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Assessing High-Value Bioproducts from Seaweed Biomass: A Comparative Study of Wild, Cultivated and Residual Pulp Sources

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Featured Application: The biochemical and bioactive profiles of wild and cultivated kelps, including industrial residual pulp, highlight their potential for use in Blue Biotechnology. These biomasses can serve as sustainable sources of high-value compounds such as polyunsaturated fatty acids, proteins, and polyphenols.

Abstract: This study aimed to evaluate the biochemical composition and bioactivities of biomass derived from wild (*Laminaria ochroleuca* and *Saccorhiza polyschides*) and cultivated (*Macrocystis pyrifera*) kelps, as well as industrially extracted residual pulp from *M. pyrifera*, to assess the potential production of high value bioproducts. All samples had a low lipid content, with *M. pyrifera* having the lowest (1.3% dw) and *S. polyschides* showing the highest value (3.3%). Fatty acids such as palmitic acid (C16:0), oleic acid (C18:1n-9 cis), alpha-linolenic acid (ALA, C18:3n-3), and eicosapentaenoic acid (EPA, C20:5n-3) were present, with the residual pulp having a distinctive n-6/n-3 ratio of less than one. The protein content was around 9% dry weight (dw) in all samples. The residual pulp and *S. polyschides* had the highest polyphenol content, with 4.86 and 4.94 mg GAE·g^{-1} extract, respectively. Hydroethanolic extracts (30–70%) confirmed Fe^{3+} reducing activity (FRAP) in all samples. These findings offer valuable insights on the biochemical composition and bioactivity of the kelp species under study and the residual kelp biomass (pulp), underlining their significant potential for applications in the field of Blue Biotechnology.

Keywords: *Laminaria ochroleuca*; *Macrocystis pyrifera*; *Saccorhiza polyschides*; lipid profile; protein content; antioxidant activity

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1. Introduction

The use of seaweed is ancient, dating back at least to the Neolithic period, over 8000 years ago [1]. Traditionally, seaweeds have been harvested by Asian populations for food and medicinal (or healing) purposes [2]. Only recently have Western European countries become widely interested in the use of seaweed, owing to advances in science and technology that allow the extraction and isolation of valuable natural compounds [3].

Most seaweeds are benthic organisms, attached to a substrate, and are exposed to a variety of abiotic and biotic factors. As a form of protection, these organisms adapt their metabolism by producing a wide range of metabolites. These include proteins, polysaccharides, fatty acids, sterols, pigments, phenolic compounds and many other secondary metabolites, in varying proportions, which can be extracted and used in food matrices, as well as in cosmetics, pharmaceuticals, and other biotechnological applications [4]. The biochemical profile of seaweed depends on many factors such as taxon, geographical location, harvesting season, environmental factors, cultivation conditions, life cycle stage and processing method [5,6]. Among the diverse seaweed species, brown seaweeds (Phaeophyceae) are particularly important for their high-value polysaccharides, such as alginic acid [7]. Alginic acid can provide rigidity and flexibility, allowing seaweed to withstand ocean currents and resist desiccation at low tide. Alginates are widely used as thickening, gelling and stabilizing agents, with applications in various industries: food (salad dressings, ice cream, beer and dairy drinks), cosmetics (shampoo, lipsticks, creams), pharmaceuticals, and even paper and paint production [8,9].

Brown algae are also well known for their content of fucoxanthin, the dominant accessory photosynthetic pigment xanthophyll responsible for their yellowish-brown colour. In addition to fucoxanthin, other carotenoids such as violaxanthin and β-carotene [10,11] can also be found, along with chlorophyll *a* and chlorophylls *c*1 and *c*2. Fucoxanthin has antioxidant, anti-inflammatory, anti-obesity, neuroprotective and anti-cancer properties [9,11], in addition to its potential to replace synthetic colourings in various industries such as food and cosmetics [12].

The brown seaweed *Saccorhiza polyschides* (Figure 1A), known as furbelows, is distinguished by a flattened stipe, with helical and wavy margins in adult individuals, growing from a large bulbous and rough attachment organ and eventually widening into several blades [13]. Unlike true kelps, *S. polyschides* is a semi-annual species, which has earned it the common name "false kelp" [14]. According to Salland et al. [15], the sporophyte phase can last between 12 and 18 months. It is characterized by rapid growth in spring and summer, with the cycle ending in autumn [16]. *S. polyschides* has a wide distribution along the northeastern Atlantic coast and can be found in the shallow intertidal and subtidal zones, up to 19 m deep on rocky bottoms, from Norway to Ghana [17]. It is traditionally known to be edible, has biofertilizer potential [18] and may also have antioxidant and neuroprotective properties [19–21].

(**A**) (**B**) (**C**) (**D**)

Figure 1. Samples of kelp used in this study. (**A**) *Saccorhiza polyschides* (distinctive stipe and bulbous base); (**B**) *Laminaria ochroleuca* (juvenile); (**C**) *Macrocystis pyrifera* by the Kelp Forest Foundation and (**D**) *M. pyrifera* residual pulp.

Laminaria ochroleuca, known as kombu, is an edible seaweed, and related species are widely consumed in Asian countries. It grows to a length of up to 3 m and has a flat blade divided into linear segments, which emerge abruptly from a rigid, smooth, cylindrical stipe, while small rhizoids attach to the substrate (Figure 1B) [17]. A distinctive feature of the species is its yellowish colour. It is a perennial seaweed, being found in temperate waters along the south and north Atlantic coasts (from the south of the UK to Morocco) as well as in the Azores and some areas of the Mediterranean ocean at depths of up to 18 m [22–24]. It is used in culinary dishes to add flavour, and is rich in minerals, particularly magnesium, calcium and iodine. The presence of laminarins gives it anti-rheumatic and anti-inflammatory properties [19].

Macrocystis pyrifera (Figure 1C), known as giant kelp, is a perennial and fast-growing species that can grow to over 40 m in length. This is a unique kelp because of its long, cylindrical, flexible and dichotomously branched stipes. The blades are rough with toothed margins, and with aerocysts at the base [25]. It has a wide geographical distribution, occurring in the southern hemisphere, in temperate zones of the Atlantic Ocean, and in the eastern Pacific Ocean [4]. This macroalgae, successfully cultivated in marine aquaculture systems, is characterised not only by its high productivity but also by its abundance of alginate and phenolic compounds with antioxidant activity [4,26]. The controlled cultivation of *M. pyrifera* enables producers to meet the industrial demand for homogeneous, high-quality biomass, promoting its potential use in high value chains such as nutraceuticals, natural cosmetics and biomedical materials. Besides, being rich in minerals such as iodine and potassium [4], *M. pyrifera* has been used in cosmetic formulations for skin rejuvenation [27], for feed enrichment [28] or as an agricultural biostimulant [29]. Its alginate is used in the food industry as an emulsifier, stabilizer and gelling agent in yoghurt and ice creams [4]. Besides, other applications as a biomaterial are being developed due to the rheological properties of this phycocolloid [30].

Marine macroalgae have attracted increasing interest by the blue economy due to their high productivity, sustainability and abundance of bioactive compounds with multiple biotechnological applications. The aim of this study was to evaluate the biochemical composition and antioxidant activity of *L. ochroleuca* (wild), *S. polyschides* (wild) and *M. pyrifera* (cultivated). Furthermore, the extraction of bio compounds from *M. pyrifera* produces a dark, dry and hard residue with unknown composition and properties (Figure 1D), which was also analysed with a view to future biotechnological applications of these biomasses.

2. Materials and Methods

2.1. Sample Collection and Preparation

Healthy blades of two species of brown algae were harvested in 2023 in central Portugal from several sites in the Peniche region. *Saccorhiza polyschides* was harvested at the end of September, at low tide, on the coast. *Laminaria ochroleuca* was collected in August, between 10 and 17 m deep.

Macrocystis pyrifera was collected in June from offshore cultivation in Lüderitz, Namibia, and provided by the NGO Kelp Forest Foundation (Zeist, The Netherlands). In accordance with the information provided by the NGO, *M. pyrifera* biomass was dried at 40 °C in a ventilated oven. To obtain the insoluble crude fraction residue, the dried biomass was subjected to aqueous extraction at 40 °C. This residue was dried at 40 °C in a ventilated oven (FD115, Binder, Tuttlingen, Germany) for 48 h, then stored for later analysis and evaluation. The solid extract residue was termed ‘residual pulp’. To simplify handling and analysis, the residual pulp was ground in a food processor (Vorwerk, Thermomixer 31-1, Wuppertal, Germany). The harvesting season and the biomass collection coordinates for each species are shown in Table 1.

Table 1. List of species studied, harvesting season and coordinates of the harvest site.

Species	Order	Harvest Season	Harvest Location	Coordinates
Macrocystis pyrifera (L.) C. Agardh 1820	Laminariales	Winter	Lüderitz, Namibia	26°38′15.8″ S 15°06′17.5″ E
Laminaria ochroleuca Bachelot de la Pylaie 1824	Laminariales	Summer	Peniche Coast, Peniche, Portugal	39°21′31.00″ N 9°21′59.05″ W
Saccorhiza polyschides (Lightfoot) Batters 1902	Tilopteridales	Summer	Casais do Baleal, Peniche, Portugal	39°22′29.61″ N 9°19′36.00″ W

Fresh samples of *S. polyschides* and *L. ochroleuca* were washed with seawater to remove impurities and epibionts and then rinsed with distilled water. Part of the sample was stored at −20 °C. The remaining sample was dried at 40 °C for 48 h in a ventilated oven (Memmert, IF110, Wuppertal, Germany). The dried samples were then ground to 200 µm, using a food processor (Breville, VBL120X Personal Active Pro, Cheadle, UK) and a grinder (SilverCrest, SKMS 180 A1, Neckarsulm, Germany). Finally, the samples were stored at −20 °C until further use.

Photographs were taken using an optical microscope (Zeiss, Axio Lab. A1, Oberkochen, Germany) equipped with a 5 MP digital colour microphotography camera (Zeiss, AxioCam 105, Oberkochen, Germany) to determine differences in the cellular structure of the *M. pyrifera* sample and the 'residual pulp'.

2.2. Biochemical Profiling Methodology

2.2.1. Ash Content and Moisture Percentage

The AOAC method was used to determine the moisture and ash content of the samples [31]. Briefly, the fresh biomass was oven-dried (Memmert, UF110, Büchenbach, Germany) at 105 °C for 48 h, and then allowed to cool to a constant weight. Moisture content was expressed as a percentage of fresh weight (fw). Ash content was determined after burning 1 g of four replicate samples in a muffle furnace (Nabertherm, B170, Lilienthal, Germany) with a heating ramp of 4 h and a plateau of 5 h at 525 °C and then allowing it to cool down. The ash content (as a percentage of dry weight) was then calculated.

2.2.2. Protein Content

Protein content was determined by the Kjeldahl method. Dried 0.5 g triplicates of each sample were placed in a digestion tube containing a catalyst tablet (VWR Chemicals, Radnor, PA, USA) and 25 mL of 97% sulphuric acid (Honeywell/Fluka, 30743, Charlotte, NC, USA), with a blank control using 1 mL of distilled water. The mixture was digested (J.P. Select bloc digest 6, Abrera, Spain) with sequential heat treatments: one at 220 °C for 30 min and the other at 400 °C for 90 min. After cooling the tubes, 8 mL of distilled water and 100 mL of sodium hydroxide were added to the distillation apparatus (Velp Scientifica, UDK 139, Usmate Velate, Italy). The distillate was collected in 30 mL of 4% boric acid and titrated with 0.1 M hydrochloric acid. The protein content was calculated as follows:

$$\%\ \text{Protein}\ (\%\ \text{dry weight}) = \frac{[(Vs - Vb) \times n \times cf \times 0.014]}{W} \times 100\%$$

where Vs is the volume of titrant used for the sample (mL), Vb is the volume of titrant used for the blank (mL), n is the concentration of HCl used in the titration (0.1 M), W is the initial sample mass (g), and cf is the conversion factor for seaweeds (5, according to Angell et al. [32]).

2.2.3. Total Lipids

Quantification of total lipids in the samples was performed according to Bligh and Dyer [33] with the adaptations by Coen et al. [34]. Briefly, dried samples (≈13 mg) were homogenized with 150 µL of a chloroform:methanol:water (1:1:1) mixture and centrifuged at 8000× *g* at 4 °C for 10 min to allow phase separation. The lower organic phase was recovered in a weighed evaporation flask through an anhydrous sodium sulphate column. Then, 100 mL of the sample was mixed with 500 mL of 97% sulphuric acid and heated at 200 °C for 15 min (Memmert, UF110, Büchenbach, Germany). After cooling, 1.5 mL of ultrapure water was carefully added to the mixture. Finally, 200 µL aliquots (in triplicate) were transferred to a 96 flat-bottom microplate (Greiner Bio-one, 655101, Frickenhausen, Germany) and the absorbance of the samples was read at 375 nm (Synergy H1 Hybrid Reader, BioTek® Instruments, Winooski, VT, USA). The absorbance of the samples was compared with the absorbance of a calibration curve using tripalmitin at 3.2 mg·mL^{-1} as a standard (Sigma–Aldrich, T5888, Darmstadt, Alemanha). Lipid content results were expressed as a percentage of dry weight (% dw).

2.2.4. Fatty Acid Profile Determination

The fatty acid (FA) profile of the samples was analysed by gas chromatography (GC). FA methyl esters (FAME) were obtained by direct acid transmethylation according to Fernández et al. [35]. Dried samples (≈50 mg) were mixed with 2 mL of methanol containing 2% sulphuric acid and heated at 80 °C (Memmert, OB14, Schwabach, Germany) for 2 h. Afterwards, 1 mL of ultrapure water and 2 mL of n-hexane (Fisher Scientific/0355/15, Waltham, MA, USA) were added to the mixture, stirred, and centrifuged at 1500× *g* for 5 min to separate the phases.

Finally, the upper hexane phase was recovered and analysed on a GC (Finnigan Ultra Trace Thermo Scientific, Waltham, MA, USA) equipped with a Thermo TR-FAME capillary column (60 m × 0.25 mm ID, 0.25 µm film thickness), an autosampler (AS 3000, Thermo Electron Corporation, Waltham, MA, USA) and a flame ionization detector (FID). Helium (1.5 mL/min) was used as the carrier gas. XCaliburTM software (Xcalibur 4.2., Thermo Scientific, Waltham, MA, USA) was used to determine the integration and peak areas. The FA content was expressed as a percentage of the total peak area (% Total FA).

2.2.5. Pigment Analysis

Photosynthetic pigments were extracted according to the methods defined by Osório et al. [36] and both fresh and dried samples were analysed. Dry powdered samples were used outright, and fresh samples were crushed in a mortar and pestle for 10 min. Fucoxanthin was extracted from both powdered and fresh samples, using a 4:1 (*v*/*v*) solution of dimethyl sulfoxide (DMSO) and ultrapure water. Chlorophyll *a* and chlorophylls *c1* and *c2* were extracted in combination from both powder and fresh samples using 90% acetone as a solvent.

For the quantification, 0.5 g of the seaweed was weighed and a 1:20 sample/solvent ratio was used. Both fresh and dried samples were transferred to a beaker with the solvent and stirred for 30 min on a digital multi-position magnetic stirrer (OVAN, MM90E, Barcelona, Spain). The sample was centrifuged at 8000× *g* for 20 min at 4 °C and then filtered through 1.2 µm glass fibre filters (VWR, 516-0869, Stockholm, Sweden). Finally, the absorbance was read in a UV–VIS spectrophotometer between 200 nm and 900 nm (Thermo Scientific, Evolution 201, Waltham, MA, USA). The following equations were used to calculate the concentrations of fucoxanthin, chlorophyll *a* and chlorophylls *c*, and the results were expressed as µg·g^{-1} [36–38]:

Fucoxanthin (μg/g) = 7.69 × (A480 − A750) − 5.5 × [(A631 − A750) + (A582 − A750) − 0.297 × (A665 − A750)] − 0.377 × (A665 − A750)

Chlorophyll *a* (μg/g) = 0.3319 × (A630 − A750) − 1.7495 × (A647 − A750) + 11.9442 × (A664 − A750) − 1.4306 × (A691 − A750) (±0.0020)

Chlorophylls *c* (μg/g) = 23.5902 × (A630 − A750) − 7.8516 × (A647 − A750) − 1.5214 × (A664 − A750) − 1.7443 × (A691 − A750) (±0.0075)

2.2.6. Quantification of Elements

Dry samples were used to quantify elements' content by X-ray fluorescence spectrometry (XRF). To ensure complete removal of moisture, the samples were placed in beakers suitable for XRF analysis and subjected to additional drying in a ventilated oven (Binder, FD23, Germany) at 60 °C for 48 h. Quantification was performed by XRF (Bruker S1 Titan, Kennewick, WA, USA), with readings taken in triplicate. Results were expressed as $mg \cdot g^{-1}$ (dw).

2.2.7. Alginate Extraction and Analysis

Alginate was extracted by adapting the method described by Trica et al. [39]. Briefly, 2 g of dried seaweed underwent a mild depigmentation in 20 mL of 70% ethanol (*v*/*v*) at room temperature for 24 h. The residual EtOH was removed by vacuum filtration (Prat Dumas, A125108, 10–12 μm, Couze-et-Saint-Front, France) and 100 mL of 0.2 M hydrochloric acid was added at room temperature and allowed to stand for 24 h. The samples were then treated with 2% calcium carbonate for the alkaline treatment and the alginate was extracted at different temperatures (25 °C, 60 °C and 80 °C) for 5 h in a water bath (Memmert, OB14, Schwabach, Germany). The alginate was precipitated by adding 2 volumes of EtOH (96%) at 4 °C, overnight. The precipitate was oven dried at 60 °C (Memmert, IF110, Büchenbach, Germany) for 48 h, and then ground in a mortar and a grinder. The alginates were analysed by Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer, Spectrum II, Buckinghamshire, PA, USA), with 64 scans, a resolution of 8 cm^{-1} and a spectral range of 4000–400 cm^{-1}. Commercial alginate (Farmaquimica Sur, Málaga, Spain) was used as a standard to validate the results of the extracted alginates.

2.3. Antioxidant Activity

2.3.1. Hydroethanolic Extraction

Hydroethanolic extraction was performed according to a dry biomass:solvent ratio of 1:10 ($g \cdot mL^{-1}$). The powdered dried extracts (50 g) were mixed in ethanol:water (70:30) at a concentration of 100 $mg \cdot mL^{-1}$. The extracts were sonicated for 15 min and kept under constant stirring for 8 h at 40 °C, protected from light. The extract was then centrifuged at 8000× *g* for 10 min at room temperature and the supernatant was freeze-dried and stored in a desiccator at room temperature until use.

2.3.2. Total Phenolic Content Assay (TPC)

The TPC assay was performed by adapting the Folin–Ciocalteu method of Singleton and Rossi [40] to microscale. Briefly, 30 μL of the hydroethanolic extracts were added to 770 μL of ultrapure water and 50 μL of Folin–Ciocalteu reagent (Sigma–Aldrich, F9252, Darmstadt, IN, USA), and the mixture was allowed to stand for 5 min at room temperature in the dark. Then, 150 μL of sodium carbonate (20% *w*/*v*) (ChemLab, CL00.1431.1000, Zedelgem, Belgium) was added and incubated for 1 h at room temperature in the dark. Then, 200 μL of each sample was transferred to flat-bottomed microplates and left to incubate for 30 min at room temperature in the dark. Absorbance was read at 755 nm (Synergy H1 Hybrid Reader, BioTek® Instruments, Winooski, VT, USA). The results were expressed as gallic acid equivalents ($mg\ GAE \cdot g^{-1}$ crude extract).

2.3.3. Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant activity of the hydroethanolic extracts was determined by a microscale adapted FRAP assay [41]. The FRAP reagent was prepared using 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) solution and 20 mM ferric chloride in a 10:1:1 ratio. Then, 30 µL of the sample and 900 µL of FRAP reagent were added. The tubes were incubated for 15 min at 37 °C, followed by 5 min in the dark at room temperature. Then, 200 µL of each sample was added to the microplate and the absorbance was read at 593 nm in a microplate reader (Synergy H1 Hybrid Reader, BioTek® Instruments, Winooski, VT, USA). The results were expressed as ferrous sulphate equivalents in mmol·100 mg^{-1} of sample.

2.4. Statistical Analysis

Results are presented as mean values ± standard deviations (SDs). Whenever homogeneity and normality tests were validated, the results were analysed using Student's *t*-tests, one-way analysis of variance (ANOVA) or two-way ANOVA, followed by a post hoc Tukey's Honest Significant Difference (HSD) test. Otherwise, the non-parametric Kruskal–Wallis test was used for the comparisons described. Statistically significant differences were considered when the *p*-value was <0.05. The statistical analysis was performed using the software SPSS Statistics 29 software (IBM Corporation, New York, NY, USA).

3. Results

3.1. Biochemical Profiling

3.1.1. Proximate Composition

The fresh seaweed had a high moisture content, with the highest value of 79.74 ± 0.50% fw recorded for *Saccorhiza polyschides*, while *Laminaria ochroleuca* had a significantly lower value (73.35 ± 1.36% fw) (Table 2). The ash content of the dried samples was higher for *Macrocystis pyrifera* and for the residual pulp, with 48.94 ± 0.70% and 28.56 ± 0.25% total dry weight (dw), respectively. The dried samples of *S. polyschides* and *L. ochroleuca* showed an ash content of 20.01 ± 0.05% dw and 22.03 ± 0.60% dw, respectively. As to the protein content, the four samples analysed had a similar result, with a small variation between 8.9 and 9.2%. *Saccorhiza polyschides* had the highest lipid content (3.41 ± 0.46% dw), followed by *L. ochroleuca* and the residual pulp (2.79 ± 0.25% and 2.37 ± 0.21% dw, respectively), while *M. pyrifera* had the lowest lipid content (1.28 ± 0.19% dw). *L. ochroleuca* had the highest carbohydrate content (56.03%), closely followed by *S. polyschides* (55.42%), whereas residual pulp had a lower value (46.41 ± 0.28%), and *M. pyrifera* the lowest carbohydrate content (29.17 ± 0.71%).

Table 2. Proximate composition of the three seaweeds and kelp pulp, expressed as percentage of fresh weight (fw) for moisture, and dry weight (dw) for other parameters. Values are presented as mean ± standard deviation (n = 4). Different superscript lowercase letters ($^{a, b, c, d}$) indicate statistically significant differences between different samples for a p-value < 0.05.

	L. ochroleuca	*S. polyschides*	*M. pyrifera*	Residual Pulp
Moisture (% fw)	73.35 ± 1.36 ^{b}	79.74 ± 0.50 ^{a}	-	-
Ash (% dw)	22.03 ± 0.60 ^{c}	20.01 ± 0.05 ^{d}	48.94 ± 0.70 ^{a}	28.56 ± 0.25 ^{b}
Proteins (% dw)	8.93 ± 0.16	8.97 ± 0.27	9.20 ± 0.04	9.08 ± 0.02
Lipids (% dw)	2.79 ± 0.25 $^{a, b}$	3.41 ± 0.46 ^{a}	1.28 ± 0.19 ^{c}	2.37 ± 0.21 ^{b}
Carbohydrates [1] (% dw)	56.03 ± 1.33 ^{a}	55.42 ± 0.30 ^{a}	29.17 ± 0.71 ^{c}	46.41 ± 0.28 ^{b}

[1] Following AOAC, carbohydrates were determined by subtraction as: Total carbohydrates (%) = 100% − (% water + % protein + % ash + % lipid).

3.1.2. Fatty Acid Profile

The fatty acid (FA) profile of the samples is shown in Table 3. Palmitic acid (C16:0) was the most abundant fatty acid in all samples, reaching levels up to 40.25 ± 1.37% of *S. polyschides*. Thus, total saturated fatty acids (SFA) were most abundant in *S. polyschides* (51.57 ± 1.57%), followed by *L. ochroleuca* (43.45 ± 0.59%).

Table 3. Percentage of total fatty acids in the three seaweeds and residual pulp. Values are presented as mean ± standard deviation (n = 3). SFA—saturated fatty acids, MUFA—monounsaturated fatty acids, PUFA—polyunsaturated fatty acids, ALA—linolenic acid, ARA—arachidonic acid, EPA—eicosapentaenoic acid, DHA—docosahexaenoic acid. Different superscript lowercase letters ($^{a, b, c, d}$) indicate statistically significant differences between samples for a p-value < 0.05.

	L. ochroleuca	*S. polyschides*	*M. pyrifera*	Residual Pulp
SFA (% Total FA)				
C14:0	6.53 ± 0.56 ^{c}	5.31 ± 0.50 ^{c}	13.29 ± 0.66 ^{a}	9.71 ± 0.82 ^{b}
C15:0	0.73 ± 0.05 ^{a}	0.49 ± 0.02 ^{b}	0.42 ± 0.02 ^{b}	0.43 ± 0.03 ^{b}
C16:0	33.9 ± 0.40 ^{b}	40.25 ± 1.37 ^{a}	21.50 ± 0.34 ^{c}	18.54 ± 0.47 ^{d}
C17:0	0.16 ± 0.01 ^{b}	0.26 ± 0.00 ^{a}	0.19 ± 0.04 $^{a, b}$	0.19 ± 0.04 $^{a, b}$
C18:0	1.43 ± 0.04 ^{b}	3.07 ± 0.12 ^{a}	1.70 ± 0.78 ^{b}	1.46 ± 0.53 ^{b}
C20:0	0.32 ± 0.01 ^{b}	1.97 ± 0.22 ^{a}	0.27 ± 0.03 ^{b}	0.32 ± 0.03 ^{b}
∑ Total SFA	43.45 ± 0.59	51.57 ± 1.57	37.66 ± 1.52	30.91 ± 0.79
MUFA (% Total FA)				
C16:1 n-7	3.58 ± 0.03 ^{a}	1.49 ± 0.06 ^{d}	1.82 ± 0.06 ^{b}	1.65 ± 0.06 ^{c}
C18:1 n-9	17.74 ± 2.09 ^{a}	14.81 ± 0.33 ^{b}	12.11 ± 0.55 ^{b}	8.64 ± 0.11 ^{c}
C20:1 n-9	6.23 ± 0.06 ^{c}	3.13 ± 0.04 ^{d}	9.07 ± 0.15 ^{b}	15.56 ± 0.10 ^{a}
∑ Total MUFA	30.79 ± 0.97	23.35 ± 0.35	28.14 ± 0.94	31.07 ± 0.30
PUFA (% Total FA)				
C18:2 n-6 cis	6.27 ± 0.12 ^{b}	9.41 ± 0.24 ^{a}	5.59 ± 0.06 ^{c}	5.53 ± 0.05 ^{c}
C18:3 n-3 (ALA)	3.38 ± 0.10 ^{c}	2.54 ± 0.06 ^{d}	5.68 ± 0.04 ^{b}	8.54 ± 0.06 ^{a}
C20:4 n-6 (ARA)	8.64 ± 0.17 ^{c}	9.24 ± 0.72 ^{c}	14.55 ± 0.50 ^{a}	10.70 ± 0.52 ^{b}
C20:5 n-3 (EPA)	5.90 ± 0.18 ^{c}	2.49 ± 0.15 ^{d}	6.80 ± 0.16 ^{b}	11.14 ± 0.50 ^{a}
C22:6 n-3 (DHA)	n.d.	0.02 ± 0.01 ^{a}	0.14 ± 0.02 ^{a}	0.11 ± 0.02 ^{a}
∑ Other PUFA	0.27 ± 0.03	0.22 ± 0.01	0.59 ± 0.07	0.92 ± 0.08
∑ Total PUFA	25.77 ± 0.60	25.08 ± 1.24	34.20 ± 0.68	38.01 ± 1.05
n-3/n-6	1.75 ± 0.02	3.92 ± 0.03	1.66 ± 0.02	0.87 ± 0.01

The most abundant monounsaturated fatty acid (MUFA) was oleic acid (C18:1 n-9), ranging from 8.64% (residual pulp) to 17.74 ± 2.09% (*L. ochroleuca*). Among the polyunsaturated fatty acids, α-linolenic acid (ALA) was predominant in the residual pulp (8.54 ± 0.06%) and was the lowest in *S. polyschides* (2.54 ± 0.06%). Arachidonic acid (ARA) was the highest in *M. pyrifera* followed by the residual pulp: 14.55 ± 0.50% and 10.70 ± 0.52%, respectively. Eicosapentaenoic acid (EPA, C20:5) was found in the residual pulp at 11.14%. Total polyunsaturated fatty acid (PUFA) ranged from 25.08 ± 1.24% in the *S. polyschides* sample to 38.01 ± 1.05% in the residual pulp. The amount of n-3 fatty acids (n3) varied among the samples, with the highest value in the residual pulp (19.86 ± 0.55%) and the lowest in *S. polyschides* (5.05 ± 0.22%). The amount of n-6 fatty acids also varied, reaching the highest value in *M. pyrifera* (21.18 ± 0.74%) and the lowest in *L. ochroleuca* (16.22 ± 0.03%). As for docosahexaenoic acid (DHA, C22:6n-3), its concentration varied between 0.02 ± 0.01% (*S. polyschides*) and 0.14 ± 0.02% (*M. pyrifera*), and it was not detected in *L. ochroleuca*. Total PUFA ranged from 25.08 ± 1.24% in *S. polyschides* to 38.01 ± 1.05% in the residual pulp. The amount of omega-3 fatty acids (n3) varied among the samples, with the highest value in the residual pulp (19.86 ± 0.55%) and the lowest in *S. polyschides* (5.05 ± 0.22%). The

amount of omega-6 fatty acids also varied significantly, reaching the highest value in *M. pyrifera* (21.18 ± 0.74%) and the lowest in *L. ochroleuca* (16.22% ± 0.29%). The values of the n6/n3 ratio were greater than one in all the samples, except for the residual pulp, which had a ratio of 0.87 ± 0.01.

3.1.3. Pigment Contents

Chlorophyll *a* was the most abundant pigment present in the fresh biomasses, with *S. polyschides* yielding 4218.74 ± 93.53 μg·g^{-1} fw and *L. ochroleuca* yielding 2599.38 ± 701.51 μg·g^{-1} fw. Regarding the results for chlorophylls *c*, fresh *S. polyschides* showed a concentration of 965.62 ± 127.39 μg·g^{-1} fw and *L. ochroleuca* 681.34 ± 159.54 μg·g^{-1} fw. Regarding fucoxanthin, the fresh *S. polyschides* yielded a result of 695.48 ± 22.51 μg·g^{-1} fw, followed by the fresh *L. ochroleuca* sample, which yielded a result of 439.93 ± 30.32 μg·g^{-1} fw (Table 4).

Table 4. Concentrations of fucoxanthin, chlorophyll *a* and chlorophyll *c* in fresh and dried samples of the three seaweed species under study and the residual kelp pulp. Data are presented as mean ± standard deviation (n = 3). Different superscript lowercase letters ($^{a, b, c, d}$) indicate statistically significant differences between different samples for p-value < 0.05.

	L. ochroleuca	***S. polyschides***	***M. pyrifera***	**Residual Pulp**
Fresh Samples (μg·g^{-1} fw)				
Fucoxanthin	439.93 ± 30.32 ^{b}	695.48 ± 22.51 ^{a}	-	-
Chlorophyll *a*	2599.38 ± 701.51 ^{b}	4218.74 ± 93.53 ^{a}	-	-
Chlorophyll *c*	681.34 ± 159.54 ^{b}	965.62 ± 127.39 ^{a}	-	-
Dried Samples (μg·g^{-1} dw)				
Fucoxanthin	142.12 ± 3.31 ^{b}	111.34 ± 4.99 ^{c}	56.71 ± 1.38 ^{d}	157.52 ± 2.71 ^{a}
Chlorophyll *a*	26.59 ± 1.93 ^{a}	22.18 ± 2.47 $^{a, b}$	19.27 ± 3.17 ^{b}	16.63 ± 2.33 ^{b}
Chlorophyll *c*	2.95 ± 0.09 ^{a}	2.66 ± 0.32 ^{a}	0.69 ± 0.07 ^{b}	0.15 ± 0.08 ^{c}

The dried biomass of the four samples was also analysed, with much lower concentrations than the fresh biomass. The highest fucoxanthin concentration was obtained in the residual pulp, which had an average value of 157.528 ± 2.717 μg·g^{-1} dw, while *M. pyrifera* had the lowest fucoxanthin concentration (56.71 ± 1.38 μg·g^{-1} dw). The concentration of chlorophyll *a* was dominant in *L. ochroleuca* (26.59 ± 1.93 μg·g^{-1} dw) and the remaining samples had values ranging from 16.63 ± 2.33 μg·g^{-1} dw (in the residual pulp) to 22.18 ± 2.469 μg·g^{-1} dw (*S. polyschides*). The concentration of chlorophylls *c* was highest in *L. ochroleuca* (2.95 ± 0.09 μg·g^{-1} dw), followed by *S. polyschides* (2.66 ± 0.03 μg·g^{-1} dw), *M. pyrifera* (0.69 ± 0.07 μg·g^{-1} dw) and the residual pulp (0.15 ± 0.08 μg·g^{-1} dw).

3.1.4. Elemental Composition

The phosphorus, chlorine and potassium contents varied significantly among the samples, with *M. pyrifera* and the residual pulp standing out with the highest values. The phosphorus content was 5.29 ± 0.03 mg·g^{-1} in *M. pyrifera* and 4.05 ± 0.26 mg·g^{-1} in the residual pulp. Chlorine showed values of 77.97 ± 0.65 mg·g^{-1} in *M. pyrifera* and 22.67 ± 1.95 mg·g^{-1} in *S. polyschides*, also with statistically significant differences ($H^2(3) = 9.974$, $p < 0.019$). As for potassium, the highest concentration was found in *M. pyrifera* (67.72 ± 0.62 mg·g^{-1}) and the lowest in *S. polyschides* (6.03 ± 0.44 mg·g^{-1}). Calcium showed the lowest concentration in *M. pyrifera* with 3.61 ± 0.05 mg·g^{-1} and the highest in *S. polyschides* with 11.7 ± 0.74 mg·g^{-1}. As far as the microelements are concerned, again there were large variations among samples, with iron showing the highest value in *M. pyrifera* with 646.33 ± 143.79 μg·g^{-1}, zinc registering the highest concentration in *S. polyschides* with 33.33 ± 2.52 μg·g^{-1}, while copper and arsenic showed the highest values in *M. pyrifera*

with 8.00 ± 1.00 μg·g^{-1} and with 55.67 ± 4.16 μg·g^{-1}, respectively. It should also be noted that arsenic was detected at high concentrations in all samples (Table 5).

Table 5. The relative percentages of macroelements (mg·g^{-1}) and microelements (μg·g^{-1}) in the three seaweed species and residual pulp. The results are reported as mean ± standard deviation (n = 3). Different superscript lowercase letters ($^{a, b, c}$) indicate statistically significant differences between different samples for a p-value < 0.05.

	L. ochroleuca	*S. polyschides*	*M. pyrifera*	Residual Pulp
Macroelements (mg·g $^{-1}$)				
Phosphorus (P)	1.18 ± 0.05 $^{b, c}$	0.83 ± 0.02 ^{c}	5.29 ± 0.03 ^{a}	4.05 ± 0.26 $^{a, b}$
Sulphur (S)	8.40 ± 0.18 ^{a}	7.50 ± 0.15 $^{a, b}$	0.59 ± 0.08 ^{c}	2.55 ± 0,13 $^{b, c}$
Chlorine (Cl)	24.84 ± 0.86 ^{b}	22.67 ± 1.95 ^{b}	77.97 ± 0.65 ^{a}	39.84 ± 0.62 $^{a, b}$
Potassium (K)	22.34 ± 0.35 $^{b, c}$	6.03 ± 0.44 ^{c}	67.72 ± 0.62 ^{a}	56.07 ± 0.70 $^{a, b}$
Calcium (Ca)	7.80 ± 0.32 $^{a, b}$	11.72 ± 0.74 ^{a}	3.61 ± 0.05 ^{c}	7.18 ± 0.11 $^{b, c}$
Microelements (μg·g^{-1})				
Iron (Fe)	72.67 ± 15.1 ^{c}	137.67 ± 10.50 $^{b, c}$	646.33 ± 143.79 ^{a}	229.00 ± 6.56 $^{a, b}$
Zinc (Zn)	15.67 ± 1.15 ^{c}	33.33 ± 2.52 ^{a}	24.67 ± 0.58 $^{a, b}$	22.00 ± 1.00 $^{b, c}$
Copper (Cu)	5.00 ± 0,00 ^{b}	3.67 ± 0.58 $^{b, c}$	8.00 ± 1.00 ^{a}	3.67 ± 0.58 ^{c}
Arsenic (As)	40.00 ± 1.73 $^{a, b}$	12.67 ± 1.53 ^{c}	55.67 ± 4.16 ^{a}	26.00 ± 1.00 $^{b, c}$

3.1.5. Alginate Content

The sodium alginate yield of the samples is presented in Table 6 and shows significant differences among the samples. The highest value was found in the residual pulp extracted at 60 °C (51.04 ± 0.75% dw) and *S. polyschides* extracted at 80 °C, while the lowest value was found in *L. ochroleuca* extracted at 25 °C (7.33 ± 0.29% dw).

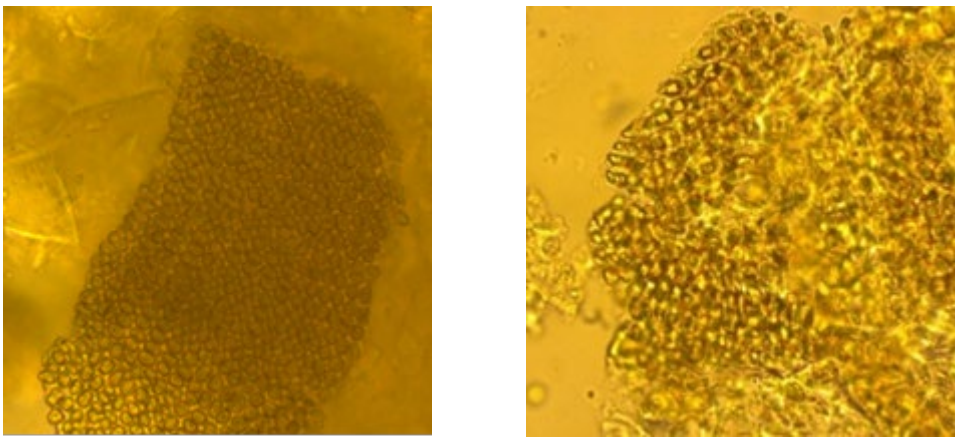
Table 6. Alginate extraction yields as % of dry matter (dw) from the three seaweeds and the residual kelp pulp after alkaline extraction at different temperatures. Values are presented in % of dry matter, as mean ± standard deviation (n = 3). Different superscript lowercase letters ($^{a, b, c, d}$) indicate statistically significant differences between samples for a p-value < 0.05.

	L. ochroleuca	*S. polyschides*	*M. pyrifera*	Residual Pulp
25 °C	7.33 ± 0.29 ^{d}	22.67 ± 0.74 ^{b}	13.34 ± 0.66 ^{c}	41.37 ± 0.50 ^{a}
60 °C	13.61 ± 0.71 ^{d}	33.32 ± 0.73 ^{b}	28.32 ± 1.05 ^{c}	51.04 ± 0.75 ^{a}
80 °C	29.91 ± 0.75 ^{c}	46.71 ± 1.02 ^{a}	31.63 ± 0.93 ^{c}	38.85 ± 0.43 ^{b}

Microscopic examination indicated that the cell wall architecture of *M. pyrifera* (Figure 2A) remained relatively intact and well-organized, in contrast to the residual pulp biomass (Figure 2B), which exhibited significant structural disruption.

Figure 3 shows the FTIR spectra obtained from the analysis of alginates extracted from different samples. The presence of water in alginate samples is related to their hygroscopicity, which means that they tend to absorb moisture from their surroundings. The broad bands at 1600–1610 cm^{-1} have been suggested to be the asymmetric stretching of the carboxylate (O–C–O). The peak between 1400 and 1454 cm^{-1} is attributed to the C–OH deformation vibration [42]. The sharp peak between 1020 and 1040 cm^{-1} can be attributed to the elongation of the C–O groups [43]. At 1200 cm^{-1}, there are slight peaks that may be related to the vibration of the sulphate group (SO_3) present in sulphated polysaccharides [9]. Between 848 and 880 cm^{-1}, the peak corresponds to the deformation vibration of the bond between carbon 1 and hydrogen (C1–H) of the mannuronic acid residues, while at 940 cm^{-1}, the peak is associated with the C–O stretching of the guluronic acid residues [42]. Our results also show that there are some differences in the peaks between the extracted

alginates and the commercial alginate, namely some noise below 800 cm^{-1}, which could be related to other compounds in the samples [9].



(**A**) (**B**)

Figure 2. Optical microscope view of (**A**) *Macrocystis pyrifera* and (**B**) residual pulp (total magnification 400×).

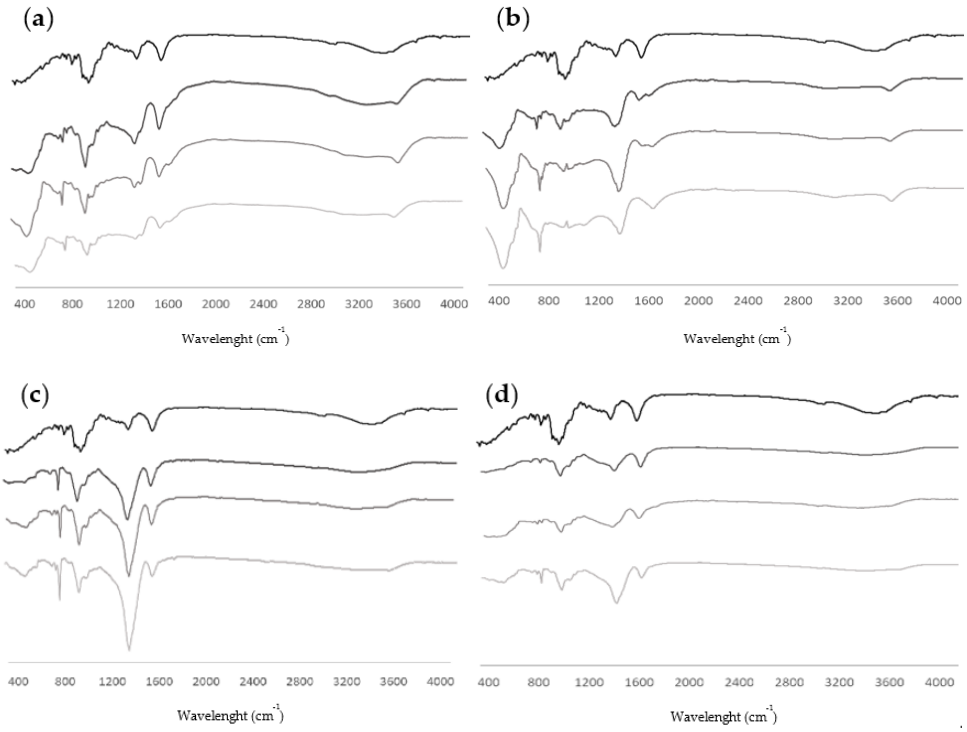


Figure 3. FTIR spectra of sodium alginate from the three seaweed species and residual pulp, extracted at different temperatures (25 °C, 60 °C and 80 °C), (**a**) *Laminaria ochroleuca*, (**b**) *Saccorhiza polyschides*, (**c**) *Macrocystis pyrifera*, (**d**) residual pulp. In each of the spectra, the four traces, correspond from top to down, to the following: commercial alginate standard, extraction at 80 °C, extraction at 60 °C and extraction at 25 °C.

3.2. Antioxidant Capacity

3.2.1. Total Phenolic Content (TPC)

The TPC determined for the three seaweeds and pulp ranged from 2.47 ± 0.33 mg GAE·g^{-1} (*L. ochroleuca*) to 4.94 ± 0.31 mg GAE·g^{-1} (*S. polyschides*), with significant differences among samples (Figure 4). The seaweed *M. pyrifera* and its residual pulp had an intermediate TPC content with 3.36 ± 0.13 mg GAE·g^{-1} and 4.86 ± 0.12 mg GAE·g^{-1}, respectively.

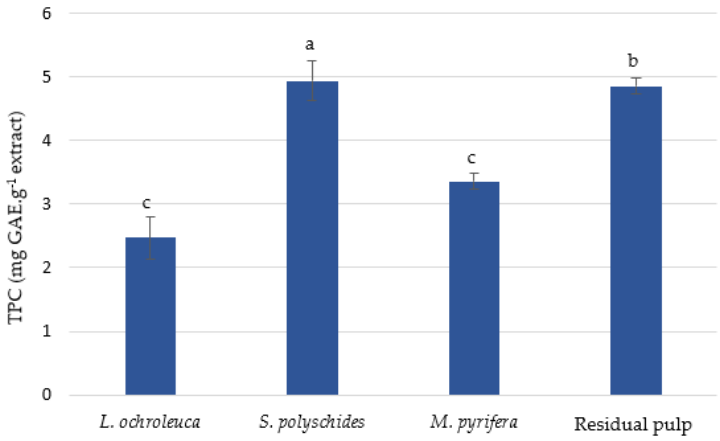


Figure 4. Total phenolic content (TPC), expressed as mg GAE·g^{-1}, in hydroethanolic kelp extracts. Data are presented as mean ± standard deviation (n = 3). Different letters above the bars indicate statistically significant differences between the extracts ($p < 0.05$).

3.2.2. Ferric Reducing Antioxidant Power (FRAP)

The residual pulp had the highest reduction potential of 61.27 ± 3.41 mmol eq Fe·g^{-1} extract, significantly higher than all the other samples, followed by *S. polyschides* with a reduction potential of 49.30 ± 5.03 mmol eq Fe·g^{-1} extract. A lower value was recorded for *M. pyrifera*, which had a potential of 34.09 ± 5.79 mmol eq Fe·g^{-1} extract, while *L. ochroleuca* showed the lowest content, 32.21 ± 5.38 mmol eq Fe·g^{-1} extract (Figure 5).

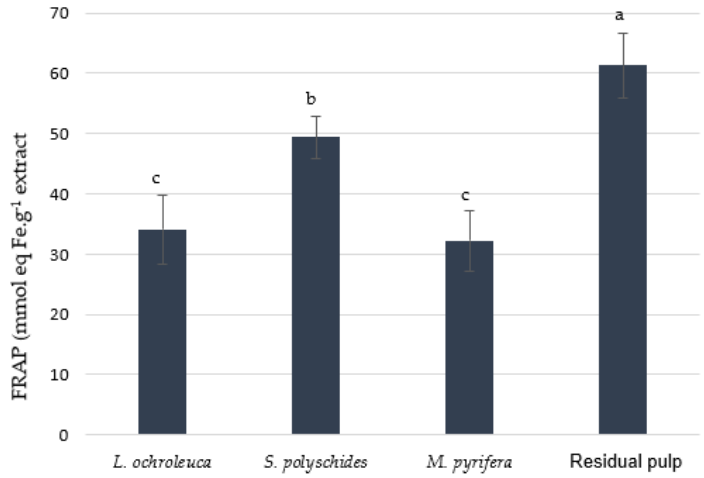


Figure 5. Ferric reducing antioxidant power (FRAP), expressed as mmol Fe^{2+} equivalents·g^{-1} of extract, in hydroethanolic kelp extracts. Data are presented as mean ± standard deviation (n = 3). Different letters above the bars indicate statistically significant differences between the extracts ($p < 0.05$).

4. Discussion

Marine seaweeds have a rich and diverse biochemical profile, containing primary and secondary metabolites such as pigments, phenolic compounds, polysaccharides, fatty acids and proteins. These compounds can be used in the development of various innovative products in many industries, particularly in the food industry. Abiotic factors such as changing nutrient levels, UV radiation, temperature, and salinity can cause environmental stress, leading to metabolic changes and variations in metabolites' production. Therefore, the biochemical profile of seaweed changes in response to these stressors [44].

Given that the aim of this study was to evaluate the biochemical composition and antioxidant activity of *L. ochroleuca* (wild), *S. polyschides* (wild), *M. pyrifera* (cultivated) and the residual pulp of cultivated *M. pyrifera*, the results show differences among the

species, which is in line with previously published data demonstrating the highly diverse biochemical composition of brown seaweeds. The fresh biomass of *Laminaria ochroleuca* and *Saccorhiza polyschides* had a high moisture content, as is usual for seaweeds, in line with or slightly higher than the values found by other authors (85–94%) [44–46]. The ash content represents the inorganic content of the sample [35]. For *S. polyschides*, large discrepancies were found between our study and previously published studies (20–44.6% dw) [44–46], indicating large environmental variations in nutrient availability between sampling sites. The remarkably high ash content found in *M. pyrifera* (48.94% dw) cultivated in Lüderitz, Namibia is even higher than that found by Lamare and Wing [36], indicating a high concentration of mineral salts and inorganic elements in the dry biomass. This may be related to the high availability of inorganic elements in the marine environment due to the Benguela Current and the seawater hydrodynamics that mobilise minerals by suspending them in seawater, making them available for absorption by the seaweed [47,48]. Finally, the reduction in the inorganic matter content of the pulp may be due to the previous aqueous extraction process. The work of Cebrián-Lloret et al. [49] showed the same downward trend during phycocolloid extraction, suggesting that inorganic matter may be lost to the liquid fraction during extraction.

The protein content of brown macroalgae usually varies between 5% and 20% of dry weight (dw), depending on species, season, and environmental conditions [29,50,51]. Specifically, *S. polyschides* has been reported to contain between 7% and 18% dw protein [52], *L. ochroleuca* exhibits values ranging from 7.5% to 18% [44], while *M. pyrifera* is known to have a protein content between 5% and 15% of its dry weight [53,54]. Our results are in line with these authors, demonstrating that seaweed biomass may be an interesting alternative protein source, suitable as a functional or supplementary food. This is particularly relevant given the growing global demand for sustainable protein sources to reduce reliance on animal-derived products.

Although brown algae tend to have a lower average protein content than red or green algae, their nutritional and functional potential remains substantial [51]. Beyond quantity, protein quality, including the essential amino acid profile, digestibility, and presence of bioactive peptides, plays a critical role in evaluating their effective value [50,55]. Several studies have demonstrated that proteins and hydrolysates derived from macroalgae exhibit antioxidant, antihypertensive, anti-inflammatory, and antimicrobial properties, highlighting their multifunctional applications in health-promoting food systems [55–57]. However, the bioaccessibility and bioavailability of algal proteins can be limited by structural barriers such as cell wall polysaccharides and the presence of anti-nutritional factors. Therefore, advances in extraction technologies such as enzymatic hydrolysis or fermentation are essential to enhance protein recovery and functionality [58].

Moreover, from an environmental and economic perspective, macroalgal cultivation and processing generally exhibit a significantly lower ecological footprint compared to conventional protein sources such as meat or soy. Their fast growth, low land and freshwater requirements, and potential for carbon sequestration make seaweeds particularly attractive for future food systems [59]. As such, brown seaweeds may contribute to more resilient, circular, and sustainable agri-food production systems.

As demonstrated by our study, brown macroalgae possess a high carbohydrate content, as has been reported by many authors [4,44,54,60–64]. A significant portion of these carbohydrates are classified as dietary fibres, primarily composed of complex, non-digestible polysaccharides such as alginate, laminarin, fucoidan, and cellulose [63]. From a nutritional perspective, these compounds are crucial for human digestive health, supporting gut microbiota, regulating bowel function, and contributing to satiety and weight management [51]. From a biotechnological perspective, they hold considerable potential across the food,

pharmaceutical, and biomedical sectors. Furthermore, these polysaccharides function as prebiotics, promoting the growth of beneficial intestinal bacteria and contributing to the prevention of metabolic disorders such as obesity, type 2 diabetes, and cardiovascular disease [65]. In addition, the viscosity and gel-forming properties of alginates make them valuable as food additives, serving as thickeners, stabilizers, and emulsifiers in functional foods and nutraceutical formulations [66]. In a broader biotechnological context, brown algal carbohydrates are being explored for a wide range of industrial applications. Alginate, for example, is extensively used in wound dressings, drug delivery systems, and tissue engineering scaffolds due to its biocompatibility and capacity to form hydrogels [67]. Fucoidans, sulphated polysaccharides with demonstrated anticoagulant, antiviral, and immunomodulatory activities, are being investigated as pharmaceutical agents and for use in targeted cancer therapies [68,69]. These applications reinforce the role of brown macroalgae as a sustainable and multifunctional biomass that aligns with the principles of a circular economy and green biotechnology. Nevertheless, challenges remain, particularly regarding efficient extraction and purification processes and variability in carbohydrate composition due to environmental factors. Overcoming these limitations through process optimization and standardization of biomass quality will be essential for the scalable exploitation of brown algal carbohydrates.

The low lipid content indicates that seaweed and residual pulp biomass are compatible with a healthy diet [70]. Compared to the results of Pacheco et al. [44] and Sánchez-Machado et al. [71], the results for *L. ochroleuca* show higher values. However, *L. ochroleuca* harvested in spring had a lipid content of 2.2% dw [45], and *S. polyschides* harvested in summer had values varying between 1.1% and 2.5% dw [44,61,62], which are closer to our results. Moreover, *S. polyschides* harvested in the late summer/early autumn [46] had a lipid content of 3.3% dw, which is also remarkably similar to our results. Conversely, *M. pyrifera* showed a lipid content (1.28%) close to that reported by Biparva et al. (1.57%) [54]. These results show that the lipid content of seaweeds not only depends on the season in which the seaweed was harvested, but also varies according to geographical location, climate and environmental conditions such as light intensity, day length, temperature, salinity and nutrient availability. All of these factors influence the metabolic activities of seaweeds and consequently the production of metabolites. The increase in lipid concentration in the residual pulp biomass is likely a consequence of the prior aqueous extraction process, which may have partially compromised the integrity of the residual pulp cell membranes. In fact, the membrane stability can be affected by processing conditions, such as temperature, pH variations and exposure to organic or polar solvents [72,73]. When cell membranes are destabilized, subsequent steps in the analysis, particularly those involving mechanical or solvent-based disruption, can lead to enhanced cell lysis and the release of intracellular components, including lipids. Therefore, the elevated lipid content detected in the residual pulp may not reflect a true increase in lipid abundance, but rather a higher extractability due to membrane permeabilization and breakdown during the initial extraction step, making lipids more readily available for quantification compared to the untreated sample.

Palmitic acid and the oleic acid were the most abundant fatty acids in the three seaweeds, a finding confirmed by several authors [44,74–76]. Besides, the amount of the polyunsaturated fatty acids linolenic acid (ALA), arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) was always higher in the residual pulp and *M. pyrifera* than in the other two species. Méndez et al. [77] found rather similar results but measured different values depending on the structure of the *M. pyrifera* biomass evaluated. The presence of EPA in both the residue and *M. pyrifera* and DHA in the residual pulp (albeit at low levels) suggests that this seaweed and the byproducts of its transformation can be used as functional ingredients in aquafeed. Both matrices contain nutrients essential

for the growth and survival of marine larvae [78,79]. Alpha-linolenic acid (ALA) and docosahexaenoic acid (DHA) were not detected in the studies by Belattmania et al. and Pereira et al. [74,76], while Méndez et al. [77] found no DHA in their seaweed samples. The n6/n3 ratio is often used to assess the quality of fats in a healthy diet. The ideal ratio for a balanced diet should be close to one, as this value reflects the recommended balance of essential fatty acids [80]. The ratios of omega-6 and omega-3 fatty acids obtained in this study for *L. ochroleuca* and *S. polyschides* (1.75 and 3.92, respectively) are higher than those reported by Sánchez-Machado et al. [71], while similar or higher ratios have been reported for *S. polyschides* in samples collected off the coast of Morocco, in Peniche (Portugal) and in the Gulf of Cádiz (Spain), with ratios of 6.62, 3.31 and 10.9, respectively [45,74,76]. Interestingly, the best ratio was obtained by the residual pulp biomass with a ratio of 0.87, which can be explained, again, by the disruption of the cell membrane, allowing some fatty acids to be extracted more easily. Lower temperatures favour an increase in the PUFA content of *Macrocystis pyrifera*, compared to those found in warmer waters (*L. ochroleuca* and *S. polyschides*) [74,81]. This increase in PUFA contributes to the greater fluidity of cell membranes in low temperature environments [64].

As previously mentioned, brown seaweeds are used in the food industry for their alginate content [82]. The extraction efficiency and properties of alginates are influenced by various parameters, such as temperature, extraction time, pH, sample size and the alkaline extraction used [83]. Statistical analysis revealed that both the extraction temperature and the seaweed sample had highly significant effects on the extraction yield of alginate ($p < 0.001$), along with a significant interaction effect. This indicates that the impact of temperature on yield is dependent on the biomass source used. Post hoc Tukey HSD tests showed that all tested temperatures (25 °C, 60 °C, and 80 °C) differed significantly in terms of extraction yield, with mean values increasing as the temperature increased. This trend suggests that higher temperatures enhance alginate solubilization from the cell wall, possibly due to increased molecular mobility and disruption of intermolecular interactions. Regarding the biomass type, all sample groups differed significantly from each other. Our extractions yielded 7.33 to 51.04% dw of alginates, with *L. ochroleuca* registering the lowest yield at 25 °C extraction and the residual pulp the highest. Lower results were obtained by Belattmania et al. [74], while a similar yield was obtained for *S. polyschides* and *L. ochroleuca* by Kaidi et al. [84]. As for *M. pyrifera*, the best values were obtained at 80 °C, which were higher than those obtained by Hernández-Carmona et al. [43]. These differences may be attributed to the extent of cell wall degradation and intrinsic alginate content. The significant interaction between temperature and biomass type further highlights that extraction efficiency is not governed by a single factor, providing a clear indication of the variation between species and the need to optimize the extraction protocols for each of them.

Specifically, regarding the *M. pyrifera* residual biomass, our results show that the highest extraction yield was obtained with an extraction temperature of 60 °C. In all the extractions, and regardless of the extraction temperature, the residual biomass always showed higher alginate extraction yield values. Figure 2A shows that the cell wall of the algal biomass of *M. pyrifera* had a well-organized cell structure compared to the structure of the residual pulp, which was disrupted due to the aqueous extraction, an aspect that is clearly visible in Figure 2B. It is likely that this cellular disintegration, resulting from the processing, fostered the release of alginate and improved the overall efficiency of the extraction.

As far as the FTIR analysis is concerned, the most prominent absorption peaks found correspond to the functional groups typically associated with sodium alginate. Between the wavelengths of 3000 and 3500 cm^{-1}, water is typically found [9,85]. Characteristic peaks

were detected around 1600 cm^{-1} and 1400 cm^{-1}, which are indicative of asymmetric and symmetric stretching vibrations of the carboxylate ($–COO^-$) groups, respectively, key functional groups in the alginate backbone [9]. These confirm the presence of uronic acids, such as guluronic (G) and mannuronic (M) acid residues, which constitute the main structural components of alginate. In the region between 3000 and 3500 cm^{-1}, a broad absorption band was observed, which is commonly attributed to the O–H stretching vibrations of hydroxyl groups and adsorbed water molecules [86]. This is typical in polysaccharide-rich samples and reflects both the intrinsic hydrophilicity of alginate and the moisture retained within the matrix or bound to its structure. The intensity of this band may also be influenced by residual humidity or hydrogen bonding within the polymer network. Overall, the FTIR spectral profile supports the presence of alginate and related polysaccharides in the sample and can also be used as a rapid tool for confirming functional group integrity following extraction processes. Moreover, minor peaks detected in other regions (e.g., 1020–1100 cm^{-1}) may correspond to C–O stretching in alcohols and polysaccharides, further substantiating the carbohydrate-rich nature of the material [87].

The natural environment provides a wide range of elements that are essential for the survival and functioning of living organisms. In addition, the environment also contains heavy metals, which can play a dual role, either supporting biological functions or causing toxicity [88]. Heavy metals are present in the environment from natural or anthropogenic sources, including industrial effluents, organic waste, waste burning, and transport and power generation. As seaweed can absorb these hazardous compounds in large quantities, their consumption can lead to the intake of these contaminants [89]. As mentioned above, the levels of arsenic found in most of our samples were rather high compared to the levels mentioned in Commission Regulation (EU) No. 465/2023 [90], which recommends a maximum level of inorganic arsenic between 10 and 300 $\mu g \cdot kg^{-1}$ of wet weight. These values are within the range reported by Yu and coworkers [91] for Phaeophyceae, usually the group with the highest arsenic concentration among seaweeds. However, the most common types of arsenic found in seaweed are arsenic sugars, which are believed to be like arseno-betaine in that they are harmless to the human body [92]. On the other hand, other authors point out that arsenic can be converted by mammals into toxic dimethylacetamide, a carcinogenic compound [93]. To assess the food safety of these algae, it is therefore necessary to obtain new data on the levels of organic and inorganic arsenic in the seaweeds and kelp pulp biomass.

Regarding macro- and microelements, the values recorded in the present study are generally in line with those reported by Circuncisão et al. [6] for brown algae, although, as mentioned above, large variations were recorded depending on the location, season and other environmental parameters. Seaweeds are usually rich in macroelements, making them valuable dietary sources of essential nutrients. These nutrients are needed to produce metabolites and cellular metabolism. However, the recommended daily intake of seaweed is between 5 and 10 g dw per day [94]. Hence, the macro- and microelements are found at lower levels than the dietary reference values [95], as only small amounts of seaweed can be consumed daily.

Like all photosynthetic organisms, brown algae contain chlorophyll *a*. These organisms also have other accessory pigments. In addition to chlorophylls *c1* and *c2*, they are rich in fucoxanthin and other carotenoids. Together, these pigmented metabolites give these algae their yellowish-brown or dark brown colour. These natural pigments, regardless of their source, can be used in the food, cosmetic and pharmaceutical industries [12] as they have antioxidant, anti-inflammatory, antidiabetic, antiobesity, neuroprotective, anticancer and photoprotective properties [75]. The quantification of these pigments hence helps to determine the biotechnological potential of these samples. The analysis of pigment profiles

in both fresh and dried macroalgal biomass revealed species-specific differences and notable changes induced by biomass drying. Fresh samples of *L. ochroleuca* and *S. polyschides* gave the best results for both fucoxanthin and chlorophylls *a* and *c* [96]. Freeze-thawing of fresh samples reduces molecular movement and promotes the formation of ice crystals, which can lead to membrane rupture due to hydrogen bridge dissociation [73]. In the fresh samples, *S. polyschides* consistently exhibited the highest concentrations of photosynthetic pigments, with chlorophyll *a* reaching 4218.74 ± 93.53 $\mu g \cdot g^{-1}$ fw, significantly higher than the value in *L. ochroleuca* (2599.38 ± 701.51 $\mu g \cdot g^{-1}$ fw). This dominance of chlorophyll *a* in *S. polyschides* aligns with previous studies showing high photosynthetic capacity in kelp and pseudo-kelp species, particularly under optimal environmental conditions [97]. Similarly, chlorophyll *c*—an accessory group of pigments involved in light harvesting—was more abundant in *S. polyschides* (965.62 ± 127.39 $\mu g \cdot g^{-1}$ fw) compared to *L. ochroleuca* (681.34 ± 159.54 $\mu g \cdot g^{-1}$ fw). Chlorophyll *c* facilitates efficient light absorption in the blue region of the spectrum, especially under low light or subtidal conditions, and its levels may reflect adaptive strategies to environmental light gradients. Variations in chlorophyll *c* concentrations could be due to several factors, including the specific methods used for extraction and quantification, and should be confirmed, in the future, by HPLC. Indeed, according to Marcheafave et al. [98], the extraction of photosynthetic pigments is influenced by a combination of abiotic and biotic factors [36,99], the type of solvent used, the state of the sample (fresh or dry) and the stage of the algae's life cycle.

Regarding fucoxanthin, fresh *S. polyschides* again demonstrated higher levels (695.48 ± 22.51 $\mu g \cdot g^{-1}$ fw) than fresh *L. ochroleuca* (439.93 ± 30.32 $\mu g \cdot g^{-1}$ fw). This supports its potential as a valuable source of bioactive compounds for nutraceutical and pharmaceutical applications [51]. Besides, the maceration process also resulted in the release of more pigments.

As to the results obtained for photosynthetic pigments in the dried samples, the lower values recorded may be related to the drying process, which can degrade chlorophylls [77]. This comparison highlights the pigment degradation or transformation post-harvest, which is consistent with the known thermal and oxidative sensitivity of these compounds. Across all pigments, concentrations in the dried samples were markedly lower. For instance, the highest fucoxanthin content was found in the residual pulp (157.53 ± 2.72 $\mu g \cdot g^{-1}$ dw), which is noteworthy given that this material is typically regarded as a byproduct. Its unexpectedly high fucoxanthin content may result from residual pigment retention in fibrous fractions or partial concentration during drying. *M. pyrifera* exhibited the lowest fucoxanthin content (56.71 ± 1.38 $\mu g \cdot g^{-1}$ dw), likely due to species-specific differences in pigment biosynthesis. Meanwhile, chlorophyll *a* remained the highest in the dry biomass of *L. ochroleuca* (26.59 ± 1.93 $\mu g \cdot g^{-1}$ dw), and intermediate values were recorded in *S. polyschides* (22.18 ± 2.47 $\mu g \cdot g^{-1}$ dw) and the residual pulp (16.63 ± 2.33 $\mu g \cdot g^{-1}$ dw). This suggests that while chlorophyll *a* is susceptible to degradation, some fraction is retained even in waste material, offering opportunities for valorisation. The pattern for chlorophyll *c* was similar, with *L. ochroleuca* and *S. polyschides* again showing the highest values (2.95 ± 0.09 and 2.66 ± 0.03 $\mu g \cdot g^{-1}$ dw, respectively). Notably, the residual pulp retained very low levels (0.15 ± 0.08 $\mu g \cdot g^{-1}$ dw), indicating greater susceptibility of chlorophyll *c* to degradation or leaching during processing.

Fernandes et al. [100] studied the photosynthetic pigments of *S. polyschides* and *L. ochroleuca*, among other algae. Despite recording differences between parts of the thalli and the harvesting site, these authors recorded a range of fucoxanthin and chlorophyll *a* values like ours. However, our chlorophyll *c* concentrations were always lower than those in the aforementioned study.

These findings highlight the biotechnological potential of *S. polyschides* and *L. ochroleuca* as primary sources of chlorophylls and fucoxanthin. In parallel, the substantial pigment content found in the residual pulp underscores the feasibility of circular economy strategies for valorising algal byproducts through integrated biorefinery approaches [101]. Overall, the results advocate for a broader utilization of both primary and residual biomass fractions in pigment extraction and functional food development.

To assess the antioxidant capacity of macroalgal extracts, Total Phenolic Content (TPC) and Ferric Reducing Antioxidant Power (FRAP) are two commonly employed methods that provide complementary insights. The TPC assay quantifies the total concentration of phenolic compounds in a sample and offer a direct indication of the extract's potential health benefits. Studies have reported a wide range of TPC values in brown algae, depending on species, season, and extraction method [102,103]. The FRAP assay evaluates the ability of antioxidants in the sample to reduce Fe^{3+} to Fe^{2+} under acidic conditions, thus measuring the electron-donating capacity of the extract [41]. This method is particularly useful for screening the antioxidant potential of complex matrices like seaweed extracts. Given the growing demand for natural antioxidants in the food, cosmetic, and pharmaceutical industries, the evaluation of macroalgal extracts using TPC and FRAP remains a vital step in bioactive compound screening. Furthermore, integrating these assays within biorefinery models enhances the sustainable exploitation of algal biomass, allowing for the valorisation of both primary and secondary metabolites.

The Total Phenolic Content (TPC) among the three seaweed species and the residual pulp showed statistically significant differences, ranging from 2.47 $\pm$ 0.33 mg GAE·g^{-1} in *L. ochroleuca* to 4.94 $\pm$ 0.31 mg GAE·g^{-1} in *S. polyschides* (Figure 4). These results highlight the species-specific variability in phenolic compound accumulation, which is known to be influenced by genetic, environmental, and seasonal factors, such as light availability, temperature, and nutrient levels [104,105]. *S. polyschides* displayed the highest TPC, suggesting a stronger antioxidant potential and increased relevance for bioactive applications, particularly in the food, pharmaceutical, and cosmeceutical industries. Regarding *S. polyschides*, previously published results obtained with methanolic extracts [20] showed a higher phenolic content (66.89 $\pm$ 0.002 mg GAE·g^{-1} extract) than ours. On the other hand, the aqueous extracts of *L. ochroleuca* and *S. polyschides* from the study by Pacheco et al. [44] had lower values than those obtained in the present study.

The relatively low TPC in *L. ochroleuca* may reflect its lower biosynthetic capacity for phenolic production or reduced accumulation due to physiological or ecological differences. *L. ochroleuca* showed a higher total polyphenol content than that obtained in the present study [75], as well as the TPC obtained by Beratto-Ramos et al. [106] for *M. pyrifera*, which was also harvested in winter. *M. pyrifera* and its residual pulp exhibited intermediate TPC values, with the pulp (4.86 $\pm$ 0.12 mg GAE·g^{-1}) containing slightly more phenolics than the raw seaweed biomass (3.36 $\pm$ 0.13 mg GAE·g^{-1}). This may be attributed to processing effects, where cell disruption and thermal exposure enhance the release of bound polyphenols from the algal matrix. It should also be stressed that the study from Beratto-Ramos et al. [106] used algae species harvested in distinct locations at different times of the year, and a residue from a previous extraction. In the subsequent hydroethanolic extraction of the residue, the temperature, polar organic solvent (ethanol) and water caused further damage to the cell membrane, and the antioxidant compounds were more easily extracted. All these factors, together with the sequential extraction, are likely to have influenced the antioxidant activity of the extracts. The residual pulp extract, therefore, showed interesting results, indicating that the primary extraction process of the biomass did not affect the whole biomass, leaving behind many of the bioactive compounds, which may be further explored.

Brown algae are generally rich in phenolic compounds, which have antioxidant properties [107]. The results obtained from the FRAP assay showed a higher antioxidant capacity of the residual pulp than the other samples, and much higher than that of the *M. pyrifera* biomass. Notably, *M. pyrifera* and *L. ochroleuca* showed markedly lower values, suggesting species-specific differences in antioxidant profiles (Figure 5). The elevated antioxidant potential of the residual pulp may be attributed to the accumulation or retention of bioactive compounds, such as phenolic acids (namely phloroglucinol) or reducing sugars, following upstream processing [25,108]. These results support previous findings indicating that algal processing residues remain rich in valuable phytochemicals, thus reinforcing their potential for reuse within circular biorefinery models [101,105]. Notably, this valorisation aligns with sustainable development goals by converting algal waste streams into functional ingredients for nutraceuticals or cosmetics [51].

The higher reducing power observed in *S. polyschides* is consistent with earlier studies reporting significant levels of polyphenols and antioxidant activity in brown algae species [109,110]. This is also in agreement with the TPC content of the species as analysed earlier.

In contrast, the lower FRAP values of *L. ochroleuca* and *M. pyrifera* may reflect differences in biochemical composition, including lower concentrations of polyphenolic compounds, as previously described, or a higher proportion of non-reducing antioxidants such as carotenoids or vitamins, which are not effectively measured by the FRAP assay [111]. This reinforces the importance of using multiple assays to comprehensively evaluate antioxidant potential, as each method has different sensitivities to various antioxidant mechanisms. In summary, the high FRAP value of the residual pulp underscores its potential as a valuable byproduct rich in antioxidant compounds, which could be further explored for functional food, cosmetic, or other applications. This finding contributes to the growing body of literature advocating for the integration of waste streams into high value bioproduct development within macroalgal processing chains.

The antioxidant profiles revealed by both the TPC and FRAP assays suggest a potential positive correlation between the total phenolic content and the ferric reducing capacity of the macroalgal samples and their byproducts. Notably, *S. polyschides* exhibited the highest TPC among the seaweeds, closely followed by the residual pulp, which also demonstrated the highest FRAP value. This parallel trend supports the widely reported observation that phenolic compounds, particularly phlorotannin's in brown macroalgae, play a significant role in antioxidant activity due to their electron-donating capacity and metal-chelating properties [110,111]. Furthermore, *L. ochroleuca*, which showed the lowest TPC, also exhibited the lowest FRAP value, reinforcing this possible correlation. Several studies have demonstrated strong correlations between TPC and FRAP values in various macroalgal species, indicating that polyphenols are key contributors to reducing power.

Overall, our findings indicate that residual pulp from seaweed processing retains substantial content of proteins, lipids, carbohydrates, pigments and phenolics, representing a promising raw material for valorisation in biorefinery and circular economy strategies, particularly in the context of sustainable extraction of antioxidant-rich compounds from industrial byproducts [112,113].

5. Conclusions

The results of the biomolecular and functional screening showed that the target seaweed species and their byproducts have high potential for various uses. This approach is in line with the concept of the circular economy, promoting the integral use of natural resources and helping to reduce environmental impact. The analysis revealed that the residual pulp and *S. polyschides* render a high yield of alginates, which are essential for

applications as thickeners and stabilisers in food, including ice creams, yoghurts, and cosmetic products. In addition, the levels of polyunsaturated fatty acids, including eicosapentaenoic acid (EPA, C20:5) and alpha-linolenic acid (ALA, C18:3), suggest applications in food supplements and balanced aquaculture feeds. The lipid profile of the residual pulp also shows a favourable n6/n3 ratio, ideal for formulations that promote human cardiovascular and metabolic health.

In terms of minerals, *S. polyschides*, *M. pyrifera* and the residual pulp have good levels of phosphorus, and both *M. pyrifera* and residual pulp have high levels of potassium, suggesting their use in agricultural fertilisers and biostimulants. Future studies could evaluate the efficacy of these compounds in agricultural systems and explore the impact of their inclusion in diets for invertebrates and fish in aquaculture systems.

Antioxidant analysis of the hydroethanolic extracts revealed high levels of polyphenols and a significant capacity to reduce iron ions, reinforcing the extracts' potential as a source of antioxidant compounds for the cosmetic and pharmaceutical industries. These compounds could be used in anti-ageing products, photo protectants and formulations designed to mitigate oxidative stress.

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