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Alginate industrial waste streams as a promising source of value-added compounds valorization

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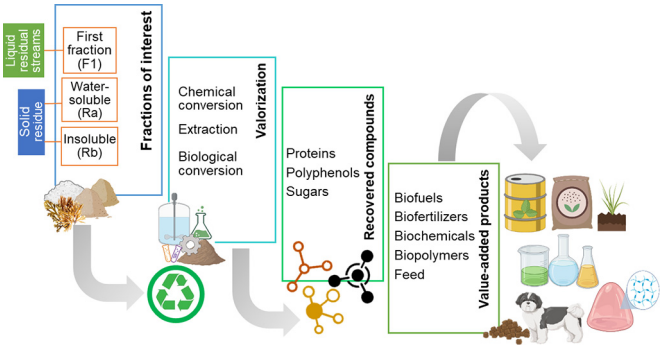
HIGHLIGHTS

- Alginate industry effluents composition varies depending on the algae species.
- Acid effluents contain significant quantities of sulfated fucoidan and polyphenols.
- Soluble protein-rich or alginate fractions recovered from the solid residue.
- Fucoidan, insoluble protein or cellulose predominates in the final residues.
- Valorisation towards bioactive, texturizing or nutritional added-value ingredients

GRAPHICAL ABSTRACT



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ABSTRACT

The alginate industry processes more than hundred thousand tons per year of algae in Europe, discarding around 80% of the algae biomass as different solid/liquid residual streams. In this work, *Saccharina latissima* and *Ascophyllum nodosum*, their generated alginates and all residual fractions generated in the process were characterized in terms of lipid, ash, protein content, and the carbohydrate composition and antioxidant capacities analyzed. The first fraction after acid treatment (ca. 50% of the initial dry biomass) was rich in phlorotannins (15 mg GAE/g) and bioactive fucoidans (15–70%), with a high sulfation degree in *A. nodosum*. Two fractions generated from the solid residue, one soluble and another insoluble (Ra and Rb, respectively), constituted 9% and 5–8% of the initial biomass and showed great potential as a source of soluble protein (30% for *S. latissima*), and cellulose (70%) or fucoidan, respectively. Valorization strategies are suggested for these waste streams, evidencing their high potential as bioactive, texturizing or nutritional added-value ingredients for cosmetic, food, feed or pharmaceutical applications.

1. Introduction

Marine biomass is highly underexploited and mostly unknown, compared to plant terrestrial biomass, and constitutes a vast renewable source for food ingredients, biomedical, cosmetic, specialty chemicals and materials in future biorefineries. Thanks to global policies fostering a "blue bioeconomy", much research and industrial efforts have been devoted to understanding marine biomass and explore potential applications. Brown algae are one of the most exploited and promising families of macroalgae, due to their high content in valuable compounds such as functional polysaccharides (e.g. fucoidan, laminarin, etc.), polyphenols (e.g. phlorotannins), protein, vitamins and minerals.

Fucoidans and fucans are a wide family of fucose-containing polysaccharides which has attracted much research attention in recent years due to abundant scientific evidence on bioactive properties, such as anticoagulant, anti-inflammatory, antioxidant, which in turn may alleviate many

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important chronic diseases (Deniaud-Bouët et al., 2017; Ponce and Stortz, 2020). Many of these bioactivities have been related to the degree of sulfation, and the ability of the sulfate groups to interact with other biological compounds or act as electron donors (Deniaud-Bouët et al., 2017; Yuan and Macquarrie, 2015). Phlorotannins are a family of polyphenols of varying molecular weight present in brown algae, with many similar ascribed bioactivities as fucoidans, linked again to their antioxidant, anti-inflammatory capacities which in turn modulate biochemical processes linked to chronic diseases (neurodegeneration, cancer, cardiovascular disease, diabetes, etc.) (Fernando et al., 2021; Shrestha et al., 2021). The interest in these compounds has prompted much research on the development of suitable extraction and purification methods towards a sustainable cascade valorization of several brown algae species (Ale et al., 2011; Allwood et al., 2020; Yuan and Macquarrie, 2015; Zhang et al., 2020).

Nevertheless, the vast heterogeneity and complexity of brown algae in terms of composition, recalcitrance, solubility and potential bioactivities is huge and poses important challenges to establish clear structure-activity relationships or create standardized processes to bring valuable products to the market (Deniaud-Bouët et al., 2017; Flórez-Fernández et al., 2018).

The main source of brown algae exploitation to date is the alginate industry, processing more than hundred thousand tons of either harvested or cultivated algae in Europe on a yearly basis (Araújo et al., 2021). Alginate is a linear heteropolysaccharide composed of 1,4-linked β-D-mannuronic acid (M) and 1,4 α-L-guluronic acid (G) and the main cell wall component of the cell wall in many brown seaweeds, representing 17–45% of the algae dry weight (Vera et al., 2011). The principal properties of alginates, including their gelling, emulsifying and film-forming abilities have made them broadly used ingredients in a number of fields such as in the pharmaceutical, cosmetic, textile, and food industries (Gomaa et al., 2018). Several species of the genus *Laminaria* (kelp) and the species *Ascophyllum nodosum* (rock kelp) are among the most heavily wild harvested species for alginate production in Europe, while *Saccharina latissima* is the most widely cultivated species (aquaculture) for direct consumption or alginate production (Araújo et al., 2021). Despite much policy efforts have been made to promote circular bioeconomy practices (Dutta et al., 2021; Khoshnevisan et al., 2021; Mak et al., 2020) and new ways of sustainable exploitation of the oceans, little has changed in the industrial processing techniques used to obtain alginate. Generally, the alginate extraction process at industrial level involves collection, transport and storage of the algae, with eventual addition of formaldehyde to avoid chemical or enzymatic reactions which might decrease the quality of the alginate. An acid pre-treatment is applied at room temperature to remove pigments, other carbohydrates and low molecular weight compounds. Then, an alkaline extraction is performed, followed by solid/liquid separation, drying and milling, to produce high quality alginate (Fawzy et al., 2017; Pawar and Edgar, 2012). This multistep extraction process generates large amounts of liquid and solid waste streams, which constitute around 80% of the initial dry biomass and are currently discarded (Mohd Fauziee et al., 2021).

While research efforts have focused on alternative processes to minimize the use of chemicals or to target extraction of bioactive fucoidans or phlorotannins, it is unlikely that current industrial alginate production practices change in the short- or mid-term. In the mean-time, the valorization of these already available waste streams into -added-value products could be a step towards a more circular bioeconomy and more sustainable practices. In fact, some of these waste streams may well still contain some the afore-mentioned valuable compounds (e.g. bioactive polysaccharides or polyphenols), together with other nutritionally valuable protein, lipid or mineral fractions. A proper characterization is nevertheless needed to ascertain their potential, which, to the best of our knowledge, is lacking. The integration of valorization strategies for these waste streams into the industrial process without altering current alginate production, would minimize waste generation and achieve a higher degree of valorization for algae biomass, while creating new cost-efficient ingredients for a wider product portfolio of the alginate processing industries.

Therefore, the focus of this work was to perform a thorough characterization of the different waste streams generated during alginate extraction from two brown algae from different families, *Saccharina latissima* and *Ascophyllum nodosum*, to create evidence for potential cascade valorization schemes which would increase the eco-sustainability of the currently used industrial process. The rationale for selecting these species in the present work was based on several reasons. First of all, they are among the most heavily exploited species for alginate production, both in aquaculture (*S. latissima*) and through wild harvesting (*A. nodosum*) and with 376 and 82,000 tons/year, respectively, in Europe only (Araújo et al., 2021). Moreover, alginate is produced from algal species in the Laminariales and Fucales families and, thus, at least one candidate from each of the families should be selected to have a broader insight into the potential residual streams derived from them. Another reason for selection was that these specific species are easily harvested with little contamination from other species, e.g. *A. nodosum* from the shoreline and *S. latissima* is cultivated in threads in controlled areas.

2. Materials and methods

2.1. Materials and reagents

The brown seaweeds *Saccharina latissima* (*S. latissima*) and *Ascophyllum nodosum (A. nodosum)* were kindly supplied by Porto-Muiños S.L. (Cerceda, A Coruña, Spain) as a dry powder (<2% water content). The practices for harvesting in this company involved manual hand-picking, which ensured the removal of other contaminant algal species or impurities. All chemicals and reagents were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA) unless otherwise stated. Mannuronic and guluronic acid standards were purchased at Biosynth Carbosynth Ltd. (Compton, UK).

2.2. Alginate extraction

The alginate extraction process was carried out mimicking conventional industrial practices according to information gathered through the BIOCARB-4-FOOD project (SUSFOOD2), in order to generate the different waste streams and residues for further analysis (Cebrián-Lloret et al., 2022). Addition of formaldehyde was avoided even though it is a common industrial practice as a preservative during transport. The alginate extraction process was divided into four steps: an acid treatment, alkali extraction, precipitation, and drying (Fig. 1). Except the drying process, all processes were performed at room temperature. Briefly, the brown seaweeds were subjected to acid treatment (0.2 M HCl) overnight at a solid/liquid ratio (S/L) of 1:25 (wt.) under mechanical stirring. The residue was recovered by centrifugation and re-suspended in 0.1 M $NaHCO_3$ (S/L 1:50) for 2 h. The pH was then adjusted to 7.5–8 with $NaHCO_3$/HCl and the solution incubated overnight with slow stirring. The suspension was centrifuged to separate the residual algae, which was freeze-dried and then washed with distilled water to separate the soluble and insoluble parts of this residue. 0.2% NaCl (w/v) was added to the solution and the sodium alginate was precipitated using different volumes of isopropanol in subsequent cycles. Finally, the last precipitate was filtered and dried overnight at 60 °C. Dried alginate samples were milled and stored in a desiccator chamber (0% HR) until use. The extraction yield was calculated from the following equation:

$$Yield\ (\%) = \frac{Mass\ of\ alginate\ (g)}{Mass\ of\ brown\ seaweed\ (g)} \times 100 \quad (1)$$

All liquid and solid residual fractions generated through the process were dried and the yield calculated analogously as for alginate.

2.3. Chemical composition

The total nitrogen content was determined using an Elemental Analyzer Rapid N Exceed (Paralab S.L., Spain). About 250 mg of each of the powdered samples were pressed to form a pellet which was then analyzed

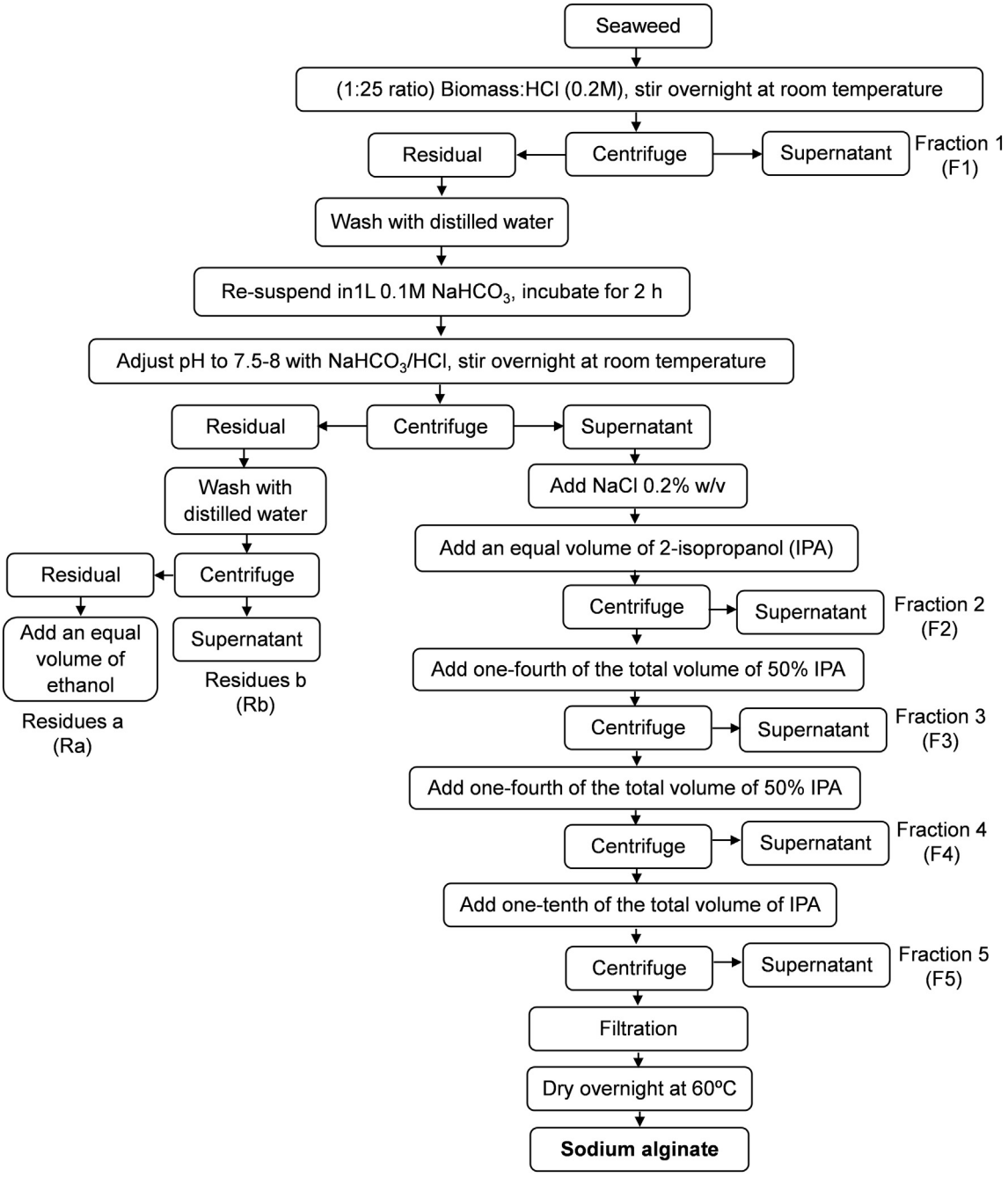

Fig. 1. Schematic diagram of sodium alginate extraction process.

using the Dumas method, which is based on the combustion of the sample and subsequent detection of the released N_2 (Wiles et al., 1998).

The lipid and ash content were determined by AOAC Official Methods 991.36 and 920.153, respectively (AOAC, 1990).

Sulfate content was calculated from the sulfur content through elemental analysis using a Thermofisher Flashsmart organic elemental analyzer. All tests were conducted in triplicate.

2.3.1. Determination of monosaccharide composition

The carbohydrate content and monosaccharide composition of the alginates and the fractions were determined after acid methanolysis as previously described Martínez-Abad et al. (2018). The monosaccharides were analyzed using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on an ICS-3000 (Dionex, Sunnyvale, CA, USA). All experiments were carried out in triplicate.

2.4. $ABTS^{\cdot+}$ radical cation scavenging activity

The antioxidant capacity of alginate was determined using the Trolox equivalent antioxidant capacity according to Re et al. (1999) with some modifications. Briefly, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as antioxidant standard. Each sample was dissolved in distilled water at a concentration of 5 mg/mL and analyzed for ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity. To this end, 20 μL of aqueous solutions of the alginate-based extracts were added to 230 μL of the $ABTS^{+}$ solution, and absorbance at 734 nm was registered after 6 min. For the calibration curve, Trolox standards of distinct concentrations were prepared, and the same procedure was followed. The results were expressed as μmol Trolox equivalents (TE)/g extract. All determinations were carried out in triplicate.

2.5. Phenolic content

The total phenolic content was determined, in triplicate, by the Folin-Ciocalteau reagent as described by Singleton et al. (1999) with a slight modification. Briefly, Folin-Ciocalteau reagent was diluted 1:10 with distilled water and 1 mL mixed with 0.2 mL of each sample (5 mg/mL). 0.8 mL of Na_2CO_3 (75 mg/mL) were, then, added and the samples were heated up to 50 °C for 30 min. Finally, the absorbance values were read at 750 nm. The calibration curve was generated by using gallic acid as the standard, and the total phenolic content was expressed as mg of gallic acid equivalents (GAE)/g extract.

2.6. β-Carotene–linoleic acid assay

The β-carotene–linoleic acid model system was assessed, in triplicate, as previously described by Koleva et al. (2002). A stock solution was prepared with 0.5 mg of β-carotene in 1 mL chloroform, and it was added to 25 μL linolenic acid and 200 μL of Tween 40. The mixture was placed in a rotary evaporator under vacuum to remove the chloroform. Distilled water (100 mL) was added, and the resulting mixture was vigorously stirred. Aliquots (200 μL) of the β-carotene–linoleic acid emulsion were transferred into 50 mL of each of the fractions in triplicate at different concentrations and incubated at 50 °C for 2 h. The absorbance was measured at 470 nm. The antioxidant activity was expressed as inhibition percentage according to the following equation:

$$\beta - carotene - bleaching\ inhibition\ (\%) = \left[1 - \frac{A_0 - A_t}{A_0' - A\ddot{E}_t}\right] \times 100 \quad (2)$$

where A_0 and A'_0 are the absorbance of the sample and control, respectively, measured at time zero, and A_t and A'_t are the absorbance of the sample and the control, respectively, measured after 2 h.

2.7. Fourier transform infrared spectroscopy (FT-IR)

All samples were analyzed by FT-IR (FTIR; Bruker, Vertex, USA) using the attenuated total reflectance (ATR) mode. The spectra were recorded at 4 cm^{-1} resolution in a wavelength range between 400 and 4000 cm^{-1} and averaging a minimum of 64 scans.

2.8. Statistical analysis

The statistical analysis of experimental data was performed using IBM SPSS Statistics software (version 23, IBM Corp., USA). One-way analysis of variance (ANOVA) was done to determine if differences between sample means were significant at a significance level of $p < 0.05$. The mean tests were performed using Tukey's Test.

3. Results and discussion

3.1. Characterization of the raw seaweeds and their alginate extracts

The proximate and carbohydrate composition of the *Saccharina latissima* and *Ascophyllum nodosum* raw seaweeds and their alginate extracts was first analyzed, and the results are summarized in Table 1. In general, the compositional values for the raw seaweeds are in agreement with available literature on the composition of *S. latissima* (Bikker et al., 2020) and *A. nodosum* (Rioux et al., 2007; Tabassum et al., 2016) and the slight compositional differences can be ascribed to the environmental factors and harvest season variations which have been widely reported to affect seaweed composition (Samarasinghe et al., 2021). The total lipid content in both algae species was relatively low (<4%) confirming these algae are not a good source of lipids. These results are in agreement with Susanto et al. (2016) and Zhang et al. (2020), who reported a lipid content in the range from 1.7–7 wt% in brown seaweeds. The ash contents are quite high, indicating the presence of significant amounts of diverse mineral components in the seaweed biomass. As expected, the protein content varied between 9 and 11% depending on the species, being lower on average than that in red or green seaweeds (10–30% dry weight) (Burtin, 2003).

The carbohydrate contents of the seaweeds comprised 60–65% of the total dry weight, similarly to other reported values for brown seaweeds (Guangling et al., 2012; Morrissey et al., 2001; Rioux et al., 2007). The monosaccharide composition revealed fucose and glucose as the main carbohydrate units for *A. nodosum* and *S. latissima*, respectively. The high sulfur content in *A. nodosum* points towards sulfation of fucoidan structures (Deniaud-Bouët et al., 2017). This evidences the important differences in the cell wall architecture, with higher abundance of sulfated fucoidan/fucan structures or cellulose, depending on the species. The high alginate

Table 1
Proximal composition of raw seaweed and alginates from *Saccharina latissima* and *Ascophyllum nodosum*.

	Seaweed		Alginates		
Composition (dry wt%)	*Saccharina latissima*	*Ascophyllum nodosum*	*Saccharina latissima*	*Ascophyllum nodosum*	Commercial*
Yield	n/a	n/a	11.2 ± 0.9[a]	13.8 ± 0.9[b]	n/a
Ash	25.9 ± 0.1	18.3 ± 0.0	24.2 ± 0.3[c]	21.2 ± 0.2[a]	22.45 ± 0.2[b]
Lipid	2.6 ± 0.8	3.0 ± 0.3	n/a	n/a	n/a
Protein	7.3 ± 0.1	9.0 ± 0.9	3.6 ± 0.3[a]	4.1 ± 0.3[a]	<0.1
Sulfate	2.0 ± 0.0	5.1 ± 0.1	0.6 ± 0.2[a]	1.8 ± 0.1[b]	<0.1
Carbohydrates[1]	61.1 ± 2.0	64.2 ± 2.5	68.2 ± 2.1[a]	68.9 ± 1.7[a]	72.0 ± 2.8[a]
of which (μg/mg dry basis)					
Fucose	3.8 ± 0.3	25.8 ± 0.6	1.1 ± 0.1[a]	2.7 ± 0.2[b]	<0.1
Galactose	0.8 ± 0.1	1.5 ± 0.1	0.5 ± 0.1[b]	0.3 ± 0.1[a]	0.9 ± 0.3[c]
Cellulose[2]	23.1 ± 0.7	6.3 ± 2.0	<0.1	<0.1	<0.1
Glucose[3]	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.1[b]	0.2 ± 0.1[a]	1.0 ± 0.2[c]
Mannose	0.1 ± 0.0	1.4 ± 0.1	<0.1	<0.1	<0.1
Xylose	0.3 ± 0.0	3.2 ± 0.2	0.3 ± 0.1[a]	1.8 ± 0.7[b]	1.1 ± 0.1[b]
GulA	9.2 ± 0.5	7.9 ± 0.2	23.7 ± 1.6[a]	23.7 ± 0.7[a]	23.0 ± 1.6[a]
GlcA	0.8 ± 0.1	1.3 ± 0.0	0.7 ± 0.2[a]	1.6 ± 0.2[b]	0.7 ± 0.1[a]
ManA	16.2 ± 0.3	13.6 ± 0.2	41.2 ± 1.3[b]	38.6 ± 1.4[a]	45.3 ± 2.5[c]
Mannitol	5.9 ± 0.2	3.8 ± 0.2	<0.1	<0.1	<0.1

The values report means (n = 3) ± standard deviation. For alginate samples, different letters in the same row indicate significant differences between the samples ($p < 0.05$). GulA: guluronic acid. ManA: mannuronic acid. GlcA: glucuronic acid.

* Commercial alginate from Sigma-Aldrich (PHR1471).

[1] Total carbohydrate content calculated as the sum of all monosaccharides detected by HPAEC-PAD.

[2] The crystalline cellulose content was determined as the difference between the typical Saeman sulfuric hydrolysis (Saeman, 1945) and the non-crystalline glucose determined after acid methanolysis (Bertaud et al., 2002; Willför et al., 2009).

[3] Glucose contribution from the non-crystalline fraction.

content in these species is reflected in the sum of GulA and ManA units, with 21.5 wt% and 25.4 wt% alginate in the raw seaweeds, respectively.

The industrial production of alginate always follows an acid and alkaline treatment as described in Fig. 1 and may differ in the recovery of the soluble sodium alginate after the alkaline treatment by different precipitation or flocculation methods. The most common method is the addition of sodium chloride and isopropanol in varying quantities to promote the flocculation of alginate to the upper phase or its precipitation. In this work, this common procedure was followed, and alginate was centrifuged and washed with 50% isopropanol and pure isopropanol twice to ensure recovery of alginate with high purity (Fig. 1). Yield values of the alginate extracts (11–14%) were significantly higher ($p \leq 0.05$) for *A. nodosum* than for *S. latissima,* which can be ascribed to differences in the cell wall structure of both species (Deniaud-Bouët et al., 2017). In general, similar or only slightly higher alginate yields have been reported by other authors for some well-known worldwide alginophytes, including *A. nodosum* (18% dw) (Yuan and Macquarrie, 2015), *S. polyschides* (16% dw) (Jard et al., 2013), *F. vesiculosus* (16.2% dw) (Mohd Fauziee et al., 2021) and *P. pavonica* (17.5% dw) (Okolie et al., 2020) pointing out that slight differences in the washing/precipitation steps or salt/solvent concentrations used do not highly affect the yield. As expected, the main sugar constituents were ManA and GulA, with no significant differences between the two seaweeds, and the protein content was relatively low (3–5%), all of which evidences the relatively high purity of the alginate extract, in line with commercial alginate samples (Table 1). The mannuronic acid:guluronic acid (M/G) ratio was 1.7 and 1.6 for *S. latissima* and *A. nodosum*, respectively. These are within the range for alginate ratios previously reported from species of the same genus (Fertah et al., 2017; Torres et al., 2007; Zrid et al., 2016). These M/G ratio results were also confirmed by NMR, as shown in Fig. S1 from the Supplementary Material, evidencing HPAEC-PAD also as a useful tool for determining the M/G ratio. Considering the alginate content in the raw material as the sum of mannuronic and guluronic acid units, these results point out the relative inefficiency in the industrial process, which aims at the extraction of a high-quality alginate leaving a significant alginate content in the residual streams, besides other potentially valuable compounds.

3.2. Compositional analysis of the different waste stream fractions

Instead of collecting the waste streams from the industry, a common industrial alginate extraction process was mimicked in the lab and the waste streams produced in a controlled manner. This not only prevented deterioration of the fractions during manipulation and transport from the industries to the lab, but also guaranteed no other additives (such as formaldehyde) were present through the process. Waste streams generated through the sequential alginate extraction process for both seaweed species were quantified and characterized in terms of their composition in order to evaluate their potential for further valorization (see Fig. 1). Two species from different families (Laminariales and Fucales) with distinct cell wall organization and recalcitrance (Deniaud-Bouët et al., 2017) were deliberately selected to evidence differential valorization possibilities. The yield, protein, ash, and sulfate content, as well as the carbohydrate composition of each fraction is shown in Table 2 and Fig. 2, respectively.

In order to assess the potential of the waste stream fractions generated in the production of alginate, the solid yield is a crucial parameter and was calculated from the initial algae solid biomass, considering the addition of acid, alkali or sodium chloride and bicarbonate salts through the process. The fraction with highest yield was the acid treated first waste stream fraction (F1), representing ~50% of all solids for both species, followed by fraction F3 (20–25%). A significant part of the initial algae materials (13–20%) was also recovered from the solid precipitate, either as a water soluble (Ra) or insoluble (Rb) waste fraction. The rest of fractions were recovered in lesser quantities (<3%), which may already hamper their potential valorization.

F1, the fraction with highest solid yield in both seaweed species, was mainly composed by salts, carbohydrates and minor amounts of protein. Minerals and carbohydrates were the main constituents in this fraction with similar contribution. The significant amount of carbohydrates (43–48%) present in F1 highly differed depending on the algae species. Fucose containing polysaccharides were predominant in *A. nodosum* (Fig. 2) typical of more soluble sulfated low-molecular weight (LMW) fucoidan fractions. A very high degree of sulfation was found in this polysaccharide rich fraction (Table 2), which is usually desirable as it provides hydrogen-donating ability to the polysaccharides, which in turn is ascribed to their antioxidant activities (Yuan and Macquarrie, 2015) as well as many other bioactivities commented in the introduction. However, a fraction of the sulfate content could arise from de-sulfation of fucoidan or other polysaccharide populations not extracted in this first step. *S. latissima*, on the other hand, showed additional significant amounts of glucose (Fig. 2), while sulfate content was drastically lower in comparison. The relative lower ash content in F1 from *S. latissima* compared to *A. nodosum* is probably attributed to the relative increase in glucose. This glucose can arise from the potential presence of laminarin, which highly depends on harvesting season (Manns et al., 2014; Schiener et al., 2015), from amorphous cellulose released during the acid treatment and from free glucose present in species from the Genus "*Saccharina*" (sugar), as previously reported by Ravanal et al. (2017).

The second waste stream fraction in terms of solids content was F2 which was generated from the supernatant upon addition of a volume of 50% isopropanol. The proximate analysis revealed this fraction to be mainly salts (75–80%) with minor quantities of carbohydrates or protein (Table 2). The addition of 50% isopropanol might explain the massive

Table 2
Chemical composition of alginate extracts, the different waste stream fractions obtained during the extraction process.

Algae	Sample	Yield* (%)	Protein (%)	Ash (%)	Carbohydrate (%)	Sulfate (%)
Saccharina latissima	F1	45.1 ± 0.9^{i}	5.0 ± 0.7^{g}	42.5 ± 0.9^{e}	42.5 ± 1.6^{d}	$1.5 \pm 0.0^{c,d}$
	F2	19.9 ± 1.2^{g}	$3.9 \pm 0.6^{e,f}$	77.8 ± 0.6^{k}	$12.9 \pm 1.6^{a,b}$	0.2 ± 0.0^{a}
	F3	2.7 ± 0.5^{c}	1.3 ± 0.7^{a}	75.9 ± 0.2^{j}	$17.4 \pm 2.3^{a,b,c}$	$0.6 \pm 0.3^{a,b}$
	F4	$1.1 \pm 0.0^{a,b}$	$3.4 \pm 0.1^{d,e,f}$	66.9 ± 0.9^{h}	24.4 ± 2.6^{c}	$0.5 \pm 0.5^{a,b}$
	F5	0.3 ± 0.1^{a}	$2.4 \pm 0.5^{b,c}$	58.9 ± 0.5^{f}	27.5 ± 2.3^{c}	$1.4 \pm 0.3^{c,d}$
	Ra	9.1 ± 0.3^{f}	31.5 ± 0.3^{k}	14.6 ± 0.7^{b}	$40.2 \pm 1.1^{d,e}$	6.1 ± 0.1^{f}
	Rb	$8.2 \pm 0.2^{e,f}$	14.7 ± 0.2^{i}	12.6 ± 0.4^{a}	68.9 ± 2.2^{f}	$4.1 \pm 0.0^{e,f}$
Ascophyllum nodosum	F1	44.9 ± 0.8^{i}	$4.4 \pm 0.1^{f,g}$	39.8 ± 0.8^{d}	$48.1 \pm 1.9^{d,e}$	7.5 ± 1.0^{h}
	F2	21.8 ± 0.2^{h}	$3.1 \pm 0.7^{c,d,e}$	80.2 ± 0.3^{l}	12.2 ± 1.6^{e}	$1.1 \pm 0.4^{b,c}$
	F3	2.6 ± 0.1^{c}	$1.5 \pm 0.1^{a,b}$	70.2 ± 0.2^{i}	$23.8 \pm 1.1^{b,c}$	5.3 ± 0.2^{g}
	F4	1.4 ± 0.2^{b}	$3.3 \pm 0.0^{c,d,e}$	70.1 ± 0.7^{i}	$23.7 \pm 1.0^{b,c}$	4.4 ± 0.0^{e}
	F5	1.3 ± 0.2^{b}	$2.7 \pm 0.1^{c,d}$	62.6 ± 1.0^{g}	25.2 ± 1.8^{c}	$1.5 \pm 0.3^{c,d}$
	Ra	8.0 ± 0.3^{e}	15.7 ± 0.6^{j}	19.5 ± 0.4^{c}	45.7 ± 1.6^{e}	7.4 ± 0.2^{h}
	Rb	5.6 ± 0.7^{d}	11.6 ± 0.3^{h}	15.8 ± 0.3^{b}	67.3 ± 2.0^{f}	7.7 ± 0.0^{h}

For a full description of the procedure to obtain the different fractions refer to Fig. 1.
The values reported are their means ($n = 3$) ± standard deviation. Different letters in the same column indicate significant differences between the samples ($p < 0.05$).
* The yield expresses the mass fraction of the specific waste stream divided by the total algal biomass in dry weight.

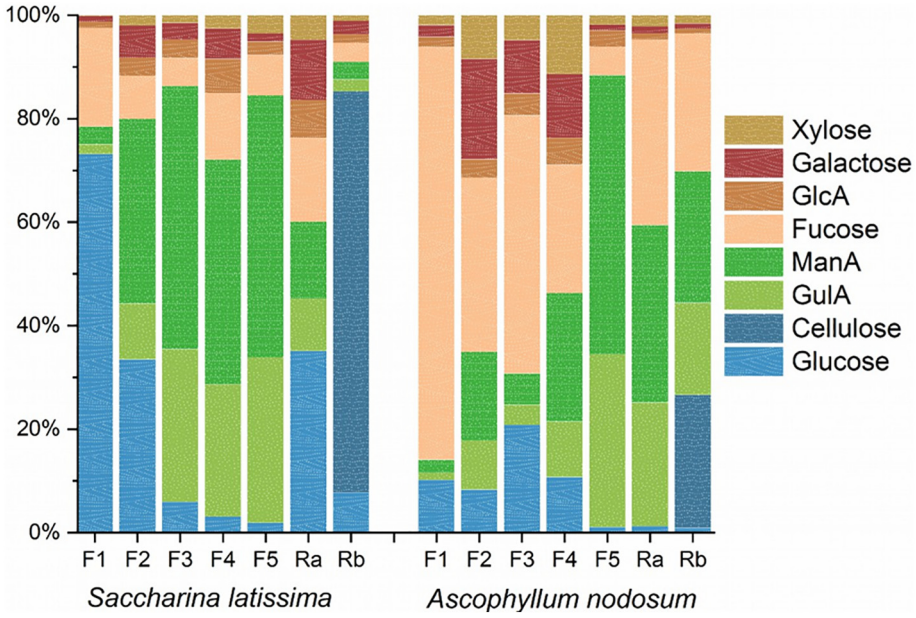

Fig. 2. Relative monosaccharide composition of the carbohydrate fractions of all waste streams (refer to Fig. 1 for nomenclature). Crystalline cellulose and glucose from non-crystalline polysaccharides were calculated as in Table 1.

precipitation of salts and confirms the need for this step to remove them and purify alginate. In the case of *S. latisima,* these low amounts of carbohydrates mainly correspond to alginate, probably of a very low molecular weight and not contributing to the desired rheological properties of commercial alginate (Fig. 2). With subsequent washings steps (F3–F5), overall solids and minerals recovered from these residual fractions gradually decreased while these very minor residual low molecular alginate is enriched. For *A. nodosum*, on the other hand, fucoidans with a high degree of galactose, xylose and glucuronic acid contribution predominate in F2–F4, but the proportion of alginate rises in the negligible last washing steps (F4–F5; Fig. 2). Although removal of these residual mineral and low molecular polysaccharides contributes to further purify an alginate with excellent gelling properties, the low yields of F4 and F5 suggest that these steps may be omitted in the process.

As commented above, a significant amount of the initial biomass remained in the solid residue after alkaline treatment (fractions Ra and Rb). In this work, this solid residue was further washed with distilled water to separately investigate those compounds which were solubilized through the alkaline treatment (Ra), from those insoluble in water (Rb). Interestingly, the water-soluble fraction (Ra) showed higher yields and was found to be very rich in protein, values being twice higher in *S. latissima* than in *A. nodosum*. Apart from soluble protein, Ra fractions also contained a significant amount of salts (15–19%) and polysaccharides. As to the composition of this polysaccharide fraction, fucan and alginate were found in *A. nodosum*, whereas fucoidan and alginate were predominant in Ra from *S. latissima* (Fig. 2). Other low molecular weight compounds or degradation products might be present in this soluble fraction which are not accounted for in a proximate analysis (Table 2). The water insoluble solid waste stream fraction (Rb) consisted mainly of polysaccharides (67–69%) although significant amounts of both insoluble protein and salts were also found (Table 2). The main insoluble carbohydrate fraction was, as expected, arising from the cellulose present in the algae (Fig. 2), which was not significantly extracted throughout the process and, thus, enriched in the Rb fractions. A much lower cellulose content has been reported for *A. nodosum* than for *S. latissima* (35% and 18% for the raw seaweeds (Cebrián-Lloret et al., 2022); which explains the lower yield of Rb in *A. nodosum* (Table 2) and a lower glucose content in the carbohydrate composition (Fig. 2). Instead, a recalcitrant fucoidan population remains in Rb from *A. nodosum*, which was not found in *S. latissima*, evidencing big differences in the cell wall architecture of these two seaweed species from the Fucales and Laminariales families and encouraging future research on the elucidation of these distinct fucoidan populations in *A. nodosum*. It is also notable to observe a significant alginate fraction which still remained in this residual fraction (around 25–75% of Rb for both species; Fig. 2). Unexpectedly, this residual alginate displayed a similar M/G ratio than the purified alginate. This may be positive for its potential valorization; as high G content is usually associated to better gelling properties (Hu et al., 2021).

FT-IR analyses were also carried out to assess the molecular organization and confirm compositional differences. Fig. 3a depicts the spectra from the alginates produced in this work as compared with a commercial one. The spectra from the liquid waste stream fractions F1–F5 for *S. latissima* and *A. nodosum* are displayed in Fig. 3b and c, respectively, while waste streams generated from the solid residue are shown in Fig. 3d and e. No notable differences are observed between all three alginates, again confirming the reproducibility of the mimicked industrial process and the purity of the samples (Fig. 3a). For both seaweeds, fractions F3-F5 showed similar spectra as that from the alginate, confirming the presence of this residual alginate as main component in these low yield fractions. This is reflected in the bands at around 1615–1626 cm^{-1}, corresponding to the asymmetric carbonyl stretching in the carboxylate anion (COO^-), the bands at 1080 and 1000 cm^{-1}, associated to the C—C and C—O stretching vibrations of the pyranose ring (Mateos-Aparicio et al., 2018), and the fingerprint or anomeric region (950–750 cm^{-1}) (Chandía et al., 2004; Gómez-Ordóñez and Rupérez, 2011). The absence of carbohydrates is patent in F2, with these bands having much less intensity, while a broad band around 1412 cm^{-1} has been associated to symmetrical stretching of the –C = O from inorganic carbonates (Leal et al., 2008). In F1, signals from the pyranose rings are present but not carboxylate signals, typical from neutral sugars, in agreement with the compositional results. In the fractions generated from the solid residue (Ra and Rb; Fig. 3d and e), both C—C and C—O stretching vibrations of the pyranose ring and carbonyl from carboxylate anions denote the presence of neutral sugars and alginate in this carbohydrate components, especially in Rb fractions. In the case of Ra, amide I and amide II signals at 1650 $cm^{-1}$and 1525 cm^{-1}, respectively, evidence the presence of protein. In the case of Ra from *S. latissima*, these bands are especially predominant, in agreement with the compositional results. The presence of sulfate was difficult to assign, as the S═O stretching band is partially overlapped by C—O stretching bands from sugars around 1200–1260 cm^{-1} (Gómez-Ordóñez and Rupérez, 2011; Palanisamy et al., 2017).

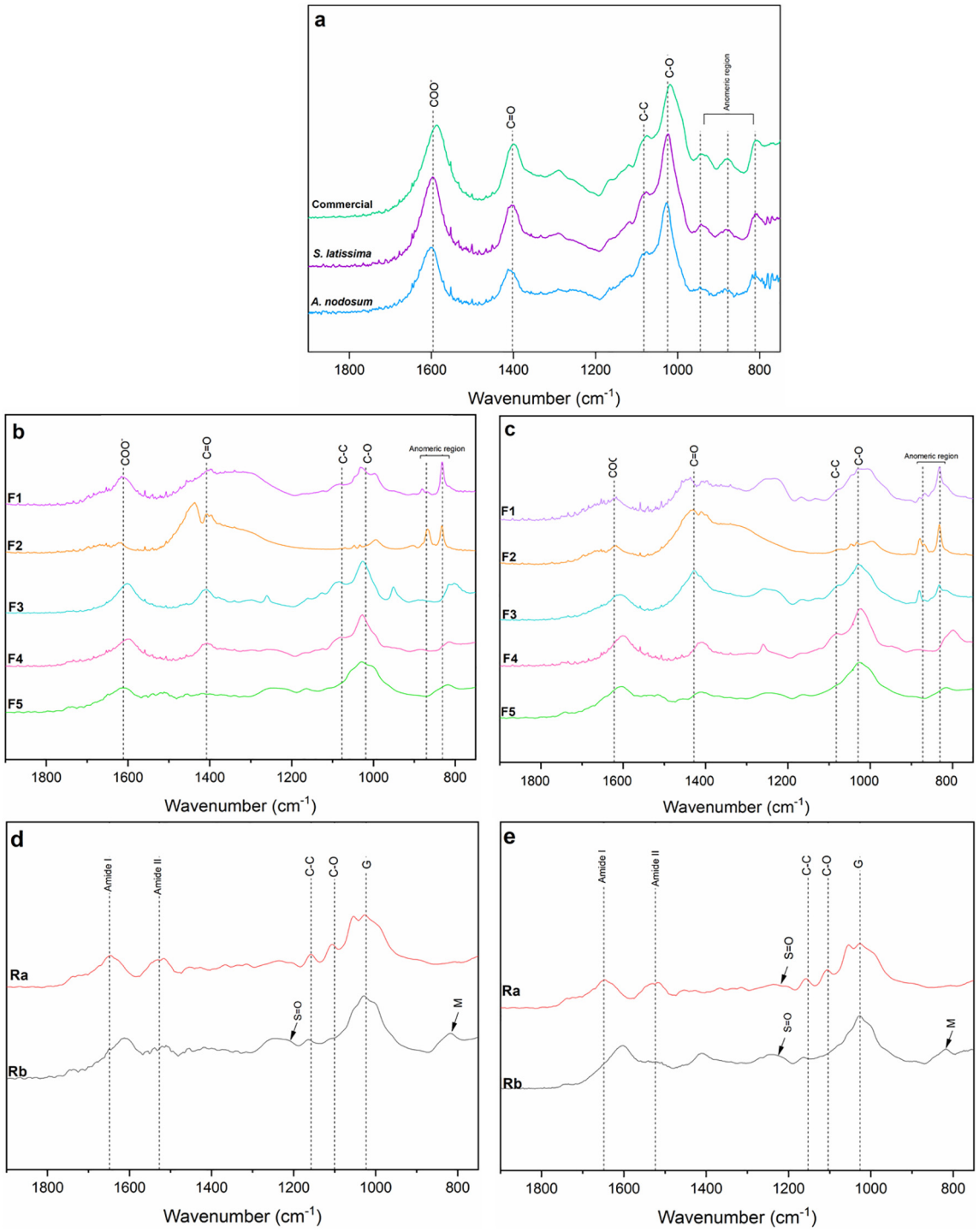

Fig. 3. FT-IR spectra of alginates (a), liquid residual streams (b) and (c), and fractions obtained from the solid residue residues (d) and (e) from *S. latissima* and *A. nodosum*, respectively. For a full description of the procedure to obtain the different fractions and their nomenclature refer to Fig. 1. G: guluronic acid; M: mannuronic acid.

3.3. Polyphenol content and antioxidant properties of the different fractions and residues

Many bioactive properties in brown algae extracts are related to their capacity to interfere with several biochemical mechanisms that regulate oxidative stress and inflammation which, in turn, has been mostly ascribed to the antioxidant or radical scavenging capacity of either the phlorotannin or sulfated polysaccharide content (Araújo et al., 2021). The determination of the ABTS radical scavenging activity or beta-carotene bleaching assay can be complementary methods to indirectly evidence a potential bioactive capacity in unknown samples, as they cover both the antioxidant and the radical scavenging capacity of either insoluble or soluble compounds, respectively (Méndez et al., 2022). Fig. 4 shows the polyphenol content, which complements previous compositional analyses, as well as the radical scavenging and antioxidant capacities of all waste streams from both seaweeds. This activity assays further back up and relate to the previous compositional results.

Interestingly, the highest polyphenol content was found in the waste streams from the solid residue after alkaline extraction, Ra and Rb, followed by F1, for both seaweeds (Fig. 4a). The polyphenol content of fractions F2

to F5 and extracted alginate did not show significant differences for both seaweed species and were significantly lower ($p < 0.05$) than the values obtained for F1, Ra or Rb. Higher total polyphenol content (TPC) values were found here for *A. nodosum,* which can be explained by a higher TPC content in the pristine algae compared to kelp species such as *S. latissima* or *A. esculenta* (Li et al., 2020; Schiener et al., 2015). Phlorotannins have also been described to be linked to the fucoidan or fucan structures, much more abundant in *A. nodosum.* This would explain not only an overall higher abundance in *A. nodosum*, but also an evidently higher contents of polyphenols in Rb compared to *S. latissima*'s cellulose-rich Rb fraction (Fig. 2). Another possible explanation is that the weaker cell wall of *A. nodosum* might have suffered greater cellular damage after the acid treatment, favoring the subsequent release of bound phenolic compounds (the Folin method mostly detects unbound ones). Apart from phlorotannins, previous studies have also reported the presence of some specific carotenoids (fucoxanthin, chlorophyll-*a*, or phycoxanthin) commonly forming complexes with protein in thylakoids and working as a light-harvesting system (Blanco-Pascual et al., 2014; Khajouei et al., 2018), which could be released upon acid/alkali treatments and further add up to the polyphenol and antioxidant activities (Sappati et al., 2019). In order to assess the antioxidant capacity of the different waste streams, both the ABTS method and β-carotene bleaching assay were performed and the results are displayed in Fig. 4b and c, respectively. Again, the results show that F1, Ra, and Rb had the highest antioxidant activity for both seaweeds, with significantly ($p < 0.05$) better results for *A. nodosum* than for *S. latissima*. This is consistent with the results on polyphenol content (Fig. 4a). The antioxidant capacity of fractions F1 was nevertheless higher than expected by the polyphenol content, suggesting this activity might also arise from the high content in highly sulfated fucoidan of this fraction (Fig. 2). The antioxidant activity of polysaccharide components from brown seaweed may depend on different factors such as molecular weight, sulfation level, and sugar residue composition (Jiménez-Escrig et al., 2011). In general, similar bioactive properties have been ascribed to both sulfated fucoidan and phlorotannins, e.g. preventing cardiovascular disease, obesity, neurodegeneration, cancer, used as food prebiotics or preservatives, etc. (Jiang et al., 2021; Meng et al., 2021). Some authors raised a controversy when ascribing functional properties to either sulfated fucoidan or phlorotannins (Imbs and Ermakova, 2021). Indeed, most studies focus in ascribing a specific functional activity to either a specific phlorotannin-rich or a sulfated fucoidan-rich extract, while neglecting the possible polyphenol-carbohydrate complexes and potential synergistic effects of both components. Further research is needed to accurately establish these structure-activity relationships. Regardless of the real source of the antioxidant or other bioactivity, this work evidences a high antioxidant potential, not only for the first acid effluents (F1), but also for the solid waste streams even after acid/alkaline treatment.

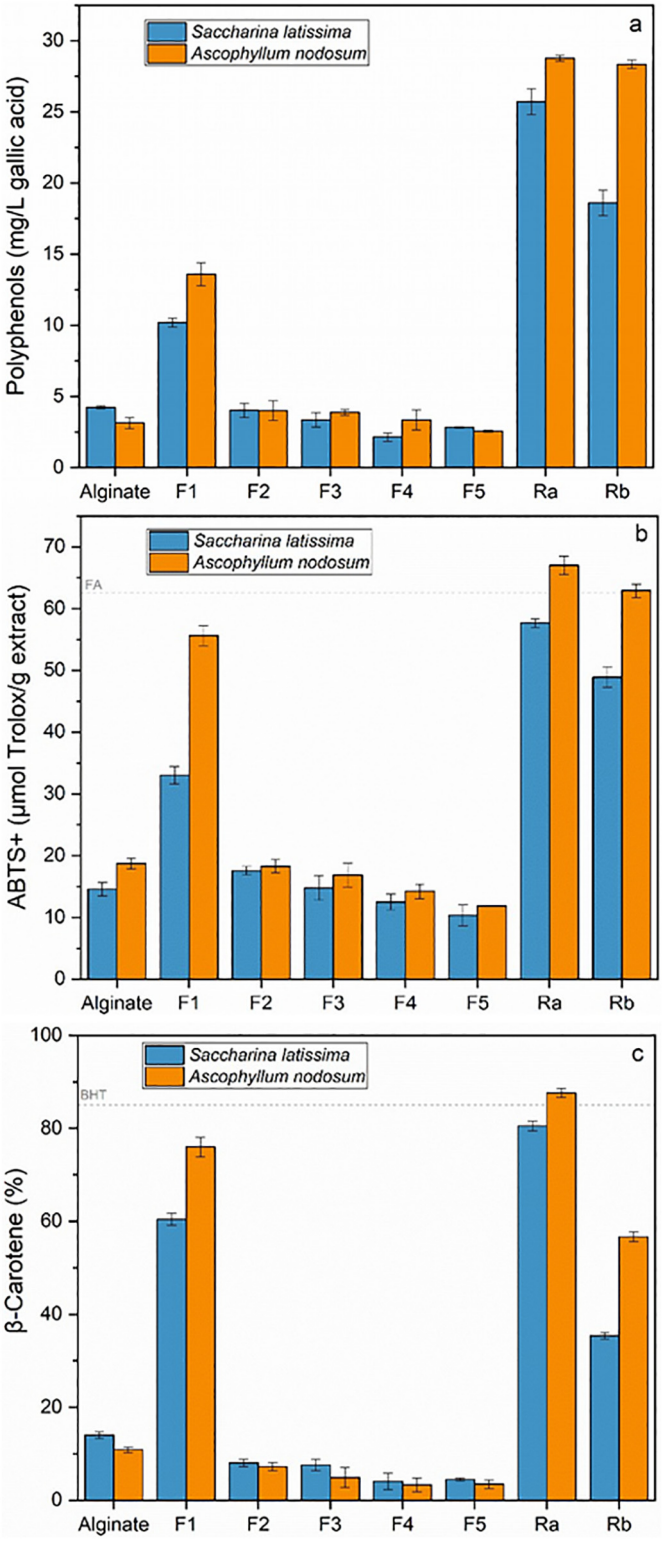

Fig. 4. Polyphenol content (mg GAE/g) (a), ABTS + antioxidant capacity (b), and β-carotene-bleaching inhibition (%) of alginates and waste streams from *S. latissima* and *A. nodosum* (see Fig. 1). The antioxidant activity of pure ferulic acid (FA) and butylhydroxytoluene (BHT) is added in a dotted line as comparison.

To confirm the antioxidant properties of the fractions and residues and to investigate how they are affected during the extraction process, ABTS + radical scavenging and β-carotene-bleaching inhibition assays were carried out on all extracts. Antioxidant activities are in the range of 40–70 μmol TEAC, which are relatively high values, in line with food natural compounds widely recognized as good antioxidants, such as caffeic acid (13.3 μM TE/g) or ferulic acid (60.8 μM TE/g) (Rodrigues et al., 2019). Through the beta-carotene bleaching assay, antioxidant activities of F1 or Ra fractions were found to be slightly lower than that of butylhydroxytoluene (BHT), a typical food additive used as strong antioxidant. Differences in the antioxidant response in Rb, or even F1, for both methods, might arise from the different solubility and availability of the antioxidant compounds when dispersed in an emulsion. These results pose both research and industrial challenges as to elucidate structure-activity and potential valorization strategies for these residues. As commented in Section 2.2, formaldehyde is often added to the harvested seaweed through transport as a preservative and to improve the quality of the extracted polysaccharide. Apart from being toxic, allergenic and possibly carcinogenic, formaldehyde can also cross-link with polyphenols (Cebrián-Lloret et al., 2022; Davis et al., 2004). This should be taken into account when designing a valorization strategy in this direction and it was one of the reasons it was not used in this study.

3.4. Potential valorization strategies for the waste streams

The compositional results and antioxidant activity studies of some of the waste stream fractions (F1, Ra and Rb) pose great potential pathways for

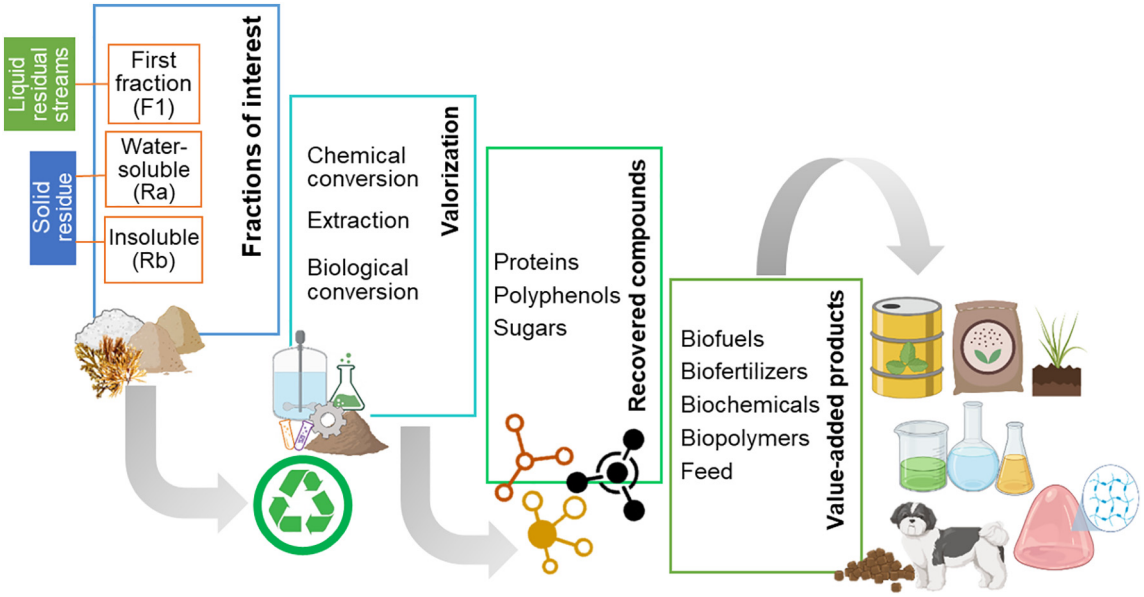

Fig. 5. Possible valorization strategies for specific waste streams generated in the alginate industry into novel added-value products.

their valorization. These promising results encourage further research efforts for the evaluation of the nutritional value of potential vegan protein and soluble fiber ingredients or mineral components and an in-depth analysis of specific bioactive properties of the fucoidan/polyphenol rich waste streams. The high yields of the soluble F1 and Ra fractions could effectively ensure a constant feedstock for the production of hundreds of tons of these high value vegan nutritional or cosmetic ingredients. Although many other valorization pathways are possible, a schematic overview of some examples is depicted in Fig. 5 and discussed below. Although alginate producing species in the same family, such as kelp species in the Laminariales family or typical nearshore algae in the Fucales family, have similar cell wall architecture and thus similar results are expected, compositional analyses to the specific waste streams should be confirmed before designing future valorization schemes.

Fraction F1, representing around 50% of all biomass, is a mostly water-soluble fraction containing high amounts of bioactive carbohydrates and minerals. In the case of *A. nodosum*, the abundance of highly sulfated fucoidan and polyphenols, together with excellent antioxidant properties, suggest this stream a firm potential candidate for the development of bioactive ingredients. For certain skin care applications, which are one of the main current market applications for soluble algae extracts, the presence of naturally occurring sea minerals may actually be positive to the formulation. For other applications, such as food or pharmaceutical ingredients bound to be ingested, simple purification steps, such as ultrafiltration or solvent precipitation, might highly purify these high-value ingredients. In the case of F1 from *S. latissima*, the high amounts of soluble glucans and free glucose makes it an interesting candidate for fermentation into biofuels or other platform chemicals. Indeed, these brown algae have been explored as a carbon source for the fermentation process into bioethanol (Albers et al., 2021; Sharma et al., 2018) or to produce terpenes, or methyl ethyl ketones (Peralta-Yahya et al., 2012; Scullin et al., 2015). Current exploitation of *A. nodosum* in Europe would allow a constant feedstock of thousands of tons/year of this up-cycled residual. Laminarin has also been ascribed a strong prebiotic effect (Nguyen et al., 2016), together with further antitumor or immunomodulating properties (Menshova et al., 2014) and has also been explored as a feed supplement (Walsh et al., 2012).

Fraction Ra, produced in this work by a simple water washing of the solid residue constitutes around 9% of all biomass, and could have as such a great potential as a food ingredient, owing to its high soluble protein content. In *S. latissima*, soluble protein in this fraction accounted for >30%. Given the high demand for soluble protein as texturizing/nutritional ingredients and the current trend towards removing animal-based texturizers, such as gelatin, from food ingredients, this fraction could be explored as a food additive in vegan or other food formulations.

On the other hand, a significant fraction of the insoluble protein is enriched in fractions Rb. This insoluble protein could be recovered by common protein extraction/purification methods and separated from the cellulose/fucoidan rich residue. This protein, even if partially denatured or degraded when extracted, might have use in the preparation of peptones for biotechnology purposes or used as a feed protein ingredient. This would help in decreasing Europe's dependence on protein import in line with current Horizon Europe R&D strategies. On the other hand, a substantial amount of alginate (nearly half of its initial content in the raw algae) also remains in the residual Rb fraction (also in Ra for *A. nodosum*). Although this alginate may not be extracted as a high molecular weight alginate with excellent gelling properties, it could still be valorized as partially degraded insoluble fiber or lower value thickener, depending on its molecular weight and rheological properties. The prebiotic activity of low molecular weight alginate has already been demonstrated (Li et al., 2020; Okolie et al., 2020). After recovery of these protein and polysaccharide fractions, the cellulose enriched residue could then be valorized as absorbents or food packaging materials as already reported by (Cebrián-Lloret et al., 2022).

As commented above, fraction F2 is also a significant waste stream in the process, made up of mostly minerals. In this case, further analysis of the mineral composition should ascertain its suitability for soil amendment or any other supplements (González-López et al., 2012). It may also be possible that this fraction contains most of the bicarbonate and chloride salts added to the process, which would definitely hamper valorization.

Due to their low yield, the rest of fractions could have limited use, as they are composed mainly of minerals, with very low quantities of soluble carbohydrates or protein.

4. Conclusions

In this work, the common alginate extraction process used in the industry was mimicked for two brown algae with different cell wall architecture, *A. nodosum* and *S. latissima,* and the different solid and liquid waste streams currently not valorized by the industry were characterized to explore potential valorization strategies. Produced alginates had similar composition or M/G ratio as commercial alginates, confirming the reproducibility of the industrial process. As for the waste streams, the first waste stream fraction after acid treatment (F1) showed a great potential as a bioactive ingredient,

due to a high yield in sulfated fucoidan and polyphenols, which demonstrated a high antioxidant efficiency by both ABTS and beta-carotene bleaching assays. A simple water washing of the solid residue after alkaline treatment (Ra) produced relatively high yields of a fraction with good antioxidant properties and rich in soluble protein (30%wt for *S. latissima*), with great potential as a texturizing vegan/functional ingredient. The solid residue (Rb) still contained a significant amount of insoluble protein and alginate, the valorization of which could be explored as feed supplement or, after further down-stream processing, as peptones or thickener ingredients, respectively. The distinct cell wall architecture of the algae was reflected in the composition of the fractions for both species. In the case of *S. latissima*, >30 wt% glucose/glucan content was found in F1, higher protein content in Ra and higher cellulose content in Rb. Comparatively, for *A. nodosum*, significantly higher antioxidant capacities were observed in F1, Ra and Rb (50–70 μM TE/g) and higher yields (>45%wt%) of a highly sulphated fucoidan were recovered in F1. These results provide the basis for a potential valorization of residual waste streams from alginate production and open up the possibility of using these fractions for the extraction ofadded-value ingredients for different applications within the cosmetic, pharma, food or feed industry, such as alternative protein sources, bioactives or texturizers. Considering the huge quantities of algae processed by the alginate industry, the transformation of these waste streams into added-value products would constitute a significant step towards a more circular and sustainable economy.

Credit authorship contribution statement

Hylenne Bojorges: Data curation, Methodology, Formal analysis, Investigation, Writing – original draft. **Maria José Fabra:** Conceptualization, Data curation, Formal analysis, Methodology, Resources, Supervision, Writing – review & editing. **Amparo López-Rubio:** Conceptualization, Data curation, Formal analysis, Methodology, Resources, Supervision, Writing – review & editing, Funding acquisition. **Antonio Martínez-Abad:** Conceptualization, Data curation, Formal analysis, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156394.

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