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Characterization of the invasive macroalgae *Rugulopteryx Okamurae* for potential biomass valorisation

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A R T I C L E I N F O

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A B S T R A C T

This study aimed to examine the composition and properties of the invasive macroalgae *R. okamurae* and explore potential applications. The results showed that the seaweed biomass is mainly composed of structural carbohydrates, with alginate being the main constituent, accounting for 32 % of its total composition and with a mannuronic and guluronic acid ratio (M/G) ratio of 0.93. It also has a relatively high concentration of fucose, related to the presence of fucoidans that have important biological functions. Among the mineral contents, a high magnesium and calcium (7107 and 5504 mg/kg) concentration, and the presence of heavy metals above legislated thresholds, were notable. *R. okamurae* also contained a high lipid content of 17 %, mainly composed of saturated fatty acids, but with a significant fraction of n3 polyunsaturated fatty acids (18 %) resulting in a low n6/n3 ratio (0.31), that has health benefits. The protein content of *R. okamurae* was 12 %, with high-quality proteins, as essential amino acids (mainly leucine, phenylalanine and valine) constitute 32 % of the total amino acids. It also showed a high polyphenol content and outstanding antioxidant properties (106.88 mg TE/g). Based on these findings, *R. okamurae* has significant potential as a sustainable source of bioactive compounds that can add value to different sectors, including food, feed, pharmaceuticals and cosmetics.

1. Introduction

The introduction of invasive species is one of the biggest threats to biodiversity due to the huge ecological impact it causes. In this respect, seaweeds are considered to be one of the greatest problems due to their contribution to marine primary production and their role as habitat providers for many wildlife species (Navarro-Barranco et al., 2019). There are currently more than 400 species of invasive marine seaweeds worldwide, 50 of which are found in the Iberian Peninsula (Patón et al., 2023).

Rugulopteryx okamurae (*R. okamurae*) is a brown seaweed of the *Dictyotacea* family and native of the Japan, Taiwan, China, Korea and North America area. It was previously registered as *Dilophus okamurae* and it is also known as *Dictyota marginata* or *Dilophus marginatus*. Its presence in Europe was first reported in a French harbour (Thau Lagon) in 2002. Later, in 2015, it was first seen in the Strait of Gibraltar, in Ceuta, but it was not until 2016 when it appeared in Tarifa (Cádiz, Spain). To this date, its presence has also been reported in the Alboran sea, the Provence (France) and in the Portugal coast also reaching the Azores Islands (Faria et al., 2022).

This is a case of cryptic invasion, where the invasive species is so similar to the native one that it can be difficult to identify, in this particular case it may be mistaken for seaweeds from the Dictyota genera (Altamirano et al., 2019). *R. okamurae* is highly competitive, reaching a level of coverage close to 90 % on rocky bottoms (García-Gómez et al., 2020), causing a displacement of the local biota and modifying the communities. It has also been reported to be dangerous for some species of gastropods, echinoderms or even some fish communities due to the production of toxic substances such as terpenoids and other secondary metabolites that prevent its predation (Bernal-Ibáñez et al., 2022). The current presence of this invasive macroalgae not only causes ecological problems, but also socio-economical ones, affecting fishing activity, beach management and tourism (Altamirano et al., 2019). In fact, to prepare for the tourist season, towards the end of spring, massive deposits of *R. okamurae* are usually removed from the coasts, which implies additional costs for the municipalities. For this

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reason, possible applications of this species are being studied as an alternative to its removal to landfills (García-Gómez et al., 2020).

Seaweeds are known to contain many interesting compounds, including polysaccharides, polyunsaturated fatty acids (PUFAs), bioactive peptides or alkaloids (Shepon et al., 2022). The multiple applications of these compounds to different industries has led to a rise on the market value. Specifically, in brown seaweeds the presence of fucoidans, alginates and phenolic compounds such as phlorotannins is remarkable (Bojorges, Martínez-Abad, et al., 2023; Olsson et al., 2020). These biomolecules are currently being used in the food, nutraceutical and pharmaceutical industry, having a great value due to their technological, functional and bioactive properties. Regarding *R. okamurae*, some studies have highlighted its potential for various applications such as bioplastic production or composting (Barcellos et al., 2023). However, to the best of our knowledge, none of them have focused on a detailed analysis of the actual composition of this particular species, as they typically rely on general information about brown seaweeds composition.

Therefore, this study aims to undertake a comprehensive examination of the biomass of the invasive macroalgae *R. okamurae*, including amino acid, fatty acid, carbohydrate and mineral with the overarching objective of acquiring an in-depth understanding of the seaweed's composition and properties and identifying potential added-value compounds. This is aimed at transforming a current threat and the waste generated into an opportunity, exploring potential valorisation pathways or higher added value market applications of the species in different sectors.

2. Materials and methods

2.1. Raw materials

R. okamurae was collected from seaweed upwelling, which migrates to the beaches of Costa Tropical in Granada, specifically in the Alboran Sea. The seaweed biomass was washed to remove sea salts and sand and dried for 24 h at 60 °C. The dried seaweed was then ground to a powder with a particle size of less than 250 μm and kept in a 0 %RH chamber before processing.

2.2. Compositional analysis of R. Okamurae

All analyses were performed at least in triplicate.

2.2.1. Carbohydrate profile

The carbohydrate content and composition of *R. okamurae* was estimated using acid methanolysis, which is a technique that cannot break down crystalline polysaccharides but has been found to be a good compromise between the lability of guluronic acid and complete scission of most glycosidic linkages (Bojorges et al., 2023). As brown seaweeds contain cellulose, a two-step sulfuric acid hydrolysis was also performed to determine the total glucose content, and the difference was attributed to the crystalline cellulose content. The sample was then subjected to analysis using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) with an ICS-6000 system (Dionex) equipped with a CarboPac PA1 column (4 × 250 mm, Dionex) at 30 °C and a flow rate of 1 mL min − 1. Neutral sugars were eluted in water for 16 min with post-column addition of 0.5 mL min − 1 of 300 mM sodium hydroxide after a preconditioning isocratic step with 260 mM sodium hydroxide and 68 mM sodium acetate (7 min) and 5 min equilibration time in water prior to injection. Uronic acids were eluted in a gradient of 100 to 200 mM sodium acetate in 10 mM sodium hydroxide over 20 min. Mixtures of fucose, glucose, galactose, arabinose, xylose, mannose, mannitol, guluronic acid, mannuronic acid, and glucuronic acid were used. Commercial microcrystalline cellulose and sodium alginate were also used as reference controls.

2.2.2. Protein content

An Elemental Analyser Rapid N Exceed (Paralab S.L., Spain) was used to analyse the total nitrogen concentration of *R. okamurae***.** Around 100 mg of the sample powder was pressed forming a pellet and then analysed by using the Dumas method, which relies on the detection of the released N_2 after the combustion of the sample. The nitrogen content obtained was then multiplied by a factor of 5 to estimate the total protein content, following previous findings for other brown seaweeds.

2.2.3. Amino acid profile

The total amino acids were determined after acid hydrolysis with HCl 6 N at 110 °C for 24 h. The analysis was carried out in a Biochrom 30 series Amino Acid Analyser (Biochrom Ltd, Cambridge, UK) equipped with a cation-exchange column. The post-column derivatization was achieved by mixing the eluent column with ninhydrin and by passing this mixture through a high temperature reaction coil. Finally, absorption was measured at 440 and 570 nm.

2.2.4. Lipid extraction

Lipids were extracted by the Folch method with slight modifications. Briefly, 30 mg of sample resuspended in 200 μL of MilliQ water were placed into a 10 mL tube to which 1660 μL of methanol were added and stirred at 1400 rpm for 10 min. Next, 3320 μL of dichloromethane was introduced into the tube and stirred at 1400 rpm for 20 min. 1000 μL of 20 mM acetic acid were added and stirred at 1400 rpm for another 10 min. The tube was centrifuged, and the lower organic phase was transferred to another tube while the aqueous phase was washed with 1660 μL of dichloromethane and then, the centrifugation and phase separation process was repeated. After pooling of the organic phase, the contents of this tube were filtered with a glass syringe using a 0,45 μm filter The organic solvent was evaporated, and the total lipid content was determined gravimetrically.

2.2.5. Fatty acid profile

Fatty acid methyl esters (FAME) were prepared using methanolic sodium methoxide, applying the direct transesterification method described by (Golay et al., 2007). Briefly, the internal standard mixture was prepared by dissolving equal amounts of tritridecanoin and methyl undecanoate (150 ± 0.1 mg) in *n*-hexane. Derivatization was carried out at room temperature for 3.5–4 min after the addition of 5 mL of the internal standard solution and 5 mL of 5 % methanolic sodium methoxide to the sample, followed by continuous stirring for 10 s. The reaction was stopped by the addition of 2 mL of *n*-hexane and 10 mL of an aqueous solution of disodium hydrogencitrate (at a concentration of 0.1 g/ml) and sodium chloride (at a concentration of 0.15 g/ml). After phase separation, the supernatant was diluted prior to analysis.

An Autosystem chromatograph (PerkinElmer, Beaconsfield, UK) equipped with a flame ionization detector (GC-FID) was used to perform the FAME analysis, following the method described in (Calvo et al., 2020). Response factors for individual FAME were determined using anhydrous milk fat with a certified composition (BCR-164, European Community Bureau of Reference, Brussels, Belgium) as a reference, and tritridecanoine (Sigma, St. Louis, MO) was employed as internal standard.

2.2.6. Total phenolic content

The Folin-Ciocalteu technique was used to determine the total phenolic content of *R. okamurae*. For this purpose, the dried material was dissolved at a concentration of 5 mg/mL for this colorimetric assay. 200 μL of the sample was combined with 1000 μL of the Folin-Ciocalteau reagent (diluted 1:10 with distilled water). After adding 800 μL of sodium carbonate (75 mg/mL), the sample was heated for 30 min at 50 °C. The absorbance value was measured at 750 nm wavelength. Gallic acid was used as a reference to create the calibration curve. The total phenolic content was expressed as mg of gallic acid (GA)/g extract.

2.2.7. Dietary mineral and heavy metal composition

An Inductively Coupled Plasma Mass Spectrometry (ICP-MS) equipment model ICPMS7900 manufactured by Agilent Technologies was utilized to conduct a quantitative analysis of the minerals and metals present in *R. okamurae.* Sample preparation consisted of a sample digestion process in a microwave oven.

2.2.8. Ash content

The mineral content of the dried seaweeds was determined using the TAPPI T211 om-07 method. To achieve this, around 0.25 g of dry substance was placed in a pre-weighed crucible, which was then weighed. The crucible was combusted in a muffle furnace at 550 °C for 24 h, and the resulting ash content was measured gravimetrically.

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

The dried seaweed ABTS$^{\cdot+}$ radical cation scavenging capacity was assessed according to (Re et al., 1999). In order to generate the ABTS$^{\cdot+}$ radical cation, 0.192 g of ABTS were first dissolved in 50 mL of PBS at a pH of 7.4 and then combined with 0.033 g of potassium persulfate overnight in the dark. The ABTS$^{\cdot+}$ was diluted with PBS for an initial absorbance of ~ 0.700 ± 0.02 at 734 nm at room temperature, before to use in the test. Free radical scavenging activity was evaluated by combining 20 μL of sample (dissolved in distilled water at a concentration of 5 mg/mL) with 230 μL of diluted ABTS$^{\cdot+}$ and observing the change in absorbance after 6 min. The calibration curve was developed by using 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox). The antioxidant capacity was expressed as mg Trolox equivalents (TE)/g extract.

3. Results and discussion

3.1. Characterization of the seaweed biomass

To valorise the invasive alga *R. okamurae,* the gross composition of the dried biomass was analysed, since, to our knowledge, no previous work has reported on it, and the results are compiled in Table 1.

3.1.1. Lipid composition of R. Okamurae

The proximate composition of macroalgae in general is subjected to variations based on several factors, including the environmental conditions, water temperature, nutrient availability, and light intensity (Olsson et al., 2020). However, it is generally known that the lipid content is relatively low, typically ranging from 1 % to 5 % of the dry weight of the seaweed biomass, even though some species of the *Dictyota* gender may reach up to 20 % (Sun et al., 2018). When compared to other seaweeds of the same order, it is noted that *R. okamurae* has a greater content (ca. 17 %) than others species such as *Dictyopteris australis* (ca. 8.5 %), *Dictyota bartayresiana* (ca. 12 %) or *Stypopodium zonale* (ca. 11 %) (Gosch et al., 2012). It is also well known that brown seaweeds exhibit significant quantities of terpenoids, primarily diterpenes, sesquiterpenoids, and meroditerpenes. *R. okamurae*, in particular, stands up for its elevated levels of sesquiterpenes, which have facilitated the spread of this invasive species as a result of their function as feeding deterrents against predators (Patón et al., 2023). Moreover, the presence of omega-3 polyunsaturated fatty acids (n3 PUFA) in brown algae has attracted significant interest due to sustainability issues related to the increasing demand of n3 PUFA for human consumption (Shepon et al., 2022). The high lipid content of *R. okamurae* makes it an excellent source for the extraction and isolation of these compounds with proven health benefits.

In this study, the fatty acid (FA) composition of *R. okamurae* was determined (Table 2), with the aim of gaining a comprehensive understanding of their potential benefits and applications across different industries. The results show that saturated fatty acids (SFA) were predominant (ca. 61 %) in the lipid profile of *R. okamurae*, followed by PUFA (ca. 24 %) and monounsaturated fatty acids (MUFA) (ca. 15 %). The main FAs present in *R. okamurae* were palmitic (C16:0) and myristic (C14:0), accounting for 32 % and 15 % of the total FA content, respectively. Such C16:0 content, is consistent with previous findings in other species of brown seaweeds (Belattmania et al., 2018; Gosch et al., 2015). Regarding C14:0, it has also been detected in other brown seaweed species, but it typically constitutes less than 10 % of total FAME (Belattmania et al., 2018). The consumption of these two SFA has traditionally been linked to an elevated risk of cardiovascular disease, insulin resistance, and inflammation. Nonetheless, recent research has investigated the potential of myristic and palmitic acids in reducing inflammation and protecting against oxidative stress. *R. okamurae* also possesses a significant amount (13 %) of oleic acid (C18:1 *cis*-9), being the main MUFA. This is in agreement with oleic acid concentrations detected in other brown algal species, with values ranging from 7 to 24 % of the total FA content (Belattmania et al., 2018).This is of particular interest since oleic acid is well-known to contribute to the promotion of

Table 2
Fatty acid composition (% of total fatty acid methyl esters) of oils obtained from *R. okamurae.*

Fatty acid	
C4:0	2.79 ± 0.66
C5:0	1.50 ± 0.42
C8:0	0.58 ± 0.14
C13 *iso*	0.69 ± 0.09
C14:0 *iso*	0.27 ± 0.15
C14:0	15.15 ± 0.29
C15:0	1.48 ± 0.15
C16:0 *iso*	0.68 ± 0.20
C16:0	31.75 ± 0.39
C17:0 *aiso*	0.76 ± 0.29
C18:0	3.13 ± 0.22
C22:0	1.60 ± 0.14
C24:0	0.72 ± 0.14
∑ SFA	61.12 ± 1.59
C16:1 *trans*-9	<0.3
C16:1 *cis*-9	1.62 ± 0.07
C18:1 *cis*-9	12.64 ± 0.25
C18:1 *cis*-11	0.83 ± 0.08
∑ MUFA	15.26 ± 0.30
C16:4 n3	7.82 ± 0.14
C18:2 n6	1.68 ± 0.12
C18:3 n3	4.09 ± 0.91
C18:4 n3	3.17 ± 0.78
C20:2 n6	1.58 ± 0.18
C20:3 n6	<0.3
C20: n6	2.18 ± 0.10
C20:4 n3	0.90 ± 0.34
C20:5 n3	0.70 ± 0.13
C22:5 n3	1.30 ± 0.18
∑ PUFA	23.62 ± 1.70
∑ n6	5.65 ± 0.44
∑ n3	17.98 ± 1.32
n6/n3	0.31 ± 0.01

Data are presented as mean values of duplicate samples ± SD (n = 2).

Table 1
Proximate composition and antioxidant capacity of the *R. okamurae* biomass (dry weight).

Lipids (%)	Proteins (%)	Carbohydrates (%)	Polyphenols (%)	Antioxidant capacity ABTS (mg TE/g sample)	Ashes (%)
17.3 ± 3.2	12.2 ± 0.2	60.4 ± 5.1	4.5 ± 0.3	106.88 ± 0.05	11.30 ± 0.08

a healthy blood lipid profile, mediate blood pressure, and have a favourable effect on insulin sensitivity and glycaemic control. Noteworthy is the high presence of hexadecatetraenoic acid (C16:4 n3), which makes up almost 8 % of total fat. This n3 FA is known to be found in certain types of fish and marine organisms like krill (Yamada et al., 2017), however, to our knowledge, it has only been reported to be present in some macroalgae species like *Ulva spp*, in which it represents up to 12 % of the total FA (Premarathna et al., 2022). Unlike other n3 FA like eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3), it has received less research attention. Studies have proposed that hexadecatetraenoic acid has anti-inflammatory properties and may help prevent chronic diseases like cardiovascular disease. Nonetheless, additional research is necessary to fully comprehend the potential health benefits of this FA. Although to a lesser extent, the n3 PUFAs alpha-linolenic acid (ALA) (C18:3 n3) and stearidonic acid (SDA, C18:4 n3) are also present at 4 % and 3 %, respectively. The role of these n3 FAs in improving cardiovascular functions, regulating systolic and diastolic blood pressure, and enhancing neurological disorders has been previously described, thus possessing antiarrhythmic and anti-inflammatory properties (Gogna et al., 2023). It is important to take note that the overall n6/n3 ratio stands at 0.31. This ratio is of great significance because it reflects the balance between n6 and n3 PUFA) which is known to have a great impact on health through the diet. A high ratio (15 and greater) has been linked to the development of chronic diseases like inflammatory and cardiovascular diseases, while a lower n6/n3 ratio can help preventing these diseases and provide anti-inflammatory benefits (Ferreira et al., 2022). This oil, with such a low n6/n3 ratio is not very common in nature and, therefore, the oil from *R. okamurae* would have high biological and commercial value. In this line, oils from certain wild berries, including crowberries, blackcurrants, and raspberries show low n6/n3 ratios, that has been related to their neuroprotective action, as well as anti-inflammatory and antioxidant properties, which may affect various bodily systems, including the skin, cardiovascular, nervous, and pulmonary systems. Therefore, these results suggest that *R. okamurae* has the potential to be a highly effective sustainable source for the extraction of lipid compounds which may have important implications for a wide range of industries, including food, feed, pharmaceuticals and cosmetics.

3.1.2. *Protein profile of R. Okamurae*

The protein content of brown seaweeds exhibits significant variation, as reported in previous literature. Within the *Dictyotacea* family, protein content has been observed to range from 1.7 % to 27.6 % (Bogaert et al., 2020; Olsson et al., 2020). Therefore, the protein content value obtained for *R. okamurae,* 12.3 %, falls within the observed range of variability. To assess the potential of this seaweed species as a food source and to determine the quality of its proteins, a comprehensive analysis of its total amino acid profile was conducted and is presented in Fig. 1. As observed, essential amino acids make up 32 % of the total amino acids, similar to that found in animal sources such as eggs (32 %), casein (34 %) or milk (39 %), (Gorissen et al., 2018) which means that the proteins of this seaweed species can be considered to be of high quality. This is of particular interest since each of these amino acids plays a crucial role in various physiological processes, such as protein synthesis, muscle growth and repair, immune function, and hormone regulation. The most abundant essential amino acids in *R. okamurae* are leucine and phenylalanine, each comprising 6.8 %, closely followed by valine (5.4 %), threonine (4.5 %), and to a lesser extent, isoleucine (3.3 %), lysine (2 %), methionine (1.6 %), and histidine (1.4 %). Depending on the species, different essential amino acid contents for brown seaweeds have been reported in the literature (Astorga-España et al., 2016). The amino acid profile does differ much from that of other previously reported species of the same family, such as *Dictyota dichotoma* (Bogaert et al., 2020). Among the non-essential amino acids, aspartic acid represents the most dominant one, constituting 12.9 % of the total amino acid content. This is followed by glycine at 12.6 %, alanine at 12 %, and glutamic acid at 11.8 %. Other amino acids present in significant amounts include serine at 7.7 % and proline at 5.8 %. On the other hand, the quantities of tyrosine, arginine, and cysteine are relatively low, with percentages of 2.4 %, 2.2 %, and 0.5 % respectively. Seaweed species are typically characterized by the high content of aspartic acid and glutamic acid, which contribute to the unique flavour and aroma associated with seaweed and marine products (umami) (Astorga-España et al., 2016). Additionally, the presence of glycine and alanine also imparts a sweet taste (Ramu Ganesan et al., 2020). As in *R. okamurae,* in other species of brown seaweeds such as *Dictyota dichotoma*, cysteine is typically present in very small quantities that are almost undetectable, while tyrosine and arginine tend to be a little more abundant (Bogaert et al., 2020). In fact,

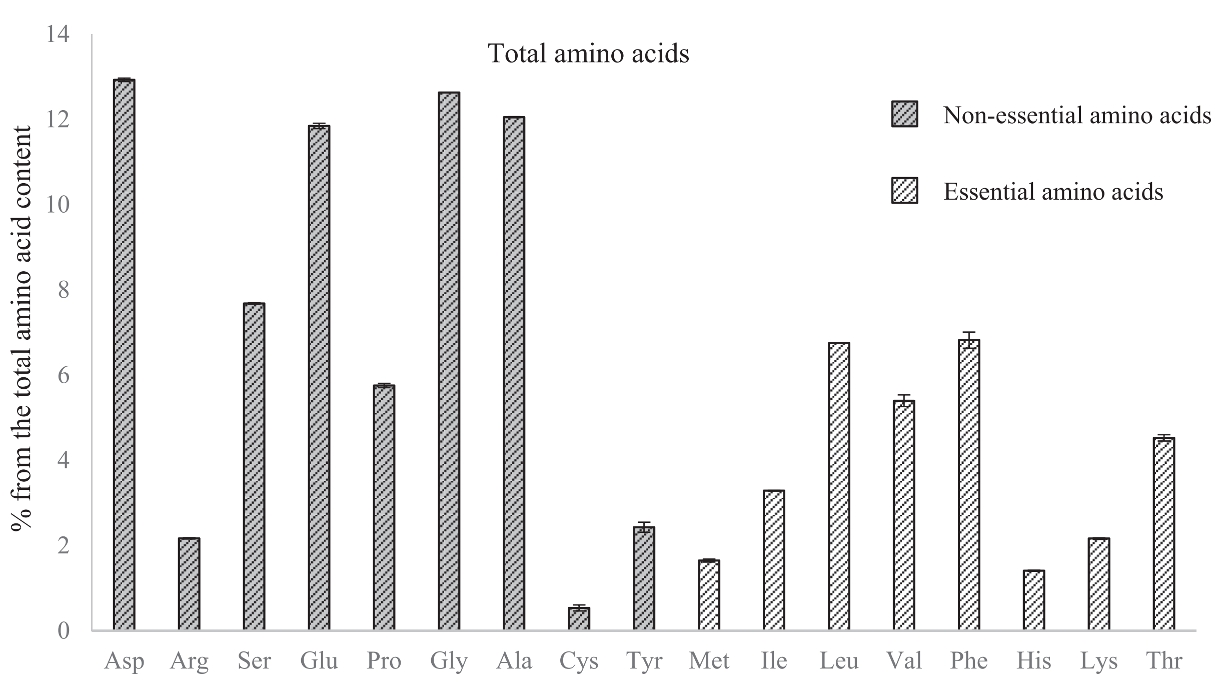

Fig. 1. Amino acid profile of *R. okamurae* (expressed as % from the total amino acid content).

several investigations have reported that lysine, threonine, tryptophan, histidine and sulphur amino acids (cysteine and methionine) are considered the limiting amino acids in algal proteins, despite their elevated levels compared to those present in land-based plants (Fouda et al., 2019). Even so, the protein derived from this seaweed exhibits a nutritionally compelling profile of amino acids (AAs) and contains a higher content of essential amino acids (EAAs) compared to alternative non-animal sources. Consequently, *R.okamurae* could potentially contribute to minimizing the deleterious environmental ramifications that arise due to animal protein production. As such, algal proteins have garnered recognition as a feasible protein source for the coming times.

3.1.3. Carbohydrate profile of R. Okamurae

Seaweeds are generally characterized by the presence of structural carbohydrates, which constitute the primary component of their cell walls, providing the necessary strength and rigidity to withstand environmental stresses such as mechanical pressure and wave action. In *R. okamurae,* carbohydrates constitute roughly 60 % of its composition on a dry weight basis, with alginate being the principal constituent, making up approximately 32 % of its total composition, which is similar to that previously reported for typical alginate sources such as the *Laminaria* (Schiener et al., 2015) and *Ascophyllum* (Cebrián-Lloret et al., 2022) genera, with a 40 and a 30 % of alginate respectively (Fig. 2). Alginate is a linear polymer composed of two types of monomers, namely guluronic acid (G) and mannuronic acid (M). The proportion of these monomers, or M/G ratio, may vary according to the seaweed species, affecting the physical and chemical properties of alginate (Cebrián-Lloret et al., 2022). In the case of *R. okamurae*, the M/G ratio was determined to be 0.93, which is lower than the M/G ratios previously reported for alginates extracted from other brown seaweed species, such as *S. latissima* (M/G ratio 1.7) and *A. nodosum* (M/G ratio 1.6) (Bojorges et al., 2023). It is known that alginates with a lower M/G ratio result in stronger gel structures, as G-blocks have a higher affinity for calcium ions than M−blocks (Bojorges et al., 2023). Therefore, investigating the potential applications of alginate from *R. okamurae* would be of great interest. Moreover, the carbohydrate profile of *R. okamurae* exhibits a higher concentration of fucose (8 %) compared to other brown seaweed species such as *Saccharina japonica* (3–4 %), *Stephanocystis crassipes* (6 %), and *Fucus vesiculosus* (4.5 %) (Obluchinskaya et al., 2022). However, the fucose levels in *R. okamurae* are lower than those observed in other species belonging to the *Fucales* order, where fucose can make up more than 70 % of total carbohydrates (Ponce & Stortz, 2020). The presence of fucose in *R. okamurae*, along with other sugars such as galactose and xylose, is of particular interest since it is linked to the presence of fucoidans (e.g. fucogalactans, galactofucans or fucoglucuronans), which have shown significant biological functions such as anticancer, antiviral, antiallergic, anticoagulant, antioxidant, anti-inflammatory, immunostimulant, cardioprotective, and hepatoprotective properties. The seaweed biomass was composed of 7 % glucose arising from cellulose. This cellulose content is in line with that observed in other brown seaweed species within the same order, such as *Dictyota bartayresiana* with 9.3 % or *Dictyota dichotoma* with 9.5 % (Siddhanta et al., 2013). Despite this, it has been reported that the cellulose content of brown seaweed biomass typically falls within the range of 2.2 % to 10.2 % (Baghel et al., 2021). Some studies demonstrate that cellulose could be economically extracted from phycocolloids industrial waste (Baghel et al., 2021; Cebrián-Lloret et al., 2022). Therefore, the possibility of valorising distinct added-value polysaccharides, such as fucoidans, alginate and cellulose in *R. okamurae*, portends great potential for this species in a variety of commercial applications.

3.1.4. Potential functional activity of R. Okamurae

To obtain a better understanding of the potential properties of the seaweed biomass, a functional characterization was also conducted, which included an evaluation of the total phenolic content and antioxidant capacity. The results (shown in Table 1) evidenced that *R. okamurae* presents a high polyphenol content (4.5 %) compared to other brown seaweed species of the *Dictyotaceae* family, such as *Dictyota ciliolate*, which has a total polyphenol content of 0.08 %, *Dictyopteris polypodioides* with 0.09 %, and *Stypopodium zonale* with 1.22 % (Mekinić et al., 2019), or even other seaweed species. The most significant type of polyphenols found in brown seaweeds are phlorotannins (Sathya et al., 2017). Several research studies have indicated that the phlorotannins obtained from various species of brown seaweeds exhibit robust antioxidant properties that may be related to their unique molecular structure (Ford et al., 2019; Sathya et al., 2017). The several antioxidant capabilities of polyphenols are closely linked to the existence of phenolic rings, which function as electron traps to neutralize harmful peroxy,

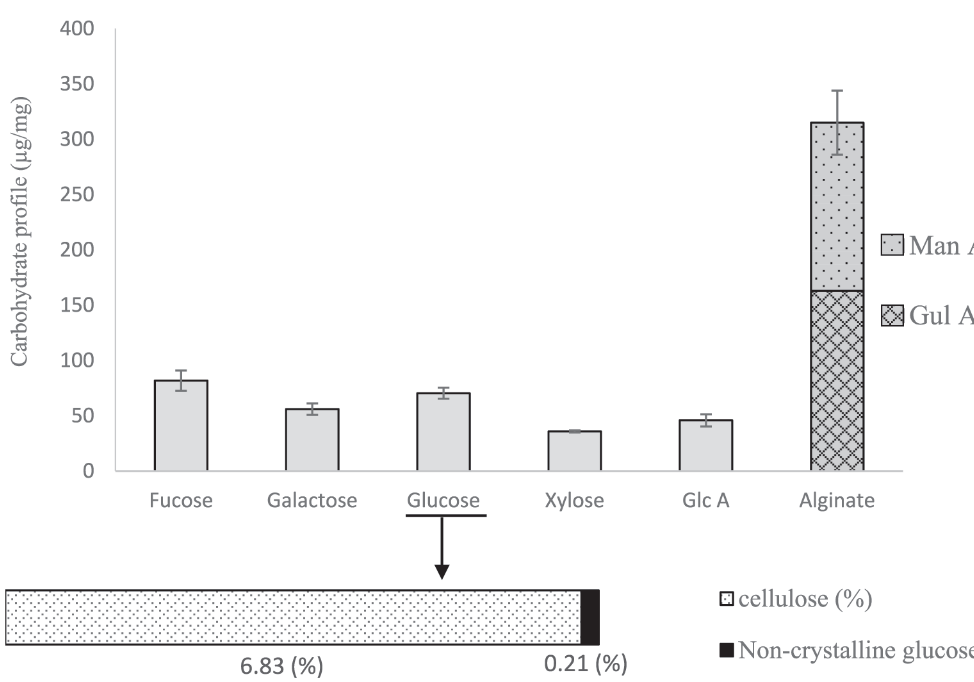

Fig. 2. Carbohydrate profile of *R. okamurae*.

superoxide, and hydroxyl radicals (Sathya et al., 2017). This, combined with the presence of sulphated polysaccharides, is consistent with a notoriously high antioxidant activity of *R. okamurae* (106.88 mg TE/g), which is much higher than some commonly used organic antioxidants such as ginger (43.6 ± 0.86 mg TE/g) and turmeric (27.5 ± 0.40 mg TE/g) (Hossain et al., 2008). Phlorotannins have been shown to possess defensive properties against predators by suppressing digestive enzymes, which, together with a high terpenoid content, could explain the large proliferation of this invasive seaweed in recent years. Thus, *R. okamurae* has the potential to serve as a plant-based alternative to conventional sources of phenolic compounds and antioxidants for use in the food, pharmaceutical, and cosmetic sectors.

3.1.5. Mineral and heavy metal composition of R. Okamurae

Since ash represented up to 11 % of the overall weight of the seaweed biomass, a quantitative elemental analysis was conducted to identify the mineral composition of *R. okamurae* and the results are compiled in Table 3. This analysis mainly focuses on human consumption of seaweed, as the levels of certain elements may indicate both potential benefits and risks associated with its consumption. It is noteworthy that the consumption of seaweeds is a relatively new trend in Europe, thus, as of now, there are no regulations in place that control the maximum levels of all toxic elements found in seaweeds, but only for some specific metals such as cadmium, which is limited to a maximum of 3 mg/kg for food supplements composed predominantly or solely of dried seaweed. (EC, 2021). Consequently, *R. okamurae*, which contains a cadmium content of 0.38 mg/kg, complies with the established limits. With regards to mercury, Regulation (EC) No. 396/2005 of the European Parliament and of the Council currently imposes a default maximum residue limit (MRL) of 0.01 mg/kg for seaweeds and prokaryotic organisms (EC, 2017). So, *R. okamurae* with 0.03 mg/kg exceeds the established limits. The lead content of *R. okamurae*, which is 2.24 mg/kg, also exceeds the regulated maximum content of 0.1 mg/kg wet weight (ww) for vegetable consumption. (EC, 2006). Similarly, the arsenic level in *R. okamurae* is over established legislative limits, as it is 11.16 mg/kg, although its content is still lower than that reported for other brown seaweed species such as *Ascophyllum nodosum* with 21.5 mg/kg or *Saccharina latissima* with 54.4 mg/kg (Olsson et al., 2020), Also, the iodine contents (416 mg/kg) was relatively high, but certain members of the *Laminariales* order, such as *Saccharina latissima*, may have as much as 7200 mg/kg (Roleda et al., 2018). As a possible solution against the presence of heavy metals, a previous investigation conducted by Noriega-Fernandez et al. demonstrated that exposing the brown seaweed species *L. hyperborea* to a combination of ultrasound (US) and ethylenediaminetetraacetic acid (EDTA) at 50 °C for 5 min resulted in a significant reduction of 32 % for As, 52 % for Cd, and 31 % for iodine content (Noriega-Fernández et al., 2021). Consequently, to ensure the suitability of this seaweed species for food-related applications, the implementation of this metal elimination protocol would be necessary.

Table 3
Minerals and elements present in *R. okamurae (*dry weight).

Element	Concentration (mg/Kg) dw	Element	Concentration (mg/Kg) dw
Na	2556.00	Cd	0.38
Mg	7107.00	Ti	40.11
Al	1020.00	P	22.54
Cl	1140.00	V	7.69
K	2590.00	Mn	12.71
Ca	5504.00	Co	0.40
Sr	1570.00	Rb	2.83
I	416.04	Ba	31.36
Br	513.45	La	1.70
Fe	686.01	Ce	1.99
Hg	0.03	W	2.96
Pb	2.24	B	41.63
Ni	5.98	Pt	0.02
Cr	4.24	Au	0.04
As	11.16	Ag	0.55
Cu	4.74	Y	0.72
Zn	34.73	Zr	1.40

Seaweeds exhibit high variability in essential mineral content depending on the species. Typically, brown seaweeds contain substantial amounts of calcium, with *Laminaria digitata* reaching levels as high as 12400 mg/kg, and magnesium, with some species such as *Ascophyllum nodosum* containing values of up to 8700 mg/kg (Olsson et al., 2020). *R. okamurae* contains relatively high quantities of essential minerals, such as magnesium (7107 mg/kg), calcium (5504 mg/kg), potassium (2590 mg/kg), iron (686 mg/kg), and to a lesser extent, zinc (34.73 mg/kg), phosphorus (22.54 mg/kg), and manganese (12.71 mg/kg). This indicates that *R. okamurae* could potentially serve as a dietary supplement, fortifying or nutraceutical ingredient, leading to a potential market for such products.

3.2. Potential valorisation strategies

The objective of this study was to conduct a comprehensive analysis of the biomass composition of the invasive macroalgae *R. okamurae*, with a focus on exploring potential valorisation strategies for the different compounds. *R. okamurae* was mainly composed of structural carbohydrates (60 %), with alginate being the main constituent, accounting for 32 % of its total composition. Apart from being a rich source of alginate, comparable to other currently used brown algae sources, the alginate of *R. okamurae* showed a M/G ratio of 0.93 equal of even lower than common industrial alginates. *R. okamurae* also contains a relative high concentration of fucose (8 %) which is related to the presence of fucoidans with important biological functions.

The oil obtained from *R. okamurae* is characterized by a high content of n3 PUFA and extremely low n6/n3 ratio (0.31), indicating a good balance between n6 and n3 fats, which is truly beneficial from a nutritional standpoint. The presence of hexadecatetraenoic acid in relevant amounts makes *R. okamurae* a sustainable source worth evaluating. The content of n3 PUFA found in *R. okamurae* (18 %) is comparable to those in widely utilized microalgae species like *Chlorella vulgaris* (Maltsev et al., 2021). According to the previous literature, n3 PUFA exhibit potential applications in treating various diseases such as atherosclerosis, Parkinson's, and Alzheimer's. In addition, *R. okamurae* could also be used for biofuel production, to generate renewable biofuels that are considered third-generation fuels.

The seaweed biomass was found to contain 12.2 % protein and the analysis of its amino acid profile indicated that the proteins present an interesting amino acid profile, with essential amino acids comprising 32 % of the total amino acids, with leucine being the most abundant. Non-essential amino acids, such as aspartic acid and glutamic acid, are also present, contributing to the unique flavour and aroma of seaweeds and seafood products. Depending on the source, vegetable proteins may be deficient in some essential amino acids. Some legumes such as soybeans, beans or peas are often deficient in sulphur amino acids such as methionine, while cereals tend to have low levels of lysine (Otero et al., 2022). This is why *R. okamurae* could serve as an alternative to animal protein sources and could complement the amino acid profile of existing vegan products.

R. okamurae also shows a significant polyphenol content of 4.5 %, and outstanding antioxidant properties. In fact, its antioxidant activity clearly surpasses that of commonly utilized organic antioxidants like ginger or turmeric, revealing extraordinary nutritional potential.

Last, the seaweed biomass also contains essential minerals such as magnesium, calcium, potassium, iron, zinc, phosphorus, or manganese, which exert beneficial effects on the human organism, allowing, among others, normal bone development, good muscle and immune system functioning or blood pressure regulation. While certain foods like peanuts are recognized for their elevated essential mineral, especially magnesium, content, this remains considerably lower than that found in *R. okamurae*. Consequently, *R. okamurae* emerges as a viable alternative for sourcing essential minerals to produce dietary supplements.

Based on the findings of this research, it can be asserted that the invasive macroalgae *R. okamurae* could be a sustainable source of functional, nutritional, technological or bioactive compounds which exhibit promising potential for their utilisation in various industrial sectors, such as food, feed, pharmaceuticals and cosmetics, transforming the threat into an opportunity within the circular economy.

4. Conclusions

The aim of this study was to investigate the composition of the invasive macroalgae *R. okamurae,* so as to find potential applications for its complex matrix. The high quantities and low M/G ratio of alginate prompt further research on their technological properties and potential extraction strategies. It also contains a relatively high concentration of fucose, which is related to the presence of fucoidans with important biological functions. For a macroalgae, a relatively high lipid content of 17 % was found, with significant amounts of n3 PUFA (13 %) and low n6/n3 ratio, indicating a good balance between essential fatty acids. It also contained alternative vegan protein with good nutritional potential, including essential amino acids. Additionally, *R. okamurae* showed significant polyphenol content and outstanding antioxidant properties, surpassing the antioxidant activity of commonly used organic antioxidants. While the seaweed biomass contained essential minerals, such as magnesium, calcium, potassium, iron, zinc, phosphorus, and manganese, it also presented concerning levels of toxic elements like lead and arsenic that exceed regulated limits for human consumption and must be considered prior to marketing. Overall, this study suggests that *R. okamurae* could be a sustainable source of various functional, nutritional, technological, or bioactive compounds, with promising potential for removal of this invasive species and utilization in various industrial sectors.

CRediT authorship contribution statement

Vera Cebrián-Lloret: Data curation, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. **Sara Cartan-Moya:** Data curation, Methodology, Formal analysis. **Marta Martínez-Sanz:** Validation, Writing – review & editing, Supervision, Project administration. **Pilar Gómez-Cortés:** Resources, Conceptualization, Formal analysis, Investigation. **María Visitación Calvo:** Resources, Funding acquisition, Writing – review & editing, Project administration, Funding acquisition. **Amparo López-Rubio:** Resources, Funding acquisition, Writing – review & editing, Project administration, Funding acquisition. **Antonio Martínez-Abad:** Conceptualization, Methodology, Writing – review & editing, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

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