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Red algae as ingredients in solid skin care products

Final Report SSNV 2021

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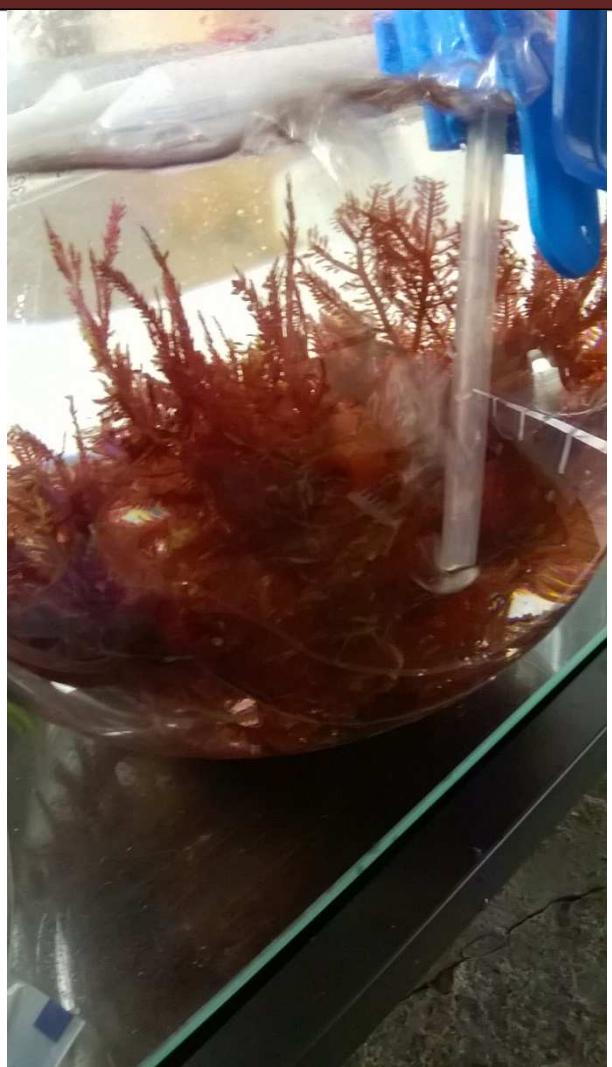

Report Summary

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Title	Red algae as ingredients in solid skin care products	
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Summary: Overall, 12 northern Icelandic red algae from the BioPol indoor culture collection were tested for their potential to accumulate sulfated polysaccharides (sPS) under exposure to different stress conditions. Utilizing the one-factor-at-a-time principle, the test algae were exposed to a) 20°C, b) 45 PSU, c*)* 2000 µmol photons m^{-2} s^{-1}, d) very strong bubbling and e) a photoperiod of 24:0 h. Accelerated light intensities and extended photoperiods did not show any significant changes of the total carbohydrates (TC) or 3,6-anhydro-galactose (AnGal) contents, while above normal temperatures, salinities and velocities let to an increase of both in all tested species. The highest TC contents were found for *Porphyra umbilicalis* [species acronym: Pu], *Palmaria palmata* [Pp] and *Plumaria plumosa* [Ppl] in the high velocity assays as well as for *Porphyra umbilicalis* in the 20°C exposure assay. In addition, *Porphyra umbilicalis* and *Heterosiphonia plumosa* [Hp] accumulated high total carbohydrate contents in response to salinity stress. Meanwhile, AnGal contents were the highest for *Porphyra umbilicalis* and *Palmaria palmata* in high velocity assays. Overall, six samples were chosen for further analysis of the sPS, considering contaminant proteins, monosaccharide compositions, total phenolic and sulfate contents, antioxidant activities, elastase and collagenase inhibitory activities, as well as effects on human keratinocytes viabilities in MTT assays; and effects on the viability of 3T3-L1 cells tested in adipocyte differentiation assays. From the six analysed samples, all three of the *Porphyra umbilicalis* sPS extracts exhibited the most interesting features and were therefore integrated into solid lotion bars with defined features such as moisturizing, anti-inflammatory and anti-aging properties. In each case, three different concentrations of the extracts were tested and the final receipes for all three types of the solid skin care product are included in the results part.

The introduced approach to gain bioactive sulfated polysaccharides from seaweeds, namely the utilization of only long-term cultivated species, provides the option of controlled environmental conditions and reliable repeatability of the produced sPS quality and quantity, including the avoidance of ecological harmful effects of wild harvests. Future investigations will include further optimization of the extraction procedures, triggering of the biosynthesis and accumulation of the sPS and finally the goal to integrate the process into a biorefinery concept to meet the United Nations Sustainable Development Goals.

Keywords: *Red algae, inducement of sulfated polysaccharides, solid skin care products*

Contents

1. Introduction

With the current societal paradigms regarding youth and beauty that have emerged new concerns about appearance, encouraging millions of consumers to use cosmetic/personal care products as part of their daily routine. Hence, cosmetics have become a global and highly competitive market in a constant state of evolution. This industry is highly committed to finding natural sources of functional/bioactive-rich compounds, preferably from sustainable and cheap raw materials, to deliver innovative products and solutions that meet consumers' expectations (Pimentel et al. 2018).

Micro- and macroalgae (=seaweeds) are an excellent example of a natural resource that can fit these requirements due to their richness in minerals, polysaccharides, proteins, lipids, and secondary metabolites such as phenolic compounds, terpenoids, halogenated compounds, sulfur and nitrogen derivatives (e.g., Peng et al. 2015). The incorporation of algal-derived ingredients in cosmetics has been growing, as more and more scientific evidence reports their skin health-promoting effects (Rucco et al. 2016). Specifically, it is proven that algae provide the skin with moisture, promote blood circulation, activate the cell renewal and metabolism, regulate the sebaceous gland function, drain the tissues, have an anti-inflammatory effect, and increase the skin's resistance (e.g., Joshi et al. 2018). In this context sulfated polysaccharides (sPS) and their lower molecular weight oligo-saccharide derivatives from marine red algae (Rhodophytes) have been shown to possess a variety of biological activities including anticoagulant, antioxidant, antiviral, and immuno-inflammatory activities that might find relevance in cosmetic/cosmeceutical applications (e.g., Usov 2011, Sebaaly et al. 2012, Pereira 2018).

SPS represent an important class of biopolymers and are recognized to possess a number of biological activities including anticoagulant, antiviral, and immuno-inflammatory activities that could find relevance in cosmeceutical applications (Jiao et al. 2011, Pereira 2018). They are a class of compounds which are abundant components of marine seaweeds and microalgae (De Borba Gurpilhares et al. 2016), comprising complex, heterogeneous molecule mixtures, with a key structural feature being hemi-ester sulfate groups on a polysaccharide backbone (Mollah et al. 2009). These sPS can substantially vary in their composition depending on the source material (e.g., algal species), environmental parameters, as well as the process of extraction and purification. This variation may impact their pharmacological activities and consequently also their efficacy (Grünewald et al. 2009). Studies have demonstrated that the structure of sPS varies from species to species, within the same algae, as and depending on its localization in the tissue (Grünewald & Alban 2009).

Red algae (Rhodophyta) are one of the oldest groups of eukaryotic algae (Lee 2008). The Rhodophyta also comprises one of the largest phyla of algae, containing over 7,000 currently recognized species with taxonomic revisions ongoing (Guiry & Guiry 2016). They range from simple single celled organisms to complex multicellular plant like organisms, including many notable seaweeds (Yoon et al. 2017). Rhodophyta are divided into subphyla and orders, with the subphylum *Eurhodophytina* containing macroalgae divided into two classes: Florideophyceae (6751 species) and Bangiophyceae (198 species). Florideophyceae regroups many orders such as: Ahnfeltiales (10 species with the genus *Ahnfeltia*), Ceramiales (2655 species with the genus *Bostrychia*), Corallinales (721 species with the genera *Corallina* and *Serraticardia*), Gelidiales (207 species with the genus *Gelidium*), Gigartinales (879 species with the genera *Chondrus*, *Dilsea*, *Eucheuma*, *Furcellaria*, *Kappaphycus*, and *Solieria*),

Gracilariales (251 species with the genus *Gracilaria*), Halymeniales (313 species with the genera *Grateloupia* and *Pachymenia*), Nemaliales (276 species with the genera *Nemalion*, *Galaxaura*, and *Nothogenia*), Palmariales (45 species with the genera *Palmaria*, and *Rhodothamniella*), and Rhodymeniales (382 species with the genus *Rhodymenia*). Bangiophyceae is a taxonomic unit less diversified. Among the two orders constitutive of the class, the order Bangiales regroups 194 recognized species with specimens of the genera *Bangia* and *Porphyra*. Many red algae, such as members of the orders Gigartinales, Gelidiales, Gracilariales, and Bangiales, commonly grow on rocky and sandy shores along the intertidal and subtidal zones of temperate or tropical oceans but are also abundant in sub-arctic coastal habitats (Mols-Mortensen et al. 2012) (Fig. 1). Some members, such as *Palmaria palmata* (dulse) and *Porphyra* sp. (nori), are used as food for humans. Other genera are used for the extraction of phycocolloids, eg, *Chondrus crispus* (pioka) in some temperate countries and *Eucheuma*/*Kappaphycus* in Indopacific countries, particularly in Indonesia (Stiger & Deslandes 2016).

Figure 1. Red algal species on the shoreline. (https://metchosinmarine.ca/strand-line-of-rhodophytes-red-algae/)

Basically, polysaccharides serve mainly as storage and structural molecules in algae. In red seaweeds, the structural cell-wall polysaccharides usually consist of an outer amorphous mucilage matrix, commonly made up of linear sulfated galactanpolymers (carrageenans, agarans, and alginates) and an inner rigid component, the cellulose fibrils (Arad & Levy-Ontman 2010). In contrast, in red microalgae, the cell walls lack this cellulose microfibrillar component; rather they are encapsulated within sPS in

the form of a gel (Bold & Wynn 1985). During growth in a liquid medium, the external part of the polysaccharide undergoes dissolution from the cell surface into the medium (soluble fraction) (Ramus 1986, Arad 1988), whereas most of the polysaccharide (50–70%) remains attached to the cell (bound fraction). This polysaccharide supplies the cells with environmental protection: the gel structure protects against desiccation; its stability to temperature, pH, and salinity (Ucko et al. 1989, 1999) protects against environmental extremes, and its antioxidant activity is probably used as a free radical scavenger (Tannin-Spitz et al. 2005, Chen et al. 2009).

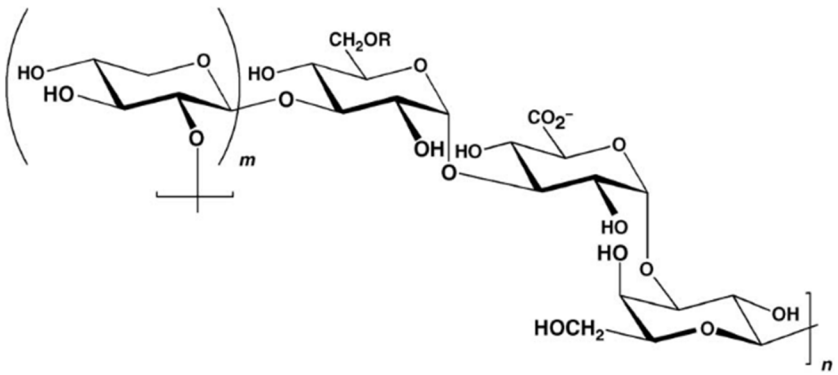

Figure 2. Proposed structure of a linear building block in the *Porphyridium* sp. polysaccharide (Geresh et al. 2009).

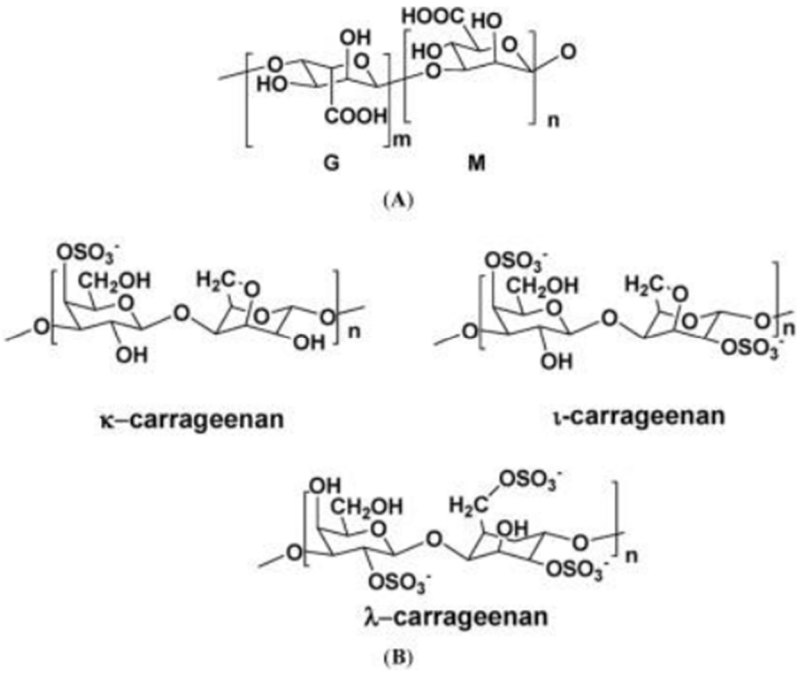

Figure 3. Examples of structures of PS from red seaweeds (**A**) Repeating units suggested for the structure of not-sulfated alginates; (**B**) Repeating units of some carrageenans (Fedorov et al. 2013).

The soluble cell-wall polysaccharides of different red microalgae species (e.g., *Porphyridium cruentum*, *Dixoniella grisea, Rhodella reticulata*) have a common structural feature: galactan heteropolymers (molar mass 2–7 x 10^6 g mol^{-1}) that contain sulfate residues (e.g., Dubinsky et al. 1992). The polysaccharides are anionic due to the presence of GlcA and half-ester sulfate groups. In the species

studied, the main sugars of the polymers are xylose, glucose, and galactose, but in different ratios. Additional minor sugars (methylated sugars, mannose, arabinose, and ribose) have also been detected. The polymers have different sulfate contents (1–9%, w/w), with the sulfate groups being attached to glucose and galactose in the 6 or 3 position (Lupescu et al. 1991).

In particular, the polysaccharide of *Porphyridium* sp. (Fig. 2) was found to have anti-inflammatory, anti-irritating (Matsui et al. 2003), and antioxidant (Tannin-Spitz et al. 2005) activities. Due to these bioactivities, this sPS has already been introduced into a wide range of cosmetic products of a leading global cosmetics company (Arad & Levy-Ontman 2010).

The major constituent (sugar) in red macroalgae is galactose, being designated as galactans (gal) (Pereira et al. 2005). Red seaweeds contain large amounts of sPS, mostly agaroids and/or carrageenans, with alternating repeating units of 1,3-α-gal and 1,4-β-d-gal (Usov 2011), and/or 3,6-anhydrogal (3,6-Agal) (Pomin 2012). Substituents can be other monosaccharides (mannose), sulphate, methoxy and/or pyruvate groups. The pattern of sulphation divides carrageenans into different families, for example, in C-4 for κ-carrageenan, and in C-2 for λ-carrageenan (cf. Fig 3). In addition, the rotation of gal in 1,3-linked residues divides agaroids (l-isomer) from carrageenans (d-isomer) (Jiao et al. 2011). Apart from agarans, found in species of *Porphyra*, *Polysiphonia*, *Acanthophora*, *Goiopeltis*, *Bostrychia* or *Cryptopleura*, red seaweeds are also good sources of κ-carrageenan (*Eucheuma spinosa*, *Kappaphycus alvarezii*), λ-carrageenan (*Chondrus* sp, *Gigartina skottsbergii* and *Phillophora*) (Anderson et al. 1973), ι-carrageenan (*Eucheuma spinosa*) (Funami et al. 2007), and other heterogalactans with mannose and/or xylose comprising part of the main sugar chain. These include, xylogalactans in *Nothogenia fastigiata* (Damonte et al. 1994), and xylomannans in *Sebdenia polydactyla* (Ghosh et al. 2009).

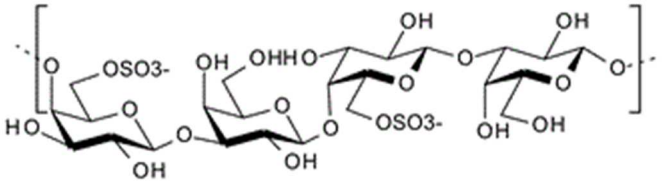

Figure 4. Porphyran a sPS from *Porphyra* sp.

These polysaccharides are complex heterogenous natural polymers and marine algae are known to be one of the most important sources of sPS, with a wide variation in structure depending on the species and environmental conditions (e.g., Manlusoc et al. 2019). The complexity in the structure of these compounds is due to a variety of linkages between the monosaccharides and the distribution of sulfate groups. Therefore, each sPS may possess unique structural conformation and thus exhibits different biological activities (Boisson-Vidal et al. 1995, Haroun-Bouhedja et al. 2000, Manlusoc et al. 2019). The virtually ubiquitous occurrence of sPS in/on the cell walls and intercellular spaces of marine algae is proposed to be an adaption to diverse environmental stress conditions in their habitat (e.g., Lühn et al. 2014).

There are several well-known red seaweed sPS used as cosmeceuticals. For instance, sulfated galactans, such as carrageenans, have been utilized in cosmetic products as a stabilizer, emulsifier and moisturizer due to its chemical and physical properties (Kim et al. 2018). Furthermore, Porphyran (Fig

4) which constitutes a well-studied class of sPS obtained by aqueous extraction from *Pyropia* and *Bangia* species (order Bangiales), has shown potential skin whitening, anti-inflammatory, analgestic and antiulcer properties (Jiang et al. 2016). It has a linear structure formed by alternating units of glycosidically substituted β-D-galactopyranose at carbon 3 and α-L-galactopyranose units substituted at carbon 4 in a repeating alternating disaccharide arrangement which may be represented by the folmula $[\rightarrow 3)$-β-D-galactopyranose-$(1\rightarrow 4)$-α-L-galactopyranose-$(1\rightarrow]_n$ (Ishihara et al. 2005). In contrast, branched sulfated xylogalactans found in the red seaweed *Delesseria sanguinea* (Hudson) Lamouroux (Ceramiales, Delesseriaceae) are not yet used in cosmetic products. *D. sanguinea* has been shown to contain non-gelling sPS, exhibiting a pharmacological profile indicating anti-inflammatory and anti-skin aging potential in sufficient amount for industrial applications (Grünewald & Alban 2009).

2. Objectives and Aims of the Study

Considering the harsh environmental conditions in northern Icelandic coastal areas, including rapid fluctuations in temperatures and salinities during low tide as well as strong velocities during storm events, it was assumed that red algal species originating from these habitats have evolved an array of until now undescribed sPS, with a range of potential uses. Utilizing 12 northern Icelandic red algal species from our indoor culture collection, the current project is conducted to induce the accumulation of sPS by exposure of the test algae to extreme environmental parameters such as high temperatures, velocities, salinities, light intensities and photo periods, and to extract, screen and analyse the polysaccharides of selected samples (cf. overview Fig. 5). Beside others, the selected samples will be analysed for their:

- chemical composition of the sPS, including for instance total carbohydrate and monosaccharide composition as well as total phenolic and sulfate contents.
- antioxidant activities.
- elastase and collagenase inhibitory activities.
- effects on human keratinocytes viabilities in MTT assays; and
- effects on the viability of 3T3-L1 cells tested in adipocyte differentiation assays.

In the second part of the project, tests will be conducted with the goal to integrate selected aqueous polysaccharide extracts into solid skin care products to facilitate specific properties such as moisturizing, anti-inflammatory and/or anti-aging.

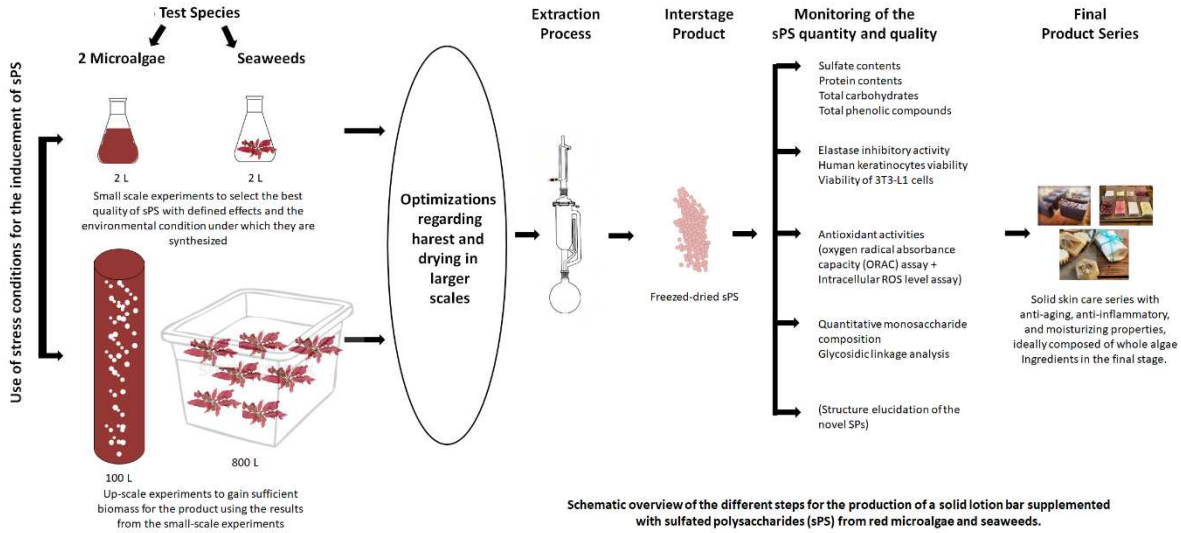

Figure 5. Overview of the present project.

3. Material and Methods

If not otherwise mentioned, all chemicals used in this study were of the highest purity from Sigma/Aldrich.

3.1 Algal Strains and Culture Conditions

Besides two red microalgae species (*Porphyridium sp.* and *Rhodella sp.*), overall, ten northern Icelandic seaweed species (Rhodophyceae) were chosen for the present project, namely *Palmaria palmata*, *Gracilaria gracilis*, *Dumontia contorta*, *Plumaria plumosa*, *Osmundea hybrida*, *Heterosiphonia plumosa, Porphyra umbilicalis, Pyropia thulaea, Delesseria sanguinea* and *Odonthalia dentata*.

Table 1. Optimum culture parameter for the red microalgae. Abbreviation: MP, Modified Provasoli's

Culture Conditions	***Porphyridium sp***	***Rhodella sp***	**Additional Remarks**
Temperature [°C]	12	10	
Light [µmol photons m^{-1} s^{-1}]	30	30	Use of full spectra day light bulbs
Photoperiod (light:dark)	12:12 h	12:12 h	Use of timer
Salinity	30	32	
Seawater	TMC	TMC	Tropic Marin Classic©
Nutrient Media	MP	f/2	

Table 2. Optimum culture parameter for red algae gametophyte and sporophyte culture systems adapted to northern Icelandic climate conditions. Abbreviation: PES, Provasoli´s Enriched Seawater

Culture Conditions	**Gametophyte Phase**		**Sporophyte Phase**	**Additional Remarks**
	Release and settlement of Spores	Sporophyte formation	Juvenile Sporo-phytes	
Temperature [°C]	5-8	8-10	8-12	
Light [µmol photons m^{-1} s^{-1}]	30	30	50	Use of full spectra day light bulbs
Photoperiod (light:dark)	8:16	12:12	12:12	Use of timer
Salinity	30	30	32	
Seawater	TMR	TMR	TMR	Tropic Marin Reef Salt©
Nutrient Media	PES 0.25 strength	PES 0.5 strength	PES 0.75 strength	
Aeration level	low	medium	strong	pump

While the cultivation of sporophytes was conducted in 2 L Erlenmeyer flasks (Fig. 6), gametophytes were first grown in sterile Petri disks and later transferred into 50- and 100-mL flasks. Cultures were supplied with fresh medium in bi-weekly intervals. For medium preparation, the artificial seawater salt (seaweeds: Tropic Marin Reef Salt®; microalgae: Tropic Marin Classic Salt®) was dissolved in tap water (salinity of 30 and 32 Practical Salinity Units (=PSU), pH 8.2) and supplied with defined nutrient concentrations (Provasoli's Enriched Seawater (PES) for seaweeds https://www.assemblemarine.org/assets/ASSEMBLE-JRA1-Protocol-11.00.pdf; Modified Provasoli's (MP) for Porphyridium sp. https://www.ccap.ac.uk/wp-content/uploads/MR_MP_Mod_Provasoli.pdf; f/2 for Rhodella https://www.ccap.ac.uk/wp-content/uploads/MR_f2.pdf) (Table 1). For the light intensities, full spectra daylight bulbs (Phillips, Germany, Master TL-D 18W/840) were used. Conductivity, temperature, dissolved oxygen and pH were measured using handheld probes (YK-31SA, YK-200PATC, YK-200PCD and YK-2001PH, SI Model 33, Engineered Systems and Designs-Model 600, Philips W9424). Photosynthetic active radiation (PAR, 400–700 nm) was measured with an underwater spherical quantum sensor LI-193SA connected to a Licor Data Logger LI-250A. The optimum cultivation parameter for gameto- and sporophytes of the northern Icelandic representatives of the Rhodophyceae are given in Table 2.

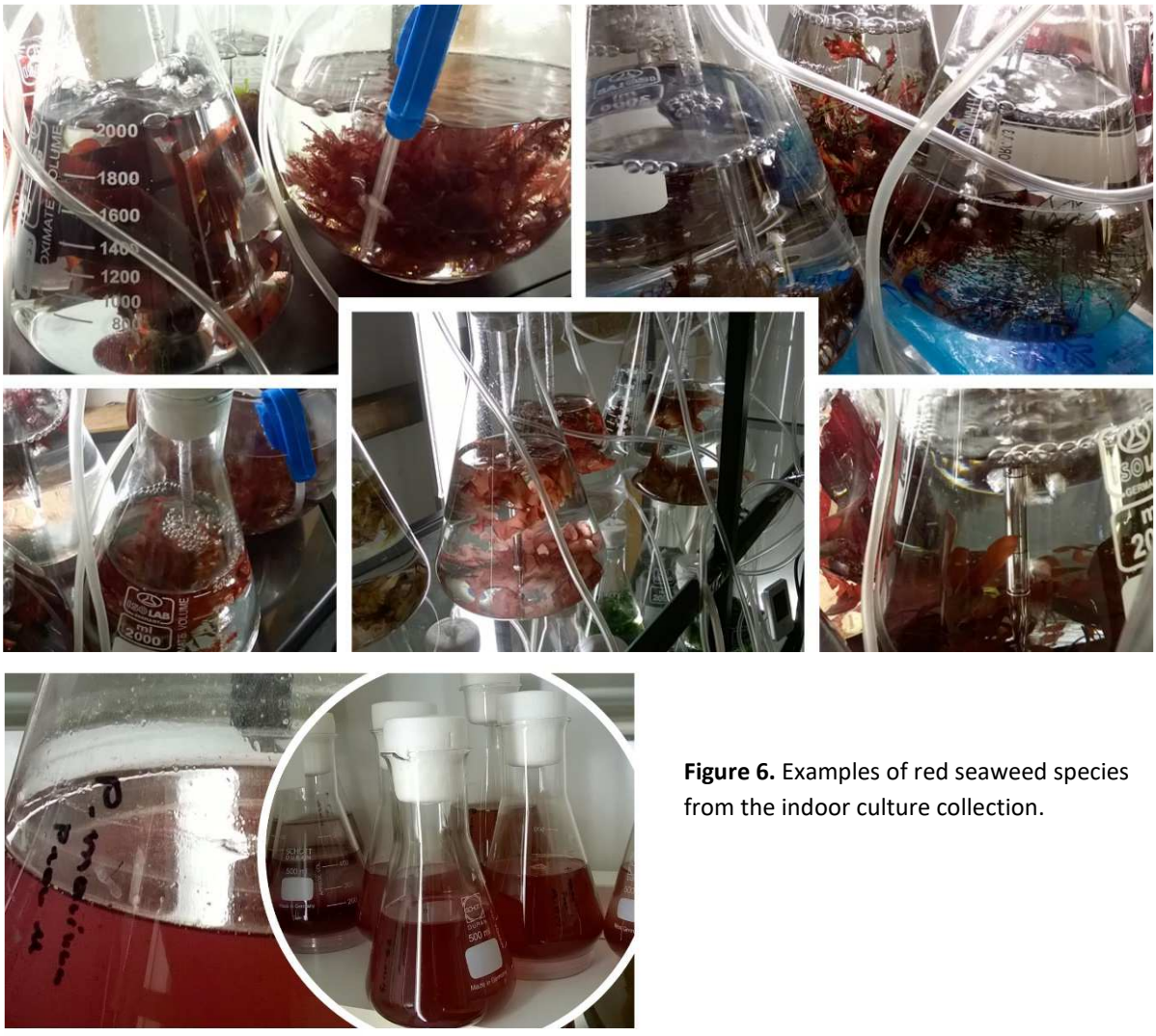

Figure 6. Examples of red seaweed species from the indoor culture collection.

3.2 Preservation and Identification of Seaweeds

For preservation and identification of seaweeds, specimens were washed in a tray containing fresh water (half filled) and were spread on paper sheets with the help of a brush to minimize the overlapping of the specimen. In the next step, the sheets were removed, and the seaweeds were properly arranged using forceps or needles if required. To blot dry, sheets were placed on carton sheets to remove the remaining water from the herbarium. Then a plastic cloth was placed on the top of the individual seaweed in such a way that it covered the entire specimen. After that, another sheet was placed over the herbarium sheet. Once, all the seaweeds were prepared, the herbaria were piled one above the other and then placed into a wooden press. After 24 h drying at room temperature the papers were replaced. This process was repeated until the seaweeds were free of moisture. This method still allows to extract DNA even after preservation. The following literature was used for identification of the taxa: Brodie & Irvine (2003), Dixon (2011), Irvine (2011a, b) and Maggs (2013).

3.3 Determination of Growth

Seaweed growth rates was calculated for each species (replicated) by the increase in wet weight and presented as percentage growth per week using the formula of Penniman et al. (1986):

$$SGR = \% [(Gt/Go)1/t - 1] \times 100$$

SGR = specific growth rate (% in wet weight per week), Gt = weight after t weeks,
Go = initial weight, t = time in weeks

Microalgae growth was monitored by conducting cell counts and by measuring dry matter. Counting of cells during the experiments were performed under a light microscope (Axiophot, Carl Zeiss AG, Oberkochen, Germany), using a Neubauer improved counting chamber with 0.1 mm depth (LO Laboroptik GmbH, Germany). During the long-term experiments, cell counts were conducted every second day. Only cells were counted, which exhibited red fluorescence under ultraviolet light. At least 500 cells were counted in each sample at a 400× magnification. The specific growth rate (μ) was calculated with the following equation:

$$\mu = \frac{\ln_{c1} - \ln_{c0}}{t_1 - t_0}$$

where c_1 and c_0 are the number of cells at time t_1 and t_0.

Dry cell weight (DCW) or dry weight was measured in triplicates by using 0.45 µm cellulose acetate filters (WHA10404006 Whatman®). Filters were pre-dried for 10 min at 105°C to remove any moisture. Subsequently 40 mL of the algal culture was filtered and dried for 24 h at 70°C and then weighed to measure the dry weight, then expressed as grams per litre.

3.4 Experimental Design

The inducement of sulfated polysaccharides was conducted by variation of culture conditions, utilizing extreme stress parameters such as for instance high temperatures and salinities. Specifically, the experiments were conducted in 2 L Erlenmeyer flasks for 336 h, utilizing 4-10 plants (depending on sizes and wet weights) per assay in triplicates. Using the one-factor-at-a-time principle, the eight rhodophytes were exposed in different runs to a) 20°C, b) 45 Practical Salinity Units (=PSU), c) 2000 µmol photons m^{-2} s^{-1}, d) very strong bubbling and e) a 24:0 light:dark cycle. As control standard cultivation conditions were used (10°C, 50 µmol photons m^{-2} s^{-1}, 30 PSU, 12:12 light:dark cycle), resulting in overall 72 samples (without triplicates). Experiments were started by filling in 1000 mL fresh prepared culture medium (cf. 3.1) into the Erlenmeyer flasks with the rhodophytes, which were weighted prior to each experiment.

3.5 Harvest and Processing

After 336 h, the seaweeds were harvested and washed twice with distilled water, whereas the microalgae were harvested by centrifugation at 4000 rpm at 10°C for 10 min. Subsequently the specimens and pellets were dried, utilizing a horizontal dryer with hot air flow at 60°C (Tray dryer, model no. FDTHQQZ).

The enzymatic extraction of polysaccharides from red algal biomass samples was performed according to the methodology of Farias et al. (2000), with some modifications described by de Castro et al. (2018). The dried tissue/pellets (5 g) was suspended in 250 mL of 0.1 mol·L^{-1} sodium acetate buffer (pH 5.0), containing 1 g of papain, 5×10 -3 mol·L^{-1} EDTA, and 5×10-3 mol·L^{-1} cysteine, and incubated at 60°C for 30 min. The incubation solution was then filtered through a nylon membrane, and the homogenate was retained. The polysaccharides in solution were precipitated with 16 mL of 10% cetylpyridinium chloride (CPC) solution. After 24 h at 25°C, the mixture was centrifuged at 2,560 × g for 20 min at 20°C. The polysaccharides were washed with 500 mL of 0.05% CPC solution, dissolved with 100 mL of a 2 mol·L^{-1} NaCl-ethanol (100:15, v/v) mixture, and the excess of salts was removed by precipitation and wash with 200 mL of absolute ethanol. After 24 h at 4°C, the precipitate was collected by centrifugation (2,560 × g for 20 min at 20 °C), washed extensively with ethanol-80%, then absolute ethanol. After this, the polysaccharides (PLS) were washed with acetone, which was followed by hot air drying (60°C) until all the acetone was removed.

3.6 First Screening for Polysaccharides

Polysaccharides derived from red seaweed are usually composed of repeating disaccharide units of alternating 1,3-linked galactose (Gal) and 1,4-linked 3,6-anhydro-galactose (AnGal) residues. The biological properties of red seaweed polysaccharides are highly dependent on the amount of AnGal, which is a key bioactive monomeric sugar of red algae (e.g., Zheng et al. 2020, Xie et al. 2020). Thus, in the present project, the determination of total carbohydrate content as well as the content of AnGal were used as pre-selection tool. The total carbohydrate content was determined by the anthrone-sulfuric acid method with minor modification described by Xie et al. (2020). Each sample solution

(1 mL, 1 mg/mL) was placed in tubes and 0.2 mL distilled water added. After adding 1 mL of anthrone reagents (0.2 g anthrone was mixed with 100 mL of 80% (v/v) sulfuric acid before colorimetric) followed by homogeneous mixing, samples were allowed to react at 80°C for 20 min. Next, tubes were cooled for 5 min in an ice water bath, before the total absorbance was measured three times at 640 nm. Total sugar level was quantified using a calibration curve plotted with Gal standards.

The content of AnGal was determined by resorcinol method with a minor modification (Yaphe & Arsenault 1965). Resorcinol reagent was prepared within 3 h before the colorimetric assay. Briefly, resorcinol reagent was prepared with 9 mL resorcinol solution (1.5 mg/mL), 1 mL acetaldehyde solution (0.04%, v/v) and 100 mL concentrated hydrochloric acid. Next, 0.03 mL aliquot of the sample solution (1 mg/mL) was added to a centrifuge tube followed by the addition of 0.2 mL distilled water. After placing in an ice bath for 5 min, 1 mL of resorcinol reagent was added, mixed homogenously in ice bath, and then placed at room temperature for 2 min. The mixture was incubated for 10 min at 80°C followed by cooling for 5 min in an ice bath. The absorbance of AnGal was measured at 555 nm and the concentration of AnGal calculated using a calibration curve with Gal standards. All samples were analysed in triplicates, with Gal content in samples calculated by the total content of carbohydrate minus the content of AnGal.

3.7 Characterisations of selected sPS samples

Out of the 72 samples generated by the exposure experiments (cf. 3.4), overall, six samples with the highest total carbohydrate (TC) and AnGal contents were chosen for further analysis.

3.7.1 Chemical Compositions

3.7.1.1 Protein

Contaminant proteins (CPs) were measured using the Lowry method as described by Herbert et al. (1971) with bovine serum bumin as a standard. In brief, 5 mL 1.0 N NaOH was added to the biomass aliquot and incubated for 5 min in a boiling water bath (95°C, Typ 1083, GFL mbH, Burgwedel, Germany). After cooling, 2.5 mL of the reactive mixture (5% Na_2CO_3 + 0.5% $CuSO_4$ $5H_2O$ in 1.0% Na-K-Tartarate; ratio 25:1 v/v) were added and incubated for 10 min at room temperature. This was followed by the addition of 0.5 mL Folin-phenol reagent (1.0 N) and incubation for another 15 min. After centrifugation (Omnifuge 2.0 RS, Heraeus Sepatch, Osterode, Germany), the intensity of the resulting blue colour was determined at 650 nm.

3.7.1.2 Total Phenolic Compounds

The total phenolic content (TPC) of each sample was determined according to the method of Gutfinger (1981). Each sample (1.0 mL) was mixed with 1.0 mL of 10% Na_2CO_3 and allowed to stand for 3 min. Then, 1.0 mL of 50% Folin-Ciocalteu reagent was added to each mixture. After incubation at room temperature, the resulting mixtures were centrifuged at 13,400 *g* for 5 min. Absorbances were measured with a spectrophotometer (TI Unicam 5625 UV/VIS Spectrometer, Richmond Scientific Ltd., Chorley, United Kingdom) at 750 nm, and the total phenolic contents were expressed as gallic acid equivalents.

2.7.1.3 Monosaccharide Compositions

Samples of the polysaccharides extracted from the rhodophytes (5 mg) were hydrolysed with 5 mol·L^{-1} trifluoroacetic acid for 4 h at 100°C, reduced with borohydride, and the alditols were acetylated with acetic anhydride:pyridine (1:1, v/v). The alditols acetates were dissolved in chloroform and analysed in a gas–liquid chromatograph/mass spectrometer (GCMS-QP2010 Shimadzu, Japan) with a DB-5ms column (Agilent) (Kirchner 1960, de Castro et al. 2018).

3.7.1.4 Sulfate Contents

The presence of sulfate groups in algal polysaccharides have been attributed to their biological activities (Barahonaa et al. 2014, Wang et al. 2016). The sulfate contents of the sPS were determined according to the method given by Terho & Hartiala (1971) utilizing standard operation protocols developed by our partner GlycoMar. The method is based on the use of sodium rhodizonate which forms a coloured compound in the presence of barium ions. This colour is reduced when sulfate is present, due to the formation of barium sulfate. Briefly, samples were hydrolysed using HCl, dried and resuspended in water. For the assay, to 50 µL of each sample or control (blanks, heparin and chondroitin sulfate), 50 µL de-ionised water and 400 µL ethanol were added and mixed thoroughly. 125 µL of each ethanol-added sample, standards (final concentration 0.048 to 0.48 µg Na_2SO_4), and controls were pipetted into a 96-well microplate in triplicate. $BaCl_2$ buffer and sodium rhodizonate solution were added, mixed and incubated at room temperature (18°C) in the dark for 10 min. The colour intensity was then measured spectrophotometrically at 520 nm. The % of sulfate in each sample was then calculated from the standard curve.

3.7.2 Activity Assays

3.7.2.1 Evaluation of Antioxidant Activity

It has been assumed that an antioxidant with a high potential to defend ROS and deplete oxidative stress, thus, a compound with a strong antioxidant activity additionally facilitates the skin protection against oxidative damages along with delaying the skin aging process (Palmer & Kitchin 2010, Chanda et al. 2015). Therefore, two antioxidative activity tests were conducted in the present project. The first was the DPPH scavenging activity assay which was performed as described by Nanjo et al. (1996). Briefly, DPPH reagent was dissolved in methanol for a solution concentration of 1.5×10^{-4} M. One hundred microliters of DPPH reagent was mixed with 100 µL sample in 96-well plates. After incubation at room temperature for 30 min, the absorbance was measured 517 nm. The second test carried out was the Hydrogen peroxide scavenging activity test which was conducted according to the method given by Müller (1985) with slight modification introduced by Jiratchayamaethasakul et al. (2020). A 100 µL of 0.1 M PBS buffer (pH 5) was added into a 96-well plate. Each 20 µL of sample and 20 mM hydrogen peroxide (H_2O_2) were added to mix with the buffer, and then incubate 37 °C for 5 min. After the incubation, a 30 µL of 1.25 mM ABTS and peroxidase (1 unit/mL) were added to the mixture and then incubated at 37 °C for 10 min. The absorbance was read at 405 nm.

3.7.2.2 MTT Assay

The MTT assay was used to examine the effects of the sPS on human keratinocytes viability. Human keratinocytes were cultured in a 96-well plate (1×10^4 cells/well) for 24 h at 37°C with 5% CO_2. Next,

the cells were treated with the rhodophyte extracts (200 µg/mL) for 48 h and then incubated with 100 µL of MTT reagent (5 mg/mL) for 1 h. Then, the reaction medium was removed and the insoluble formazan remaining in the keratinocytes was dissolved in 100 µL of DMSO at room temperature for 15 min. The absorbance of each well was measured at 540 nm. The viability of the extract-treated cells was expressed as a percentage of the viability of untreated cells.

3.7.2.3 Adipocyte Differentiation (AD)

3T3-L1 cells were maintained at 37°C in a humidified, 5% CO_2 atmosphere for all procedures. The 3T3-L1 preadipocytes were seeded in a 12-well plate and cultured for 3∼4 days, until confluency. Two-day post confluent 3T3-L1 preadipocytes were maintained in DMEM supplemented with 10% FCS and 100 units/mL penicillin-streptomycin, and the medium was replaced every 2 days. Two days after reaching confluency (Day 0∼2), the preadipocytes were treated with DMEM supplemented with 10% FBS and 100 units/mL penicillin streptomycin (FBS-medium) and containing 500 µM IBMX, 5.2 µM dexamethasone, and 167 nM insulin (differentiation medium; DM). The 3T3-L1 cells were treated with 200 µg/mL doses of the rhodophyte extracts until 2 days post-confluence. After Day 2, the medium was changed to FBS-medium with 167 nM insulin for additional 2 days (post-differentiation medium; Post-DM). Thereafter, the 3T3-L1 adipocytes were cultured in FBS-medium. The cells were harvested at Day 7 and stained for matured adipocytes.

After adipocyte differentiation, the cells were stained with Oil Red O staining (Inazawa et al. 2003), an indicator of cell lipid content. Briefly, cells were washed with phosphate-buffered saline, fixed with 10% buffered formalin and stained with Oil Red O solution (0.5 g in 100 ml isopropanol) for 10 min. After removing the staining solution, the dye retained in the cells was eluted into isopropanol, and OD_{540} was determined.

3.7.2.4 Enzyme Assays

Solar radiation, or UV radiation, is the major stimulator that accelerates the overproduction of reactive oxygen species (ROS) which leads the endogenous oxidative stress in the epidermis (Kim et al. 2016). The accumulated ROS after skin exposure to photoaging stressors can indirectly activate dermal enzymes such as collagenase and elastase which basically break down and degrade collagen as well as elastin, respectively (Sahasrabudhe & Deodhar 2010, Popoola et al. 2015, Chatatikun & Chiabchalard 2017). Thereby, the synthesis of elastase and collagenase promotes premature skin aging as evidenced by signs such as wrinkles, freckles, sallowness, deep furrows or severe atrophy, laxity, and leathery appearance (Peres et al. 2011, Ding et al. 2018).

Each test was performed in triplicate using standards reported in the literature, known for their high inhibitory activity, which have been used in this study as positive controls to validate each assay. Negative controls were prepared by adding water instead of inhibitor. The quantification of the inhibitory effect of the SPs, and those of the reference compounds, were calculated using the following equation:

$$\% \text{ Inhibition} = [(A_n - A_s)/A_n] \times 100$$

Where A_n is the absorbance of negative control and A is the absorbance of the sample measured at the wavelength indicated for each test.

3.7.2.4.1 Collagenase inhibitory Assay

The enzyme assay was based on the method reported by Madhan et al. (2007), with some modifications. For the enzymatic hydrolysis an 8 mg/mL solution of collagen was used as substrate. As enzyme, a stock collagenase solution of 3.6 x 103U/mL was prepared in a 0.1 M Tris-HCl buffer (pH 7.4) containing 0.006 mM $CaCl_2$. Inhibitors and enzyme were incubated for 30 minutes at 25°C before adding the substrate. The 1 mL reaction mixture containing 902.83 U of enzyme, 2.4 mg of substrate and 250 µL of the sPS at different final concentrations (2.5, 1.25, 0.63 and 0.25 mg/mL) was incubated for 8 hours at 37°C. Finally, samples were centrifugated at 8500 rpm for 15 minutes at 4°C and hydroxyproline was detected in 500 µL of supernatant by the method of Kolar (1990) adapted for a microplate reading. To validate the method, we compared the effect of the sPS with the caused by a positive control containing EDTA at a concentration of 0.25 mg/mL. The spectrophotometric measurements were made at a wavelength of 558 nm.

3.7.2.4.2 Elastase inhibitory Assay

This test was based on the protocol described by Sachar et al. (1955). The principle of the method is the elastin-orcein hydrolysis, which is initially insoluble in water but when it is digested by elastase, it generates soluble products which can be determined spectrophotometrically at a wavelength of 590 nm. For this assay, an elastase solution of 0.399 U/mL dissolved in Tris-HCl buffer (pH 8.8) and a substrate solution at a final concentration of 4.7 mg/mL were used. The experiment began by adding 500 µL of the sPS extract (2.5, 1.25, 0.63 and 0.25 mg/mL) to 500 µL of enzyme and then incubating for 30 minutes at 25 °C. Then, the reaction was obtained with the addition of the substrate and then the reaction mixture was incubated at 37 °C for 4 hours. Subsequently, all reaction mixtures were centrifugated for 15 minutes at 8500 rpm and 4 °C, and then the absorbance of the supernatant was read at 590 nm, using a solution containing the same amount of substrate dissolved in the buffer as blank. To validate the test, the inhibition caused by the sPS was compared to those caused by EDTA (disodium salt) at 15 mg/mL. At this concentration EDTA exhibits a high inhibition of elastase.

3.8 Statistical Analysis

Measures were carried out in triplicate (*n* = 3), and the results are given as mean values and standard deviations. The results were statistically analysed using a one-way ANOVA with a statistical difference of 5% and the Tukey TSD test of the IBM SPSS software version for multiple comparisons.

3.9 Development of The Solid Skin Care Product Series

Out of the six selected sPS samples, three were used in the development of the solid skin care products. Basically, solid skin care products are made by combining butters (solid) with carrier oils (liquid) and essential oils (to scent). In the present project, shea and cocoa butter were tested, combined with coconut and olive oil as carrier oil. In all cases raw, cold-pressed butters and oils – free of chemicals that are used in bleaching and refining – will be utilized. In addition, beeswax was also utilized to stabilize the solid skin care bars (Fig. 7). Targeted were combinations which exhibited

- moisturizing,
- anti-inflammatory and
- anti-aging properties,

utilizing overall, three different concentrations of three pre-selected sPS extracts. To obtain a firmer lotion bar, more beeswax was used. Conversely, for a smoother lotion bar, less beeswax and more coconut oil or olive oil was utilized.

Figure 7. Some of the ingredients used for the solid lotion bars in the present project.

4 Results

4.1 Impacts of Environmental Stress Factors on the amount of accumulated Polysaccharides

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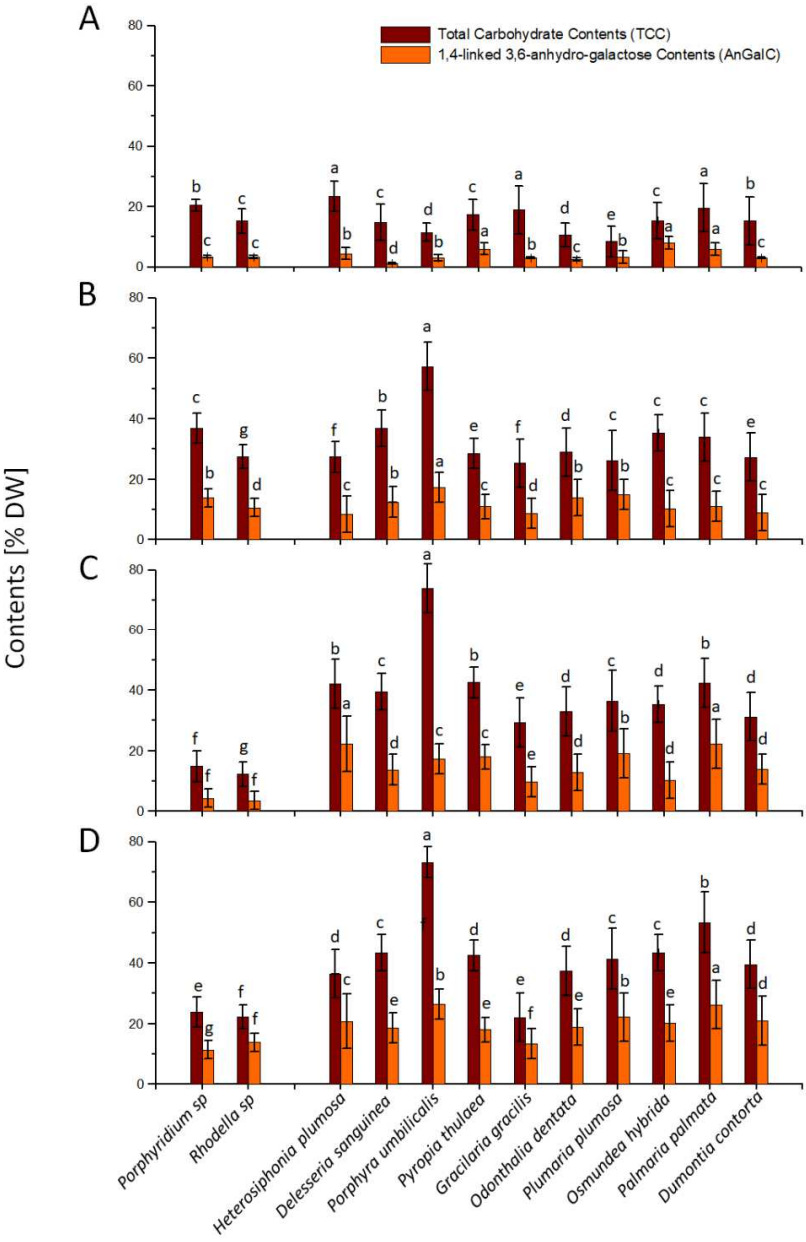

Figure 8a. Results of the first screening for high PS accumulations in 12 northern Icelandic rhodophytes, exposed to 20°C (B), 45 PSU (C) and high velocities (bubbling, VE) (D) in comparison to the control assays (10/12°C, 50 µmol photons m^{-2} s^{-1}, 30 PSU, 12:12 light:dark cycle) (A). Values correspond to the average of triplicates ± standard deviation. Letters indicate significant differences between stress condition and normal condition ($p<0.05$).

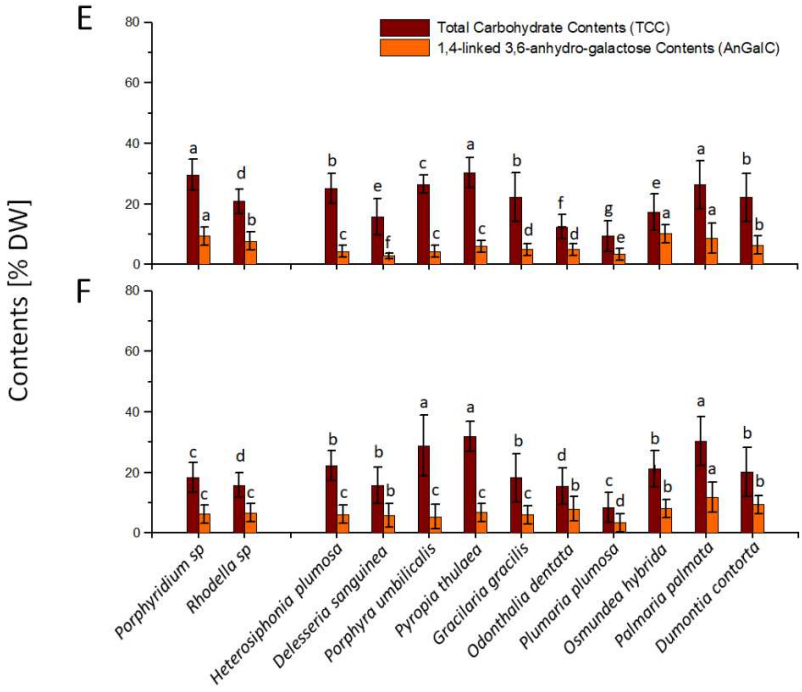

Figure 8b. Results of the first screening for high PS accumulations in 12 northern Icelandic rhodophytes, exposed to 2000 µmol photons m^{-2} s^{-1} (E) and a 24:0 light:dark cycle (F). Values correspond to the average of triplicates ± standard deviation. Letters indicate significant differences between stress condition and normal condition ($p<0.05$).

Overall, 12 long-term cultured northern Icelandic red algae species were tested for their potential to accumulate polysaccharides under exposure to selected environmental stress parameters in controlled laboratory conditions for two weeks (Figs. 8a, b), utilizing the microalgae *Porphyridium sp.* and *Rhodella sp.*, and the seaweeds *Palmaria palmata* [Pp], *Gracilaria gracilis* [Gg], *Dumontia contorta* [Dc], *Plumaria plumosa* [Ppl], *Osmundea hybrida* [Oh], *Heterosiphonia plumosa* [Hp], *Porphyra umbilicalis* [Pu], *Pyropia thulaea* [Pt], *Delesseria sanguinea* [Ds] and *Odonthalia dentata* [Od]. The designations in square brackets indicate the species code for the samples chosen according to the results in this paragraph (HV = high velocity; T = temperature stressed; S = salt stressed).

As expected, total carbohydrate and 3,6-anhydro-galactose (AnGal) contents remained the lowest in the control assays (Fig. 8A), whereas the highest values were reached in the high velocity assays (Fig. 8D) followed by the exposure trials to temperature (Fig. 8B) and salinity stress (Fig. 8C). Total carbohydrate contents were found to be significantly increased for *Porphyra umbilicalis* [species acronym: Pu], *Palmaria palmata* [Pp] and *Plumaria plumosa* [Ppl] in the high velocity assays (74.2 ± 4.9 % [sample acronym: Pu-HV], 53.4 ± 8.9 % [Pp-HV], 41.4 ± 8.9 % [Ppl-HV]) as well as for *Porphyra umbilicalis* in the 20°C exposure assay (57.3 ± 5.5 % [Pu-T]). In addition, *Porphyra umbilicalis* and *Heterosiphonia plumosa* [Hp] accumulated high total carbohydrate contents in response to salinity stress (73.9 ± 7.9 % [Pu-S], 42.5 ± 5.7 % [Hp-S], Fig. 8C). Meanwhile, AnGal contents were the highest for *Porphyra umbilicalis* and *Palmaria palmata* in high velocity assays with 26.4 ± 4.4 % and 26.3 ± 7.8 %, respectively, dry weight of samples (Fig. 8D). The samples just introduced were chosen for further analysis of the sPS (cf. 4.2).

4.2 Characterisation of selected sPS samples

4.2.1 Chemical Compositions

4.2.1.1 Protein and Total Phenolic Contents (TPC)

Table 3. Protein and total phenolic contents of the six chosen samples. Values correspond to the average of triplicates ± standard deviation. Letters indicate significant differences between stress condition and normal condition ($p<0.05$).

Sample	Protein [%]	TPC [%]
Pu-HV	4.01 ± 0.19^{a}	n.d.
Pu-S	3.59 ± 0.26^{b}	n.d.
Pu-T	3.30 ± 0.02^{b}	n.d.
Pp-HV	4.03 ± 0.15^{a}	0.08 ± 0.02^{b}
Ppl-HV	0.31 ± 0.08^{d}	n.d.
Hp-S	0.85 ± 0.05^{c}	2.9 ± 0.3^{a}

n.d., below detection limit (0.001)

The contaminant proteins (CPs) content of *Porphyra umbilicalis* and *Palmaria palmata* samples [Pu-HV, Pp-HV] obtained from the high velocity assays were significantly higher compared to the sPS isolated from samples from *Heterosiphonia plumosa* and *Plumaria plumosa* [Ppl-HV, Hp-HV] (Table 3, $p < 0.05$). Furthermore, out of the six samples tested, only two gave positive results in the TPC assay [Pp-HV, Hp-S].

4.2.1.2 Monosaccharide Compositions and Sulfate Contents

Table 4. Monosaccharide composition of sPS detected in the six selected samples, including sulfate contents. Values correspond to the average of triplicates ± standard deviation. Letters indicate significant differences between stress condition and normal condition ($p<0.05$).

Sample	Monosaccharides [%]							Sulfate content
	Gal	Rib	Xyl	Ara	Rha	Glu	Man	[%]
Pu-HV	8.78^{d}	1.83^{a}	0.09^{a}	1.41^{a}	n.d.	1.16^{b}	n.d.	11.11 ± 0.63^{a}
Pu-S	11.38^{a}	1.55^{c}	0.06^{c}	1.17^{c}	0.08^{d}	1.53^{a}	0.04^{b}	9.76 ± 0.18^{b}
Pu-T	9.59^{b}	1.63^{b}	0.08^{b}	1.23^{b}	0.19^{c}	1.01^{c}	0.01^{b}	10.85 ± 0.44^{a}
Pp-HV	7.87^{d}	1.11^{e}	1.01^{a}	1.09^{d}	n.d.	0.89^{d}	0.22^{a}	5.41 ± 0.39^{c}
Ppl-HV	9.09^{c}	1.32^{d}	0.09^{a}	n.d.	0.33^{a}	0.93^{c}	n.d.	0.06 ± 0.008^{e}
Hp-S	9.43^{b}	1.68^{b}	0.01^{d}	n.d.	0.26^{b}	1.04^{c}	n.d.	0.19 ± 0.06^{d}

Abbreviations: Ara = arabinose; Rib = ribose; Gal = galactose; Glc = glucose; Man = mannose; Rha = rhamnose; Xyl = xylose; n.d., below detection limit (0.001)

The monosaccharide composition of the polysaccharide extracted from the chosen samples was determined based on gas chromatography/mass spectrometry analysis of the alditol acetates formed after acid hydrolysis. The monosaccharide composition of the sPS revealed that they may contain heterogeneous polymers due to the variation of monosaccharides. Furthermore, the analysed polysaccharides contained varying levels of sugars (Table 4). The major monosaccharide detected was galactose in all six samples. No other sugar was detected up to a limit of < 2% as % of dry weight, ensuring the purity of the material. Similar, sulfate contents showed also varying values, being the highest for all *Porphyra umbilicalis* samples [Pu-HV, Pu-T, Pu-S].

4.2.2 Activity Assays

Table 5. Results of the activity screenings of the six selected sPS samples.

Sample	Antioxidant Capacities		MTT	AD	Enzyme Assays	
	DPPH radical [%[	Hydrogen peroxide [%[	Limit capacity of keratino-cytes to reduce MTT [%]	Reduction of lipid droplets [%]	Collagenase inhibition [%[	Elastase inhibition [%[
Pu-HV	83.80 ± 0.21^{a}	32.44 ± 0.44^{b}	n.e.	13.6 ± 1.15^{b}	29.73 ± 0.18^{c}	16.62 ± 2.09^{d}
Pu-S	22.65 ± 0.75^{e}	21.22 ± 4.19^{c}	n.e.	22.5 ± 0.83^{a}	33.82 ± 0.52^{b}	70.93 ± 2.31^{a}
Pu-T	83.52 ± 1.38^{a}	36.32 ± 2.49^{b}	n.e.	11.4 ± 0.77^{b}	90.21 ± 0.05^{a}	62.37 ± 3.43^{b}
Pp-HV	76.28 ± 2.08^{b}	34.82 ± 2.80^{b}	n.e.	0.94 ± 0.09^{c}	n.d.	32.23 ± 11.43^{c}
Ppl-HV	34.38 ± 1.15^{d}	65.81 ± 3.61^{a}	n.e.	12.3 ± 1.11^{b}	31.70 ± 2.91^{b}	31.59 ± 2.71^{c}
Hp-S	56.64 ± 1.58^{c}	9.22 ± 2.09^{d}	n.e.	14.9 ± 1.05^{b}	13.25 ± 0.05^{d}	n.d.

Values correspond to the average of triplicates ± standard deviation. Percent calculated compared to untreated controls. Letters indicate significant differences between stress condition and normal condition ($p<0.05$). The final concentration of tested samples was 1 mg/ml. Abbreviations: n.e., no effect; n.d., not detected

4.2.2.1 Antioxidant Capacities

In this study, there were two antioxidant methods used to assess antioxidant activities, namely the DPPH free radical and the hydrogen peroxide scavenging assay. As depicted in Table 5, all six extracts exhibited antioxidant activities to a varying degree, ranging from 22.65% and 9.22% to 83.80% [Pu-HV] and 65.81% [Ppl-HV] in the DPPH free radical and hydrogen peroxide assays, respectively.

4.2.2.2 MTT Assay and Adipocyte Differentiation (AD)

To investigate the cytotoxic effect of the selected extracts, human keratinocytes were treated with a concentration of 200 µg/mL of the tested extracts and cellular viability was assessed via MTT assay. Treatment for 48 hours did not affect the capacity of keratinocytes to reduce MTT (Table 5). As shown in Table 5, incubation of 3T3-L1 cells with 200 µg/ml of extracts significantly decreased the lipid droplets by 22.5% compared to the adipocyte control, suggesting that the sPS sample from *Porphyra umbilicalis* Pu-S can reduce adipogenesis in 3T3-L1 cells ($p < 0.05$).

4.2.2.3 Anti-Collagenase and Anti-Elastase Activities

The collagenase and elastase inhibition effects of all six sPS extracts at the final concentration of 1 mg/ml were determined and elucidated as shown in Table 5. It was notable that the highest collagenase inhibitory effect was exhibited by the Pu-T extract (90.21 ± 0.05%) and it also showed an above-average effect in the anti-elastase activity (62.37 ± 3.43%). Conversely, the highest anti-elastase activity was recorded in the Pu-S extract with 70.93 ± 2.31%.

4.3 The Solid Skin Care Product Series

Due to the former results given in 4.2, all three *Porphyra umbilicalis* sPS extracts [Pu-HV, Pu-S, Pu-T] were integrated in the solid skin care products. In the following the final receipes of the products are presented.

4.3.1 Moisturising Lotion Bar

- 50 g raw organic shea butter
- 25 g unrefined coconut oil
- 3 mL olive oil
- 5 mg beeswax
- 2 mL aqueous sPS extract Pu-S (2.5 mg/mL]
- 200 µL of essential oils (100 µL chamomile, 75 µL geranium and 25 µL orange)

All ingredients, except the essential oils and sPS extracts, were slowly melted down in a sturdy glass bowl over a small pan of boiling water. After melting the bowl was removed from the heat and the sPS and essential oils were added. The mixture was then poured into silicone molds and left to cool at room temperature.

4.3.2 Anti-Inflammatory Lotion Bar

- 70 g beeswax pastilles
- 1 cup unrefined cocoa butter
- 1 cup extra virgin coconut oil
- 2 Tbsp. extra virgin olive oil

- 3 Tbsp dried calendula flowers
- 4 Tbsp dried rose petals
- 3 Tbsp dried lavender buds
- 3 mL aqueous sPS extract Pu-HV (1 mg/mL)
- 200 µL essential oils (lavender, geranium)

Calendula, rose petals, lavender, cocoa butter, and coconut oil were placed together into a small pan and heated (medium heat). The temperature was kept at around 75°C for 5 h. Next, the mixture was strained out through a nut milk bag into a clean saucepan. Then beeswax was added and warmed over medium heat until the beeswax was melted. The mixture was allowed to cool briefly before olive oil, sPS and essential oils were added and mixed. After pouring the mixture into silicone molds the lotion bars were left to cool and harden at room temperature.

4.3.3 Anti-Aging Lotion Bar

- 70 g raw organic shea butter
- 28 g beeswax pellets
- 112 g Macadamia nut oil
- 42 g cocoa butter
- 3 mL Sea Buckthorn Oil
- 20 drops Frankincense essential oil
- 3 mL aqueous sPS extract Pu-T (1.5 mg/mL)
- 15 drops essential oil

All ingredients, except for the Sea Buckthorn oil, sPS and the essential oils, were slowly melted down in a sturdy glass bowl over a small pan of boiling water. As soon as the oils and butters were melted, the bowl was removed from the heat and the Sea Buckthorn oil, sPS and the essential oils were quickly added and mixed. The mixture was poured into silicone molds the lotion bars were left to cool and harden at room temperature.

5 Discussion

A close relationship between environmental factors and the accumulation of sulfated polysaccharides (sPS) in seaweeds is proposed by several authors, demonstrated by the observation that the amount and quality of sPS varies seasonally in wild harvested seaweeds (e.g., Grünewald & Alban 2009, Lühn et al. 2014, Sfriso et al. 2017). While individual studies focusing on the effects of specific environmental parameters on seaweed sPS are scarce, investigations utilizing higher land plants suggest that the presence of sPS in plants is an adaptation to high salt environments, which may have been conserved during plant evolution from marine green algae (Aquino et al. 2011). But changes in seasonal conditions are far more than only salinity variations, comprising also alterations of temperature, light intensity and photoperiods beside other parameters. Thus, overall, 12 northern Icelandic red algae from the BioPol indoor culture collection were tested in the present study for their potential to accumulate sPS under exposure to different stress conditions in controlled laboratory trials. While light intensities and extended photoperiods did not show any significant accelerations of the total carbohydrate (TC) or AnGal contents, above normal temperatures, salinities and velocities led to an increase of both contents in all tested species. Comparisons of the obtained data to values given in the literature showed only in some cases relatively good accordance (e.g., for *Palmaria palmata* or *Porphyridium*). In contrast, for most of the tested species either no equivalent data were available at all (e.g., *Dumontia contorta*) or the data showed huge differences to the obtained values in the present study. This may be due to the use of field collected seaweed specimens in most published studies, whereas the rhodophytes tested in the present investigation were already integrated into the culture collection for several years and well adapted to the culture conditions described in 3.1. Physiological and phenotypical changes of algal species in adaptation to long-term culture conditions are well known to science (e.g., Schlüter et al. 2016) and may be the main cause in difficulties to compare results. Also, the methods used for sPS analysis in the literature differ substantially. Currently, the main method used for quantitative analysis of red seaweed polysaccharides involves hydrolysis of the polysaccharides to release monosaccharides followed by colorimetric assay or chromatographic analysis (Cheong et al. 2015, Sudharsan et al. 2018, Cui et al. 2019). Unfortunately, the AnGal residues are unstable in acidic conditions, as these are easily converted into Gal residues or 5-hydroxymethyl-furfural by common acid hydrolysis procedures. This is one of the main reasons for some studies to report or show the monosaccharide compositions of red seaweed polysaccharides without being able to detect AnGal (Seedevi et al. 2017, Sudharsan et al. 2018, Xu et al. 2018a, b). In the colorimetric determination of total carbohydrates (reducing and nonreducing sugars), the anthrone method or phenol–sulfuric acid assay method (Yemm & Willis 1954, Khan et al. 2019, Zhang et al. 2020) is used. These assays are based on the hydrolysis of the red seaweed polysaccharide, followed by intramolecular dehydration of all monomers in acidic conditions. The resulting furfural derivatives then react with anthrone or phenol to form chromogenic products, which have maximal absorption wavelength at 640 nm and 490 nm, respectively. Where the Resorcinol reagent is chosen for the direct determination of AnGal (Yaphe & Arsenault 1965, Wang et al. 2012, Khan et al. 2020), the method involves treatment with thymol in the presence of ferric chloride and hydrochloric acid, with the released monosaccharides forming coloured compounds. The difference in the rate of colour formation with the anthrone reagent between Gal and AnGal has also been used to study the simultaneous determination of a mixture of these monosaccharides in red seaweed polysaccharides.

For a chromatographic method to be used to quantify AnGal, the acid-labile problem must be remedied with the use of a methylmorpholine–borane complex (MMB) under acidic conditions. This method first involves the reductive hydrolysis of AnGal-containing polysaccharides, followed by acetylation of the alditols and, finally, analysis of alditol acetates by gas chromatography (Navarro & Stortz 2003, Xu et al. 2019). In this context, also the relatively low recovery of monosaccharides, in particular galactose, in the present study (Table 4) is noteworthy and demand a different analytical approach in the future.

Although, the sPS in the present investigation were not further structurally elucidated, from the literature it is known that for instance porphyrans extracted from *Porphyra umbilicalis*, have 3-β-d-Galp units methylated or sulfated at the C-6 and that 50% of the B units are 4-α-l-Galp-6-sulfate residues (Delattre et al. 2011). These sPS belong to the polymer class of agaroids and are known to exhibit weakly gelling characteristics with a structure close to agars (Hentati et al. 2020). Particularly, porphyran has been found to have positive effect on lifespan and vitality of *Drosophila melanogaster* (Zhao et al. 2008). In addition, porphyran was shown to significantly decrease the lipid peroxidation, increase total antioxidant capacity and the activity of superoxide dismutase (SOD) as well as glutathione peroxidase (GSH-Px) in all organs tested in aging mice (Zhang et al. 2004). However, the protective effect of porphyran on cell damage and the possible mechanisms of cytoprotection remain unclear. In fact, cellular senescence is a complex process that is characterized by physiopathological changes including irreversible proliferation arrest, enlarged and flattened cell morphology, increased senescence-associated β-galactosidase (SA-β-gal) activity, and enhanced senescence-associated heterochromatin foci (SAHF) formation (Kosar et al. 2011). Chemical or physiological preconditioning has been reported to be a practical prophylactic pharmacologic strategy to increase tolerance to oxidative stress (Das & Maulik 2006, Zhang et al. 2009). Zhang et al. (2018) showed that porphyran was able to restore morphological changes, decrease SA-β-gal activity and inhibit p53-p21 pathways in H_2O_2-treated WI-38 cells, suggesting the promising role of porphyran as an attractive and biosafe agent with the potential to retard senescence and attenuate senescence-related diseases. In the present study, particularly the high anti-collagenase and anti-elastase activities of one of the *P. umbilicalis* extracts, specifically the Pu-T extract, point to interesting anti-aging features.

Previous studies have found that porphyran has diverse physiological activities, including antitumor, immunomodulating, antioxidant, antihyperlipidemic, and hyperc-holesterolemic effects (e.g., Ishihara et al. 2005, Inoue et al. 2009). A study by Jiang et al. (2012) also demonstrated that porphyran prepared from *Porphyra yezoensis* inhibits nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 mouse macrophages in a concentration-dependent manner, through the prevention of nuclear factor kappa-light-chain enhancer of activated B cells (NF-ķB) activation, suggesting that porphyran should have anti-inflammatory activity (Isaka et al. 2015). *P. umbilicalis*, being a rich source of polyphenols, has a broad range of biological activities including anti-inflammatory, immune-modulatory, antioxidant, cardiovascular protective, and anti-cancer actions (López-López et al. 2009, Hussain et al. 2016). Interestingly, in these studies none of these activities were related to the presence of sPS (López-López et al. 2009, Hussain et al. 2016, Santos et al. 2019). In contrast, the sPS extracts from *P. umbilicalis* in the present investigation showed no notable phenolic content at all (Table 3) but exhibited relatively high anti-inflammatory activities in the DPPH free radical and hydrogen peroxide assays (Table 5) pointing to pronounced anti-inflammatory activity in the tested extracts Pu-HV, Pu-S and Pu-T.

Furthermore, the use of the sPS as ingredients in solid skin care products is to date completely new and untested, although non-sulfated alginates are employed mainly in the cosmetic and pharmaceutical industries for their thickening and gelifying character (Hentati et al. 2020). In the present project, besides shea and cocoa butter mainly beeswax was used to stabilize the lotions bars which can be replaced by plant-based waxes for vegan users. Plant waxes are generally the waterproofing components found in an amorphous layer on the outer surface of the plants. They are essential for plants as barrier protection against environmental stress. Many of them have gained GRAS (Generally Regarded as Safe) status approved by FDA (U.S. Food and Drug Administration), allowing for extensive food and cosmetics applications. Typical plant waxes include candelilla wax, carnauba wax, rice bran wax as well as sunflower wax, etc (McIntosh et al. 2018). Finally, a further advantage is the nature of the solid product itself, since the developed skin care bars can be simply wrapped in environmentally friendly paper or carton and addresses thereby the problematic of plastic pollution by avoidance of unnecessary packaging.

6 Conclusions and Perspectives

Sulfated polysaccharides are structurally diverse and heterogeneous, which makes studies of their structures challenging, and may also have hindered their development as therapeutic agents to date. The production of a standardized commercial product based on algal sulfated polysaccharide constituents will be a challenge since their structural and pharmacological features vary depending on species, location and time of harvest. In the present study, only long-term cultivated species were utilized, providing the option of controlled environmental conditions and reliable repeatability of the produced sPS quality and quantity, including the avoidance of ecological harmful effects of wild harvests. Unfortunately, only a minor part of possible environmental culture conditions with the potential to trigger the biosynthesis and accumulation of sPS were tested in the present project due to the restricted timeframe, pointing to future opportunities for more investigations considering parameter such as nutrient conditions and/or further variation of already tested abiotic stress factors. Finally, the development of novel extraction and purification methodologies of algal polysaccharides in the future can further facilitate the turning event toward an industrial utilization. Also, to comply with the United Nations Sustainable Development Goals and bioeconomy key principles such as food security, sustainable use of resources, and reduced climate impact, it would be important to combine the sPS extraction with the gain of multiple products in a seaweed biorefinery approach.

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