Universidade de Lisboa
Faculdade de Farmácia

Exploring the potential of using marine-derived ingredients in skin products: an overview

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Monografia orientada pela Professora Doutora Catarina Pinto Reis,
Professora Auxiliar da Faculdade de Farmácia da Universidade de Lisboa
e coorientada pela Professora Doutora Luísa Custódio, da Faculdade de
Ciências e Tecnologia da Universidade do Algarve

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Resumo

A Indústria Cosmética tem um histórico de constante modernização, com cosméticos cada vez mais sustentáveis e bem tolerados pelos consumidores. Nos últimos anos, o crescente entendimento e conhecimento do potencial das espécies marinhas, bem como a aplicação da "biotecnologia azul" têm motivado o aparecimento de novas soluções inovadoras nesta área.

As espécies marinhas são fontes importantes de compostos com possível aplicação em cosméticos, com atividades farmacológicas que incluem a atividade despigmentante, a atividade anti-envelhecimento ou a anti-bacteriana, especificamente contra a acne, mas também enquanto promotores e facilitadores de formulação. Vários métodos podem ser utilizados na extração destas moléculas, mas estes não se encontram desprovidos de desafios e implicações – possíveis contaminações, quer química como biológica, e problemas de reprodutibilidade dos vários lotes, associada à variabilidade intraespecífica dos exemplares, devem ser consideradas. A par destes, o enquadramento regulamentar e normativo, que é ainda relativamente superficial no que toca a este tipo de cosméticos, encontra-se cada vez mais desenvolvido e nota-se uma preocupação crescente com esta situação.

A aplicação de fundamentos de sustentabilidade na produção de cosméticos deve respeitar uma abordagem de ciclo de vida, analisando criticamente cada etapa. Neste sentido, a "biotecnologia azul", em crescente evolução, juntamente com a utilização de espécies invasoras ou de lixo marinho para a obtenção de novos ingredientes ativos, surgem como soluções inovadoras e sustentáveis para a Indústria Cosmética do futuro.

Palavras-chave: cosméticos; marinho; extratos; algas; sustentabilidade

Abstract

The Cosmetic Industry has a history of constant modernization, with cosmetics that are increasingly sustainable and well tolerated by the customers. In the last years, the growing understanding and knowledge of the potential of marine species, as well as the application of "blue biotechnology" have been motivating new innovative solutions in this area.

Marine species are important sources of compounds that may be applicable in cosmetics, with pharmacological activities that include depigmentant activity, anti-aging activity or anti-bacterial activity, specifically against acne, but also as formulation promoters and facilitators. Several methods can be used to extract these molecules, but these are not free of challenges and implications – possible biological and chemical contamination, and problems with batch reproducibility, which can be associated to the intraspecific variability of the different specimens, should be considered. Alongside these, the legislative and normative framework, which is still relatively superficial regarding this type of cosmetics, is increasingly developed and there is a growing concern with this situation.

The application of sustainability fundamentals in the production of cosmetics must respect a life cycle approach, critically analyzing each stage. In this sense, "blue biotechnology", in constant evolution, together with the use of invasive species or marine waste products to obtain new active ingredients, emerge as innovative and sustainable solutions for the future's Cosmetic Industry.

Keywords: cosmetics; marine; extracts; algae; sustainability

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List of abbreviations

AKT - protein kinase A
AP-1 - activator protein-1
α-MSH - α-melanocyte stimulating hormone
BAX - Bcl-2-associated X protein
BSE - bovine spongiform encephalopathy
cAMP - cyclic adenosine monophosphate
CEN - European Committee for Standardization
CITES - Convention on International Trade in Endangered Species of Fauna and Flora
CMC - critical micelle concentration
COX-2 - cyclo-oxigenase 2
CREB - cAMP response element-binding protein
CRP - C-reactive protein
DNA - deoxyribonucleic acid
ECM - extracellular matrix
ERK - extracellular signal-regulated kinase
EU - European Union
GAGs - glycosaminoglycans
GSH - glutathione
HMF - high molecular weight fucoidans
IMTA - integrated multi trophic aquaculture
iNOS - inducible nitric oxide synthase
ISO - International Organization for Standardization
IUCN - International Union for Conservation of Nature
JNK - c-Jun amino-terminal kinase
LCA - life cycle approach
LMF - low molecular weight fucoidans
MAPK p38 - MAP-kinase p38
MC1R - melanocortin 1 receptor
MCPs - marine collagen peptides
MITF - melanocyte inducing transcription factor
MPO - myeloperoxidase
mRNA - messenger ribonucleic acid

NF-kB - nuclear factor kB

NO - nitric oxide

NOX4 - NADPH-oxidase 4

p75NTR - P75 neurotrophin receptor

PAR-2 - protease activator receptor-2

PBT - persistent, bioaccumulative and toxic

PCOLCE - procollagen C proteinase enhancer

PGE2 - prostaglandin E2

PKA - protein kinase A

PPAR - peroxisome proliferator-activated receptor

PUFA - poliunsaturated fatty acids

RAS - recirculating aquaculture systems

R&D - research and development

ROS - reactive oxygen species

ROV - remotely operated vehicles

SC-CO2 - supercritical carbon dioxide

SFC - surfactant-free cosmetics

SOD - superoxide dismutase

SLES - sodium laureth sulfate

SLS - sodium lauryl sulfate

TIMP - tissue inhibitors of matrix metalloproteinases

TLR4 - Toll-like receptor 4

TRP1 - tyrosinase related protein 1

TRP2 - tyrosinase related protein 2

TNF-α - tumor necrosis factor-α

UN - United Nations

UV - ultraviolet

vPvB substances - very persistent and very bioaccumulative substances

XPF - xeroderma pigmentosum factor

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1 Main marine-derived cosmetic ingredients

1.1 Skin-whitening agents

Skin whitening products have been used across the world, with an especially significant market share in Asia (1).

When skin cells are irradiated by UV radiation, damage of DNA is induced, with increase of cAMP and, consequently, MITF, which results in the initiation of transcription of pigmentation genes, including melanin (2). Inhibiting the activity of tyrosinase, which catalyzes the rate-limiting step of skin pigmentation (1), is the most common among the different strategies to promote hypopigmentation (1,3,4). Tyrosinase catalyzes, through a first reaction, the formation of DOPA and, in a second phase, the oxidation of DOPA to dopaquinone (5).

Hydroquinone's toxicity and adverse effects (5,6), such as contact dermatitis and exogenous ochronosis (6), together with a growing tendency for sustainable and biological solutions, has motivated research for skin-whitening agents derived from marine products (7). Azelaic acid, kojic acid and phomaligol A are some of the already marketed examples (7,8).

Brown algae are sources of skin-whitening agents (5,6), which are believed to be safer than conventional skin whiteners (9). Fucoidans and fucoxanthin possess tyrosinase inhibition activity, suppress tyrosinase-related protein 1 (TRP1) (6), and reduce pigmentation (10). The oral consumption of fucoxanthin is able to repress COX-2, p75NTR, EP1 and MC1R-mRNA expression, reducing melanogenesis (6).

Furthermore, phloroglucinol and its derived polymers phlorotannins (6), such as 7-phloroeckol (3,5), fucophloroethol (11), fucodiphloroethol (11), fucotriphloroethol (11) and dieckol (11,12), found exclusively in brown seaweeds (6,13), exhibit anti-tyrosinase activity (3,5,9,10). Eckol and dieckol have reduced melanin synthesis in B16F10 cells (from a murine melanoma cell line) and dieckol was reported as having an activity three times higher than kojic acid, with an IC50 2.16 µg/mL (6). Isolated from *E. stolonifera*, dioxinodehydroeckol, another phlorotannin, downregulated melanogenic enzymes, such as tyrosinase, TRP1 and TRP2 (6).

SNA077, an extract of marine *Streptomyces* sp., has a potential to act as a potent and effective whitening agent, as it showed to inhibit melanogenesis in the

MNT-1 human melanoma cell line and in the mouse melanocyte cell lines Melan-a and B16, by downregulating melanogenic proteins in the cAMP/PKA/CREB signaling pathway (14). Meroterpenoids isolated from *Sargassum serratifolium*, like sargahydroquinoic acid, sargachromenol and sargaquinoic acid, also decrease melanogenesis activated by α-MSH, influencing CREB signaling pathways (15).

Another important, less common, strategy to promote hypopigmentation is through preventing the maturation or intracellular trafficking of tyrosinase, inhibiting melanosomal transfer. An example is niacinamide, a vitamin B3 derivative, which is found in fish (4).

1.2 Anti-aging activity

Skin aging is a complex process involving both intrinsic and extrinsic mechanisms (5,16,17,18), resulting in a loss of structural and physiological function (30), a loss of sensibility and a thinning of epidermal and dermal skin layers (33). Therefore, xerosis, wrinkles, fine lines, laxity, vasculature prominences and the appearance of benign neoplasms, such as seborrheic keratoses, are some of the macroscopic consequences of skin aging (5,9,17,19).

The inevitable genetic and physiological changes that occur over time (5,11,16,17,18), including ethnicity and hormonal and anatomical variations (16), make up the intrinsic mechanism of skin aging, in which production of progerin increases (5). On the other hand, controllable and environmental variables, such as exposure to pollution, smoke, UV radiation and infections agents (16,18), are related to extrinsic skin aging (5,11,16,17,18), in which DNA alteration and damage occurs (5,17). A photoaged skin and a skin that has aged mostly due to intrinsic mechanisms are different morphologically and physiologically, as **Table 1** shows.

Table 1. Extrinsically-aged (or photoaged) skin and intrinsically-aged skin compared, regarding morphology and cellular and physiological changes (16).

Extrinsically-aged skin (photoaged skin)	Intrinsically-aged skin
Pronounced increase in metabolic processes	Slowing down of the metabolic processes
Irregular skin pigmentation	Pallor skin
Leathery skin, profound wrinkles	Smooth skin, fine wrinkles
Mature collagen degradation	Mature collagen degradation, but more stable
Marked elastogenesis, followed by massive degradation	Gradual decline in the elastin production
Pronounced inflammation, with infiltrate	No inflammation

1.2.1 Photoaging and photo-protective activity

UV radiation, in particular UVA radiation because of its ability to penetrate the dermis (16), is estimated to account for about 80 to 90% of the skin aging process (16,17).

Globally, exposure to sunlight is responsible for a series of biochemical and physiological outcomes, such as disruption of the ECM turnover and of the dermal fibers network, DNA damage, ROS formation, increase in inflammatory mediators and activation of signaling pathways (5,16,17,18).

In a photo-aged skin, the disruption of the ECM turnover process, characterized by an upregulation of MMPs (collagenases, gelatinases and stromelysins-1) and a downregulation of TIMPs in keratinocytes and fibroblasts (17,20), results in the deterioration of collagen, elastin and other ECM components (16,17,18,21). Collagen fibrils, elastic fibers, glycoproteins and glycosaminoglycans (GAGs) are disorganized into a dermal-spreaded agglomeration (18). Together with an increased production of XPF (17), wrinkles appear in the aged skin.

UV radiation also damages the genetic material – through dimerization of pyrimidine (UVB) and formation of ROS or free radicals (UVA) (16,17). Mitochondrial, peroxisomal, membranal and cytosolic ROS are particularly important in the extrinsic skin aging process (18), since they influence numerous cellular processes, including the activation of MAP-K p38/JNK/ERK/AP-1 signaling pathway that leads to the already mentioned upregulation of MMPs (16,22) and, consequently, increased degradation of the ECM (16). Simultaneously, NF-kB and AP-1 play an important role in the balance between proliferation and apoptosis (16).

Additionally, UV radiation interferes with enzymes that participate in the DNA repair process, as well with the immune system's T cells and Langerhans cells, further damaging the skin in an indirect manner (16).

MAAs (mycosporine-like amino acids, such as porphyra-35, porphyra-334 or shirorine) and scytonemin are examples of natural algae-based UV filters (9,11,15,21,23), which constitute alternatives to the other available UV filters, whose use is controversial due to their environmental impact, sensitizing properties and potential endocrine disrupting effect (21). MAAs absorb UV radiation in wavelengths ranging between 310 and 362 nm, presenting with a better protection efficiency when located outside the cell, whereas scytonemin has a maximum absorption at 386 nm (21).

Erebusinone, a tryptophan derivative isolated from the Antarctic sponge *Isodictya erinacea*, showed photoprotective properties, absorbing UVA radiation with a peak absorbance of 370 nm (24).

Isolated from cod eggs (*Gadus morhua* L.), gadusol and gadusolate are structurally similar to mycosporines (25), sharing some chemical and mechanistic features that make these compounds prone to be included in sunscreen formulations in the future (21,25).

Marine bacteria, especially extremophiles, also produce photoprotective compounds. For instance, *Klebsiella aerogenes* produces extracellular semiconductor particles of cadmium sulfide (CdS) in response to environmental stress. These crystallites absorb UV radiation (22).

1.2.2 **Inhibition of MMPs (matrix metalloproteinases)**

As stated before, matrix metalloproteinases (MMPs), which include interstitial collagenases, gelatinases and stromelysin (5), regulate, among other, the tissue remodeling process, the synthesis and secretion of cytokines and cell adhesion molecules and the degradation of components (5,26). Suppressing and modulating MMPs activity in skin cells, either directly or via signaling pathways, is one of the strategies used nowadays in anti-aging formulations (5).

Phlorotannin (from *Eisenia bicyclis*) and phloroglucinol derivatives, such as eckol, dieckol, dioxinodehydroeckol and bieckol, are responsible for the inhibition of MMPs in human fibroblasts (1,11,15). Joe et. al. identified that eckol and dieckol from *E. stolonifera* is able to inhibit MMP-1 expression, by interfering with the expression of NF-κB and AP-1 (5,27). Additionally, phlorofucofuroeckol A, also a phloroglucinol derivative, in *Eisenia bicyclis* showed an inhibitory effect on hyaluronidase's activity (1,15).

Fucosterol, fucoidan and, to a lesser extent, fucoxanthin also present themselves with anti-MMP activity. Fucosterol, a phytosterol present in *Hizikia fusiformis*, modulates AP-1 and TGF-β1 signaling, leading to a less expressive activation of MMP-1 (28). In a similar manner, fucoidan, as other sulfated polysaccharides, not only inhibit the production of MMP-1 mRNA when fibroblasts are induced with UVB radiation, through ERK pathway, but also increase the expression of type I procollagen synthesis (11,15). Also by inhibition of the ERK/JNK pathway, *Streptomyces* sp.'s derived sarmentosamide is another promising anti-aging agent that works by reducing the expression of UVB-induced MMP-1 in normal human dermal fibroblasts (NHDFs) (29).

Sargachromanol E, present in the extract of brown macroalgae *Sargassum horneri*, activates tissue inhibitors of metalloproteinase 1 (TIMP-1) and 2 (TIMP-2), inhibiting MMPs expression, with a higher effect than retinoic acid (5,15).

Besides their potential as UV filters, the aforementioned MAAs also have anti-MMP activity, either by suppressing the expression of UVA-induced MMPs or by modulating the expression of procollagen C proteinase enhancer (PCOLCE) and elastin genes (11).

1.2.3 **Antioxidant activity**

Oxidative stress, which is related to ROS production (superoxide anion, hydroxyl radical and hydrogen peroxide), plays an important role in the skin aging process (5,9,18), especially in extrinsic aging, where UV radiation is the main factor (18).

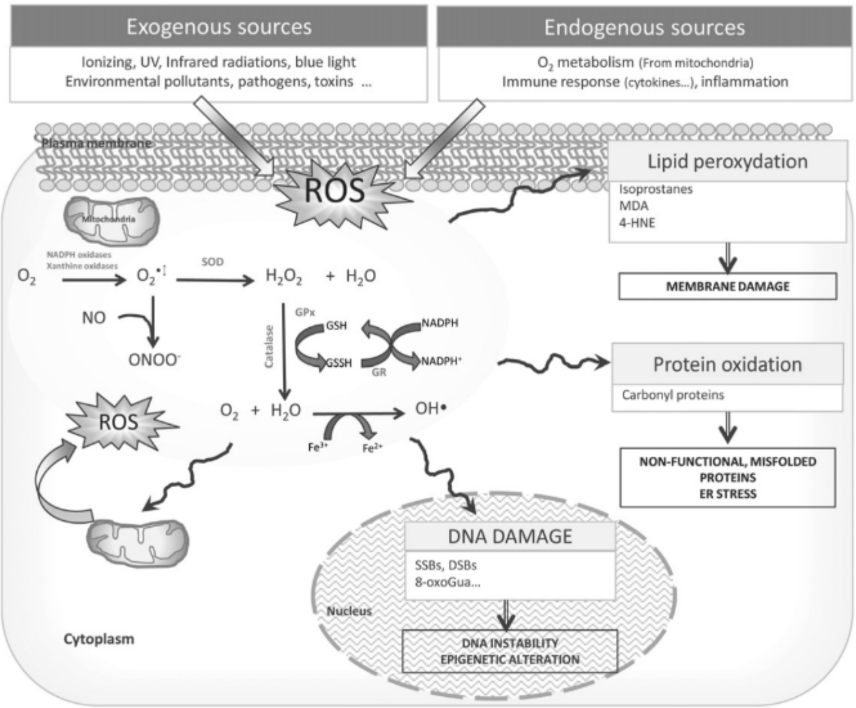

Figure 1. ROS-induced cellular damage. SOD (superoxide dismutase), GPx (glutathione peroxidase) and reduced GSH (glutathione) act as ROS scavengers, avoiding further and worse DNA damage and protein and lipid peroxidation. Adapted from Berthon et al., 2017 (11)

These ROS cause damage to cellular components, including proteins, DNA and membrane lipids (5,9), the latter related to lipid peroxidation (5) (**Figure 1**), and activate numerous signaling pathways, such as MAPK/AP-1/NF-κB/TNF-α/IL-6-mediated inflammation-induced aging and p53/BAX/cleaved caspase-3/cytochrome c-mediated apoptosis-induced aging (30). The production of ROS also activates the production of MMPs (30).

1.2.3.1 Carotenoids

Some of the most known antioxidants are carotenoids, such as β-carotene, largely found in microalgae (5), astaxanthin, lycopene, torulene and torularhodin (31), which can be found in marine sources (3,31). All have an excellent scavenging activity and an important capacity to inhibit and prevent the formation of ROS (3,31), thus being used to prevent UV-induced damage (30).

Astaxanthin is widely distributed among crustaceans (shrimps, crawfish, crabs and lobsters) and algae, showing stronger antioxidant properties than β-carotene (11). It scavenges ROS (3,11,31), inhibits lipid peroxidation (32) and the activation of the NF-jB transcription factor (11) and blocks the production of pro-inflammatory cytokines, COX-2 and nitric oxide (11,32). According to Lyons et. al. (15,22,33), extracts containing astaxanthin caused significant changes in SOD and GSH, as well as a protective effect on UVA-induced DNA damage. Extracts of *Haematococcus pluvialis*, the richest source of astaxanthin (2,21), show improvements in the skin's macroscopic appearance, regarding wrinkle and texture, displaying a relevant protection against photooxidative damage (11). These extracts are already being used in cosmetics (3).

Isorenieratene, renieratene and renierapurpurin are other less known carotenoids isolated from marine sponges, that show similar oxygen scavenging and lipid peroxidation inhibitory activities as those of astaxanthin (9).

Another important pigment with established antioxidant activity, β-carotene, is also in use nowadays, specially the one isolated from microalga *Dunaliella salina*, as provitamin A (3).

1.2.3.2 **MAAs, fucoxanthin and pyropheophytin A**

Aside from the already mentioned anti-MMPs and photo-absorbing activities, MAAs (mycosporine-like aminoacids) scavenge ROS, such as superoxide anion, and prevent lipid peroxidation (9). Notably, mycosporine-glycine offers a fast protection against oxidative stress, even prior to the intervention of endogenous antioxidant enzymes (24). The same goes for sargachromanol E – it already has a well established anti-MMPs activity, but research shows suppression of UVA-induced intracellular formation of ROS and inhibition of lipid peroxidation (11).

Isolated from the brown algae *Hijika fusiformis*, fucoxanthin also showed potent antioxidant activity against DPPH radical scavenging (11).

Cahyana et. al. (34) showed a strong antioxidant activity of both acidic and neutral fractions of the extract of *Eisenia bicyclis*, identifying pyropheophytin A as one of the contributors. This newly isolated compound showed a higher antioxidant activity than α-tocopherol (22).

1.2.3.3 **Polysaccharides and oligosaccharides**

Regarding marine-derived polysaccharides, fucoidans, carrageenan and ulvans also present antioxidant properties.

A great superoxide radical and hydroxyl radical scavenging activity in fucoidan isolated from *Laminaria japonica* and a considerable ferric reducing antioxidant power (FRAP) in fucoidan from *Fucus vesiculosus* are some examples (35).

Isolated from red algae, carrageenan has an established use in cosmetic products as a stabilizer, emulsifier and moisturizer (36). However, it also shows an interesting antioxidant activity, with κ-carrageenan exhibiting the highest DPPH reducing capability and, consequently, the highest ROS scavenging potential (35,36).

Extracted from the green algae *Ulva pertusa*'s cell wall, sulfated heteropolysaccharides called ulvans have revealed important biological properties, such as antioxidant activity and the ability to chelate ferrous ions. The higher the sulfate content in ulvans, the higher was the antioxidant activity (35).

Lastly, agarose-derived oligosaccharides (AOSs), obtained from chemical and enzymatic hydrolysis of agar, showed some antioxidative potency, especially regarding the radical scavenging capacity in DPPH assays. Agarohexose showed the best results, but agarobiose, agarotetrose and agarohexaose also demonstrated antioxidant properties. In particular, agarohexaose can protect against ROS-associated cell damage with substantial quality (35).

1.2.4 **Anti-inflammatory and wound healing ingredients**

Exposure to sunlight leads not only to microvascular changes and transendothelial migration of leukocytes (5), but also to the activation of proinflammatory genes, resulting in an inflammation cascade that triggers ROS (5,11), which activate COX-2 and PGE2 (11).

In parallel, a transcription factor that regulated higher oxidative stress called NFjB is activated by ROS, which stimulates the expression of proinflammatory cytokines such as TNF-α, IL1a, IL-1b, IL-6, IL-8, IL-10, iNOS and COX-2 (5,11). These molecules are produced in keratinocytes and are regulated by NF-κB, a transcription factor that regulates, among others, telomerase gene expression, inflammation and angiogenic activity (5).

1.2.4.1 **Polysaccharides and oligosaccharides**

Different sulphated polysaccharides have shown *in vivo* anti-inflammatory activity in different studies (36). Fucoidan is the most cited one for these properties, thus being the one in which we will focus the most.

Purified from *Fucus vesiculosus*, fucoidan reduces the production of pro-inflammatory molecules, such as nitric oxide (NO), prostaglandin E2 (PGE2), IL-1β and TNF-α, and disrupts the MAPK and AKT signaling pathways (36). Also isolated from brown algae *Turbinaria ornate*, fucoidan shows a declination of inflammatory biochemical markers, such as cathepsin D, myeloperoxidase (MPO) and C-reactive protein (CRP), somewhat comparable to dexamethasone (36), following a reduction of leukocytes' recruitment to the site of inflammation (36).

Fucoidan can be presented in two main isoforms: low molecular weight fucoidan, hereinafter referred to as LMF, and high molecular weight fucoidan (HMF). LMF has a better bioavailability in tissues (9). As stated by the study conducted by Park et. al. (37), *Undaria pinnatafida*'s LMF is expected to act as a "wound-healing accelerator" (36,37), due to its anti-inflammation and angiogenesis activities (9). In this study, LMF enhanced the wound healing process, not only by reducing the recruitment of leukocytes, but also by promoting re-epithelization (37).

The use of fucoidan in topical cosmetic after-sun formulations (10) and in preventive and therapeutic agents against atopic dermatitis, through inhibition of several chemokines (35), is also justified by the widely described wound healing and anti-inflammatory properties.

1.2.4.2 **Phlorotannins**

Dieckol isolated from *Ecklonia cava* suppresses, in a dose-dependent way and with no cytotoxicity, the production of nitric oxide (NO) and prostaglandin E2 (PGE2), but also of other pro-inflammatory cytokines (IL-1β and TNF-α). The inhibition of NF-κB and p38-MAPK signaling pathways and, consequently, the inhibition of ROS production is another mechanism in which dieckol can prevent inflammation (38).

Other phloroglucinol derivatives, including phlorofucofuroeckol A and B, which downregulate iNOS and PGE2, inhibiting NO production, and diphlorethohydroxycarmalol, which also downregulates iNOS, COX-2 and NF-κβ, have also been described as anti-inflammatory compounds (5).

1.2.4.3 **Coral-derived pseudopterosins**

Corals are also a source of cosmetically interesting compounds and molecules. The most relevant ones are the *Pseudopterogorgia elisabethae*-derived pseudopterosins A, B, C and D, a group of diterpene glycosides that possess various biological activities including anti-inflammatory, analgesic, antibacterial and anti-acne

(9). These glycosides are already being commercialized as Resilience® by Estée Lauder (9).

Pseudopterosin A is the most studied compound. Its anti-inflammatory properties differ from those described so far, as it inhibits phagosome formation and triggers the G-receptor-mediated release of intracellular calcium ions (9).

1.2.4.4 **Sea cucumbers-derived fatty acids**

Sea cucumbers are known in folk medicine for the treatment of wound healing, being used to accelerate the wound contraction rate (9).

Stichopus hermanni-based hydrogel has numerous advantages, mostly due to the immobilization of biological active compounds for a longer period in the matrix, which creates a controlled release system that easily interacts with the wounds and facilitates the healing process at a later stage. This hydrogel showed enhanced histological reorganization and modulation of the inflammatory responses, with a significant reduction of pro-inflammatory cytokines (such as IL-1α, IL-1β and IL-6) (39).

Globally, studies have been corroborating that aqueous extracts of sea cucumbers are much more cosmetically interesting than organic extracts, because of their content in fatty acids and antioxidants, the latter playing an important role in controlling ROS production at wound sites (9). For example, the aqueous extract of *Stichopus chloronotus* has a higher concentration of docosahexaenoic acid (DHA) than the organic extract, which has been linked to the stimulation of pro-inflammatory cytokine production at wound sites, leading to a stimulation of the migration and proliferation of skin cells and to a breakdown of ECM proteins. (9)

Moreover, the major fatty acids in sea cucumbers, both DHA and EPA (eicosapentaenoic acid), also stimulate the production of resolvins (inhibiting IL-1β) and protectins (inhibiting TNF-α and IL-1β) via COX-2 and 5-LOX pathways (9). Saponins present in the extracts of sea cucumbers have also been linked to the prevention of TNF-α production by NF-κB (9).

The improvement of the levels of TNF-α after the incorporation of sea cucumber extracts into Carbopol® gel base in diabetic foot ulcer patients, as described by Haryanto et. al. (40), is a practical example of the benefits of these marine organisms in wound healing and inflammation.

1.2.5 **Marine-derived collagen**

Collagen protein, the main structural protein in the ECM, plays a structural role in supporting the formation, tensile strength and flexibility of joints (41). The different types of collagen, namely types I, II, III, V and XI, organize themselves into fibrils that allow for support and resistance to mechanical stress in connective tissues (41).

One of the common sources of collagen is bovine and porcine skin. However, a series of bovine spongiform encephalopathy cases, as well as religious issues, limit its use (41,42). Therefore, marine collagen arises as an important alternative, commonly isolated from fish, jellyfish and sponges (41).

Aside from accelerating wound healing, through increased vascularization and epidermal growth, and regenerating bone (41), marine collagen has been showing anti-aging properties, through reduction of wrinkles and improvement on the skin elasticity, structure and appearance (41). Furthermore, marine-derived collagen has also shown ROS-scavenging activity, with antioxidant properties (43).

Collagen derivatives, namely marine collagen peptides (MCPs), have also shown advantages regarding skin and bone repair (43). These MCPs are obtained via enzymatic digestion of collagen (43,44), using trypsin for example (44).

MCPs are considered anti-aging compounds, since they promote photoprotection and immunomodulation, as well as improvement of premature senescence of the skin cells (43). However, there is a risk of increased oxidative stress, due to an increase of hydroxyproline levels, associated with collagen synthesis, as well as an activation of immune-mediated TLR4 and NOX4 (43).

In particular, Pozzolini et. al. (44) suggests that MCHs (marine collagen hydrolysates) derived from the marine sponge *Chondrosia reniformis* can be used in drug and cosmetic formulations, because of their antioxidant and proliferative properties.

1.3 **Anti-acne activity**

Although there are numerous marine-derived ingredients with anti-bacterial potential, in this paper, we will only focus on the anti-acne activity, as it is the most represented in the cosmetic field.

Acne vulgaris is the most common skin disease, characterized by the chronic inflammation of the pilosebaceous unit (9,45). It is a multifactorial disorder, in which hormonal, microbiological and immunological mechanisms can be taken into account, and exacerbated sebum production, hyperkeratinization of the follicles and bacterial proliferation are some of the main factors that contribute to acne's severity and progression (9,45). Microcomedones, the primary type of acne lesions, result from the occlusion of the pilosebaceous ducts due to follicle blockage and accumulation of sebum. These can be closed, resulting in white heads, or open, making up black heads (45).

Regarding bacterial proliferation, *Propionibacterium acnes* and *Staphylococcus epidermidis* are the main microbiological targets, because these stimulate an inflammatory environment through the release of ROS and cytokines and the activation of TLR (Toll-like receptors) both in early-stage and late-stage acne inflammation (9,46). (**Figure 2**) *P. acnes* also releases lipases that digest the excess skin oil and sebum, resulting in local inflammation (9).

However, defensins, immunocompetent cells, peptidases, PPARs and pro-inflammatory neuropeptides also play an important role in acne-derived inflammation (46). (**Figure 2**)

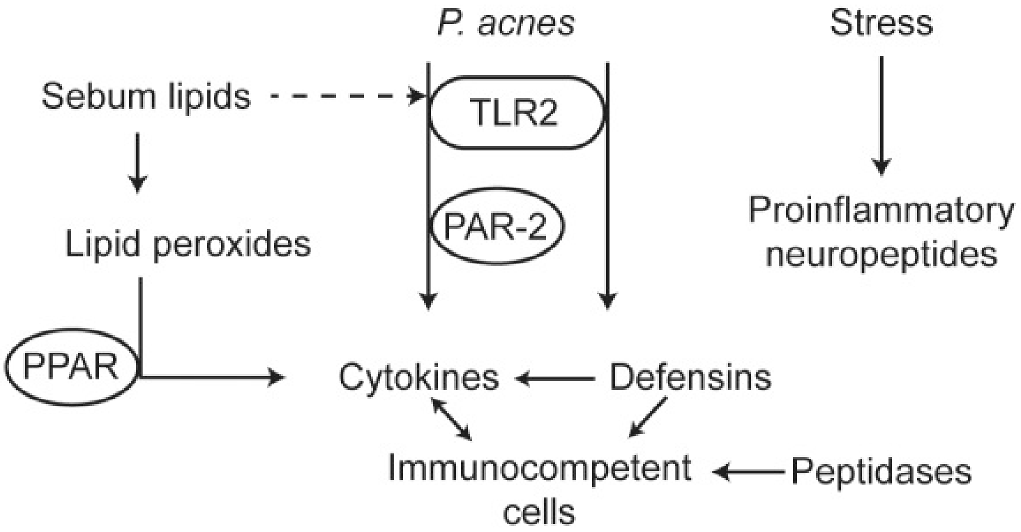

Figure 2. Mechanisms of inflammation in early- and late-stage acne. *P. acnes* activates TLR2 (Toll-like receptor 2) and PAR-2 (protease-activated receptor), resulting in the release of cytokines and defensins which, together with peptidases and lipid peroxides (derived from ROS-induced oxidative stress), contribute to the inflammation of immunocompetent cells. PPAR = peroxisome proliferator-activated receptor. Adapted from Tanghetti, E.A., 2013 (46)

Sargafuran, derived from *Sargassum macrocarpum*, has an antibacterial activity against Propionibacterium acnes, with a minimum inhibitory concentration (MIC) of 15 µg/mL (9), which may be useful in new skincare cosmetics to prevent acne (5,47).

On the other hand, *E. bicyclis*-derived phlorotannins also present with an effective inhibitory activity against *P. acnes*, *Staphylococcus aureus* and *S. epidermidis*. The latter is also inhibited by carrageenan (from red algae) with a MIC of 0,325 mg/mL and by sulfated galactan also from red algae (15).

Diterpenes, originated from soft corals, including cembrene diterpenoids, carry many biological properties of interest to the cosmetic industry. Wei Chen et. al. (48) demonstrated that sinulariolides from *S. flexibilis*, such as SC-2, SC-7 and sinularin (SC-9), inhibit keratinocyte over-proliferation and anti-NO production properties. SC-9 also inhibited sebum secretion. Overall, these compounds show great potential to be integrated in anti-acne formulations.

Brominated compounds, isolated from *Rhodophyta* species (red algae), constitute a wide group of anti-acne molecules. Ranging from simple compounds like bromophenols or bromoform, to more complex molecules, such as organobromine compounds, these exhibit anti-bacterial activity against *P. acnes* and *S. epidermidis*. *Symphyocladia latiuscula* contains high amounts of bromophenols, which are toxic to some bacteria, showing ability to inhibit *C. acnes* growth. *Osmundaria serrata*'s lanosol ethyl ether, another brominated phenol, is highly bacteriostatic and mildly bactericidal, with a MIC of 0,08 mg/mL (45). Apart from lanosol, brominated nonterpenoid metabolites such as acetogenins, bromoform, brominated monoterpenes and indoles also present with anti-bacterial activity. Finally, *Asparagopsis armata*'s organobromine compounds also have shown an important inhibition in a *P. acnes* culture (45).

Regarding *S. epidermidis*, brominated compounds also seem to play an important role in its inhibition, including those isolated from *Polysiphonia fibrillosa*, *Polysiphonia denudata*, *Rhodomela genus* and *Rhodomela confervoides* (45).

1.4 **Formulation promoters and facilitators**

So far, we have been discussing the different uses of marine-derived products as cosmetic active substances.

However, the marine environment also provides interesting excipients and ingredients that facilitate and promote more cosmetically appealing formulations for consumers. Excipients are also indispensable for the product's long-term stability and microbial resistance to contamination (49).

1.4.1 **Sea water**

There is a very limited amount of cosmetics that don't have water in their formula - only powders, lipsticks and nail polishes. Given that fresh water is a limited resource, sea water can be an interesting alternative, since it is a well-known source of minerals, such as chlorides, magnesium, sodium, calcium, potassium, bromides, sulfates and bicarbonate, with benefits in inflammatory skin disorders, such as atopic dermatitis (21).

Dead Sea water, for example, has a 7 to 10 times higher salinity (approximately 345 g minerals/liter) than water from oceans, presenting as a natural humectant (21).

1.4.2 **Polysaccharides as gel forming agents and viscosity controllers**

Polysaccharides and oligosaccharides include carrageenans, alginates, agar, laminarin, fucoidan, xylans and mannans.

In **Table 2**, the cosmetic properties of the different marine-derived polysaccharides are shown.

Table 2. Marine-derived polysaccharides and their cosmetic properties. Source: COSING.

Polysaccharides	Cosmetic properties according to COSING database	Species where these PS are found (e.g.)
Alginates and their salts (calcium, sodium, magnesium, ammonium and potassium)	Binding Emulsion stabilizing Film forming Humectant Viscosity controlling	Brown macroalgae (e.g. *Ascophyllum nodosum*, *Laminaria hyperborea*, *Laminaria digitata*) (49)
Carrageenans (including hydrolyzed carrageenan)	Binding Emulsion stabilizing Film forming Skin conditioning Viscosity controlling	*Kappaphycus* and *Eucheuma* genera (49)

Agar and agarose	Binding Fragrance Viscosity controlling Skin conditioning	Red macroalgae (*Gelidium* and *Gracilaria* species) (49)
Fucoidan	Skin conditioning Skin protector	Brown macroalgae (e.g. *Sargassum stenophyllum*, *Fucus vesiculosus*) (49)
Xylans	Film forming Skin conditioning	Green macroalgae (*Bryopsidales* order) (49)
Mannans	Film forming Skin conditioning	Green macroalgae (*Bryopsidales* order) (53)

Alginates and its derived salts can be obtained from brown marine macroalgae (49), with their cosmetic properties (**Table 2**) being linked to physical properties, biocompatibility and biodegradability (21). Their role as viscosity controllers requires the presence of a divalent cation, normally Ca2+, which is not advisable in some cosmetic forms, limiting their use (21). Furthermore, the application of alginates in microencapsulating materials for new cosmetic formulations, including those involving a controlled release, is also important, especially at a pH higher than 3-4 for better stability (21,49). At a low pH, these polysaccharides also solidify and stabilize emulsions in a highly efficient manner (10).

Carrageenans are already widely used in cosmetics, such as creams, air freshener gels, shampoos, shoe polishes, sticks, sprays and foams (10,49), with gel-forming, emulsifying, thickening and stabilizing properties, besides those described in **Table 2** (49). These can form single and double helices, which make up their gelling capacity (49), depending on the concentration, temperature, presence of other solutes and the type of carrageenan used (21).

Agar, mainly formed by agarose and agaropectin, is found in the cell wall of red macroalgae and acts as a gelling, emulsifying and suspending agent (49). The use of agar as an excipient has been suggested in creams, lotions, deodorants and anti-aging and anti-acne formulations (10). Also in sustained-release preparations, agar has shown a good profile as a dispersing agent and as a vehicle in several *in vivo* studies (49).

Agarose, a natural polymer of galactose also extracted from red seaweed, is also a candidate for an emulsifier and thickener used in cosmetics. However, due to its strong hydrophilic and gel properties, algae-derived agarose needs to be chemically modified in order to gain hydrophobicity, reducing its gel strength and giving it amphiphilicity (50). In an attempt to better design surfactant-free cosmetics (SFCs), Xiao et. al. (50) used agarose stearate-carbomer940 as a stabilizer and rheology modifier. The final results showed SFCs with good appearance and sensation, with a satisfactory gel-like behavior and a rheological behavior similar to commercial cosmetic creams.

Finally, fucoidan, extracted from brown macroalgae, can be used as a drug carrier for controlled release systems in skin formulations, as a wall component of a number of pharmaceutical forms, such as microparticles, nanoparticles, hydrogels and nanocapsules (49).

1.4.3 Carotenoids, chlorophylls and phycobilins as hair dyes

Natural colorants and dyes benefit from a better reputation and acceptance by consumers than the artificial ones, due to the latter's negative impact on human health and environment (49). Macroalgae and cyanobacteria are sources of colorant molecules, such as carotenoids, chlorophylls and phycobilins, with a lower side effects profile and a variety of colors covered – blue, yellow, orange and red (21,49).

Phycobillins, chemically tetrapyrroles, constitute the main photosynthetic accessory pigments from some algae of Glaucophyte, Crystophyte and Rhodophyte groups and are covalently bound to proteins, forming phycobiliproteins (21).

However, the potential use of these compounds comes with some formulation challenges. Chlorophylls and carotenoids are lipophilic molecules, requiring organic solvents for their extraction (e.g. methanol, acetone or DMS), which are incompatible with cosmetics (49). On the other hand, water can be used in extracting phycobiliproteins, as they are polar molecules (49), but it will ultimately alter the organoleptic properties of some formulations, as water should not be used in make-up (21).

1.4.4 Marine biosurfactants

Marine biosurfactants are byproducts of marine microorganisms' metabolism – in bacteria, for example, these molecules are produced so that they can use substrates that are not water-soluble (36). These amphiphilic molecules, containing both hydrophilic and hydrophobic domains, allow for an easier solubilisation of hydrophobic substances in water, by reducing the interfacial tension. Biosurfactants have low critical micelle concentrations (CMC), which allows for lower concentrations of these compounds when compared to chemically produced surfactants (36,51).

Biosurfactants can be classified in high molecular weight (HMW) biosurfactants or bioemulsifiers and in low molecular weight (LMW) biosurfactants, the latter including fatty acids and lipoaminoacids. (51) Especifically, mannosylerythritol, sophorolipids and rhamnolipids (**5** in **Figure 3**, e.g.) have emulsifying and solubilizing properties, among others, justifying their potential use in cosmetic formulations (13). Advantages in the use of marine biosurfactants include the low irritancy to the skin, when compared to their synthetic counterparts (13,94), and their low eco-toxicity, showing an acceptable environmental impact (22,94).

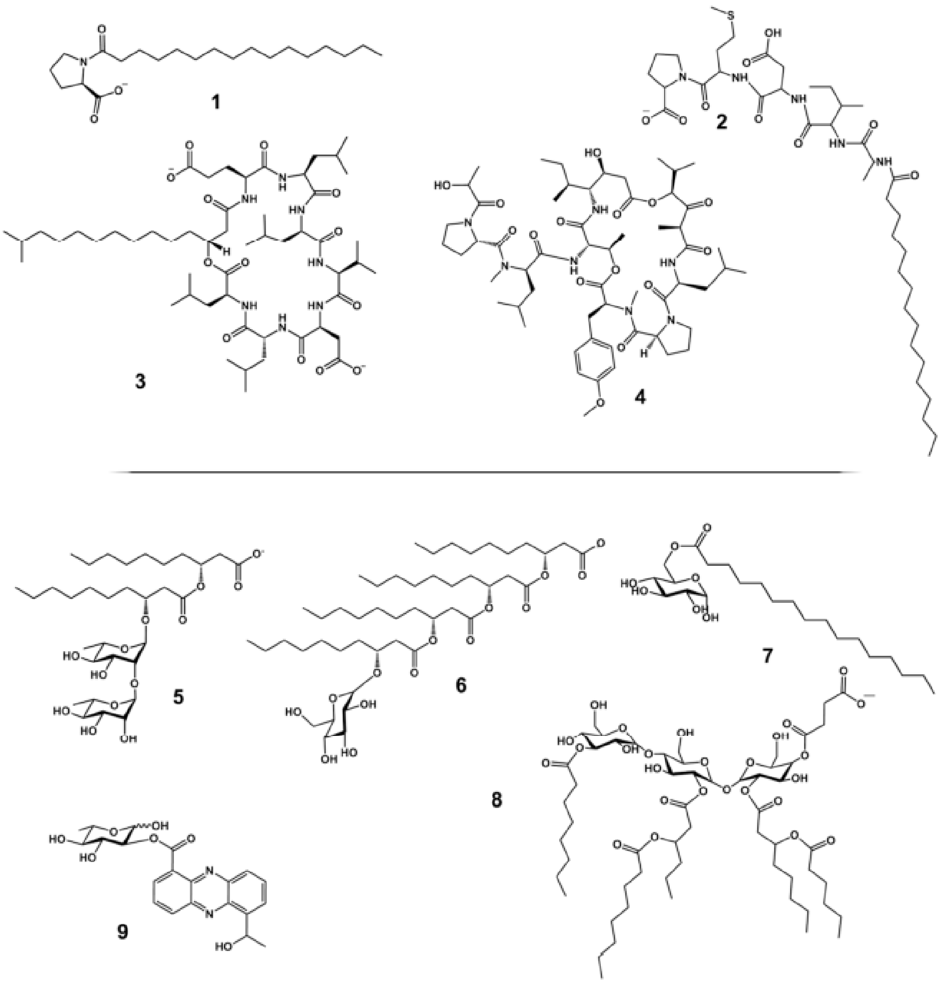

Figure 3. Structural diversity of LMW (low molecular weight) biosurfactants. 1. proline lipid (from *Brevibacterium luteolum*); 2. rhodofactin (from *Rhodococcus* sp.); 3. surfactin (from *Bacillus siamensis*); 4. didemnin B (from *Bacillus*

***amyloliquefaciens*); 5. di-rhamnolipid (from *Pseudomonas aeruginosa*); 6. glucose lipid; 7. glucosyl palmitate (from *Serratia marcescens*); 8. tri-glucose-tetraester; 9. 2-l-quinovose-phenazine ester (from *Streptomyces* sp.). Adapted from Kubicki et.al., 2019 (51)**

1.4.5 **Odorous components**

Terpenoids, carotenoids, fatty acid derivatives and sulfur compounds are the most common odorous components found in algae and cyanobacteria (21).

Geosmin, found in cyanobacteria such as *Oscillatoria*, *Lyngbya*, *Symploca* and *Anabaena*, is derived from farnesyl diphosphate, through a cyclisation reaction catalyzed by geosmin synthase. β-cyclocitral, with a tobacco odor, and β-ionone, with a woody and fruity odor, are also found in *Ulothrix fimbriata*, a green alga (21).

Filamentous cyanobacteria, such as *Phormidium* sp. and *Rivularia* sp., produce strong odoriferous PUFA (polyunsaturated fatty acid derivatives), which are characterized by a fishy, rancid or cucumber odor. Some examples are 1,3,3-trimethyl-2,7-dioxabicyclo (2,2,1) heptane (TDH) and 2(E),4(Z)-heptadienal (21).

Regardless, even though macroalgae have not been associated with a common source of aromas, the volatile compounds in *Capsosiphon fulvescens* (C. Agardh) Setchell and N.L. Gardner and *Fucus serratus* L. have been already studied (49).

1.4.6 **Flavonoids as preservatives**

Polyphenols like flavonoids, found in several species of algae, have a significant antibacterial activity.

Gracilaria dendroides, for example, contains high concentrations of rutin, quercetin and kaempferol (10.5 mg/kg, 7.5 mg/kg and 15.2 mg/kg, respectively), which are able to successfully inhibit *E. coli*, *P. aeruginosa*, *S. aureus* and *E. faecalis* (21).

2 Extraction of marine-derived cosmetic ingredients: challenges and implications

Globally, the production of algal extracts consists of several unit operations, including upstream processing (preparation for cultivation), cultivation in photobioreactors and downstream processing, in which cell harvesting, rehydration, extraction and ultrafiltration are included (52).

Throughout this chapter, different extraction methods and their challenges and implications will be discussed, addressing the necessity for appropriate, quick, cost-effective and environmentally friendly methods of extraction of compounds of interest without loss of their activity. **Figure 4** shows an overview of the existing methods of extraction, some of which will be mentioned in this paper.

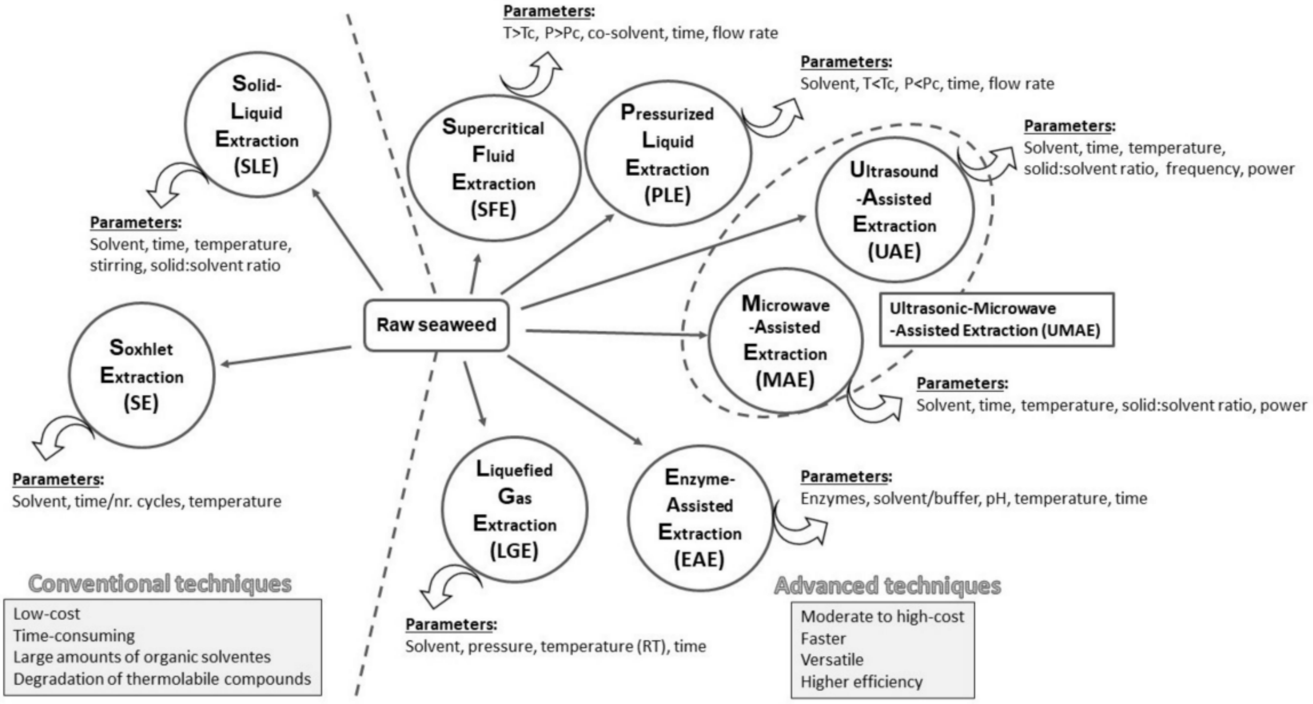

Figure 4. Schematic representation of the different conventional and advanced/alternative extraction methods, with their characteristics and parameters to control. Overall, advanced techniques are faster, more efficient and more environmentally friendly. T = temperature. Tc = critical temperature. P = pressure. Pc = critical pressure. Adapted from Quitério et al., 2022 (53)

2.1 Methods of extraction

2.1.1 Conventional methods of extraction

Conventional methods of extraction include infusion, percolation, Soxhlet extraction, maceration, steam distillation, among others. These constitute less environmentally friendly processes, requiring many hours of extraction and large amounts of chemical solvents and raw material (54).

Sometimes, due to the difficult penetration of the solvents inside cells, cell disruption methods are therefore used in order to improve the efficiency of algal protein extraction. Physical processes, such as grinding with a homogeniser and promoting osmotic stress, have been reported to improve the extraction efficiency of a number of active ingredients, especially algal proteins (55).

The choice of solvent depends on the solubility of the target substance (56), impacting selectivity and allowing for a high efficiency in the extraction process – for instance, phycocolloids are extracted in hot water due to its water solubility (54). The heating process usually involves convection through the solvent and conduction from the surfaces to the nucleus of the matrix particles (54). Other properties influence the yield of extraction, such as the diffusion coefficient, solvent viscosity, rate of mass transfer of the extracted substance and conditions under which the extraction is conducted – time, temperature and pressure (56).

2.1.1.1 Solid-liquid extraction

Solid-liquid extraction includes different processes, such as maceration, infusion and percolation, among others (53,57). It is the most common extraction method for antioxidant material and has been successfully used to extract polyphenolic compounds (53). Different variables are evaluated, including biomass-solvent ratio, percentage of solvent, time and temperature of incubation. (57) The solvents utilized in the different maceration processes are usually organic (53) and include methanol (MeOH), acetone (AcO) and ethanol (EtOH) at different percentages, with ethanol as the preferred solvent (57).

Using a suitable liquid phase, this technique allows the isolation of desired soluble constituents from a solid or semisolid matrix, during which the solute migrates from the solid matrix into the solvent. Removing the excess water and grounding samples into powder are essential to improve the process yield, as it will, respectively, increase recovery of non-polar compounds and increase their surface area (53).

Therefore, SLE is a high-energy technique, besides being very simple and relatively low cost (53).

2.1.1.2 Soxhlet-assisted extraction

Soxhlet assisted extraction (SAE) is extensively used, especially for the extraction of molecules with limited solubility in a solvent and in the presence of insoluble impurities. Through the continuous passage of the solvent through the sample matrix, using boiling at temperatures below the solvent's boiling point and condensation, the compounds of interest are transferred from the solid to the solvent (53,57).

The polarity of the solvent, as well as its capacity to dissolve the desired analytes, are critical aspects that affect the efficiency of extraction – the most used are organic liquids, such as ethanol, methanol, acetone and hexane (53).

SAE is widely used for the recovery of lipids and phenolic antioxidants, such as carotenoids, from seaweeds (53,56).

2.1.2 **Alternative methods of extraction**

Advanced, alternative or contemporary extraction methods include supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE) and electro-technologies (5,53,54,57).

These methods of extraction are developed under a "green chemistry" approach, with a reduction of labor and energy needed, with safer, more environmentally-friendly solvents and less production of waste and gas emissions (56).

2.1.2.1 Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) is the most cost-effective used technology at the laboratory scale nowadays (54) and a preferred method for extracting ulvan and rhamnan sulfate due to the possibility of eliminating toxic solvents (5). This technique uses microwave irradiation – nonionizing waves with a frequency between 300 MHz and 300 GHz –, generating heat in a few seconds through collisions and frictions between molecules (54) and is environmentally friendly and economically appealing because of its reduced extraction time and solvent consumption (5).

MAE can be operated in open and closed vessels, with the first being safer, effective and able to process larger samples (5).

In this method, pressure and temperature are key factors and offer some selectivity to the extraction process – when high temperatures and pressures are applied, the yield of the phenolic compounds seems to be reduced; on the other hand, a lower yield of polysaccharides is obtained using low pressure for a longer period of time (5).

Fucoidan, fucoxanthin, phenols and polysaccharides are some examples of compounds that have already been extracted from marine products using MAE (58).

2.1.2.2 Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) uses high-frequency sound waves (>20 KHz), which create bubbles and zones of high and low pressure (53,54).

The passage of these waves induces cavitation in the solvent and the formation of cavitation bubbles, which increase the surface area between solid and liquid phases and the mass transfer from the biological matrix (53,54,55). The violent implosion of these cavitation bubbles also facilitates algal cell wall breakdown and enhances extraction efficiency (54,55), through the creation of microscopic regions of extreme pressure and temperature (55).

This cost-effective and efficient technique is usually used for the extraction of phenolic compounds (5) and can be used as a pretreatment to SLE by destroying the biomaterial and making the target compounds more accessible (53,57), using ultrasonic bath (indirect sonication) or an ultrasonic probe (direct sonification) (53).

Pressure, temperature, intensity and frequency of the waves, surface tension and viscosity of the solvent are parameters that influence the extraction process (54), with higher temperature increasing the yield (5).

2.1.2.3 Enzyme-assisted extraction (EAE)

Enzyme-assisted extraction (EAE) is an eco-friendly approach that uses enzymes such as peptidases, glycosidases and carbohydrases to cause a disruption on the algae cell wall, allowing for a release of the intracellular metabolites (5,54). This method is analogous to the previously mentioned method of physical disruption of the cell wall (5).

Some advantages of this method include the conversion of water-insoluble materials into water-soluble materials, the preservation of the original efficacy of the compounds, a good catalytic efficiency, a high specificity and a significant process scalability (5,54). It can also be considered the greenest among all the methods, as it does not use any harmful chemicals or organic solvents (54).

On the other hand, this method shows some limitations, including the cost of enzymes, some of these with prohibitive costs, the lack of enzymes and the difficulty in maintaining bioreactor conditions (5,54). These limitations can be overcome by the use of other extraction methods coupled with EAE, such as ultrasound assisted enzymatic extraction (UAEE) or enzyme assisted high pressure extraction (5,59).

2.1.2.4 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is a technology that uses carbon dioxide and water as supercritical solvents (54).

SFE operates in the supercritical state of the solvent – above the critical pressure and temperature of the fluid, in which the supercritical fluid density is similar to a liquid and the viscosity is low in a range between liquid and gas – so that this can rapidly penetrate into solid materials and improve the extraction process yield. These conditions promote the transfer of solute from the matrix to the solvent (52,53,54).

Supercritical carbon dioxide (SC-CO2) is the most used solvent because it is non-toxic, non-inflammable, non-explosive, safe and environmentally available (53,54). Other possible supercritical solvents, like methanol or propylene, do not meet the criteria of "green chemistry" (54).

Due to its low polarity, supercritical carbon dioxide (SC-CO2) is only applied to recover non-polar or mid-polar compounds, such as fatty acids, carotenoids and essential oils (5,53). More polar co-solvents, such as ethanol, can be used together with SC-CO2 to expand the range of compounds achievable by this technology, with low toxicity, but with possible decreased selectivity (53).

Pressure and temperature affect the selectivity and solubility of the various compounds in the supercritical fluid – carbon dioxide has a relatively low critical temperature (31.1ºC) and pressure (73.8 bar), making it an excellent solvent for sensible compounds (53). In general, increasing pressure and temperature results in an increased extraction rate, due to an easier dissipation of the molecules through the matrices and a lower surface tension of SC-CO2 (53). Other factors, such as water content, particle size and extraction time, are also involved (53).

Overall, SFE is considered advantageous, but also expensive due to high equipment costs and energy consumption, making the scale-up process yet unfeasible (54). Some of the already mentioned applications for SFE include the extraction of astaxanthin, fatty acids, fucoxanthin and lipids (58).

2.1.2.5 Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE) is based on pressurized solvents under values of temperature and pressure ranging between 50-300ºC and 35-200 bar, respectively (53,54). The solvent is heated above its normal pressure and temperature boiling point, but below its critical point, resulting in a decreased solvent viscosity and surface tension and a better penetration of the matrix, with an improved mass transfer to the solvent and extraction rate (53,54).

Temperature influences the extraction's efficiency and selectivity greatly, with higher temperatures being linked to better extraction yields. Nevertheless, this technique cannot be used for thermolabile compounds (53).

When water is used, this technique is also called *subcritical water extraction (SWE)*. Water is the most used solvent, as it does not generate toxic waste. As water's affinity towards less polar compounds is increased with temperature, the combined high temperature and pressure allows for an increased desorption of target compounds from the material, overall increasing the extraction kinetics (54).

Therefore, PLE allows for a high extraction efficiency, less solvent consumption and a shorter extraction time, with phenolic compounds, polysaccharides and aminoacids as some examples of extracted compounds (54,58).

2.1.2.6 Electro-technologies

Electro-technologies include pulsed electric field (PEF), ohmic heating (OH), moderate electric field (MEF) and high-voltage electric discharges (HVED) in which the synergistic effects of temperature and electric fields are applied (54,60).

Pulsed electric field (PEF) has been used as a cell disruption technique in algae (55), as it enhances cell membranes' permeability by inducing reversible or irreversible electroporation (54,55), through the application of very short pulses of very high voltage electric fields (54). Electroporation enables the introduction of foreign components to cells, including DNA, proteins and drugs (55).

PEF is extremely versatile, efficient, requires low energy and water consumption, does not require chemicals and generates low heat. PEF is being used to induce stress and thus extract compounds more easily (54). Numerous studies have shown PEF’s capacity of potentially increasing the yield of extraction of several compounds, including with lipids, carbohydrates, carotenoids and chlorophyll (55).

Ohmic heating (OH) is another electro-technology, in which a moderate electric field is applied to heat a sample. A homogeneous and instantaneous transmission of thermal energy and the fast heating enhances cell membrane permeability, making it possible to recover high-value molecules (55). Besides the thermal effects, electroporation of cellular tissues, with membrane damage and solutes diffusion, has also been reported, thus facilitating the extraction of bioactive compounds (55).

Moderate electric field (MEF) exposes the sample matrix to low electric fields (between 1 and 1000 V/cm), where electric frequencies in the range of Hz up to tens of kHz are applied. High-voltage electric discharges or HVED typically apply 40-60 kV/cm for 2–5 µs electrical property (60).

2.2 Challenges regarding the extraction and utilization of cosmetic ingredients

Despite all the benefits and potentialities that come with the utilization of marine-derived ingredients in cosmetics, it is important to consider the different biological, technical and supply challenges, as well as problems regarding contamination and reproducibility of extracts obtained from raw materials.

2.2.1 Biological and technical challenges

It is undeniable that marine biodiversity is both an advantage and a disadvantage for the cosmetic industry and manufacturers – on the one hand, there is a considerable amount of compounds that can be explored in cosmetic formulations, but identifying and exploring this diversity in a sustainable and safe manner is yet very hard (61).

Obtaining marine raw materials remains very difficult, still relying on robotic and engineering advances regarding the access to the ocean, especially the deepest places. Thus, most studies are conducted in shallow coastal waters, due to difficulties in reaching deeper spots (normally more than 30 m), leading to gaps in biodiversity and chemo-diversity knowledge. ROVs (remotely operated vehicles) are an interesting solution to this problem, but are very expensive and unattractive in developing countries from the tropical and subtropical regions where these natural products are most likely unknown (61).

Access to biodiversity on natural resources is now under the Convention of Biological Diversity (CBD), in which “the potential role of access and benefit-sharing to contribute to the conservation and sustainable use of biological diversity” is acknowledged (62).

The use of marine products for pharmacological purposes implies a correct taxonomic identification and classification – an incorrect taxonomic identification and classification can, on the one hand, compromise the entire cosmetic discovery project and also lead to problems in the reproducibility of the extracts obtained. Nowadays, this is considered a challenge for the use of marine-derived products, both for the lack of taxonomic knowledge and for the large number of undiscovered and undescribed species (61).

2.2.2 **Sustainable supply**

As natural products, different organisms contain a high biological and biochemical variability, especially when it comes to the amount and type of metabolites produced according to the environmental conditions in which they are cultivated (61).

On the other hand, a sustainable supply is also challenging regarding the utilization of natural products, whether due to the low amount of the compound of interest present in the raw material sample or to the difficulty in isolating those compounds. Hence, large quantities of raw material are necessary (61). Advances in molecular biology and aquaculture technologies, the latter including IMTA (Integrated Multi Trophic Aquaculture) and RAS (Recirculating Aquaculture Systems), have been trying to solve the problem of sustainable supply, but it is still a relevant challenge (61,63).

Therefore, the development of synthetic or hemisynthetic analogues has been applied. Regardless, the complexity of naturally-derived molecules, with several stereocenters, and of the purification processes makes this approach complicated and difficult to perform. The need for virtual screening of molecules and the misassignment of natural products are also key points (61).

2.2.3 Reproducibility of extracts and challenges in the scale-up process

Reproducibility of extracts and the expansion of cultivations from a laboratory scale to an industrial unit are some of the most important challenges industries face when dealing with natural products in general.

Conventional methods show low reproducibility, because of the lack of automation. Therefore, using advanced methods of extraction can be beneficial, both due to better reproducibility between extract batches and also due to sustainability (52).

As stated before, natural products change their levels of metabolites and growth rates according to environmental conditions as a protective response (64). Temperature, light availability, oxygen levels, mixing, nutrient levels, risk of contamination or infestation, biomass film formation and loss of bioreactor's transparency are some well-known key factors that influence algal growth (65). For example, monitoring *P. palmata*'s protein levels throughout a year ranges from 9 to 25%, reaching a peak in May (55). Strategies to harmonize metabolites' production in algae has been used due to rising research on this topic – for example, red light photons have shown to accelerate cell cycle and green light has shown to increase lipid production (64).

The scale-up process is crucial in process development (65) and is usually linked to significant productivity losses – optimal conditions determined in a laboratory scale are drastically different from those at an industrial scale (64,65). Moreover, large-scale algal cultivations benefit the environment by capturing carbon and feeding on atmospheric nitrogen oxides, thus reducing greenhouse effect (65).

Monitoring temperature and pH of each photobioreactor in real time is an important approach to standardize and optimize the quality of the produced biomass. Management of the cooling system and harvest during the semi-continuous phase on a daily basis are also strategies to overcome set challenges, allowing for different growth conditions, whether optimal or to increase the production of certain metabolites, and bigger consistency between batches (64,65).

Before the full implementation of the commercial plant, data obtained from the scaling-up stage must be subjected to rigorous analysis and consideration and the economical aspects of the process should be considered (65).

2.2.4 Potential contamination of raw material and extracts

The contamination of raw material and, consequently, extracts is an important feature when using natural products in the formulation and production of cosmetics. Biological and chemical contamination are two types of contamination that will be further addressed below.

2.2.4.1 Biological contamination

Biological contamination can be divided into two groups – a cell-growth affecting contamination and a protein accumulation-affecting contamination. The latter is related to the contaminant's ability to reduce or consume the molecules produced by the organism of interest. Aquatic pollution, air pollution and the accumulation of nutrients, salt and other organisms in the blind angles of the bioreactor are some of the identified sources of biological contamination (66).

In order to reduce these problems, optimization of growth conditions and control and the application of standardized procedures can ensure a constant and repeatable level of contaminants-free, microbiologically-pure components in each raw product unit (56).

The choice of media is also fundamental for this purpose. An artificial media can, for instance, substitute supplemented municipal domestic wastewater in a culture of algae, reducing the probability of water-related contamination. When wastewater is effectively used in the growth media, pH control can prevent grazers' contamination (such as zooplankton) (65).

The use of detergents or phenols can also be an alternative for bacterial contamination (65).

2.2.4.2 Chemical contamination

Arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn) are the most commonly found heavy metals in the water environment, with significant environmental and evolutionary toxicity (21,67).

Algae, especially macroalgae, absorb heavy metals from marine water, through a bioaccumulation process (21,67,68). For example, arsenic is found in its organic form – as arsenosugars, such as dimethylarsinyl ribose derivatives –, which are non-toxic (68).

Cadmium (Cd), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), zinc (Zn) and mercury (Hg) were some of the heavy metals found in five species of seaweeds (67). Chang et. al. (68) also demonstrated the presence of arsenic (3.9 ppm), iron (14.9 ppm) and zinc (3.0 ppm) in *E. cottonii*, from the eight tested elements.

In a study developed with seaweeds from the Venice lagoon, a group of investigators found high contamination levels of lead in *Ulva sp.* and, to a lesser extent, in *Gracilaria sp.*, whereas *Cystoseira sp.* was highly contaminated with arsenic (69).

Besides heavy metals, iodine (I) is also present in algae and other marine products (52). However, both heavy metals and iodine are prohibited in cosmetics according to the EU regulation on cosmetic products (70).

Although the presence of heavy metals in marine products is consensual, there is still a lack of information regarding their presence in cosmetic products. However, these chemical elements are expected to be found in their extracts, which will later be incorporated in cosmetic products.

Periodically, and due to oil spills for example, contamination of seawater by VOCs (volatile organic compounds) such as various alkanes and benzene compounds are also possible and they have already been found in *Cystoseira corniculata* and *Jania rubens* (21).

2.3 Public policy and regulatory framework

The use of marine-derived products in cosmetic products still needs further development regarding its regulatory framework, even though its use is increasingly common in the cosmetic industry.

CosIng, EU's cosmetic ingredient database (71), along with Regulation 1223/2009/EC (70), contains all legal requirements and restrictions on each substance. There are several allowed marine-derived cosmetic ingredients in this database, such as fucoidan and carrageenan (71,72). In the EU, a clear science-based regulatory environment is established, where cosmetic claims need to be supported by scientific and adequate evidence and address certain determined criteria, as shown in **Table 3** (72).

Table 3. Common criteria for claims used in cosmetic products. (74)

Legal compliance	"Claims that indicate that the product has been authorized by a competent authority within the EU should not be allowed since a cosmetic product is allowed on the EU market without any governmental approval."
Truthfulness	"Neither the general presentation of the cosmetic product nor individual claims made for the product should be based on false or irrelevant information."
Evidential support	"Claims for cosmetic products, whether explicit or implicit, should be supported by adequate and verifiable evidence regardless of the types of evidential support used to substantiate them, including where appropriate expert assessments."
Honesty	"Presentations of a product's performance should not go beyond the available supporting evidence."
Fairness	"Claims for cosmetic products should be objective and should not denigrate the competitors, nor should they denigrate ingredients legally used."
Informed decision-making	"Claims should be clear and understandable to the average end user; should contain information allowing the average end user to make an informed choice."

ISO standard guidelines, such as ISO 16128-1:2016, regarding natural and organic cosmetic ingredients, and ISO 16128-2:2017, regarding the quality criteria for ingredients and products, are also important tools for the cosmetic industry, even though they don't have legal and regulatory power (72).

Besides EU's regulatory documents and ISO guidelines, CEN, the European Committee for Standardization, elaborated several technical reports (TR) about the use of algae in cosmetics – CEN/TR 454 and CEN/TR 17611 (74,75). The latter will be further discussed below.

CEN/TR 454 (standards for algae) considers that algae-derived raw materials should be treated in the same way as plants-derived materials, addressing possible quality and contamination issues with specific standards (75).

2.3.1 CEN/TR 17611 Algae and algae products - Specifications for cosmetic sector applications

This technical report was developed in January 2021, after CEN was requested by the European Commission to develop a standardized guideline for this matter.

In this technical report, several important aspects are mentioned regarding product characteristics, product information documents, traceability, sustainable development and labelling (74).

Regarding purity, for example, the presence of GMO (Genetically Modified Organisms) material and non-organic material in algae and algae products is considered as impurity. These impurities can be determined with macroscopical/microscopical characterization and other identification tests. All powdered materials should be analyzed through microscopical characterization (74).

Heavy metal contamination, physical contamination with e.g. plastic fragments and microbiological contamination are some of the addressed subjects in this technical report. Attention for long-term safety aspects are needed, especially regarding local toxicity on skin and eye irritation and sensitization. Dioxins, PAHs (polycyclic aromatic hydrocarbons) and other xenobiotics are also some of the discussed contaminants (74).

3 Sustainability and marine biotechnology in the cosmetic industry

3.1 Ensuring sustainability in marine-derived cosmetics: a life cycle approach

Out of 9.2 billion tonnes of plastic produced between 1950 and 2017, 7 billion tonnes became plastic waste, which ended up in landfills or dumped. These amounts of plastic can alter habitats and endanger ecosystems (76).

Even though sustainability is increasingly becoming a trendy topic nowadays, it is absolutely necessary for our planet's environment to address this concern, due to the ecological, economical and social impact the cosmetic industry has – whether we refer to the packaging, the production methods or the costs to the population. Marine natural products (MNP) are in high demand for skin care and it is absolutely necessary to ensure a sustainable and environmentally friendly use of these resources (61).

3.1.1 Fundamentals of sustainability and its application to the cosmetic industry

According to the UN's 1987 Brundtland Report on Environment and Development, sustainability features a balanced consideration of three dimensions – social, economic and environmental (77,78,79,80). (**Table 4**) Sustainable development (80) is thus defined as it follows:

> *"Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs."*

Innovation and technology are considered important drivers of sustainability and it can concern products or processes (78,81).

Table 4. Three pillars of sustainability applied to the cosmetic industry in a life cycle approach, according to Good Sustainability Practice (GSP) for the Cosmetic Industry (77).

Social	Cosmetics contribute for the population's sanitary conditions, good hygiene and self-esteem, e.g. cosmetics for oncology patients. The cosmetic companies can also encourage volunteer work and the working conditions of employees throughout the product's life cycle should be addressed. (77)
Economic	Cosmetics companies should stimulate economic development throughout the product's life cycle, with business ethics and fair relationships with the stakeholders. (77)
Environmental	An environmental management system (EMS) should be used by cosmetic industry companies, together with an environmental risk assessment (ERA) conducted to determine an ingredient's potential hazard (77,78). EMS, in a life-cycle approach, combines regulatory requirements and systems to identify wastes and emissions, through the application of ISO 14001. (77)

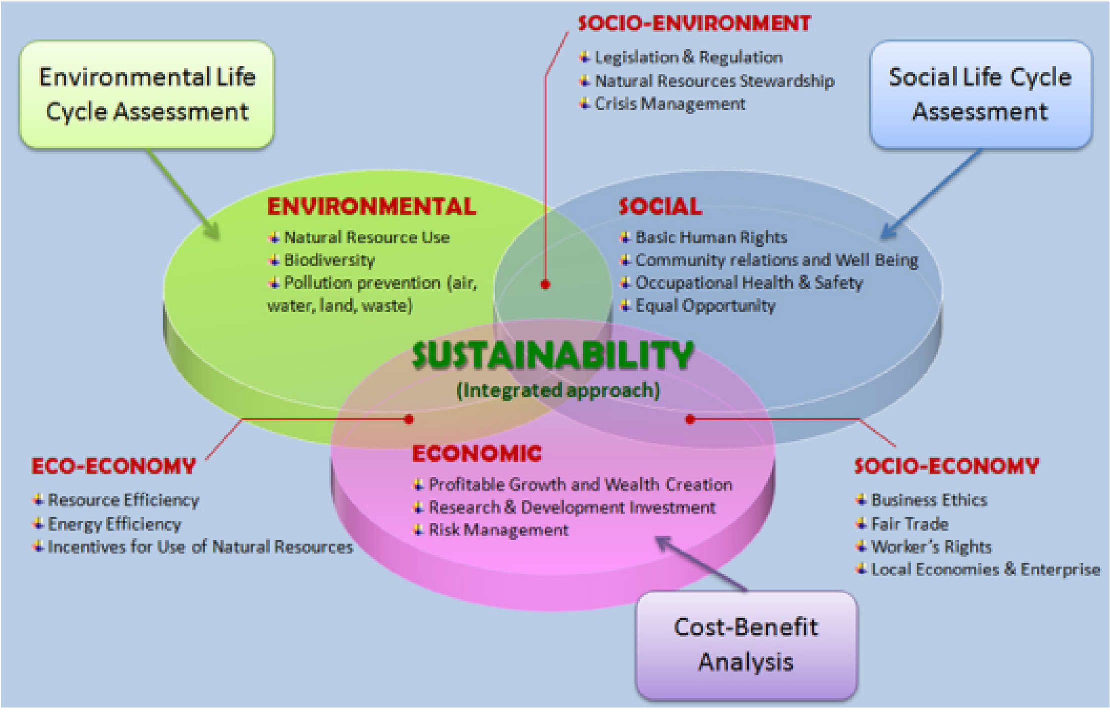

Figure 5. A life cycle approach (LCA) methodology considers three pillars of sustainable development. All three pillars complement and are related to each other. Unlike the environmental LCA, the evaluation of social and economical aspects has not been standardized. Adapted from Murray et.al., 2013 (82).

The rise of ‘eco-friendly’ cosmetics and a special concern with the use of clean technologies and the rational use of natural resources has led to the use of **life-cycle approaches (LCA) (Figure 5)** (78,79,83). A LCA comprises three steps (77):

- the definition of goal, scope and process system boundaries;
- the construction of a life-cycle inventory, with inputs and outputs of relevance throughout the entire product’s life cycle;
- impact assessment of each life cycle phase, including areas for improvement.

LCA covers the product’s full life cycle process, in a holistic way and where the different stages are interdependent, initiating in the extraction of raw materials and also including the production, formulation and recycling steps (78,79,82).

It is important to take into consideration that there are no materials that can be considered 100% sustainable – the environmental impact of using certain materials occurs throughout the whole cosmetics supply chain (84).

3.1.2 **Environmental aspects in a cosmetic’s life cycle**

In this paper, we will focus on the environmental aspects of a sustainable cosmetic’s life cycle, due to its importance and significance for the marine world. That includes product formulation, manufacturing, packaging, distribution, consumption and a post-use phase (77,78).

3.1.2.1 Product formulation

In the EU, cosmetic products’ ingredients must be in compliance with the requirements of the cosmetics legislation – Directive 76/768/EC and Regulation 1223/2009 (70,77,78). The product formulation phase should respect the CITES Regulation (EC) N.° 338/97 (endangered/protected species) for raw materials, which is particularly important when using raw materials from marine sources in cosmetics (77).

Besides legal requirements, companies can progress further and integrate other sustainability aspects, using a life-cycle approach and assessing the environmental profile of all cosmetic ingredients. Attention should be paid to volatile substances and PBT/vPvB substances, problems that should be addressed through a preliminary risk assessment (77).

Using a new sustainability calculator and applying it to different cosmetic formulations, Bom et. al. (84) concluded that the phase of a cosmetic's life cycle with the highest impacts on sustainability is product formulation. For example, among the assessed emollients, mineral derivatives and silicone oils are the raw materials with the biggest impact on sustainability.

True sustainability in this life cycle's phase can also be achieved through the creation of partnerships and cooperation with fair-trade organizations that supply the different raw materials (77).

3.1.2.2 Manufacturing

Water and energy consumption during the manufacturing processes, as well as an effort to reduce carbon footprint, should be premises for the cosmetics industry. A review on the current input, processes and output, replacing old equipment by new and more efficient electrical devices, and an optimization of production planning are some of the suggested measures (77).

The same study conducted by Bom et. al. (84) estimated that the use of a non-renewable energy source on the production process has an impact of 13% versus an impact of 5% if a renewable energy source is used. Furthermore, biodiversity issues, which were approached already, have an approximate impact on the cosmetic's sustainability of 16% (84).

3.1.2.3 Packaging and distribution

Packaging is usually a critical aspect in cosmetics, not only for marketing reasons, but also for the application, function and durability of the final product. In the EU, packaging is regulated under the Packaging and Packaging Waste Directive, in which a concern for minimizing waste and noxious or hazardous substances is present (77).

When developing a packaging system, performance and the choice of materials are important considerations, as well as the ability to be reused or recycled. The use of a biodegradable material is highly encouraged (77). Glass packaging, for example, has an estimated impact on sustainability of 11% (84).

Distribution also plays an important component of cosmetic products' life cycle, where a greater use of technology and other innovative solutions can help reduce gas emissions and fuel consumption (77). Load utilization, frequency of deliveries and choice of fuel are some parameters that can be considered for the evaluation of sustainability (77,84).

3.1.2.4 Consumption and post-use phase

Assessing sustainability during the consumption phase might not be that obvious, but, depending on the cosmetic we are referring to, sustainability impacts should be considered when applying a life-cycle approach. For example, cleansing products, such as shampoos or hand washes, exhibit a significant impact on the environment due to water heating, bathing and washing (77).

Although consumption-related waste relies mostly on consumers, companies should also adopt a didactic and informative role in order to decrease negative impacts, such as providing information on how to take the product or how to reduce water consumption or temperature (77).

After the use of cosmetics, waste is generated, especially concerning packaging. Waste management techniques, which include reuse, recycling, incinerating with energy recovery or composting, will depend on consumer habits and processing systems, for example. Therefore, companies should provide products that fit in the local requirements for post-use collection (77).

Refill should also be considered whenever possible and, in those cases, packaging needs to be designed to withstand a certain number of refills before being recovered (77). This is an especially important way to reduce the production of plastic waste, as it is estimated that the cosmetic industry adds up to 120 billion units of plastic packaging each year (85).

3.2 “Blue biotechnology” and sustainability

3.2.1 Biotechnology, marine biotechnology and “blue biotechnology”

OECD defines biotechnology as the “application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services.” On the other hand, marine biotechnology is defined as “[encompassing] efforts that involve marine bio-resources, as either the source or the target of biotechnology applications” (86).

Biotechnology can be considered an “umbrella term” (79,86), as it includes other sectors, such as “red biotechnology” (or medical, health and pharmaceutical biotechnology), “green biotechnology” (related to agriculture), “yellow biotechnology” (or environmental biotechnology) and “white biotechnology” (related to industry) (86).

“Blue biotechnology” is related to the use of marine resources as source materials, which is a unique case among the other biotechnology sectors. Therefore, “blue biotechnology” is only applied to the first part of the development pipeline, including the sampling, discovery and bioprospecting, R&D and initial product development phases (86,87). As represented in **Figure 6**, “blue biotechnology” ends in the early stages of product development; the subsequent stages are then inserted in the other sectors of biotechnology (86).

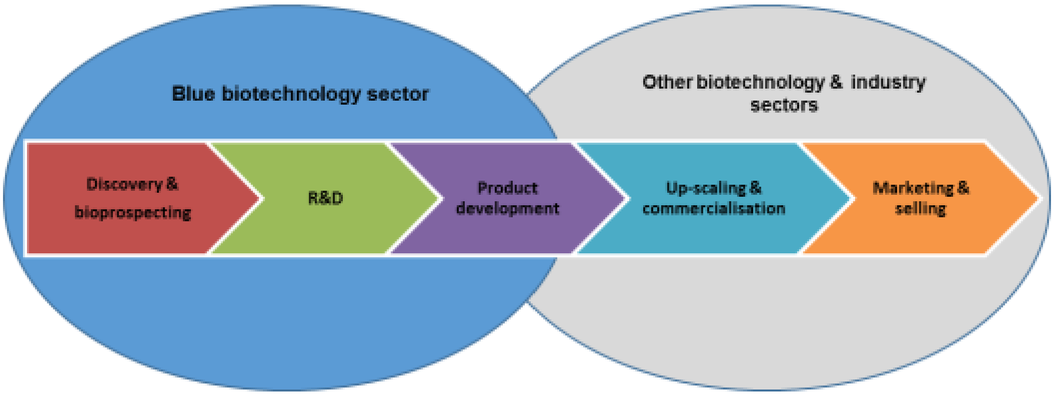

Figure 6. “Blue biotechnology” sector as a part of the product development pipeline. Discovery and bioprospecting involves the investigation of environments and collecting living organisms. In the R&D phase, active molecules are characterized and synthetic strategies are discussed. Finally, in the product development stage, a LCA is adopted and sustainable production strategies are developed. Adapted from Collins et.al., 2018 (86)

3.2.2 Sustainability assessment of “blue biotechnology” processes

The EU Green Deal, which aims for climate neutrality by 2050, together with the consumers’ preferences with eco-cosmetics, have produced an important environmental awareness among the cosmetics industry (83).

Using a LCA approach and considering that “blue biotechnology” is only applied to the initial steps of a cosmetic’s life cycle, it is important to assess the different biotechnological processes regarding their sustainability.

Pagels et. al. (83) carried out an environmental evaluation of the processing of *Fucus vesiculosus* and its comparative profile with antioxidants vitamin C and green tea extracts, using a life cycle approach. They demonstrated that the use of sea-harvested seaweed requires a lower human intervention and a much higher growth rate, without the use of terrestrial fertilizers, when compared to terrestrial plants. Furthermore, seaweeds have a similar environmental load when compared to ascorbic acid, even presenting an advantage regarding marine eutrophication and water consumption (65,83).

To minimize seaweed waste, while potentiating all seaweed's applications, a biorefinery concept is suggested by Pagels et. al. – firstly an extract is produced, for cosmetics, and the remaining matter is used as a fertilizer (as a biostimulant and biopesticide) or in the production of biogas (83).

Besides antioxidants, the production of astaxanthin from *Haematococcus pluvialis* can also have remarkable benefits, whether economical, social and environmental. As demonstrated by Pérez-López et. al. (79), most of the environmental impact was associated with the cultivation stage, especially due to the production of electricity for artificial illumination and air supply. This process was economically favorable, with high profitability and low payback time, even considering less favorable conditions and a considerable level of uncertainty.

3.2.3 **"Blue-biotechnology"-based applications to the cosmetic industry**

3.2.3.1 Marine viruses as a source of ceramides

Despite being not that obvious, marine viruses outnumber any living organism in the sea and have an enormous potential in biotechnology, including in the cosmetic industry (88).

English researchers from the Plymouth Marine Laboratory and the Sanger Institute in Cambridge discovered that EhV-86, a marine virus, contains a cluster of at least 7 genes that encodes several components of sphingolipid biosynthesis after the virus' genome had been sequenced. These components then lead to the formation of ceramides, which have been increasingly used in the cosmetics industry as active ingredients due to their skin protection and hydration properties (88).

The use of ceramides from marine sources have several advantages, because, similarly to collagen, the animal sources used nowadays have a risk of contamination with pathogenic agents such as bacteria or prions (BSE, for example) (88).

3.2.3.2 Marine-derived biosurfactants

Surfactants and active surface agents are one of the fundamental components of a cosmetic formulation, due to their cleansing, emulsification, foaming, solubilization and conditioning properties (36).

Although marine biosurfactants have already been addressed in this paper, we will now discuss several sustainability-based benefits of using these, as well as some examples.

Synthetic surfactants, such as sodium lauryl sulfate (SLS) and sodium laureth sulfate (SLES), are synthesized from petroleum derivatives, which end up in bioaccumulation, biodegradability and biocompatibility issues, both for the environment and human health. The latter one is especially important because several studies have shown that SLS and SLES can cause skin damage and irritation (36).

These molecules have been already applied in different contexts. A biosurfactant obtained from *Nocardiospsis* VITSISB (a marine actinobacteria) allowed for a more pH-optimum toothpaste formulation than the one formulated with SLS. Liposan and Yansan, biosurfactants from *Yarrowia lipolytica*, are also other examples (36).

3.2.3.3 Immobilized lipases from Antarctic fungi and yeasts

Lipases are enzymes which catalyze the hydrolysis of carboxylic ester bonds in hydrophobic compounds, under natural conditions. These proteins are compatible with fatty, non-aqueous media and emulsions (i.e. cosmetics) and have a broad substrate acceptance (89).

In cosmetic products, lipases can be used both as active ingredients or as biocatalysts in the synthesis of specific cosmetic ingredients (89).

Facial cleansing, anti-cellulitis and body slimming are some examples of applications of lipases as active ingredients. These molecules are responsible for a mild skin peeling, as they affect the *stratum corneum*'s keratinocytes and break down fat deposits (89).

Using their hydrolytic, esterifying and acylating properties, lipases can also be used as biocatalysts of numerous cosmetic ingredients, as part of a "green chemistry" strategy. Enzymes like lipases are a part of this strategy due to their selectivity and stability and do not usually involve the use of organic solvents, which can be environmentally hazardous. Furthermore, enzymatic reactions reduce energy consumption, by-product formation (which is itself biodegradable) and the time for heating, with both environmental and economic advantages and a reduction in the production costs (89).

Marine Antarctic fungi and yeasts were isolated to discover low temperature active lipolytic enzymes. Enzymes like lipases were isolated from the phylum *Basidiomycota*, from sea urchin-derived *Cryptococcus laurentii* and from *Palmaria decipiens* and *Geomyces* sp. (82).

3.3 Invasive species as a source of compounds of interest

Climate change is a 21st century issue, which is reflected in the change of marine species' behavior, abundance, diversity and distribution (90). Together with environmental changes in the "recipient" ecosystem, all these conditions favor the development of IAS (invasive alien species) or NIMS (nonindigenous marine species) (90,91).

These invasive species most often have facilitated and successful invasions through high growth rates, vegetative propagation, innovative growth strategies, high levels of sexual reproduction and broad environmental tolerance (91).

A sustainable approach to counteract these species is to promote their valuableness for new product development, including cosmetics, thus answering consumers' demands (90). Next, we will present two examples of invasive species with cosmetically-appealing properties that can (and should) be used in a sustainable way, reducing the impact on the ecosystems.

Sargassum muticum

S. muticum occurs in several European coastlines, in high amounts, with a highly invasive profile. Using this brown macroalgae in the cosmetic industry will not only allow for a better mitigation of this spreading species, but also the retrieval of several cosmetically-important active ingredients (90).

Susano et. al. (90) concluded that *S. muticum*'s extract has anti-aging, anti-acne and anti-UV radiation properties, allowing for the maintenance of skin's microbiome homeostasis.

Undaria pinnatifida

U. pinnatifida is a brown seaweed from Japan, Korea and China, which has developed an invasive behavior through other parts of the globe including Europe and North America (90).

As discussed before in Chapter 1, fucoidans from *U. pinnatifida*, especially LMF (low molecular weight fucoidans), act as “wound-healing accelerators” (36,37), promoting re-epithelization (37) and angiogenesis and reducing inflammation (9).

3.4 Marine waste products as a source of compounds of interest

Fishing, farming and processing fish and other sea products are huge generators of leftovers and waste (93), which are normally disposed of directly into the environment without any treatment (93,94). The utilization of this sea waste not only allows for new important cosmeceutical ingredients, but also endorses a zero-waste strategy, pursuing the Sustainable Development Goals (SDGs) of the UN (93).

3.4.1 Chitin and chitosan

By deacetylation, in an alkaline environment, chitosan is originated from chitin. The first has medical and pharmaceutical applications, with constraints due to its molecular weight and viscosity (93). Not only chitosan is biocompatible, biodegradable and non-toxic, but also has antibacterial and antifungal properties (94).

Crab and shrimp shells are the most known sources of these compounds (93).

In cosmetics, chitosan has been used as a carrier and stabilizer in sunscreen preparations, in the form of nanoparticles, with good storage and color stability in stability studies (93). These chitosan-based nanoparticles can also be used as carriers of other cosmetic ingredients, such as anti-aging molecules (94). Furthermore, a good cosmetic mask has been developed using a chitosan film. This mask showed good flexibility, good water retention characteristics and is compatible with other active ingredients (93).

3.4.2 Collagen and collagen derivatives

Collagen is one of the most used active ingredients in cosmetics and it is biodegradable and biocompatible (95). It is a structural protein, being found in the various connective tissues of the human body (95). Animal-derived collagen is the most often choice for sources of collagen, but religious beliefs and ethnicity, as well as the presence of different diseases (such as BSE) make it a disadvantage (95). Therefore, marine-derived collagen (and its derivatives) can be an effective and sustainable option, especially when derived from waste (93,95).

The effectiveness of fish collagen on skin is different when comparing mammal-derived collagen and fish-derived collagen – fish collagen contains less proline and hydroxyproline, which decreases stability and cross-linking compatibility (95). Furthermore, the human body has difficulties in absorbing high molecule peptides – marine hydrolyzed collagen should thus be better absorbed by the human skin (93).

Fishbone, skin, scale and swim bladders are rich in a collagen matrix, especially type I collagen (95). Collagen can absorb water in a potent way, showing good moisturizing effects with no skin irritation (93,95).

Guan et. al. (96) studied the cosmeceutical properties of silver carp skin collagen (SCSC), showing good foaming and emulsifying properties, as well as better water absorption capacity and oil absorption capacity than other proteins from terrestrial sources.

3.4.3 **Natural calcium phosphates**

Natural calcium phosphates (CaP) are found in the bone and teeth of vertebrates, being used in skin care, hair care, deodorants and oral care cosmetic products in an increasing way (93,97).

Fisheries' waste, such as fish bones, are a source of these CaPs, including hydroxyapatite that showed effective absorption of the full-range of UV radiation (97), showing particular interest in broad-spectrum sunscreens (93).

A study conducted by Right et. al. (97) concluded that the use of natural calcium phosphates is environmentally more beneficial than the use of zinc oxide nanoparticles, especially in regards to eutrophication and ecotoxicity. Furthermore, these nanoparticles do not exhibit adverse effects on the four aquatic species tested.

4 Conclusion

Decades of R&D have shown the enormous potential of marine-derived products for cosmetics – whether it is for the multiple pharmacological properties these products present or for the fact they are a natural, more sustainable way of producing cosmetics. Nowadays, sustainable cosmetics and natural ingredients are trending, as concerns about the cosmetics' environmental and human health impact rises.

Throughout this paper, we have demonstrated two sides of the same coin – not only the advantages and the positive aspects of using marine-derived ingredients, but also the challenges and implications related to the use of set ingredients.

Anti-aging, anti-tyrosinase, anti-inflammation, wound healing – these are some of the advantages we can find in marine species, ranging from the most obvious algae to the less known sea cucumbers, without forgetting fish and crustaceans.

However, and in regards to the future, the extraction methods need to be further improved, as there is not only a risk of biological and chemical contamination, which can be, nonetheless, attenuated by several techniques, but also a relatively high sustainability burden due to the considerable amount of raw material needed to obtain the compounds.The current regulatory framework for marine-derived cosmetics is also discussed. The Regulation 1223/2009/EC, together with ISO guidelines and CEN technical reports, make up most of the regulatory documents on display regarding the use of marine-derived ingredients in cosmetics. As the use of this type of ingredient increases, an update to the regulatory and normative framework set in place is demanded in the following years. The presence of iodine in most marine species is also a challenge, as it is one of the substances prohibited in cosmetic products.

Finally, "blue biotechnology"-based solutions can be and should be an answer for more sustainable cosmetics. A lower impact on the environment and society is a key advantage in using these scientific breakthroughs. The exploration of invasive species, through which it is possible to counteract some of their negative impacts, as well as recycling sea waste are other creative ways of minimizing the cosmetics' impact, while ensuring new and cutting-edge cosmetics to consumers. A better understanding when applying the sustainability fundamentals to the cosmetic's life cycle is also needed, especially to minimize the associated carbon footprint, as well as the emission of greenhouse gases.

The use of marine-derived cosmetic ingredients is not something we believe to be in the future, it is something that is already in the present. Only through innovation, creative thinking and technology, where “blue biotechnology”-based solutions are included, can the cosmetic industry face the challenges ahead regarding this topic.

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