lactobacillus acidophilus* FTDC3871 as the best, obtaining a 76% of deproteinization and yielding 57.7% of chitin, also obtaining 183.9 μg/g of carotenoids extracted and 21.3 g/L of lactic acid produced (Tan et al. 2020).

2.1.2. Solid-state fermentation

Solid-state fermentation (SSF) is defined as a bioprocess in which microorganisms are grown in a solid substrate with the specific amount of water needed to carry out microbial growth and metabolites synthesis, but with the absence of free water (López-Cruz et al. 2023). SSF is often employed to valorize agro-industrial by-products such as peels, seeds, pomace, husks, or bagasse to generate or extract bioactive compounds due to their composition, because of their remaining sugars these substrates are suitable to support microbial growth and enzyme production (Bhattacharya et al. 2023). From the point of view of marine sources, algae strains (micro- and macroalgae) are known to produce bioactive compounds such as pigments, fatty acids, and polyphenols which are valuable for the industry (Muñoz-Miranda and Iñiguez-Moreno 2023). However, algae strain possesses a particular rigid cell wall which is overall composed of polysaccharides, polyphenols, and proteins which has been a challenge to overcome for the extraction. A known marine source rich in polyphenols and other bioactive compounds is the macroalgae *Ulva lactuca,* it is reported that this alga can contain polysaccharides, pigments such as chlorophyll, minerals, vitamins, fatty acids such as omega-6 fatty acid, and polyphenols such as flavonoids (quercetin and kaempferol) and tannins (Pérez-Alva et al. 2022; Xu et al. 2024). Besides, this is a fast-growing macroalga that favors its use for production and harvests sustainability, it is known for its uncontrolled growth even becoming a problem to the environment

called “green tide” which treats marine life, being the exploitation of this source for bioactive compounds extraction a more than feasible alternative (Liu et al. 2024; Xu et al. 2024). Nowadays the fermentation of *U. lactuca* is scarcely reported (Pérez-Alva et al. 2022), until now, there has been used in submerged fermentation to produce nutritional mycoprotein from the marine fungi *Paradendryphiella salina* using *U. lectica*’s alginate and other polysaccharides as a substrate for the fungi growth (Landeta-Salgado et al. 2021). Given the valuable composition of *U. lactuca* is very suitable for exploration utilizing reported fungal species that can degrade cellulose and other polysaccharides from algae as well to extract polyphenols from this underutilized source. SSF has also been applied for the extraction of bioactive peptides. To valorize the remaining grounds from katsuobushi fish flakes used to prepare bonito broth, SSF was carried out with the activity of endo- and exopeptidases of *Aspergillus sydowii* to produce amino acids and bioactive peptides via protein hydrolysis which display antioxidant capacity (Takenaka et al. 2023). SSF is a very useful alternative tool for bioactive compound production and/or extraction. Advantages of the use of SSF include reduced energy consumption, low-cost media (because of the use of cheap raw materials) lower risk of contamination because of the reduced moisture content, and simpler technology which has reduced operational cost.

2.2. Enzyme-assisted extraction (EAE)

EAE is another alternative strategy to improve HVBC extraction attempting to reduce the extensive use of solvents. EAE method refers to a green extraction method in which a single enzyme or a mixture of enzymes are used in a certain ratio to hydrolyze polymeric components in cell walls, improving extraction efficiency (Inamuddin 2021; López-Cruz et al. 2023). EAE displays a series of advantages in HVBC extraction over conventional methods which are the potential to improve extraction efficiency, recovery rate and specificity of desired compounds

(purity), reduction of solvent volumes and extraction times, preservation of bioactivity from the compounds, and biotransformation of bound insoluble compounds into free soluble compounds.

Cellulolytic enzymes are often applied to break down a plant's cell wall; however, these enzymes can have a very well performance on microalgae cell walls because of their nature. Enzymes from *Trichoderma reesei* have been applied to *Nannochloropsis sp*. biomass for a cost-effective extraction of microalgae´s oil rich in eicosapentaenoic acid allowing a recuperation of 369.4 ± 4.6 mg of total fatty acids per g of dry weight after 12 h of enzymatic treatment (Bhattacharya et al. 2023). On the other hand, glycosidases and proteases have been successfully applied in the extraction of plant growth regulators from the red seaweed *Solieria chordalis*. Compared to water extraction the EAE with proteases and glycosidases resulted in a more abundant content of plant regulators such as indole 3-acetic acid, 1-amino-1-cyclopropane- carboxylic acid, and salicylic (Choulot et al. 2023). Brown algae *Ecklonia maxima* is another marine source that is gaining attention for its bioactive compound content and potential valorization. A framework for its valorization was proposed in a biorefinery approach, the wasted biomass from polyphenols extraction was submitted to commercial enzymes Cellic® Ctec 2 and Viscozyme® L to release alginate and fucoidan which are valuable compounds with valuable functional properties into the food, cosmetic, and pharmaceutical industries. As a result, different fractions (polyphenols, alginate, and fucoidan) were obtained achieving a complete biomass valorization, demonstrating the potential of this approach to implement sequential extraction methods to implement biomass biorefinery (Mabate and Pletschke 2024).

2.3 Molecular tools

Molecular tools play a pivotal role in harnessing the potential of marine ecosystems for the extraction of HVBCs. These tools leverage advanced scientific techniques to explore, identify, and

cultivate marine organisms, offering opportunities for industrial applications, and sustainable resource utilization (Inamuddin 2021; Kim et al. 2022). DNA Barcoding is a fundamental tool in marine biotechnology, enabling the identification of marine organisms based on specific gene regions. By sequencing short DNA fragments of an unidentified species, the information can be compared with the reference library of DNA markers. DNA barcoding makes use of different gene areas. The most widely utilized enzyme for species identification is cytochrome c oxidase I (COI or COX1), which is present in mitochondrial DNA 1. This region has been used to identify 12 new species of fish in Bangladesh. By having a proper identification of the organisms, it is possible to promote the biosynthetic obtention of HVBCs from marine sources (Akter et al. 2023). Otherwise, metagenomics involves the genomic analysis of entire microbial communities present in marine environments. Metagenomics allows to have the complete genetic information of diverse microorganisms, including those that are challenging to culture. This is a key tool for the discovery of novel genes and metabolic pathways in marine microbes, leading to the identification of enzymes, antibiotics, and bioactive compounds with potential industrial applications. Omics technologies (proteomics and metabolomics) help in the understanding of metabolic pathways and functions, ultimately leading to the identification of HVBCs, being of particular importance in the elucidation of pathways involved in the production of compounds with pharmaceutical or industrial relevance, such as in the phytochemical derivatives from *Rhizophora mucronata* and *Avicennia marina* from mangroves (Rajivgandhi et al. 2024). Finally, CRISPR-Cas9 has revolutionized genetic engineering by enabling precise gene editing in marine organisms, making possible the modification of specific genes for enhanced compound production. The application of CRISPR-Cas9 in marine biotechnology has resulted in improved yields of valuable compounds, showcasing its potential for sustainable resource utilization. For example, CRISPR-Cas9 was used

to promote a mutation in the histone deacetylase gene (*rpd3*) leading to the activation of several new chemicals, none of which are generated by the wild-type strains of *Aspergillus* sp. SX7S7 and *Spiromastix* sp. SCSIO F190 (Chen et al. 2022).

Table 1. Molecular tools used to produce marine bioactive compounds.

Biotechnological tool	**Basis**	**Advantages**	**Limitations**	**Applications**	**Examples of extracted compounds**
Metagenomics	Genomic analysis of entire microbial communities	Capture of diverse genetic information	Computational challenges	Discovery of novel genes and pathways, functional metagenomics	Enzymes, antibiotics, bioactive compounds
CRISPR-Cas9 Technology	Precision gene editing in marine organisms	Targeted modification of specific genes	Off-target effects	Genetic modification for enhanced compound production	Improved yields of valuable marine compounds
Omics Technologies (Proteomics, Metabolomics)	Comprehensive analysis of molecules	System-level insights into cellular processes	Data interpretation challenges	Understanding metabolic pathways, functional analysis	Identification of bioactive compounds, pathway elucidation

2.4 Organisms cultivation

Marine microbial cultivation implies the isolation and cultivation of specific microorganisms from marine environments. By this method, it is possible to control and promote the synthesis of antibiotics, enzymes, pigments, and secondary metabolites by optimizing the culture conditions (Alem et al. 2020; Nisticò et al. 2022). In comparison, algae cultivation represents a sustainable approach to continuous and reliable compound extraction like omega-3 fatty acids, pigments, and polysaccharides. Enabling to address the concerns related to the overexploitation of marine resources by providing a controlled and renewable method for compound production, particularly regarding hydrocolloid obtention for the food industry (Zhang et al. 2024).

3. Downstream processing and purification

HVBC cannot be used directly from the natural source or the fermentation and enzymatic process. Usually, HVBCs are part of a solid matrix (whole organisms) or found as soluble or insoluble portions of the medium (i.e. after the fermentation process). Depending on the target compound and the source the content can vary from a few milligrams such as in the case of pigments to several grams (proteins, peptides, polysaccharides, and so on) of the dry matter. These compounds can be extracted and purified through downstream processing methods, which involve mainly four stages extraction, separation, purification, and concentration. Not just in marine biotechnology, downstream is one of the most overlooked phases in bioprocesses. Therefore, the composition variability of the different compounds demands separation procedures with some characteristics for their extraction. In addition to this, it is usually necessary to obtain a highly pure dry product from a diluted suspension for almost all items. Downstream is the costliest step in bioprocess, particularly in the pharmaceutical sector. The proper extraction process could increase the yield and maintain the quality properties of the HVBC promoting their application in the industry. To

isolate HVBC, traditional and emerging techniques based on chemical reactions or physical phenomena are applied (Figure 1).

3.1. Sample disruption

Disruption is an early and fundamental step in the downstream process aiming to release as much as possible of the product from the matrix. The matrix properties affect the selection of the disruption method and the yield of the process. A certain proportion of the target compound may be destroyed in the process, depending on the technique chosen. In this regard, quick and efficient processes are needed; mechanical techniques are frequently chosen over enzymatic ones due to their shorter residence times, lower operating costs, and more contained operation. On the other hand, the sample disruption can produce particles of different sizes depending on the selected method and operation conditions. Regarding the chitosan extraction it has been demonstrated that the size and shape of the particles are critical to achieving a higher quality and yield of the product. In this sense, chitosan flakes were ground using zirconium oxide balls (diameter 8 mm) in a chamber at 30 °C for 2, 4, 6, 8, and 10 h. The results indicated that after 8 h of milling, the mean diameter of the chitosan was reduced to 37.53 μm, and the continued grinding at 9 and 10 h not decrease particle size. Following milling, chitosan decreased its molecular weight and average viscosity, maintaining a constant degree of acetylation. The milling process broke down the crystalline structure of chitosan and its color was intensified. UV-Vis spectra suggested that the milling procedure might result in the formation of additional C=O groups. All these modifications promoted high dissolving rates of chitosan powder in acidic solutions (Zhang et al. 2014).

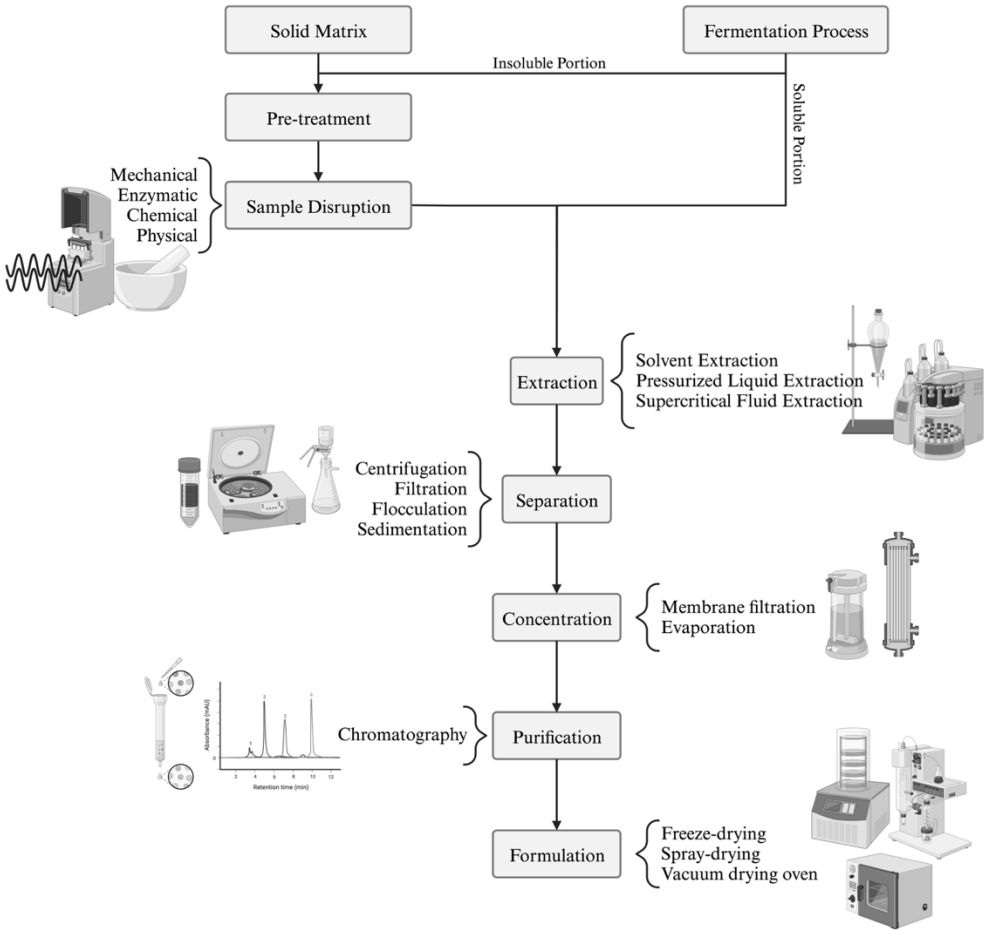


Figure 1. The main process followed for the obtention of high-value bioactive compounds from marine sources using a fermentation method.

3.2 Solvent extraction

Solvent extraction (SE) is an often-chosen technique for downstream processing of bioactive compounds from cultivation media, seawater, or sediment. SE in marine biotechnology focuses on the extraction of primary metabolites (ethanol, citric acid, acetic acid, and amino acids), which usually are present in higher concentrations than secondary metabolites (antibiotics or vitamins). Even this, secondary metabolites also can be obtained by SE, owing to they are use as therapeutics, therefore the quality requirements for the products are high, which can easily be met by SE. This

technique involves the use of solvents, usually organic solvents such as ethanol, methanol, and chloroform (López-Cruz et al. 2023). Parameters that affect SE include the polarity and thermolability of the compounds of interest, and the properties of the solvent (toxicity, volatility, viscosity, and so on). SE with dichloromethane and methanol was used for astaxanthin from shrimps (*Macrobrachium rosenbergii* and *Litopenaeus vannamei*) and prawns (*Penaeus monodon*, *Penaeus japonicus,* and *Fenneropenaeus chinensis*). Depending on the species, the yield varied from 2.91 to 19.20 μg/g, being higher for shrimp wastes (Liu et al. 2021). On the other hand, methanol, ethanol, acetone, hexane, dichloromethane, chloroform, dimethyl sulfoxide, dimethyl formamide, and diethyl ether were assessed for the obtention of chlorophyll, xanthophyll, and carotene from *Ascophyllum nodosum*, *Laminaria saccharina*, *Laminaria ochroleuca*, *Himanthalia elongata*, *Fucus spiralis*, *Pelvetia canaliculata*, *Bifurcaria bifurcata*, *Sargassum muricum*, and *Undaria pinnatifida* those are brown algae. All the algae had higher yields when they used ethanol. Information regarding possible natural dye sources for industrial use is provided by this type of research (Garcia-Perez et al. 2022). Traditional SE techniques can have low yields, be time-consuming, and require large amounts of solvents (López-Cruz et al. 2023).

Alternative and efficient extraction methods have been developed to overcome these limitations. One of them is pressurized liquid extraction (PLE), also known as accelerated solvent extraction, which involves high pressure and temperature to extract compounds from solid matrices. This technique reduces the extraction time and solvent consumption. Another alternative is supercritical fluid extraction (SFE), which utilizes supercritical fluids, such as water or carbon dioxide as extraction solvents providing two alternatives to organic solvents. Water and carbon dioxide at the supercritical state have been widely used for the extraction of HVBC from marine sources. For example, supercritical carbon dioxide with ethanol as co-solvent was used for β-carotene obtention

from *Dunaliella salina* achieving an efficiency of over 90%. The techno-economic assessment of this technology in comparison to hexane extraction revealed that the high energy requirement of SFE is compensated for by the reduction of solvent costs usage. SFE is a viable environmentally friendly substitute for industrial algal biomass extraction (Ludwig et al. 2021).

3.3 Filtration

Liquids are filtered to remove particulates by passing them through a solid support or filter media. Membrane technologies that are widely recognized and have potential uses in a variety of sectors because of their durability, economy, and standard operation include microfiltration, ultrafiltration, nanofiltration, reverse osmosis, and diafiltration. While ultrafiltration utilizes membranes with pore diameters of 1 to 100 nm to separate macromolecules weighing 1×10^3 to 2×10^5 Da, microfiltration separates particles with a molecular weight of more than 200,000 Da. However, nanofiltration can be used to produce molecules with a molecular weight of 200–1000 Da (Drioli and Cassano 2023). The water, soluble minerals, non-protein nitrogen, and water-soluble vitamins are all present in the permeate, often referred to as ultrafiltrate or nanofiltrate. Ultrafiltration and diafiltration have been used in combination for phycocyanin purification obtained by aqueous extraction from *Arthrospira maxima*. By using these filtration methods, 91.7% of the DNA in the crude extract was removed and a pigment yield of 1.17 mg/mL was achieved (Nisticò et al. 2022).

Membrane filtration enables selective and efficient obtention of a target compound, using simple equipment, being easy to handle, consuming little energy, scalable, and automatable for large production. However, membrane fouling is a prevalent issue, requiring changing of membranes often increasing the cost of the process (Praveen et al. 2019; Muñoz-Miranda and Iñiguez-Moreno 2023). The shape and charge of the compound play an important role in the separation, but

molecule size is the primary factor. It is important to keep in mind that a difference in molecular weight between two proteins does not always indicate a corresponding change in molecular size. Only substantial differences in the molecular weight of the proteins allow for the successful fractionation of proteins. Proteins that differ 10 times in molecular weight may only differ three times in size when they are folded or globular. The selective adsorption of molecules on membranes for their separation based on varying chemical behaviors is being employed to address this issue. Membrane filtration (20 and 2 μm) followed by glass fiber/filter (47 mm) has been used recently to separate the components of phytoplankton to evaluate their nutritional value and establish whether they may be considered a staple food source in the Arctic. The combination of these techniques enabled the determination of the chlorophyll and organic carbon content in micro, pico, and nanophytoplacton from different light depths (Park et al. 2022).

However, the primary obstacle to using membranes in marine biotechnology is the creation of an inexpensive method that produces long-lasting, highly effective membranes. These points are of particular interest during the extraction of HVBC from marine sources due to their high mineral content or the low concentration of the product. To resolve the problem of higher mineral content, the implementation of a preliminary desalination seems to be the best option. Another issue that may arise while employing membrane processes is the slow retentate flux, leading to the development of a dense secondary membrane owing to the sample's contact with the membrane material. This could lead to the formation of a linkage between the product and the membrane by hydrophobic interactions such as weak interactions and Van der Waals forces. Therefore, to limit the unspecific binding of the sample and to reduce this occurrence, it is required to screen a variety of membrane types when establishing a novel filtering process. Additionally, it is vital to consider modifying flow parameters, such as feed temperature and flow rates. Time-series sampling with

standard molecules in controlled research is suggested to improve the understanding of the behavior of natural chemical assemblage filtration processing.

3.4 Centrifugation

Centrifugation is a separation technique used to isolate or concentrate particles suspended in a liquid, taking advantage of the different speeds of movement depending on their shape, size, or weight when subjected to a centrifugal force. Which is the force exerted on a body when it rotates around an axis. The force's magnitude is directly proportional to the body's mass, the radius of gyration, and the angular speed. Centrifugal force accelerates particle sedimentation that tends to do so spontaneously or those whose density is lower than that of the liquid. In this sense, current technology allows us to reach forces of hundreds of thousands of times the force of gravity enabling the isolation and purification of HVBC from marine sources enhancing the purity and quality of the final product. This technique has been used for the purification of pigments such as phenazine-1-carboxylic acid, prodigiosin, and violacein from *Pseudomonas aeruginosa* (Patil et al. 2016) *Serratia marcescens* (Dozie-Nwachukwu et al. 2017) and *Janthinobacterium* sp. strain UV13 (Alem et al. 2020). In the three studies, the pigments were assessed for the development of alternatives for cancer treatment including melanoma skin, breast cancer, and cervix carcinoma.

3.5 Chromatographic techniques

Chromatography is the first method based on affinity strategy, which is currently used in many different separation processes. The main benefits of this method include high recovery rates and high purification factors (up to 1000-fold). The premise is that a reversible compound will be formed by the ligand and target, commonly using a ligand in an insoluble matrix, or immobilized.

This method uses the interactions between receptors and proteins, antigen and antibody, and enzyme and substrate to produce a selective extraction (Kavitha and Damodharan 2021). *Pelagibacter* sp. HTCC7211, a marine bacterium, produced thermophilic β-carotene 15,15′-monooxygenase $BCMO_{7211}$, which was purified using Ni–NTA affinity chromatography and Superdex–200 gel filtration chromatography. $BCMO_{7211}$ demonstrated extensive selectivity for carotenoid substrates, including α-carotene, lutein, β-cryptoxanthin, and zeaxanthin. Under optimal conditions *Pelagibacter* sp. HTCC7211 was able to produce 888.3 mg/L retinal in 60 min with an 89% (w/w) conversion rate. Suggesting that this bacterium is a viable option for enzymatical retinal production with biotechnological applications (Long et al. 2021).

To reduce the cost of the chromatography process, adsorbents such as zeolite, molecular sieve, ammonium Y zeolite, silver-exchanged zeolite, and mesoporous carbon, as well as nanoparticles made of gold, silver, zinc oxide, and graphene oxide, have been proposed as ways to purify R-phycoerythrin from marine macroalga *Gelidium pusillum*. The outcomes demonstrated the high-yield effectiveness of adsorbents and nanoparticles as high-yield replacements for R-phycoerythrin enrichment and purification (Mittal et al. 2022). By lowering the concentration of hazardous substances in marine extracts, purifying methods can guarantee safer and healthier goods promoting their incorporation into the industrial sector and consumption (Quitério et al. 2021). The downstream processing and purification of marine compounds can be complex and involve a combination of these steps depending on the specific characteristics of the target compounds, the source organism, and the extraction technique employed. However, always target is to maintain the focus on the obtention of pure, bioactive compounds with potential applications in various industries.

4. Current applications of biotechnological tools to produce marine bioactive compounds and future trends.

Different types of compounds, including gelling agents, colors, stabilizers, and natural preservatives can be found in the marine environment, and utilized in several industrial sectors. Moreover, these substances provide health advantages through their utilization of functional food additives, nutraceuticals, dietary supplements, and prebiotics. Marine pigments have well-known qualities and for this their demand is constantly growing in the food, feed, pharmaceutical, cosmetic, and medical sectors. Because of these pigments' well-known qualities, their demand is constantly growing. Currently, it has been estimated that the market for functional foods reach US $586.1 billion by 2030. However, only astaxanthin from natural sources can be used in human dietary supplements (Stachowiak and Szulc 2021). Owing to astaxanthin stereoisomers can be present in synthetic preparations, besides the natural pigment has higher antioxidant activity, stability, and assimilation than synthetic astaxanthin (Rodriguez-Amaya 2019). The production and sale costs of this pigment using *Haematococcus pluvialis* and *Xanthophyllomyces dendrorhous* can reach US $2500 and $7000 per kilogram, respectively. This means 2.5 and 2.8 times more expensive than synthetic astaxanthin (Panis and Carreon 2016). For this reason, the industrial applications of marine pigments are constrained by factors including manufacturing yields, pricing, regulatory permission, pigment purity, resistance to processing and storage conditions (pH, light, and temperature) (Kusmayadi et al. 2021; Freitas et al. 2021). Consequently, only a small number of examples are available for purchase, such as the blue colorant Linablue Spirulina Extract, which is marketed as a food product decorating element meant to replace artificial components (Freitas et al. 2021).

The main limitation in obtaining high-value compounds from marine sources is that cell walls can resist the digestion process because they are made mainly of cellulose, hemicellulose, alginate, pectin, glycoproteins, and other polysaccharides. An alternative to reduce the production cost of HVBC from these sources is the implementation of techniques based on the circular economy principle, which is based on three principles, eliminating waste and pollution, circulating products and materials (at their highest value), and regenerating nature. To break down the algal cells and extract the greatest percentages of the chemicals of interest, the use of pretreatments and cascade biorefinery processes has been assessed. Extraction of water-soluble fractions, such as minerals and phycobiliproteins, may be the first stage in the biorefinery's application in marine sources. Followed by solvent recovery of the low polar fractions, such as lipids and pigments (Geada et al. 2021). This is consistent with the fact that *Chlorella vulgaris* (NIOT-74) treated with 0.5% hydrochloric acid raised around 1.8 times the extraction of total carotenoid (60.88 mg/g) and lutein (11.56 mg/g) in comparison with the untreated control (Mary et al. 2021). Promoting the biorefinery concept of several HVBCs can be obtained by reducing production costs (Figure 2). Otherwise, the optimization of the extraction process of each HVBC from a particular microorganism should be performed to improve the yield. Nevertheless, the high carbon and energy needed for pigment production by algae makes the process an expensive alternative. An alternative to solve this drawback is the use of biowaste as a carbon source which also reduces the negative impact of agricultural wastes on the environment (Kusmayadi et al. 2021). For example, a culture medium based on cassava industry wastes, such as wastewater and residues, was formulated for the synthesis of prodigiosin with antidiabetic by fermentation using *Serratia marcescens* TNU01. The pigment extraction was performed using a C18 column using a mobile phase containing 70% methanol aqueous solution (70%, v/v) at pH 3.0 with a stable flow rate of

0.8 mL/min (Tran et al. 2021). These production or extraction systems contribute widely to the circular economy.

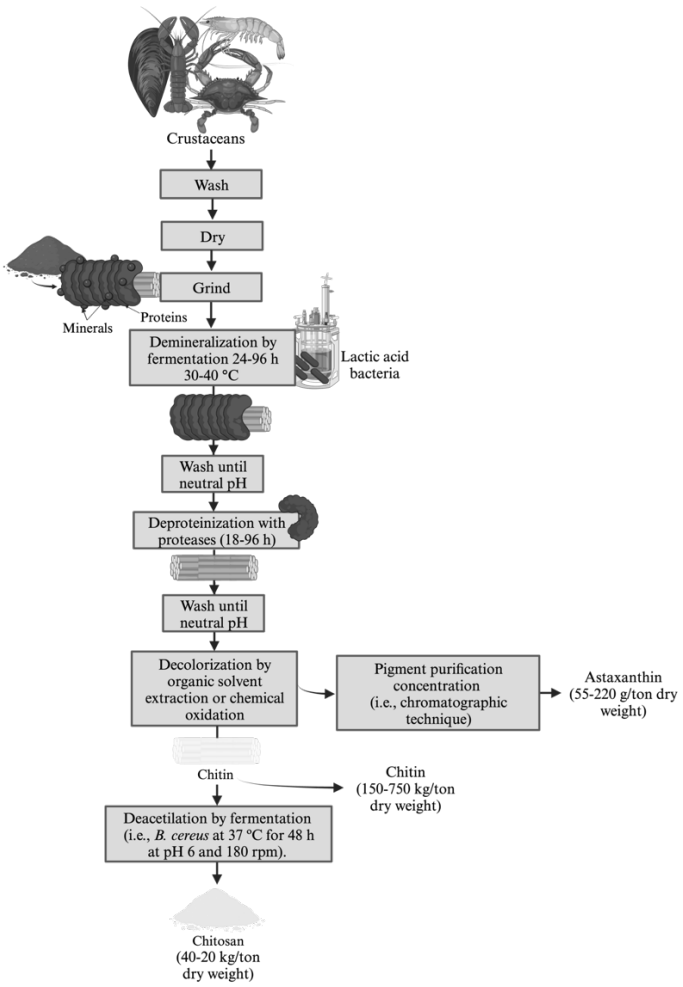


Figure 2. Potential valorization of crustaceans for the obtention of pigments and chitin or chitosan. The yield of the process will be strongly affected by the source, extraction, and downstream processes.

In addition to this, the use of molecular technologies to regulate and produce marine compounds enables the design and fast manufacture of the target compounds for a wide range of applications.

In this sense, by using synthetic biology technologies, a heterologous tyrosinase gene was used to promote melanin synthesis in *Vibrio natriegens*. The final product's physical and chemical characteristics, such as its nanoparticle structure, UV protection, and hazardous component adsorption, were like melanin produced chemically and naturally (Wang et al. 2020). However, as in all fields, there are some limitations to the use of marine compounds on a large scale, such as the low yields obtained while their extraction can imply a high cost (Nakano and Wiegertjes 2020). Furthermore, methods like encapsulation have been used to maintain the bioavailability and bioaccessibility properties of HVBC, and for addressing their instability during processing and storage. Some of the employed techniques include coprecipitation, coacervation, spray drying, electrospraying, electrospinning, vibrating nozzle, external ionic gelation by oleoresin, and freeze-drying. In addition to providing stabilization, these techniques contribute to their releasing control, increasing their potential applications in different industrial sectors (Quitério et al. 2021).

Marine organisms are a highly desirable renewable resource that can be used in a variety of ways to extract HVBC due to their capacity to provide a wide range of activities. But it's also important to consider the methods employed in their extraction and purification procedures. The buildup of toxic elements including cadmium, mercury, and lead in raw sea algae might pose health problems. These elements can cause cancer and mutagenesis in humans, thereby restricting the market's growth (Llop et al. 2015; Cherry et al. 2019). Furthermore, consuming wild seaweed regularly may put individuals at risk owing to its high iodine content, which can affect thyroid function(Llop et al. 2015). The best approach to solve this issue may be to combine different extraction and purification processes to lessen the practical challenges of a single method (Praveen et al. 2019).

5. Conclusions

The exploration of the marine environment as a source of HVBCs by using biotechnological tools is a sustainable promising alternative for the replacement of synthetic compounds in various industries. However, the successful integration of these bioactive compounds into industrial processes heavily relies on efficient production and extraction methods. In response to the drawbacks of traditional methods and t the growing demand for more natural products, biotechnological tools have emerged as viable and sustainable alternatives to produce high-quality bioactive compounds. However, their optimization and validation must facilitate their integration into large-scale industrial applications.

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Biotechnology strategies for bioactive compound extraction

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2025-02-21

Springer

https://doi.org/10.1007/978-981-96-1253-6_3

Iñiguez-Moreno M, de la Rosa-Flores OD, Melchor-Martínez EM, Parra-Saldívar R. (2025) Biotechnology strategies for bioactive compound extraction. In: Bioactive Compounds Extraction from Marine Resources and Wastes. Interdisciplinary Biotechnological Advances (IBA), Springer, Singapore, pp. 49-67

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