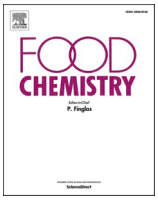
Food Chemistry 458 (2024) 140310

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Exploring the bioactive potential of algae residue extract via subcritical water extraction: Insights into chemical composition and biological activity

Esther Trigueros [a,b,*], Andreia P. Oliveira [a], Paula B. Andrade [a], Romeu A. Videira [a], Paula Guedes de Pinho [c,d], M. Teresa Sanz [b], Sagrario Beltrán [b]

[a] REQUIMTE/LAQV, Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, R. Jorge Viterbo Ferreira, n° 228, 4050-313 Porto, Portugal
[b] Department of Biotechnology and Food Science, Chemical Engineering Division, University of Burgos, Plza. Misael Bañuelos s/n, 09001 Burgos, Spain
[c] Associate Laboratory i4HB – Institute for Health and Bioeconomy, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[d] UCIBIO – Applied Molecular Biosciences Unit, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

ARTICLE INFO

Keywords:
Macroalga residue
Subcritical water
Valorization
Bioactivity
Maillard reaction products
Antioxidant activity

ABSTRACT

Gelidium sesquipedale is valued in the Spanish agar industry, but its production generates substantial waste, often discarded despite its nutritional and bioactive content. Subcritical water extraction (SWE) at 175 °C and 50 bar for 130 min was performed on this waste after agar extraction, comparing it to conventional ethanol extraction. The SWE extract exhibited superior nutritional profile, including proteins (170.6 ± 1.0 mg/$g_{freeze\text{-}dried\text{-}extract}$), essential amino acids (18.1%), carbohydrates (148.1 ± 0.3 mg/$g_{freeze\text{-}dried\text{-}extract}$), total phenolic content (57 ± 7 mg-EqGA/$g_{freeze\text{-}dried\text{-}extract}$), and also containing Maillard reaction compounds, such as 5-hydroxymethylfurfural, furfural, 2-furanmethanol, 1-(2-furanyl)-ethanone, and 5-methyl-2-furfural, influencing color, aroma and flavor. This extract showed better antioxidant and anti-inflammatory properties than the conventional extract, and higher xanthine oxidase, tyrosinase, and acetylcholinesterase inhibition activities. Toxicological assessment on human cells indicated the safety of the SWE extract. Therefore, SWE technology offers a promising method to valorize *G. sesquipedale* residue, yielding a bioactive and nutrient-rich extract suitable for food and nutraceutical applications.

1. Introduction

Nowadays, there is a growing focus on discovering natural and sustainable food sources and ingredients which not only present appealing sensory qualities but also offers beneficial effects on overall wellness and human health. Macroalgae are emerging as promising solutions to this challenge due to their rich nutritional profile, abundant in carbohydrates, proteins, lipids, vitamins and minerals, making them a compelling source of bioactive compounds with potential applications in the industry (Hans et al., 2024).

Macroalgae, as sessile organisms, have the remarkable ability to adapt themselves to harsh environmental conditions such as temperature fluctuations, salinity, exposure to UV radiation, and the presence of pollutants. Consequently, they produce a diverse range of secondary metabolites, including pigments and bioactive substances (Cikos et al., 2020). These compounds have garnered significant attention due to their positive impact on human health, mainly attributed to their antioxidant, anti-inflammatory, antidiabetic, antibacterial, antiviral, and cytotoxic properties (Khursheed et al., 2023; Lomartire & Gonçalves, 2023; Thambi & Chakraborty, 2022). Additionally, the distinctive bioactive compounds found in macroalgae distinguish them from terrestrial plants, drawing interest towards their potential application in developing nutraceuticals and functional foods (Thambi & Chakraborty, 2022). Red macroalgae are extensively utilized in the industry for the extraction of phycocolloids, notably agar and carrageenan, as primary commercial seaweed extracts (Trigueros et al., 2022), employed within the food, pharmaceutical and cosmetic industries (Lomartire & Gonçalves, 2023). *Gelidium sesquipedale* (Clemente) Thuret 1876, a type of red algae, represents the principal seaweed resource in the Spanish agar industry due to its superior quality raw material, resulting in the

* Corresponding author at: Department of Biotechnology and Food Science, Chemical Engineering Division, University of Burgos, Plza. Misael Bañuelos s/n, 09001 Burgos, Spain.
E-mail addresses: etrigueros@ubu.es (E. Trigueros), asoliveira@ff.up.pt (A.P. Oliveira), pandrade@ff.up.pt (P.B. Andrade), rvideira@ff.up.pt (R.A. Videira), pguedes@ff.up.pt (P.G. de Pinho), tersanz@ubu.es (M.T. Sanz), beltran@ubu.es (S. Beltrán).

https://doi.org/10.1016/j.foodchem.2024.140310
Received 24 April 2024; Received in revised form 18 June 2024; Accepted 1 July 2024
Available online 2 July 2024

production of highly esteemed agar within the industry. According to the 'Asociación Empresarial de Acuicultura de España' (APROMAR), the global seaweed harvest in 2019 represented 28.9% (34.7 million tons) of total aquaculture, with a first sale market value of 11,863 million euros (APROMAR OPP30, 2021). Moreover, the value of agar exports increased from 289 million dollars in 2021 to 305 million dollars in 2022, a 5.75% increase (Observatorio de Complejidad Económica - OEC World, 2022), with an expected CAGR of 5.55% for the period of 2024–2029 (Mordor Intelligence, 2023). The principal agar exporters are China, with export values of 107 million dollars, followed by Spain, with export values of 43 million dollars (Observatorio de Complejidad Económica - OEC World, 2022). Traditionally, this alga has primarily been utilized in agar production (Castejón et al., 2021); however, the industrial process involving *G. sesquipedale* to produce agar results in the generation of substantial amounts of solid residue, constituting between 15 and 40% of the original dried alga (Álvarez-Viñas et al., 2019). This residue is commonly used as fertilizer or treated as waste and discarded, thus its improper management contributing to residue accumulation and environmental concerns. Nevertheless, despite being considered a residue, this byproduct contains significant levels of nutritional and bioactive compounds, including carbohydrates, proteins, and free amino acids (Trigueros, Alonso-Riaño, et al., 2021).

Conventional methods commonly employed for extracting bioactive compounds involve lengthy extraction periods, substantial energy requirements, and the use of large volumes of organic solvents, which are hazardous and harmful to the environment. Consequently, there is a growing interest in adopting greener extraction techniques within the framework of green chemistry principles (Castejón et al., 2021). In line with this paradigm, subcritical water extraction (SWE) has emerged as a sustainable technology involving the use of hot water (100–374 °C) under sufficient pressure to maintain its liquid state. Under these conditions, water exhibits unique properties, including a higher ionization constant and lower dielectric constant, which, influenced by temperature, facilitate the extraction and hydrolysis of organic compounds (Trigueros et al., 2024).

Up to now, research on *G. sesquipedale* has mainly focused on optimizing agar extraction techniques, investigating methods such as hydrothermal or ultrasound processes as alternatives to traditional procedures (Gomes-Dias et al., 2022; Gómez Barrio et al., 2022; Martínez-Sanz et al., 2019). Additionally, various authors have explored *G. sesquipedale* as a potential reservoir of fatty acids and polyphenols with antioxidant, anti-inflammatory, and enzyme inhibitory activities employing both conventional and ultrasound-assisted extraction methods (Castejón et al., 2021; Grina et al., 2020; Matos et al., 2020). Regarding the valorization of byproducts generated from *G. sesquipedale* after agar extraction, Koçi et al. (2022) examined the alkaline solution generated after the industrial processing of 35 batches of algae, suggesting its potential application as a stimulant for plant defense mechanisms. Despite the available literature on *G. sesquipedale*, only a limited number of studies have investigated the solid waste produced from industrial agar extraction for valorization. For example, Errati et al. (2022) explored the utilization of this solid residue as organic fertilizer and for soil amendments, demonstrating its efficacy in supporting sustainable agriculture. Similarly, Martínez-Sanz et al. (2020) investigated this residue for the production of cellulosic films, while Tuma et al. (2020) utilized it to produce poly-3-hydroxybutyrate after converting its carbohydrates into monomeric sugars through saccharification.

In previous studies published by our group, the solid residue from *G. sesquipedale* after agar extraction was investigated as a source of biocompounds employing SWE. Our studies involved evaluating the capability of water under subcritical conditions to extract and hydrolyze protein and carbohydrate fractions, resulting in the release of sugars, proteins, free amino acids, and total phenolic compounds with high antioxidant activity. Subsequently, the feasibility of fractionating oligosaccharides and peptides derived from the algal hydrolysate obtained via subcritical water was investigated using an ultrafiltration-based process (Trigueros et al., 2022). Additionally, a study on the scalability of the SWE process was conducted, stating its viability in a pilot-scale industry (Trigueros et al., 2023). However, despite our previous exploration of the potential of *G. sesquipedale* solid residue and subcritical water technology to yield nutrient-rich extracts, literature lacks reports on the biological activity of these residue derived extracts. Therefore, the main goals of this work are: 1) to characterize the chemical profile of the extract obtained from *G. sesquipedale* residue through SWE, 2) to evaluate the antioxidant and anti-inflammatory potentials of the extract as well as its capacity to inhibit some enzymes, namely xanthine oxidase, tyrosinase, and acetylcholinesterase, 3) to assess its safety profile and cytotoxicity effects on human cells, and 4) to compare the results with those obtained through a conventional extraction procedure. Hence, this study aims to provide insights into the bioactive potential of *G. sesquipedale* residue extracts obtained through subcritical water technology and to advance understanding of their potential applications in the development of nutraceutical and functional foods.

2. Materials and methods

2.1. Standards and reagents

Acetoin (95%), acetamide (≥ 99%), allopurinol (≥ 99%), arabinose (99%) arginine (≥ 98%), benzyl alcohol (≥ 99%), 1-(2-furanyl)-ethanone (99%), 2-furanmethanol (98%), furfural (99%), galanthamine hydrobromide from *Lycoris* sp. (≥ 94%), gallic acid (≥ 98.0%), glucose (99.5%), galactose (99%), 1-hydroxy-2-propanone (90%), 5-(hydroxymethyl)furfural (≥ 99%), kojic acid (≥ 98.5%), 3-methyl-1,2-cyclopentanedione (99%), 5-methyl-2-furfural (≥ 98%), methylpyrazine (≥ 99%), quercetin (95%), acetylthiocholine iodide (ATCI), iron (II) sulfate heptahydrate, L-DOPA, linoleic acid, NADH, *N*-(1-naphthyl)ethylenediamine dihydrochloride, ninhydrin, nitrotetrazolium blue chloride (NBT), phenazine methosulfate (PMS), sodium nitroprusside dihydrate (SNP), sulfanilamide, trypan blue solution (0.4%), and xanthine were supplied by Sigma-Aldrich (St. Louis, MO, USA); Dulbecco's Modified Eagle Medium DMEM (1×) + GlutaMAX, AB "Pen Strep" Glico, FBS, HBSS and trypsin from GibcoTM, Thermo Fisher (Waltham, MA, USA); 5–5'-Dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) and MTT from Alfa Aesar Chemicals (Haverhill, MA, USA); ascorbic acid and 2,6-di-*tert*-butyl-4-methylphenol (BHT) (≥ 99.0%) from Fluka (Busch, Switzerland); acetic acid (≥ 99.5%) from Atom Scientific (Hyde, UK); formic acid (99%) from Carlo Erba Reagents (Val de Reuil, France); and furyl hydroxymethyl ketone from TRC (Toronto, ON, Canada). Acetylcholinesterase from *Electrophorus electricus*, lipoxygenase from *Glycine* max (soybean), tyrosinase from mushroom, and xanthine oxidase from bovine milk, all supplied by Sigma-Aldrich (St. Louis, MO, USA), were the enzymes used for the in vitro enzymatic assays. Human colorectal adenocarcinoma Caco-2 (ATCC HTB-27), and human neuroblastoma SH-SY5Y (ATCC CRL-2266) cell lines were obtained from American Type Culture Collection (Barcelona, Spain).

2.2. Sample

The raw material utilized in this work comprises the solid residue derived from the red alga *G. sesquipedale* after industrial agar extraction process, and it was generously provided by Hispanagar (Burgos, Spain). To reduce moisture content to below 6%, the residue underwent oven-drying at 45 °C for 24 h. Subsequently, it was subjected to milling (Retsch mill SM100, 1.5 kW, 1500 rpm) and sieving (Cisa Sieving Technologies) to collect the fraction with particles smaller than 500 μm, herein referred to as DMR (dried macroalga residue).

2.3. Extraction procedures

2.3.1. Subcritical water extraction

Subcritical water extraction (SWE) was conducted using a

laboratory-scale reactor with a maximum volume of 500 mL, operated in a discontinuous mode. Optimal conditions were determined based on prior findings utilizing the same raw material (Trigueros et al., 2023), resulting in the selection of 175 °C and 50 bar for 130 min of extraction, employing a DMR:water ratio of 1:20. In summary, 15 g of DMR was introduced into the reactor along with 300 mL of distilled water, and mechanical stirring ensured suspension during extraction. The mixture was heated to the desired temperature using a ceramic heating jacket (230 V, 4000 W) covering the reactor, and pressurized with nitrogen gas to prevent oxidation. Equipment specifications are detailed in Trigueros et al. (2023). Upon completion of the extraction period, the solid residue was discarded, and the liquid phase was subjected to freeze-drying (Labconco Freeze Dry System) at 0.15 mbar for a minimum of 48 h to obtain a dry powder extract. This extract, referred to as DMR-SWE throughout this manuscript, was stored at −20 °C until further use. For analysis, the extract was reconstituted in distilled water to achieve a known concentration of 70 mg/mL. A WTW 330i pH-meter was employed to measure the pH of the extract after SWE.

2.3.2. Ethanolic extraction

SWE was compared with conventional extraction methods using organic solvents to evaluate its efficacy and propose it as an alternative green extraction to traditional procedures. For this purpose, a conventional extraction method employing ethanol (EE) was selected within the comparative study due to human safety concerns, considering the potential use of the extract as a food ingredient. A solvent mixture of ethanol:water (80:20, *v*/v), maintaining the same DMR:(ethanol:water) ratio as in the SWE process, was employed. The extraction temperature was set at 25 °C based on previous research on brown seaweed (Shipeng et al., 2015). Although previous conventional extraction experiments in our group were conducted for 60 min, the ethanolic extraction in this study was extended to 190 min to ensure complete solubilization of materials into the solvent medium and to be comparable with the SWE conditions. In brief, 10 g of DMR was transferred to an Erlenmeyer flask, and 200 mL of solvent was added. Mechanical stirring (400 rpm) was sustained throughout the extraction process using an orbital shaker (RSLAB-7). After the extraction process was finished, the solid residue was discarded, and the ethanol and part of the water in the liquid fraction was removed from the extract by using a rotary evaporator (Buchi R-114) at 30 °C and pressure below 80 mbar, followed by freeze-drying to obtain a dry powder extract. This extract, referred to as DMR-EE throughout this manuscript, was stored frozen until use. For analytical procedures, the extract was reconstituted in the extraction solvent to achieve a known concentration.

2.4. Chemical characterization of the extracts

2.4.1. Nutrient profile

Total Soluble Sugars. The total soluble sugar content in the extracts was assessed using the phenol-sulfuric method with slight variations (Nielsen, 2010). The absorbance of a solution containing 100 μL of sample, 100 μL of a phenol solution, and 500 μL of 6.5% sulfuric acid was measured at 490 nm. Sucrose was employed as the standard for constructing the calibration curve.

Total Carbohydrates. Carbohydrates were quantified in the DMR-SWE extract by high-performance liquid chromatography (HPLC), employing a Bio-Rad Aminex-HPX-87H column, a variable wavelength detector (VWD), and a refractive index detector (RID). A mobile phase of sulfuric acid 5 mM was used. According to the NREL protocols (Sluiter et al., 2010), monomeric sugars were directly analyzed in the liquid fraction obtained after the extraction process. However, to estimate oligomeric sugars, the difference between total and monomeric sugars was calculated. For quantifying total sugars, an acid-hydrolysis process of the sample was necessary to hydrolyze the oligomeric sugars into monomeric form.

Total Protein. The total protein content in the extracts was assessed by measuring the total nitrogen content and applying the corresponding nitrogen factor. Total nitrogen was quantified using a TOC/TN analyzer (Shimadzu TOC-V CSN analyzer), while the nitrogen factor for the macroalgae residue utilized in this study as raw material was previously established as 4.9 based on its amino acid profile determined by GC-FID (Trigueros, Sanz, et al., 2021).

Total free amino acids. The profile of free amino acids in the DMR-SWE extract under the specified conditions (175 °C, 50 bar, 130 min) is detailed in a previous work (Trigueros et al., 2023). Free amino acids were quantified employing the EZ:faast Phenomenex method, involving initial solid extraction, subsequent derivatization, and liquid/liquid extraction steps. In this study, the overall content of free amino acids and essential amino acids content has been calculated from the sum of the respective individual amino acids.

Protein Hydrolysis Degree (DH). The protein hydrolysis degree, defined as the percentage of peptide bonds hydrolyzed, was estimated for the macroalgae residue extract obtained by SWE and determined using the ninhydrin method protocol from Sigma-Aldrich (Saint Louis, MO, USA) with some modifications. Briefly, 500 μL of the extract was mixed with 500 μL of ninhydrin solution, prepared in acetone and sodium acetate buffer (100 mM, pH 5.0), and then incubated in boiling water for 10 min. After completion of the incubation, the mixture was allowed to cool, and upon adding 1 mL of ethanol the absorbance was measured at 570 nm. Arginine was employed as the standard for constructing a calibration curve. The DH was calculated as the ratio between the number of equivalent hydrolyzed peptide bonds and millimoles of free amino acids per gram in the non-hydrolyzed protein, estimated from the amino acid profile (Adler-Nissen et al., 1983).

Total Phenolic Content (TPC). TPC was determined following the method outlined by Singleton et al. (1999). In summary, 1 mL of extract was combined with 1 mL of Folin-Ciocalteau reagent, 2 mL of 25% sodium carbonate, and 3 mL of distilled water. Then, the absorbance was measured at 750 nm after incubation in darkness for 60 min. Gallic acid was used as standard, and the results are expressed as mg equivalent of gallic acid per gram of extract.

2.4.2. Volatile organic compounds analysis

Volatile organic compounds (VOCs) were quantified using headspace-solid phase microextraction (HS-SPME). Solid phase microextraction is a widely employed technique for directly extracting VOCs from the headspace. It involves using a silica fiber coated with a polymeric stationary phase, facilitating the extraction and separation process. The analysis was conducted using a 436-GC system (Bruker Daltonics, Fremont, CA, USA) coupled with a SCION Single Quadrupole (SQ) mass detector. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber was utilized. Extraction process occurred at 45 °C for 30 min under continuous stirring at 250 rpm after a 5 min incubation period. The carrier gas, helium C-60 (Gasin, Leça da Palmeira, Portugal), was set at 1 mL/min of flow rate, and a silica capillary column (30 m × 0.25 mm × 0.25 μm) from RESTEK Corporation (Bellefonte, PA, USA) was employed for compounds' separation. The oven temperature was initially set at 40 °C for 1 min, followed by an increase to 250 °C at a rate of 5 °C/min, maintained for 5 min, and raised to 300 °C (5 °C/min) and held for 1 min (Barros et al., 2012). The MS detector operated in electron impact (EI) mode at 70 eV. Transfer line, ion source, and manifold temperatures were set at 250, 260 and 42 °C, respectively. Chromatographic acquisition was performed in full scan mode within the mass range of 40 to 250 *m/z*, at a scan rate of 6 scans/s. For validation of compounds identities, Bruker Daltonics MS workstation software (version 8.2) was utilized to compare the *m/z* values and mass spectra of each peak against commercial standards with a purity above 90%. Quantification was conducted through calibration curves constructed using commercial standards at the same experimental conditions.

2.4.3. Maillard reaction products measurement

Melanoidins were assessed by measuring the browning intensity of the DMR-SWE extract according to Fan and Gao (2022), using a ten-fold dilution of the reconstituted extract. Absorbance readings were taken at 280, 360 and 420 nm to monitor products in various stages of the Maillard reaction.

2.5. Non-cellular assays

2.5.1. Nitric oxide scavenging activity

The efficacy of the extracts in scavenging nitric oxide radical ($^{\bullet}NO$) was evaluated using a previously established method (Ferreres et al., 2021). In brief, 75 μL of extract and SNP 20 mM were subjected to incubation under light for 60 min, after which 75 μL of Griess reagent was added. The Griess reagent was formulated by combining sulfanilamide and *N*-(1-naphthyl) ethylenediamine dihydrochloride in 2% phosphoric acid. Subsequently, absorbance was measured at 560 nm. Samples were compared against a control prepared with phosphate buffer (100 mM, pH 7.4) instead of sample, while quercetin was employed as positive control.

2.5.2. Superoxide scavenging activity

The scavenging capacity of extracts against superoxide anion radical ($O_2^{\bullet-}$) was determined by measuring the absorbance at 560 nm of a mixture containing 50 μL of the extract, NADH 0.166 mM, and PMS 0.0027 mM, along with 150 μL of NBT 0.043 mM (Ferreres et al., 2021). Results were expressed relative to a control prepared in a phosphate buffer solution (19 mM, pH 7.4). Quercetin, as positive control, was tested under the same experimental conditions for comparison.

2.5.3. Lipid peroxidation reduction

The effect of the extracts on the peroxidation of fatty acyl groups was conducted through the methodology described by Ferreres et al. (2012). In a mixture composed of linoleic acid, Tris-HCl (0.1 M, pH 7.5), $FeSO_4 \cdot 7H_2O$, and the sample in a volume proportion of 5:3:1:1, the peroxidation of linoleic acid was initiated by adding an equivalent volume of ascorbic acid. The mixture was then incubated at 37 °C for 1 h. Following the incubation period, 750 μL of ethanol:ether (3:1, *v*/v) was added, and the absorbance was measured at 233 nm. For comparison, BHT standard was employed as positive control.

2.5.4. Lipoxygenase inhibition

The activity of 5-lipoxygenase (5-LOX) was evaluated monitoring the oxidation of linoleic acid according to the method previously reported by Andrade et al. (2019). Briefly, a mixture of different concentrations of the extracts and 5-LOX was incubated with phosphate buffer (0.1 M, pH 9) for 5 min at room temperature. The reaction was initiated by the addition of linoleic acid, and the kinetic was followed for 3 min at 234 nm. Quercetin served as positive control.

2.5.5. Xanthine oxidase inhibition

Xanthine oxidase activity was evaluated by monitoring the conversion of xanthine to acid uric. Briefly, a mixture consisting of 50 μL of the extract, 110 μL of phosphate buffer with EDTA 5 mM (100 mM, pH 7.4), and 10 μL of the enzyme solution was incubated at 37 °C for 5 min. Extract was replaced by buffer to prepare a control. Subsequently, 80 μL of xanthine were added as the substrate, and the absorbance was measured at 295 nm (Lopes et al., 2019). Allopurinol was selected as positive control.

2.5.6. Tyrosinase inhibition

The activity of mushroom tyrosinase was assessed by monitoring the oxidation of L-DOPA recording the increase in absorbance at 475 nm over a 3-min period. Assays were conducted by combining 40 μL of extract solution, 40 μL of enzyme, along with 80 μL of potassium phosphate monobasic buffer (0.05 M, pH 6.8), and 40 μL of L-DOPA, as described by Moreira et al. (2023). Kojic acid was used as positive control in this assay.

2.5.7. Acetylcholinesterase inhibition

The macroalgae extracts' ability to inhibit acetylcholinesterase (AChE) activity was evaluated through the Ellman et al. (1961) with some adjustments. The substrate, 25 μL of ATCI 15 mM, was mixed with 125 μL of DTNB 3 mM, 50 μL of Tris-HCl buffer (50 mM, pH 8) containing 0.1% albumin, 25 μL of sample, and 25 μL of enzyme solution (0.22 U/mL). The absorbance at 405 nm was recorded to follow the formation of 5-thio-2-nitrobenzoate resulting from the reaction of thiocholine with DTNB (João et al., 2021). A control was prepared using buffer replacing the sample, and the inhibition capacity of the extracts was determined relative to the control. Galanthamine hydrobromide standard was employed as positive control.

2.6. Cellular assays

2.6.1. Cell culture

Human colorectal adenocarcinoma Caco-2 and human neuroblastoma SH-SY5Y cells were cultured in DMEM (1×) + GlutaMAX medium supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C in a humidified incubator with 5% CO_2. Upon reaching 70–80% confluence, cells were rinsed with HBSS, detached using 0.05% trypsin, and seeded onto 96-well plates at densities of 20,000 and 40,000 cells/well for Caco-2 and SH-SY5Y, respectively, being incubated at 37 °C for 24 h.

2.6.2. Cell viability assay

The impact of the DMR extract obtained through SWE on cell viability was evaluated using the MTT assay (João et al., 2021; Trigueros et al., 2024). Cells were incubated with increasing concentrations of the extract and kept at 37 °C for 24 h. Following the incubation period, the medium was aspirated, and the MTT solution was added, with absorbance readings taken at 560 nm. Cell viability in wells containing the extract was compared to a control prepared with medium instead of sample. Experiments were conducted in triplicate using cells obtained from distinct cell passages.

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.4.2. Software (San Diego, USA). All the results are expressed as the mean ± SEM of at least three independent experiments, performed in triplicate. To validate significant differences, an unpaired two-tailed Student *t*-test was conducted with a significance level of $p < 0.01$.

3. Results and discussion

3.1. Chemical characterization of the extracts

Table 1 presents the chemical composition of the freeze-dried extracts obtained from *G. sesquipedale* residue. The primary metabolites identified in the DMR-SWE extract are proteins and carbohydrates, with galactose-based sugars being the most abundant (57.8%), followed by glucans (35.2%) and arabinans (7.0%). Within the galactose sugars fraction, 87% was extracted as oligomers, with the remaining fraction as monomers. Previous findings indicated a decrease in galactose-oligomers content after 45 min of extraction due to the degradation rate surpassing the formation rate via hydrolysis, resulting in the solubilization of galactose as monomer (Trigueros et al., 2023). In contrast, no degradation reactions were observed at 175 °C for glucans, as no monomers were detected. However, arabinans exhibited fast degradation throughout the extraction process, resulting in a lower amount at the end of the treatment. Proteins constitute 43.2% of the identified compounds in the lyophilized extract, with essential amino acids comprising 18.1% of total free amino acids, including valine, leucine,

Table 1
Nutritional profiles of the macroalgae residue extracts obtained through subcritical water extraction at 175 °C, 50 bar and 130 min (DMR-SWE) and ethanolic extraction (DMR-EE), expressed as mg of component per **gram of freeze-dried extract.**

	DMR-SWE	DMR-EE
Total Soluble Sugars	164 ± 10[a]	19 ± 2[b]
Total Carbohydrates (HPLC)	148.1 ± 0.3	nd
Galactose (monomer)	11.1 ± 0.1	nd
Galactans (oligomer)	74.5 ± 0.3	nd
Glucans (oligomer)	52.1 ± 0.1	nd
Arabinans (oligomer)	10.4 ± 0.1	nd
Total Protein	170.6 ± 1.0[a]	1.24 ± 0.06[b]
Total free amino acids	3.04 ± 0.04	nd
Essential amino acids	0.55 ± 0.01	nd
Total Phenolic Content (TPC)	57 ± 7[a]	8.0 ± 0.3[b]

Results expressed as mean ± SEM ($n = 3$).
Values with different letters in each row are significantly different when applying unpaired two-tailed Student *t*-test ($p < 0.01$) nd = not determined.

phenylalanine, threonine, lysine, histidine, methionine, and tryptophan, as reported by Trigueros et al. (2023). Subcritical water has been widely reported to extract and hydrolyze the protein fraction, releasing peptide fractions of different sizes and free amino acids into the extraction medium (Marcet et al., 2016). In this study, the degree of protein hydrolysis in the DMR-SWE extract accounted for 23.2 ± 0.3%. This value surpasses those obtained using a commercial protease on the same raw material, where DH ranged from 4.2 to 9.3% depending on pH (Trigueros, Alonso-Riaño, et al., 2021), highlighting the great capability of subcritical water at 175 °C to hydrolyze the protein fraction, transforming proteins into small peptide chains and free amino acids. Additionally, compared to previous work on the same algal residue, SWE at 171 °C in semicontinuous mode resulted in nearly four times lower hydrolysis degree (Trigueros, Alonso-Riaño, et al., 2021), indicating the superior protein hydrolysis ability of the SWE design on this study. Compared to the extract obtained through conventional ethanol extraction, the subcritical water extract exhibits a significantly higher content of total sugars and proteins ($p < 0.01$) (**Table 1**). This underscores the greater efficiency of SWE in extracting and hydrolyzing these targeted bioactive compounds from the raw material compared to the low solubility of proteins and sugars in ethanol, as previously described by Kashaninejad et al. (2020). Additionally, the TPC determined in the DMR-SWE lyophilized extract (57 ± 7 mg-EqGA/g) is significantly higher ($p < 0.01$) than that in the DMR-EE lyophilized extract (8.0 ± 0.3 mg-EqGA/g).

As previously noted, TPC steadily increased throughout the entire extraction process, yielding the maximum at the highest temperature, 175 °C, selected in this study. It is widely recognized that conditions during SWE treatment, particularly high temperatures, can prompt Maillard and caramelization reactions (Trigueros et al., 2023). The Maillard reaction (MR) is a non-enzymatic browning process involving the reaction between carbonyl group from reducing sugars and amino group from amino acids, resulting in the formation of Maillard reaction products (MRPs) and melanoidins. These compounds, contributing to the color and aroma of cooked and processed foods, such as coffee, cocoa, bread, peanuts, meat, fish and potatoes (Liu et al., 2022), present multiple sensory properties, making the MR a valuable tool for creating artificial flavorings. For example, MR has been reported to produce artificial maple syrup by subjecting corn syrup and amino acids to high temperatures (Echavarría et al., 2012), and to modify the unpleasant odor of oysters by a more pleasant aroma of milk, nuts and meat (Liu et al., 2022).

The intensity of the brown color is an indicative marker of MR progression, with a higher intensity correlating to a more extensive reaction (Trigueros et al., 2023). In **Fig. S1c**, the DMR-SWE extract dissolved in distilled water displays a dark brown coloration, indicating MR occurrence during the SWE process from *G. sesquipedale* residue. Absorbance measurements at specific wavelengths serve as indicators to monitor MR development. Absorbances at 280 and 360 nm are related to the formation of primary and advanced MRPs, respectively, while absorbance at 420 nm provides a visual assessment of brown color development (Fan & Gao, 2022). The A_{420} value determined for the DMR-SWE extract is 26.8 ± 0.7 a.u., aligning with the visual observation of dark-brown coloration (see **Fig. S1c**), and indicating the significant occurrence of MR and consequent generation of MRPs and melanoidins. Additionally, A_{280} and A_{360} values measure 145 ± 2 a.u. and 65.1 ± 1.4 a.u., respectively, indicating the occurrence of MR in both early and advance stages.

Multiple chemical transformations occur during the MR, including cyclizations, dehydrations, retroaldolizations, rearrangements, isomerizations, and condensations, rendering it a complex process involving numerous compounds (Echavarría et al., 2012). The analysis of volatile compounds in the DMR-SWE extract by HS-SPME/GC–MS is presented in **Table 2** and Fig. 1. Notably, small organic acids such as formic **(1)** and acetic acids **(2)** emerge as the primary volatile compounds detected in the extract, both arising from the degradation of sugar and protein fractions under subcritical conditions. Kwak et al. (2005) established that MR typically initiates under neutral or alkaline pH conditions and gradually transitions to acid values. During SWE, the water is initially neutral, but as the extraction proceeds, the pH becomes more acidic due to the formation of organic acid. In this case, the acidic pH value determined for the DMR-SWE extract was 5.86 ± 0.01, which correlates with the progression of MR and the high content of organic acids quantified in the extract.

Other compounds present in significant concentrations in the DMR-SWE extract include 1-hydroxy-2-propanone **(3)**, acetamide **(5)**, 2-furanmethanol **(8)**, 3-methyl-1,2-cyclopentanedione **(11)**, furyl hydroxymethyl ketone **(13)**, and 5-hydroxymethylfurfural (5-HMF) **(14)**. 5-Hydroxymethylfurfural is formed during SWE via sugar dehydration reactions, particularly from hexoses, being widely recognized as an intermediate in the MR (Fan & Gao, 2022). Its concentration in the DMR-SWE extract is 92.7 ± 0.5 mg/kg, surpassing that of furfural **(7)**, which measures 3.05 ± 0.11 mg/kg (**Table 2**). The lower content of furfural, a product generated from pentoses dehydration, is comprehensive given the lower presence of pentoses in the raw material (Trigueros et al., 2023). These compounds are associated with various odor and taste descriptors, including woody, almond, sweet, fruity, flowery, bread **(7)**, fatty, buttery, musty, waxy, caramel, herbal, and tobacco **(14)** (Bento-Silva et al., 2022; Xiao et al., 2015). In the DMR-SWE extract, the content of 5- hydroxymethylfurfural does not exceed its threshold value, whereas furfural does (3000 ppb) (**Table 2**), indicating its potential to be perceived. Odor activity value (OAV), calculated as the concentration in the DMR-SWE extract to odor threshold ratio, is employed to evaluate the most impactful compounds on a food product's aroma, with values above one considered significant. Furans and furanone derivatives, originated from MR and thermal degradation of carbohydrates and amino acids, are the most common volatile compounds in heated foods, associated with caramel, fatty and nutty odors (Diez-Simon et al., 2019). 1-(2-Furanyl)-ethanone **(9)**, and 5-methyl-2-furfural **(10)** have been found in the DMR-SWE extract, along with those mentioned earlier and notable for their high content: **(8)**, **(13)** and **(14)**. 2-Furanmethanol, with a concentration of 95 ± 0.5 mg per kg of extract, contributes to weak, fermented, creamy, caramel, coffee, and roast notes (Bento-Silva et al., 2022; Xiao et al., 2015), configurating the odor profile of the DMR-SWE extract as evidenced by its OAV value (**Table 2**). Conversely, although methylpyrazine **(6)** content is low, it provides roasted, burnt, and sweet odors (Bento-Silva et al., 2022) to the extract, as its OAV value accounts for 5.

These findings imply that MR occurring during SWE could influence the color, aroma and flavor of food preparations derived from *G. sesquipedale* residue. However, excessive MR may compromise the flavor and sensory attributes of foods (Liu et al., 2022), necessitating further investigation into the effect of the combined VOCs identified in

Table 2
Volatile Organic Compounds (VOCs) in macroalgae residue freeze-dried extract obtained by subcritical water extraction at 175 °C, 50 bar and 130 min (DMR-SWE).

	Compounds	RT	Most abundant ions (m/z)	Rmatch	Concentration ($mg/kg_{extract}$)	OT (ppb)	Odor/Taste descriptors	OAV
1	Formic acid [L1]	2.17	45/46	967	697 ± 6	–	Sour	–
2	Acetic acid [L1]	2.60	43/45/60	913	504 ± 7	–	Sour, vinegar	–
3	1-Hydroxy-2-propanone [L1]	3.22	43	889	527 ± 7	–	n/a	–
4	Acetoin [L1]	3.84	43/45	894	2.1 ± 0.0	–	n/a	–
5	Acetamide [L1]	5.17	43/44/59	944	386 ± 5	–	n/a	–
6	Methylpyrazine [L1]	6.32	67/94	924	0.31 ± 0.00	60	Roasted, burnt, sweet	5
7	Furfural [L1]	6.41	95/96	947	3.05 ± 0.11	3000	Woody, almond, sweet, fruity, flowery, bread	1.0
8	2-Furanmethanol [L1]	6.97	41/53/98	971	95 ± 5	2000	Weak, fermented, creamy, caramel, coffee, roast	48
9	1-(2-Furanyl)-ethanone [L1]	8.54	95/110	897	0.49 ± 0.00	10,000	Smoky, roasty	0.05
10	5-Methyl-2-furfural [L1]	10.11	109/110	947	0.31 ± 0.01	500	Almond, sweet, bitter	0.6
11	3-Methyl-1,2-cyclopentanedione [L1]	12.04	41/55/69/112	947	56 ± 2	–	Fruity, woody, mushroom-like	–
12	Benzyl alcohol [L1]	12.38	77/79/108	890	1.56 ± 0.02	–	n/a	–
13	Furyl hydroxymethyl ketone [L1]	13.78	95	945	102 ± 6	–	n/a	–
14	5-Hydroxymethylfurfural [L1]	18.01	41/97	862	92.7 ± 0.5	1,000,000	Fatty, buttery, musty, waxy, caramel, herbal, tobacco	0.09

RT = Retention time (min); Rmatch: identification according to the NIST14 Library Database; OT: Odor Threshold expressed in ppb.; Odor/Taste descriptors collected from Bento-Silva et al. (2022), Liu et al. (2022), and Xiao et al. (2015); OAV: Odor activity value, estimated as the ratio of the concentration to OT. n/a: not applicable. [L1] Compound identified by comparison of the RT and MS fragmentation of a commercial reference standard performed under the identical analysis conditions (Viant et al., 2017). Concentration, expressed as mg of compound per kg of dry extract, was estimated from calibration with standards under the same analytical conditions.

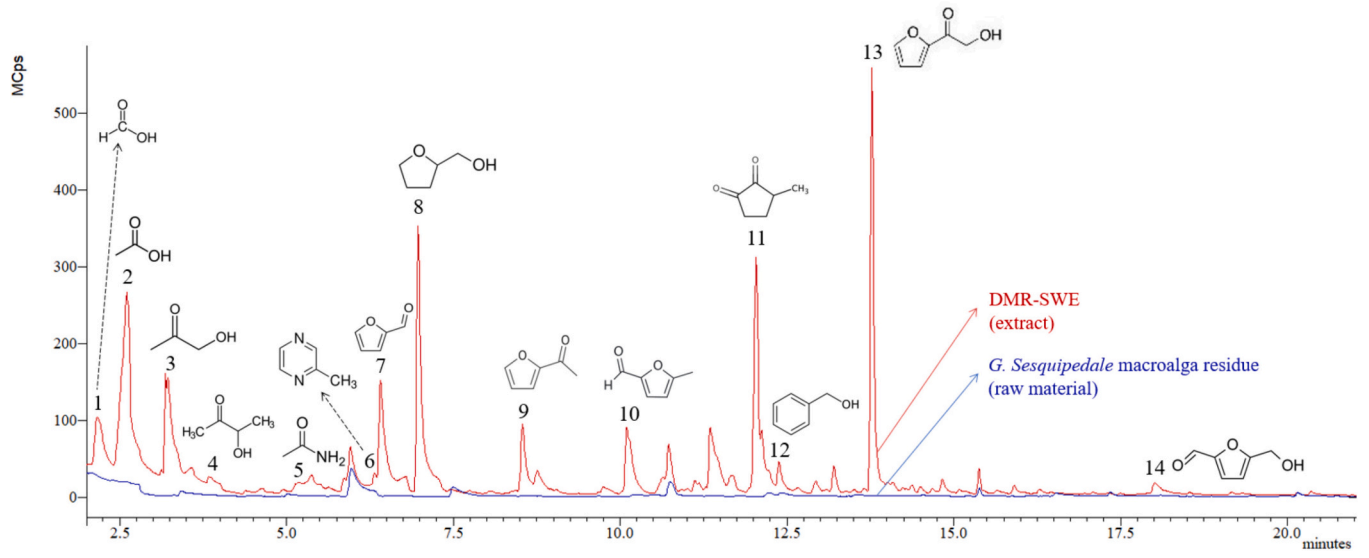


Fig. 1. HS-SPME/GC–MS chromatogram of macroalgae residue extract obtained by subcritical water extraction at 175 °C, 50 bar and 130 min (DMR-SWE), in red, and raw macroalga residue, in blue. Identity compounds **1–14** as in **Table 2**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the DMR-SWE extract and their respective odor intensities in different food formulations. Purification processes could aid in isolating and concentrating target VOCs to achieve the desired flavor profile.

3.2. Bioactivity of the extracts obtained from G. sesquipedale residue

3.2.1. Antioxidant capacity

MRPs are known to interfere in the Folin Ciocalteau assay (Trigueros et al., 2023), suggesting their role in the significantly higher ($p < 0.01$) TPC determined in the DRM-SWE extract compared to that obtained through ethanolic extraction. Additionally, MR has been linked to heightened antioxidant properties due to the contribution of MRPs (Liu et al., 2022).

$O_2^{\bullet-}$, among the most effective free radicals, serves as a precursor of reactive oxygen species, such as hydroxyl radical and peroxynitrite, contributing to cellular damage, associated with various diseases. Conversely, $^{\bullet}NO$ plays a crucial role in multiple physiological processes, but excessive production leads to biological damage, as it reacts with $O_2^{\bullet-}$ to form peroxynitrite, exacerbating oxidant stress (Valentão et al., 2010).

Fig. 2a and Fig. 2b depict the capacity of the extracts from *G. sesquipedale* residue to scavenge $^{\bullet}NO$ and $O_2^{\bullet-}$, respectively. Notably, the extract obtained through SWE demonstrates superior scavenging efficacy against both radicals compared to ethanol extraction. Specifically, the DMR-SWE extract exhibits higher activity against $O_2^{\bullet-}$, compared to the $^{\bullet}NO$, surpassing 75% neutralization at the highest tested concentration (389 μg/mL) (Fig. 2b). Conversely, the DMR-EE extract exhibits low neutralization activity, with scavenging efficacy below 25% for both radicals, making estimation of the IC_{50} unfeasible. On the contrary, IC_{50} values for the DMR-SWE extract are determined to be 237 ± 38 μg/mL and 41 ± 3 μg/mL for $^{\bullet}NO$ and $O_2^{\bullet-}$, respectively (Table 3), demonstrating superior scavenging power against $O_2^{\bullet-}$,

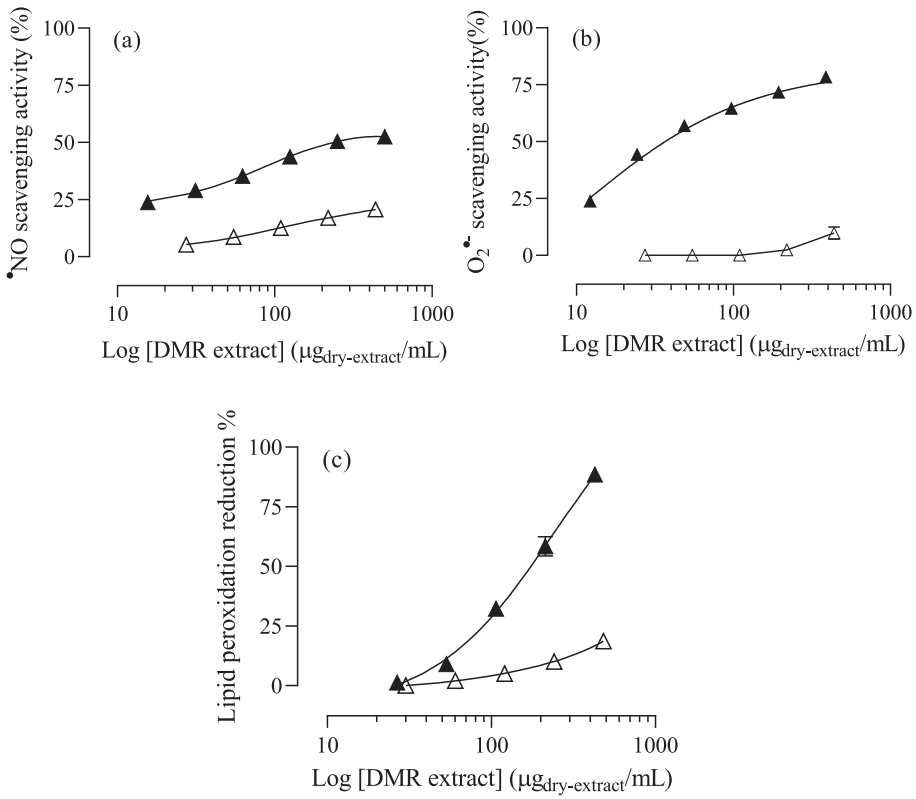


Fig. 2. Comparison of macroalgae extracts' ability, obtained via subcritical water extraction (175 °C, 50 bar, 130 min) (▲) and ethanol extraction (△), to scavenge **(a)** nitric oxide and **(b)** superoxide anion radicals, and to reduce **(c)** lipid peroxidation. Results represent the mean ± SEM of three independent experiments, performed in triplicate.

Table 3
IC_{50} values determined for the extracts obtained from dried macroalga residue through subcritical water extraction (175 °C, 50 bar, 130 min) (DMR-SWE) and through ethanol extraction (DMR-EE) in different radical scavenging and enzymatic activity assays.

	IC_{50} (μg/mL)	
	DMR-SWE	DMR-EE
Nitric oxide	237 ± 38	–
Superoxide	41 ± 3	–
Lipid peroxidation	177 ± 14	–
5-LOX	233 ± 30	-[*1]
Xanthine-oxidase	1168 ± 38	–
Tyrosinase	1148 ± 47	–
Acetylcholinesterase	-[*2]	–

Values expressed as mean ± SEM ($n = 3$).
[*1] IC_{50} could not be estimated as low inhibition rates were determined. IC_{25} value was determined as 456 ± 36 μg/mL.
[*2] In this case, although IC_{50} could not be estimated, IC_{25} value was accounted for 538 ± 4 μg/mL.

approaching the efficacy of quercetin ($IC_{50} = 22.4$ μg/mL) employed as positive control. Valentão et al. (2010) also demonstrated higher scavenging potential of aqueous macroalgae extracts against $O_2^{\bullet-}$ compared to $^{\bullet}NO$. The IC_{50} values determined for both radicals (688–737 μg/mL for $^{\bullet}NO$ and > 50 μg/mL for $O_2^{\bullet-}$) were notably higher compared to those observed in this study for the *G. sesquipedale* residue extract obtained by SWE. In other studies, carrageenan from red algae exhibited neutralization below 50% for both $^{\bullet}NO$ and $O_2^{\bullet-}$ at 250 μg/mL (Sokolova et al., 2011), while sulfated polysaccharides from *Albidum corallinum* red alga reached a maximum scavenging capacity of 40% for $^{\bullet}NO$ at 1 mg/mL of extract (Ben Saad et al., 2023). Andrade et al. (2013) evaluated the antioxidant potential of ethanolic extracts from various algae, reporting insufficient activity to estimate the IC_{50} values, with IC_{25} values ranging from 0.24 to 14.33 mg/mL for $^{\bullet}NO$ and from 0.10 to 2.94 mg/mL for $O_2^{\bullet-}$.

To further explore the antioxidant potential of the extracts, their ability to reduce linoleic acid peroxidation in the presence of Fe^{2+} and ascorbate was evaluated and illustrated in Fig. 2c. Lipid peroxidation serves as an indicator of oxidative stress in human cells and tissues (Patra et al., 2017). Notably, a substantial contrast between the two extracts is evidenced. While the DMR-SWE extract exhibits a dose-dependent response, nearly completely reducing lipid peroxidation at the highest tested concentration (426.8 μg/mL), the DMR-EE extract exhibits reduction rates below 20%. The IC_{50} value for the DMR-SWE extract is determined to be 177 ± 14 μg/mL (Table 3), similar to BHT (IC_{50} of 103 ± 7 μg/mL), used as positive control. Thus, the DMR-SWE extract effectively impedes linoleic acid oxidation, thereby preventing the formation of conjugated dienes that facilitate the generation of free radicals. Comparison with literature findings reveals that aqueous and methanolic extracts obtained from *Asparagopsis toxiformis* red alga exhibited slightly superior reduction of lipid peroxidation at 200 μg/mL (approximately 60%), whereas hydromethanolic and ethanolic extracts displayed reduction rates below 50% (Mellouk et al., 2017), lower than those observed in our study. Conversely, carrageenan extracts from various red algae reduced lipid peroxidation below 50% at 1 mg/mL (Sokolova et al., 2011), representing a notably lower reduction capacity compared to the DMR-SWE extract obtained in our study.

These findings underscore the ability of subcritical water technology to yield phenolic-rich extracts from this red algal residue exhibiting superior antioxidant capacity compared to other algae extracts documented in the literature, facilitated by the formation of novel compounds, notably MRPs, through the MR. This broadens our understanding of the unexplored biological capacity of the residue derived from *G. sesquipedale* alga, which have not been previously

investigated.

3.2.2. Anti-inflammatory activity

Seaweeds have garnered significant attention in literature as a rich source of bioactive compounds with anti-inflammatory properties (Khursheed et al., 2023; Thambi & Chakraborty, 2022). A recent review published by Meinita et al. (2023) focused on evaluating the bioactivity of algae belonging to the *Gelidium* genus, and covering the period from 2001 to 2021, revealed that the majority of studies concerning *G. sesquipedale* have centered on its antimicrobial properties. Notably, only one study, conducted by Oumaskour et al. (2013), explored the anti-inflammatory potential of a dichloromethane/methanol extract from *G. sesquipedale*, however, it lacks essential data regarding effective concentrations. To address this gap, the anti-inflammatory potential of *G. sesquipedale* after agar extraction by assessing its ability to inhibit 5-LOX was evaluated. Indeed, lipoxygenases play a pivotal role in the inflammatory cascade by catalyzing the oxidation of polyunsaturated fatty acids (Bernardo et al., 2021), 5-LOX having particular importance in neuroinflammation and neurodegeneration (Fernandes et al., 2018). Our findings, as depicted in Fig. 3a, indicate that both ethanolic and subcritical water extracts exhibit concentration-dependent inhibition of 5-LOX. Notably, the DMR-SWE extract nearly completely inhibits the enzyme, contrasting with the DMR-EE extract, which shows inhibition rates below 50% in the range of tested concentrations, not being feasible to determine its IC_{50} value. The IC_{50} value for the DMR-SWE extract was determined to be 233 ± 30 μg/mL (Table 3).

To the best of our knowledge, this study represents the first attempt to exploit *G. sesquipedale* residue after agar removal to obtain extracts containing anti-inflammatory agents. Similarly, Lee et al. (2018) observed anti-inflammatory potential in agar-free *Gelidium amansii* ethanolic extract through enhanced production of anti-inflammatory cytokines. Numerous studies have explored the anti-inflammatory potential of *Gelidium* sp. algae (Meinita et al., 2023), including the identification of sulfated polysaccharides from *G. pacificum* as anti-inflammatory agents (Cui et al., 2019), as well as investigations into glycoproteins (Rafiquzzaman et al., 2015), sulfated polysaccharides, and peptide fractions (Khursheed et al., 2023) from algae. Thambi and Chakraborty (2022) evaluated the potential of various algae to inhibit 5-LOX, reporting IC_{50} values ranging from 0.40 to 1.98 mg/mL for red algae and from 0.52 to 1.52 mg/mL for brown algae, highlighting the significantly higher potential of the extract in this study to inhibit 5-LOX and the proinflammatory cascade reaction. Moreover, while advanced glycation end-products (AGEs) transformed from MRPs are known to participate in inflammatory reactions, MRPs have been documented for their anti-inflammatory effects (Oh et al., 2017). For instance, products derived from a glucose-lysine mixture heated for 60 min exhibited higher anti-inflammatory effects compared to other MRPs (Kitts et al., 2012), which aligns with the observed decrease in lysin content in the DMR-SWE extract (Trigueros et al., 2023). Additionally, Andrade et al. (2022) reported furfural **(7)** and 2-furanmethanol **(8)** in spent coffee grounds to possess anti-inflammatory effects, while 3-methyl-1,2-cyclopentanedione **(11)** has been documented for its ability to decrease pro-inflammatory gene expression (Chen & Kitts, 2011). Furthermore, despite the negative association of 5-hydroxymethylfurfural **(14)** with human health, this compound has demonstrated antioxidant and anti-inflammatory effects, with no toxic effects at daily doses of 80–100 mg/kg body weight (Shapla et al., 2018). These compounds, except for furfural, are present in the DMR-SWE extract at concentrations above 50 mg/kg, suggesting their potential contribution to the DMR-SWE extract's anti-inflammatory profile.

In summary, this study underscores the potential of *G. sesquipedale* residue extract obtained though SWE as a potent LOX inhibitor, thereby positing its role as a preventive agent against inflammatory processes and lipid metabolism alterations, which are closely associated with Alzheimer's disease and other neurodegenerative disorders (Fernandes et al., 2018).

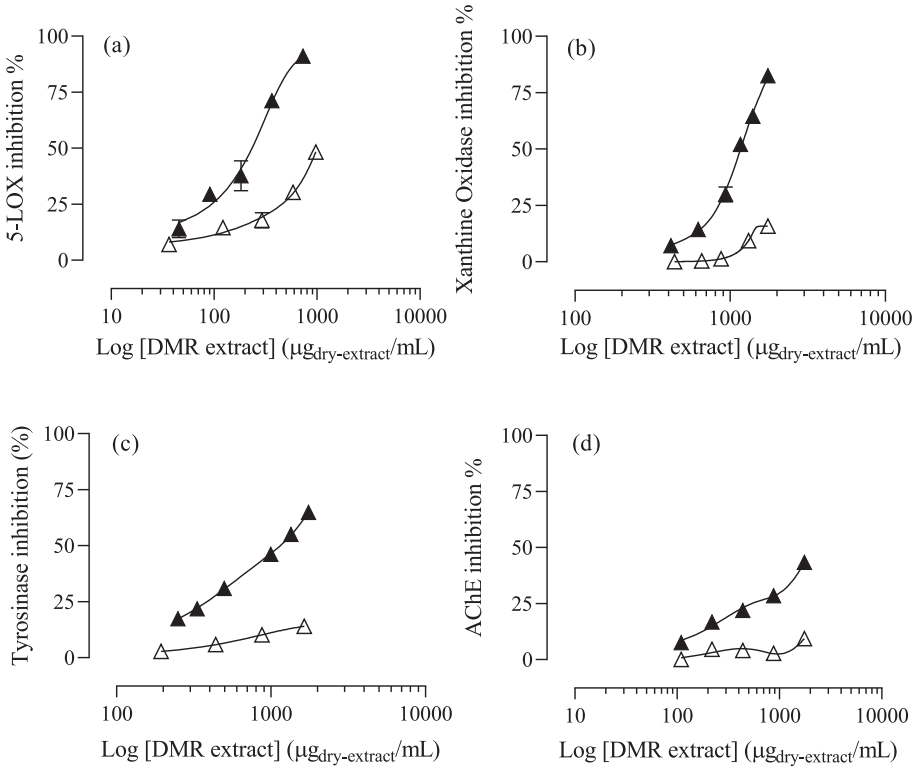


Fig. 3. Inhibitory capacity of macroalga residue extracts obtained through subcritical water extraction (175 °C, 50 bar, 130 min) (▲) and ethanol extraction (△) on the activity of **(a)** 5-lypoxygenase, **(b)** xanthine oxidase, **(c)** tyrosinase, and **(d)** acetylcholinesterase enzymes. Results represent the mean ± SEM of three independent experiments, performed in triplicate.

3.2.3. Other bioactivities

The efficacy of the extracts obtained from *G. sesquipedale* residue in inhibiting the xanthine oxidase (XO), tyrosinase, and acetylcholinesterase (AChE) was evaluated and illustrated in Fig. 3b-d. In all cases, inhibition occurred in a concentration-dependent manner, although the inhibition rates were lower compared to the antioxidant (Fig. 2) and anti-inflammatory (Fig. 3a) extracts' potential. Nevertheless, the DMR-SWE extract exhibited higher inhibition compared to the extract obtained by conventional extraction. The XO enzyme showed the highest inhibition rate, surpassing 80% inhibition at the maximum tested concentration (1.750 mg/mL), with the effect of concentration more pronounced compared to the tyrosinase inhibition assay, despite similar IC_{50} values were obtained (Table 3). In both cases, the extract obtained through SWE demonstrated significantly higher inhibition rates than the ethanol one, where inhibition rates remained below 20%. Conversely, a lower inhibition rate was observed for AChE inhibition, falling below 50% at the same highest concentration. Nevertheless, the superior effectiveness of the DMR-SWE extract over the DMR-EE extract was still apparent.

XO is a key enzyme in purine metabolism, catalyzing the hydroxylation of hypoxanthine to xanthine and then to uric acid. Elevated levels of uric acid, condition known as hyperuricemia, can lead to its crystallization, resulting in tissue damage and the development of gout. During acute gouty arthritis, the interaction between urate crystals and phagocytes triggers the secretion of inflammatory mediators like cytokines, chemokines, and interleukins. Treatments for hyperuricemia and gout aim to inhibit uric acid production and enhance its excretion; however, while synthetic XO inhibitors like allopurinol have been developed, they are associated with significant side effects such as liver damage and adverse neurological effects. Therefore, there is a growing interest in finding new and natural XO inhibitors with potent activity and low toxicity as alternative for hyperuricemia and gout treatment (Wang et al., 2023). Although previous research has explored the potential of seaweed as XO inhibitors, to best of our knowledge, the potential of *G. sesquipedale* has not been documented. Hence, this study represents the first investigation into the potential *G. sesquipedale* residue, obtained after industrial agar extraction, as a source of anti-gout compounds. The extract obtained through SWE demonstrated nearly complete inhibition of XO activity (Fig. 3b), with an IC_{50} value of 1.168 ± 38 mg/mL (Table 3), indicating a higher potential for XO inhibition compared to other red algae extracts reported in the literature, with inhibition rates below 75% (Wang et al., 2023). This might be partially attributed to the presence of 5-HMF **(14)**, previously identified as a xanthine oxidase inhibitor (Shapla et al., 2018).

Tyrosine plays a critical role in melanin biosynthesis, a pigment present in human skin responsible for protecting against UV radiation; however, excessive activity can lead to melanoma. Inhibiting tyrosinase is pursued to address various skin disorders like hyperpigmentation; nevertheless, common tyrosinase inhibitors are associated with dermatitis, cytotoxicity, and even carcinogenicity (Švarc-Gajić et al., 2019). Hence, there is a quest for natural tyrosinase inhibitors to treat hyperpigmentation disorders. As previously mentioned, the DMR-SWE extract effectively inhibited tyrosinase activity (Fig. 3c), with an IC_{50} value of 1.148 ± 47 mg/mL (Table 3). Few studies have evaluated the potential of *G. sesquipedale* to inhibit tyrosinase, none focusing on algal residue after agar removal, making this study the first. Castejón et al. (2021) assessed *G. sesquipedale* extracts, reporting no anti-tyrosinase activity for extracts obtained through traditional maceration and ultrasound-assisted extraction, with an IC_{50} value of 86.5 mg/mL for the extract obtained through a combination of both methods. On the other hand, Grina et al. (2020) obtained ethanolic extracts from *G. sesquipedale* but reported no capacity to inhibit tyrosinase, with 200 μg/mL being the highest tested concentration. Various MRPs, specifically acetamide **(5)**, furfural **(7)**, 5-methyl-2-furfural **(10)**, and 5-HMF **(14)**, have been identified in previous studies as tyrosinase inhibitors (Hashemi et al., 2023; Sharma et al., 2004), which may have contributed to the anti-tyrosinase profile of the DMR-SWE extract. Based on these findings, the extract obtained in this study through SWE has proven to be a significantly effective tyrosinase inhibitor, suggesting it as a candidate for developing skin-whitening agents.

Finally, the efficacy of the extract obtained from *G. sesquipedale* residue in aiding Alzheimer's disease (AD) therapy was investigated by examining its ability to inhibit AChE. Alzheimer, an irreversible neurodegenerative disorder, stands as the primary cause of dementia among the elderly (Andrade et al., 2013), affecting around 50 million people globally. One of the most promising therapeutic strategies for AD patients revolves around the cholinergic hypothesis (Cvetanović et al., 2018), by which cholinesterase catalyzes the hydrolysis of acetylcholine, a neurotransmitter in synapses, into acetate and choline (Castejón et al., 2021). Consequently, most drugs used in AD treatment aim to elevate acetylcholine levels in synaptic regions by inhibiting cholinesterase (Andrade et al., 2013). However, drugs like galantamine are associated with limited efficacy and adverse effects like gastrointestinal and cardiovascular events, and toxicity (Cvetanović et al., 2018; Rafiquzzaman et al., 2015).

Similar to tyrosinase inhibition evaluation, this study represents the first assessment of *G. sesquipedale* residue to inhibit AChE. Prior investigations on *G. sesquipedale* alga indicated IC_{50} values ranging between 36.3 and 94.3 mg/mL for extracts obtained through traditional maceration and ultrasound-assisted extraction (Castejón et al., 2021), while other reported no activity at 200 μg/mL (Grina et al., 2020). Andrade et al. (2013) did not observe any inhibition rate on AChE activity for the evaluated red and green algae, whereas IC_{25} values for brown algae ranged from 0.53 to 4.67 mg/mL. These findings align with the relatively lower efficacy observed for the DMR-SWE extract in inhibiting AChE when compared to other assessed bioactivities. Moreover, despite the identification of 5-HMF **(14)** as an AChE inhibitor formed during SWE due to the MR, its previously determined IC_{50} value was 1.605 mg/mL (Onoja & Elufioye, 2023). In this study, a maximum inhibition of 43.4% was observed at 1.750 mg/mL for the DMR-SWE extract. Despite the inhibition was not strong enough to estimate the IC_{50} value, it was estimated to cause 25% inhibition at 538 ± 4 μg/mL. In literature, SWE was explored for valorizing the brown alga *Padina tetrastromatica* after fucoidan extraction, yielding IC_{50} values ranging from 17.9 to 52.9 mg/mL for temperatures between 100 and 220 °C (Hans et al., 2024). Therefore, the DMR-SWE extract obtained in this study has exhibited higher efficacy in inhibiting AChE activity than others reported in literature. Furthermore, the correlation between AD, inflammation, and oxidative stress has been extensively documented, as an imbalance between oxidative stress and antioxidant defense can lead to inflammatory reactions contributing to brain inflammation and neurodegeneration (Rafiquzzaman et al., 2015). Alongside being a moderate AChE inhibitor, the DMR-SWE extract has demonstrated to be a reservoir of bioactive compounds with substantial antioxidant and anti-inflammatory potential, suggesting its plausible application in AD therapy.

3.3. Cytotoxicity of the extract obtained by SWE

As the concluding section of the study, the evaluation of the toxicological profile of the DMR-SWE extract on human cells was conducted with the aim of verifying its potential for application in the development of formulations with beneficial effects on human health. Human colorectal adenocarcinoma Caco-2 cells and human neuroblastoma SH-SY5Y cells were utilized for this purpose. Caco-2 and SH-SY5Y cells serve as common cellular models used for evaluating the impact of bioactive compounds on the gastrointestinal epithelium (Trigueros et al., 2024) and for investigating both neurotoxic and neuroprotective effects, respectively (Fernandes et al., 2018).

The cytotoxicity of the extract was assessed across a concentration range where all evaluated bioactivities exerted effects, and the results are illustrated in **Fig. S2**. The DMR-SWE extract did not induce any

significant changes ($p < 0.01$) in cell viability for either Caco-2 or SH-SY5Y cells within the evaluated concentration range, indicating the safe profile of the extract obtained by SWE. Although compounds present in the extract, such as 5-HMF **(14)**, have been extensively documented as genotoxic, mutagenic, carcinogenic and DNA-damaging agents (Shapla et al., 2018), Zhao et al. (2013) evaluated the effects of this compound on various human cancer and normal cell lines, obtaining IC_{50} values ranging from 1.00 to 3.71 mM for most cells, values significantly higher than the concentration of 5-HMF in the DMR-SWE extract (0.735 mM) (**Table 2**). Hence, initially, these findings support the utilization of the DMR-SWE extract for the development of novel compounds that positively impact on human health. However, further in-depth studies should be conducted to elucidate the bioavailability, potential interactions, and cytotoxic effects of these compounds.

4. Conclusions

Subcritical water extraction has demonstrated its efficiency compared to conventional extraction in valorizing *G. sesquipedale* residue after industrial agar extraction for yielding nutritional extracts with various bioactivities and potential applications in the industry due to their high-added value. This process yielded an extract with superior nutritional profile, comprising proteins with essential amino acids, carbohydrates, and phenolics. Analysis of absorbances and identification of volatile organic compounds indicated the occurrence of Maillard reactions during subcritical water extraction, influencing the extract's color, flavor and aroma, as well as enhancing its antioxidant capacity. The extract effectively scavenged nitric oxide and superoxide anion radicals and reduced lipid peroxidation. Furthermore, addressing gaps in the existing literature concerning the bioactive properties of this algal waste, the extract exhibited notable anti-inflammatory potential through 5-lipoxygenase inhibition and demonstrated the ability to inhibit enzymes such as xanthine oxidase, tyrosinase and acetylcholinesterase, which play key roles in various pathologies, making this extract a promising candidate for the development of functional foods or nutraceuticals with benefits in various medical therapies. As a future research direction, a comprehensive economic study should be conducted to analyze the costs involved in the valorization of this algal residue through subcritical water extraction and to evaluate the feasibility of incorporating this process at an industrial level. Moreover, in order to guarantee the biological action of the compounds present in the extract, studies on the bioavailability and interaction of these components with others should be conducted.

Funding

This work was supported by PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDP/50006/2020. This work also had the financial support from the projects UIDP/04378/2020 and UIDB/04378/2020 of Research Unit on Applied Molecular Biosciences–UCIBIO and the project LA/P/0140/2020 from the Associate Laboratory Institute for Health and Bioeconomy–i4HB, and from the Spanish Ministerio de Ciencia e Innovación [grant number PDC2022–133443-I00] and Junta de Castilla y León and ERDF [grant number BU050P20].

CRediT authorship contribution statement

Esther Trigueros: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Andreia P. Oliveira:** Writing – review & editing, Supervision. **Paula B. Andrade:** Project administration, Funding acquisition. **Romeu A. Videira:** Validation. **Paula Guedes de Pinho:** Validation, Funding acquisition. **M. Teresa Sanz:** Writing – review & editing, Project administration. **Sagrario Beltrán:** Writing – review & editing, 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

No data was used for the research described in the article.

Acknowledgements

Esther Trigueros thanks European Union-NextGenerationEU, Ministry of Universities and Recovery, Transformation and Resilience Plan, for the post-doctoral contract through a call from the University of Burgos. Andreia P. Oliveira thanks FCT (Fundação para a Ciência e Tecnologia) for funding through program DL 57/2016 – Norma transitória (DL57/2016/CP1346/CT0015). Romeu A. Videira thanks FCT for funding through the Scientific Employment Stimulus – Institutional Call (Ref. CEECINST/00136/2021). Paula Guedes de Pinho is supported by a research contract (under Scientific Employment Stimulus) CEECINST/00108/2021/CP2794/CT0001.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.140310.

References

Adler-Nissen, J., Eriksen, S., & Olsen, H. S. (1983). Improvement of the functionality of vegetable proteins by controlled enzymatic hydrolysis. *Qualitas Plantarum Plant Foods for Human Nutrition, 32*(3–4), 411–423. https://doi.org/10.1007/BF01091198

Álvarez-Viñas, M., Flórez-Fernández, N., Torres, M. D., & Domínguez, H. (2019). Successful approaches for a red seaweed biorefinery. *Marine Drugs, 17*, 620. https://doi.org/10.3390/md17110620

Andrade, C., Perestrelo, R., & Câmara, J. S. (2022). Valorization of spent coffee grounds as a natural source of bioactive compounds for several industrial applications—A Volatilomic approach. *Foods, 11*(12). https://doi.org/10.3390/foods11121731

Andrade, C., Ferreres, F., Gomes, N. G. M., Duangsrisai, S., Srisombat, N., Vajrodaya, S., … Valentão, P. (2019). Phenolic profiling and biological potential of Ficus curtipes corner leaves and stem bark: 5-lipoxygenase inhibition and interference with NO levels in LPS-stimulated RAW 264.7 macrophages. *Biomolecules, 9*(400), 1–17. https://doi.org/10.3390/biom9090400

Andrade, P. B., Barbosa, M., Matos, R. P., Lopes, G., Vinholes, J., Mouga, T., & Valentão, P. (2013). Valuable compounds in macroalgae extracts. *Food Chemistry, 138*(2–3), 1819–1828. https://doi.org/10.1016/j.foodchem.2012.11.081

APROMAR OPP30. (2021). *Informe 2021: La Acuicultura en España*.

Barros, E. P., Moreira, N., Pereira, G. E., Leite, S. G. F., Moraes Rezende, C., & Guedes De Pinho, P. (2012). Development and validation of automatic HS-SPME with a gas chromatography-ion trap/mass spectrometry method for analysis of volatiles in wines. *Talanta, 101*, 177–186. https://doi.org/10.1016/j.talanta.2012.08.028

Ben Saad, H., Frikha, D., Bouallegue, A., Badraoui, R., Mellouli, M., Kallel, H., … Ben Amara, I. (2023). Mitigation of hepatic impairment with polysaccharides from red alga Albidum corallinum supplementation through promoting the lipid profile and liver homeostasis in Tebuconazole-exposed rats. *Pharmaceuticals, 16*(9). https://doi.org/10.3390/ph16091305

Bento-Silva, A., Santos, M., Costa, C. P., Carlota, M., Patto, V., Rocha, S. M., & Ros, M. (2022). *Comprehensive two-dimensional gas chromatography volatile composition*.

Bernardo, J., Malheiro, I., Videira, R. A., Valentão, P., Santos, A. C., Veiga, F., & Andrade, P. B. (2021). Trichilia catigua and Turnera diffusa extracts: In vitro inhibition of tyrosinase, antiglycation activity and effects on enzymes and pathways engaged in the neuroinflammatory process. *Journal of Ethnopharmacology, 271*, Article 113865. https://doi.org/10.1016/j.jep.2021.113865

Castejón, N., Parailloux, M., Izdebska, A., Lobinski, R., & Fernandes, S. C. M. (2021). Valorization of the red algae gelidium sesquipedale by extracting a broad spectrum of minor compounds using green approaches. *Marine Drugs, 19*(10). https://doi.org/10.3390/md19100574

Chen, X. M., & Kitts, D. D. (2011). Antioxidant and anti-inflammatory activities of Maillard reaction products isolated from sugar-amino acid model systems. *Journal of Agricultural and Food Chemistry, 59*(20), 11294–11303. https://doi.org/10.1021/jf2031583

Cikos, A.-M., Coz-Rakovac, R., Subaric, D., Jerkovic, I., Ackar, D., & Jokic, S. (2020). Macroalgae in the food industry – Opportunities and challenges. *Engineering Power, 15*(3), 1–19.

Cui, M., Wu, J., Wang, S., Shu, H., Zhang, M., Liu, K., & Liu, K. (2019). Characterization and anti-inflammatory effects of sulfated polysaccharide from the red seaweed

Gelidium pacificum Okamura. *International Journal of Biological Macromolecules, 129*, 377–385. https://doi.org/10.1016/j.ijbiomac.2019.02.043
Cvetanović, A., Zengin, G., Zeković, Z., Švarc-Gajić, J., Ražić, S., Damjanović, A., Mašković, P., & Mitić, M. (2018). Comparative in vitro studies of the biological potential and chemical composition of stems, leaves and berries Aronia melanocarpa's extracts obtained by subcritical water extraction. *Food and Chemical Toxicology, 121*(July), 458–466. https://doi.org/10.1016/j.fct.2018.09.045
Diez-Simon, C., Mumm, R., & Hall, R. D. (2019). *Mass spectrometry-based metabolomics of volatiles as a new tool for understanding aroma and flavour chemistry in processed food products.*. https://doi.org/10.1007/s11306-019-1493-6
Echavarría, A. P., Pagán, J., & Ibarz, A. (2012). *Melanoidins Formed by Maillard Reaction in Food and Their Biological Activity., 4*, 203–223. https://doi.org/10.1007/s12393-012-9057-9
Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology, 7*(2), 88–95. https://doi.org/10.1016/0006-2952(61)90145-9
Errati, H., Bencheqroun, S. K., Aboutayeb, R., Abail, Z., Lebbar, S., Dari, K., & Hilali, L. (2022). Assessment of the red seaweed Gelidium sesquipedale by-products as an organic fertilizer and soil amendment. *Sustainability (Switzerland), 14*(21). https://doi.org/10.3390/su142114217
Fan, R., & Gao, Y. (2022). Maillard and hydrolytic reactions in subcritical water extraction of bioactive compounds from licorice. *Molecules, 27*(20). https://doi.org/10.3390/molecules27206851
Fernandes, F., Barbosa, M., Pereira, D. M., Sousa-Pinto, I., Valentão, P., Azevedo, I. C., & Andrade, P. B. (2018). Chemical profiling of edible seaweed (Ochrophyta) extracts and assessment of their in vitro effects on cell-free enzyme systems and on the viability of glutamate-injured SH-SY5Y cells. *Food and Chemical Toxicology, 116* (February), 196–206. https://doi.org/10.1016/j.fct.2018.04.033
Ferreres, F., Andrade, C., Gomes, N. G. M., Andrade, P. B., Gil-Izquierdo, A., Pereira, D. M., … Valentão, P. (2021). Valorisation of kitul, an overlooked food plant: Phenolic profiling of fruits and inflorescences and assessment of their effects on diabetes-related targets. *Food Chemistry, 342*, Article 128323. https://doi.org/10.1016/j.foodchem.2020.128323
Ferreres, F., Lopes, G., Gil-Izquierdo, A., Andrade, P. B., Sousa, C., Mouga, T., & Valentão, P. (2012). Phlorotannin extracts from Fucales characterized by HPLC-DAD-ESI-MSn: Approaches to hyaluronidase inhibitory capacity and antioxidant properties. *Marine Drugs, 10*, 2766–2781. https://doi.org/10.3390/md10122766
Gomes-Dias, J. S., Pereira, S. G., Teixeira, J. A., & Rocha, C. M. R. (2022). Hydrothermal treatments – A quick and efficient alternative for agar extraction from Gelidium sesquipedale. *Food Hydrocolloids, 132*(March). https://doi.org/10.1016/j.foodhyd.2022.107898
Gómez Barrio, L. P., Cabral, E. M., Zhao, M., Álvarez García, C., Senthamaraikannan, R., Padamati, R. B., … Tiwari, B. K. (2022). Comparison study of an optimized ultrasound-based method versus an optimized conventional method for agar extraction, and protein co-extraction, from Gelidium sesquipedale. *Foods, 11*(6). https://doi.org/10.3390/foods11060805
Grina, F., Ullah, Z., Kaplaner, E., Moujahid, A., Eddoha, R., Nasser, B., … Essamadi, A. (2020). In vitro enzyme inhibitory properties, antioxidant activities, and phytochemical fingerprints of five Moroccan seaweeds. *South African Journal of Botany, 128*, 152–160. https://doi.org/10.1016/j.sajb.2019.10.021
Hans, N., Solanki, D., Nagpal, T., Amir, H., Naik, S., & Malik, A. (2024). Process optimization and characterization of hydrolysate from underutilized brown macroalgae (Padina tetrastromatica) after fucoidan extraction through subcritical water hydrolysis. *Journal of Environmental Management, 349*(390). https://doi.org/10.1016/j.jenvman.2023.119497
Hashemi, A., Noori, M., Dastyafteh, N., Sadat-Ebrahimi, S. E., Fazelzadeh Haghighi, N., Mehrpour, K., … Mahdavi, M. (2023). Synthesis and tyrosinase inhibitory activities of novel isopropylquinazolinones. *BMC Chemistry, 17*(1), 1–11. https://doi.org/10.1186/s13065-023-00978-3
Intelligence, M. (2023). Análisis de participación y tamaño del mercado de agar tendencias y pronósticos de crecimiento (2024-2029). https://www.mordorintelligence.com/es/industry-reports/agar-market.
João, K. G., Videira, R. A., Paiva-Martins, F., Valentão, P., Pereira, D. M., & Andrade, P. B. (2021). Homarine alkyl Ester derivatives as promising acetylcholinesterase inhibitors. *ChemMedChem, 16*(21), 3315–3325. https://doi.org/10.1002/cmdc.202100265
Kashaninejad, M., Sanz, M. T., Blanco, B., Beltrán, S., & Niknam, S. M. (2020). Freeze dried extract from olive leaves: Valorisation, extraction kinetics and extract characterization. *Food and Bioproducts Processing, 124*, 196–207. https://doi.org/10.1016/j.fbp.2020.08.015
Khursheed, M., Ghelani, H., Jan, R. K., & Adrian, T. E. (2023). Anti-inflammatory effects of bioactive compounds from seaweeds, bryozoans, jellyfish. *Shellfish and Peanut Worms. Marine Drugs, 21*(10), 524. https://doi.org/10.3390/md21100524
Kitts, D. D., Chen, X. M., & Jing, H. (2012). Demonstration of antioxidant and anti-inflammatory bioactivities from sugar-amino acid maillard reaction products. *Journal of Agricultural and Food Chemistry, 60*(27), 6718–6727. https://doi.org/10.1021/jf2044636
Koçi, R., Dupuy, F., Lebbar, S., Gloaguen, V., & Faugeron Girard, C. (2022). A new promising plant defense stimulator derived from a by-product of agar extraction from Gelidium sesquipedale. *Horticulturae, 8*(10), 1–15. https://doi.org/10.3390/horticulturae8100958
Kwak, E. J., Lee, Y. S., Murata, M., & Homma, S. (2005). Effect of pH control on the intermediates and melanoidins of nonenzymatic browning reaction. *Lwt, 38*(1), 1–6. https://doi.org/10.1016/j.lwt.2003.09.011
Lee, Y., Oh, H., & Lee, M. (2018). *extracts in high-fat diet-induced obese mice., 12*(6), 479–485.
Liu, S., Sun, H., Ma, G., Zhang, T., Wang, L., Pei, H., Li, X., & Gao, L. (2022). Insights into flavor and key influencing factors of Maillard reaction products: A recent update. *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.973677
Lomartire, S., & Gonçalves, A. M. M. (2023). Marine macroalgae polyphenols as potential neuroprotective antioxidants in neurodegenerative diseases. *Marine Drugs, 21*(5), 1–18. https://doi.org/10.3390/md21050261
Lopes, G., Barbosa, M., Andrade, P. B., & Valentão, P. (2019). Phlorotannins from Fucales: Potential to control hyperglycemia and diabetes-related vascular complications. *Journal of Applied Phycology, 31*, 3143–3152. https://doi.org/10.1007/s10811-019-01816-7
Marcet, I., Álvarez, C., Paredes, B., & Díaz, M. (2016). The use of sub-critical water hydrolysis for the recovery of peptides and free amino acids from food processing wastes. Review of sources and main parameters. *Waste Management, 49*, 364–371. https://doi.org/10.1016/j.wasman.2016.01.009
Martínez-Sanz, M., Cebrián-Lloret, V., Mazarro-Ruiz, J., & López-Rubio, A. (2020). Improved performance of less purified cellulosic films obtained from agar waste biomass. *Carbohydrate Polymers, 233*(November 2019). https://doi.org/10.1016/j.carbpol.2020.115887
Martínez-Sanz, M., Gómez-Mascaraque, L. G., Ballester, A. R., Martínez-Abad, A., Brodkorb, A., & López-Rubio, A. (2019). Production of unpurified agar-based extracts from red seaweed Gelidium sesquipedale by means of simplified extraction protocols. *Algal Research, 38*(October 2018). https://doi.org/10.1016/j.algal.2019.101420
Matos, J., Gomes, A., Cardoso, C., Afonso, C., Campos, A. M., Gomes, R., … Bandarra, N. M. (2020). Commercial red seaweed in Portugal (Gelidium sesquipedale and Pterocladiella capillacea, Florideophyceae): Going beyond a single-purpose product approach by valorizing bioactivity. *Thalassas, 36*(1), 213–224. https://doi.org/10.1007/s41208-019-00181-z
Meinita, M. D. N., Harwanto, D., Amron, H., & M. A., Jeong, G. T., Moon, I. S., & Choi, J. S.. (2023). A concise review of the potential utilization based on bioactivity and pharmacological properties of the genus Gelidium (Gelidiales, Rhodophyta). *Journal of Applied Phycology, 35*(4), 1499–1523. https://doi.org/10.1007/s10811-023-02956-7
Mellouk, Z., Benammar, I., Krouf, D., Goudjil, M., Okbi, M., & Malaisse, W. (2017). Antioxidant properties of the red alga Asparagopsis taxiformis collected on the north west Algerian coast. *Experimental and Therapeutic Medicine, 13*(6), 3281–3290. https://doi.org/10.3892/etm.2017.4413
Moreira, R., Ferreres, F., Gil-Izquierdo, Á., Gomes, N. G. M., Araújo, L., Pinto, E., … Videira, R. A. (2023). Antifungal activity of Guiera senegalensis: From the chemical composition to the mitochondrial toxic effects and Tyrosinase inhibition. *Antibiotics, 12*, 869. https://doi.org/10.3390/antibiotics12050869
Nielsen, S. S. (2010). Phenol-sulfuric acid method for Total carbohydrates. In S. S. Nielsen (Ed.), *Food analysis laboratory manual* (pp. 47–53). Springer. https://doi.org/10.1007/978-1-4419-1463-7_6.
Observatorio de Complejidad Económica - OEC World. (2022). Agar agar. https://oec.world/es/profile/hs/agar-agar.
Oh, J. G., Chun, S. H., Kim, D. H., Kim, J. H., Shin, H. S., Cho, Y. S., … don, & Lee, K. W.. (2017). Anti-inflammatory effect of sugar-amino acid Maillard reaction products on intestinal inflammation model in vitro and in vivo. *Carbohydrate Research, 449*, 47–58. https://doi.org/10.1016/j.carres.2017.07.003
Onoja, O. J., & Elufioye, T. O. (2023). *Acetylcholinesterase inhibition and metal chelating potentials of fractions and compounds isolated from Cola hispida., 10*, 10–21.
Oumaskour, K., Boujaber, N., Etahiri, S., & Assobhei, O. (2013). Anti-inflammatory and antimicrobial activities of twenty-tree marine red algae from the coast of Sidi Bouzid (El Jadida-Morocco). *International Journal of Pharmacy and Pharmaceutical Sciences, 5*(3), 145–149.
Patra, J. K., Lee, S. W., Kwon, Y. S., Park, J. G., & Baek, K. H. (2017). Chemical characterization and antioxidant potential of volatile oil from an edible seaweed Porphyra tenera (Kjellman, 1897). *Chemistry Central Journal, 11*(1), 1–10. https://doi.org/10.1186/s13065-017-0259-3
Rafiquzzaman, S. M., Kim, E. Y., Lee, J. M., Mohibbullah, M., Alam, M. B., Soo Moon, I., … Kong, I. S. (2015). Anti-Alzheimers and anti-inflammatory activities of a glycoprotein purified from the edible brown alga Undaria pinnatifida. *Food Research International, 77*, 118–124. https://doi.org/10.1016/j.foodres.2015.08.021
Shapla, U. M., Solayman, M., Alam, N., Khalil, M. I., & Gan, S. H. (2018). 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: Effects on bees and human health. *Chemistry Central Journal, 12*(1), 1–18. https://doi.org/10.1186/s13065-018-0408-3
Sharma, V. K., Choi, J., Sharma, N., Choi, M., & Seo, S. Y. (2004). In vitro anti-tyrosinase activity of 5-(hydroxymethyl)-2-furfural isolated from Dictyophora indusiata. *Phytotherapy Research, 18*(10), 841–844. https://doi.org/10.1002/ptr.1428
Shipeng, Y., Woo, H. C., Choi, J. H., Park, Y. B., & Chun, B. S. (2015). Measurement of antioxidant activities and phenolic and flavonoid contents of the Brown seaweed Sargassum horneri: Comparison of supercritical CO2 and various solvent extractions. *Fisheries and Aquatic Sciences, 18*(2), 123–130. https://doi.org/10.5657/FAS.2015.0123
Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of Total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178. https://doi.org/10.1016/j.scienta.2016.11.004
Sluiter, J. B., Ruiz, R. O., Scarlata, C. J., Sluiter, A. D., & Templeton, D. W. (2010). Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. *Journal of Agricultural and Food Chemistry, 58*(16), 9043–9053. https://doi.org/10.1021/jf1008023
Sokolova, E. V., Barabanova, A. O., Bogdanovich, R. N., Khomenko, V. A., & Solov'eva, T. F., & Yermak, I. M.. (2011). In vitro antioxidant properties of red algal

polysaccharides. *Biomedicine and Preventive Nutrition, 1*(3), 161–167. https://doi.org/10.1016/j.bionut.2011.06.011

Švarc-Gajić, J., Cerdà, V., Clavijo, S., Suárez, R., Zengin, G., & Cvetanović, A. (2019). Chemical and bioactivity screening of subcritical water extracts of chokeberry (Aronia melanocarpa) stems. *Journal of Pharmaceutical and Biomedical Analysis, 164*, 353–359. https://doi.org/10.1016/j.jpba.2018.11.006

Thambi, A., & Chakraborty, K. (2022). Brown and red marine macroalgae as novel bioresources of promising medicinal properties. *Journal of Aquatic Food Product Technology, 31*(3), 227–241. https://doi.org/10.1080/10498850.2022.2035877

Trigueros, E., Sanz, M. T., Alonso-Riaño, P., Beltrán, S., Ramos, C., & Melgosa, R. (2021). Recovery of the protein fraction with high antioxidant activity from red seaweed industrial solid residue after agar extraction by subcritical water treatment. *Journal of Applied Phycology, 33*(2), 1181–1194. https://doi.org/10.1007/s10811-020-02349-0

Trigueros, E., Ramos, C., Alonso-Riaño, P., Beltrán, S., & Sanz, M. T. (2023). Subcritical water treatment for valorization of the red algae residue after agar extraction: Scale-up from laboratory to pilot plant. *Industrial and Engineering Chemistry Research, 62*(8), 3503–3514. https://doi.org/10.1021/acs.iecr.2c04132

Trigueros, E., Sanz, M. T., Beltrán, S., & Ruiz, M. O. (2022). Filtration of subcritical water hydrolysates from red macroalgae byproducts with ultraporous ceramic membranes for oligosaccharide and peptide fractionation. *Journal of Membrane Science, 660* (July). https://doi.org/10.1016/j.memsci.2022.120822

Trigueros, E., Sanz, M. T., Filipigh, A., Beltrán, S., & Riaño, P. (2021). Enzymatic hydrolysis of the industrial solid residue of red seaweed after agar extraction: Extracts characterization and modelling. *Food and Bioproducts Processing, 126*, 356–366. https://doi.org/10.1016/j.fbp.2021.01.014

Trigueros, E., Benito-Román, Ó., Oliveira, A. P., Videira, R. A., Andrade, P. B., Sanz, M. T., & Beltrán, S. (2024). Onion (Allium cepa L.) skin waste valorization: Unveiling the phenolic profile and biological potential for the creation of bioactive agents through subcritical water extraction. *Antioxidants, 13*(205), 1–18.

Tuma, S., Izaguirre, J. K., Bondar, M., Marques, M. M., Fernandes, P., Fonseca, M. M. R., & Cesário, M. T. (2020). Upgrading end-of-line residues of the red seaweed Gelidium sesquipedale to polyhydroxyalkanoates using Halomonas boliviensis. *Biotechnology Reports, 27*(27). https://doi.org/10.1016/j.btre.2020.e00491

Valentão, P., Trindade, P., Gomes, D., Guedes de Pinho, P., Mouga, T., & Andrade, P. B. (2010). Codium tomentosum and Plocamium cartilagineum: Chemistry and antioxidant potential. *Food Chemistry, 119*(4), 1359–1368. https://doi.org/10.1016/j.foodchem.2009.09.015

Viant, M. R., Kurland, I. J., Jones, M. R., & Dunn, W. B. (2017). How close are we to complete annotation of metabolomes? *Current Opinion in Chemical Biology, 36*, 64–69. https://doi.org/10.1016/j.cbpa.2017.01.001

Wang, Y., Zhou, L., Chen, M., Liu, Y., Yang, Y., Lu, T., Ban, F., Hu, X., Qian, Z., Hong, P., & Zhang, Y. (2023). Mining xanthine oxidase inhibitors from an edible seaweed Pterocladiella capillacea by using in vitro bioassays, affinity ultrafiltration LC-MS/MS, metabolomics tools, and in silico prediction. *Marine Drugs, 21*(10), 1–18. https://doi.org/10.3390/md21100502

Xiao, Z., Wu, M., Niu, Y., Chen, F., Zhang, X., Zhu, J., Song, S., & Zhu, G. (2015). Contribution of chicken base addition to aroma characteristics of Maillard reaction products based on gas chromatography-mass spectrometry, electronic nose, and statistical analysis. *Food Science and Biotechnology, 24*(2), 411–419. https://doi.org/10.1007/s10068-015-0054-7

Zhao, L., Chen, J., Su, J., Li, L., Hu, S., Li, B., Zhang, X., Xu, Z., & Chen, T. (2013). In vitro antioxidant and Antiproliferative activities of 5-Hydroxymethylfurfural. *Journal of Agricultural and Food Chemistry, 61*(44), 10604–10611. https://doi.org/10.1021/jf403098y