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Cystoseira baccata Extracts Obtained by Accelerated Solvent Extraction as Candidate for the Functional Food Industry †

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Abstract: In recent years, the interest in food products containing natural additives that claim health benefits has increased exponentially. Algae have long been traditionally used for food in certain Asian regions and today the demand for edible seaweed and new culinary trends concerning algae (new production models and products) are emerging. In this work, the fatty acid profile and the antioxidant and antibacterial activities of *Ulva intestinalis*, *Ulva lactuca*, *Cystoseira baccata* and *Himanthalia elongata* extracts obtained by accelerated solvent extraction (ASE) (ethanol 100% / 120 ºC) were investigated. Lipids were determined by gas chromatography coupled to mass spectrometry (GC-MS). *C. baccata* ethanolic extracts showed considerably higher capacity of inhibiting 50% of DPPH (1,1-diphenyl-2-picryl hydrazyl) and higher potential antimicrobial activity on *Escherichia coli* and *Staphylococcus aureus* in comparison with the other species studied, showing ASE is an efficient alternative technique for obtaining high-quality extracts from the nutritional point of view.

Keywords: Seaweeds; *Cystoseira baccata*; Accelerated Solvent Extraction (ASE); fatty acids; antioxidant activity; antimicrobial activity

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1. Introduction

Nowadays, the food sector is looking for ingredients and natural additives to develop functional foods that claim health benefits [1]. In addition, food industry is considering the new post-pandemic COVID-19 period in which consumers are concerning about ingesting products that enhance their immune systems [2,3]. To follow this trend, food companies are searching for new functional foods to comply the consumers demand, being algae one of the potential raw materials considered. Algae have been investigated as antimicrobial, anti-inflammatory, and potential antiviral agents due to the content of bioactive lipids and polyphenols [1,2,4,5].

The objective of this study was to determine the fatty acid (FA) composition of 4 common macroalgae species *Hilmanthalia elongata (H. elongata)*, *Ulva intestinalis (U. intestinalis)*, *Ulva lactuca (U. lactuca)* and *Cystoseira baccata (C. baccata)* and evaluate the antioxidant and antimicrobial bioactivities of ASE extracts from these species. These 4 macroalgae are commonly found throughout the year in the coast of Galicia (NW of Spain) and Portugal [4,6]. They are accounted for a relatively rapid and annual growth and thus, a high availability of their biomass.

Despite seaweeds are edible and consumed in many Asian countries as part of salads, with rice or bread among others, the European market of macroalgae food products is

low, and fortify other food with algae bioactive extracts can gain acceptability among consumers. Traditionally the extraction of their compounds have been performed by Solid-Liquid Extraction (SLE) methods [8]. However, SLE presents the following disadvantages like time-consuming, energy consumption and the use of solvents which are not in accordance with the current regulation [9]. Therefore, it is necessary to identify new efficient extraction processes to exploit the bioactives present in marine algae. Among others, acceleration solvent extraction (ASE) is currently considered an advanced, environmental friendly technique since it offers important benefits such as shorter extraction time, decreased solvent consumption, decreased sample handling and increased yield [10].

2. Material and Methods

2.1. Sample Origin and Fatty acid Extraction

The seaweeds *U. intestinalis*, *U. lactuca*, *C. baccata* and *H. elongata* were collected in June 2017 on the Northwest of Galicia (Atlantic coast), in Ferrol. Once in the laboratory, algae were washed with distilled water, freeze-dried and grounded (particle size < 500μm).

Extractions of algae species were performed using an accelerated solvent extractor (ASE, 350, Dionex Corp, Sunnyvale, CA, USA) equipped with a solvent controlled unit. Extractions were performed in triplicate in 10 mL extractions cells with ethanol at 120 °C and 100 bar. The amount of 1 g of algae was loaded into the stainless-steel cell with sea sand (thin grain, particlesize 250–300μm, Sigma-Aldrich, Madrid, Spain) above and below the sample to avoid any voidspaces. Then, the extraction cell was placed into the carrousel and the automatic extraction sequence began with the loading of the cell into the oven. When the cell was heated to the pre-set extraction temperature, the cell was pressurized for 10 min and then allowed to flow the extract into the collection vial. The solvent total volume used was 20 mL.

2.2. GC-MS Fatty Acid Analysis

Idenficiation and quantification of FAs were performed by modified method of Folch [11]. A known amount of lipid and extracted samples were saponified and submitted to the same trans-esterification steps as mentioned in Otero et al. [8]. FA methyl esters (FAMEs) were analysed by a GC-MS-FID using 7890A System (Agilent Technologies, (Loveland, CO, USA) comprising a split/splitless injector, electronic pressure control G4513A autoinjector,a 5975C triple-axis mass spectrometer detector and GC-MS Solution software. The column used was an Agilent HP-5MS UI capillary column (30 m×0.250 mm × 0.25μm). Helium was used as a carried gas at a constant flow of 1.8 mL/min. Oven temperature programme started at 50 °C, increased to 210 °C at 20 °C increase per min and hold for 18 min. Then, temperature was further increased to230 °C at 20 °C increase per min and kept at 230 °C for 13 min. The injection volume was 1μL in splitless mode. Inlet temperatures was set at 260 °C and MS ion source and interface temperatures were 230 °C and 280 °C respectively. Data were acquired in a full scan from 40 to 500 m/z. FA standards, linoleic acid, γ-linolenic acid, oleic acid, palmitic acid, stearic acid, myristic acid, cis-5,8,11,14,17-eicosapentaenoic acid (EPA) and arachidonic acid (ARA) were obtained from Sigma (Madrid, Spain).

2.3. Antioxidant Activity

The antioxidant activity algae extracts was determined by the DPPH scavenging assay based on a procedure described by Brand-Williams et al. [12]. This method is based on the neutralization of free radicals of DPPH (1,1-diphenyl-2-picryl hydrazyl) (Sigma–Aldrich, Spain) by the antioxidant extracts. A dilution series of the extracted samples was prepared (0.25, 0.5, 1, 1.5 mg/mL) and an aliquot (25 μL) of each one was added to 975 μL of DPPH in ethanol (23.5 μg/mL). Reaction was complete after 2 h at room temperature in the dark. Absorbance was then measured at 515 nm against the blank in the Generys 10

uv spectrophotometer reader. Ethanol was used to adjust zero and DPPH–ethanol solution as a reference sample. The DPPH concentration in the reaction medium was calculated from the following calibration curve, determined by linear regression (r = 0.9998): Y = 0.0385X + 0.0083. The percentage of remaining DPPH against the extract concentration was then plotted to obtain the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% or IC50. Thus, the lower the IC50, the higher the antioxidant power.

2.4. Antibacterial Activity

The algae extracts were individually tested against a Gram-positive, S. aureus (ATCC 25923), and Gram-negative, E. coli (ATCC 25922), bacterial strains. All tests were performed in Mueller-Hinton broth supplemented with 0.5% tween 20. The inocula of bacterial strains were prepared from overnight Mueller-Hinton broth cultures at 37 °C. Test strains were suspended in Mueller-Hinton broth to give a final density 1 × 108 cfu/mL. The algae extract dilutions in ethanol ranged from 50 to 10 mg/mL. The 96-microwell plates were prepared by dispensing into each well 185 µL of culture broth, 5 µL of the inocula and 10 µL of the different extract dilution. The final volume of each well was 200 µL. Plates were incubated at 37 °C for 24 h for each bacterium. Negative controls were prepared using 10 µL of ethanol, the solvent used to dissolve the algae extracts. Chloramphenicol (Sigma, Madrid, Spain) were used as positive reference standards to determine the sensitivity of the microbial species used. Absorbance was then measured at 620 nm at initial time and after 24 h

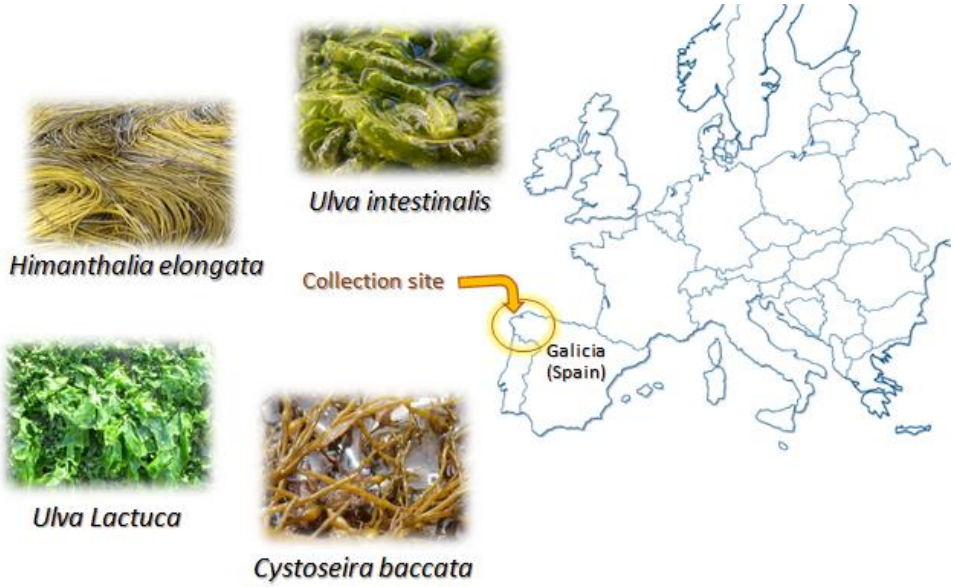

Figure 1. Collection site of Himanthalia elongata, Cystoseira baccata, Ulva lactuca and Ulva intestinalis.

3. Results

First, the lipid content of four macroalgae species was determined by the Folch method and then, FA profile was determined by GC-MS after derivatization, yielding corresponding Fatty Acid Methyl Esters (FAMEs). Figure 2 shows the lipid content and FA profile in seaweeds. Macroalgae are not accounted for high lipids yield which were ranged from 4–6%. *C. baccata* showed relatively high levels of these lipids, specially of palmitic, myristic and palmitoleic acids. Besides, it was the only specie in which arachidonic and eicosapentaenoic acids were present.

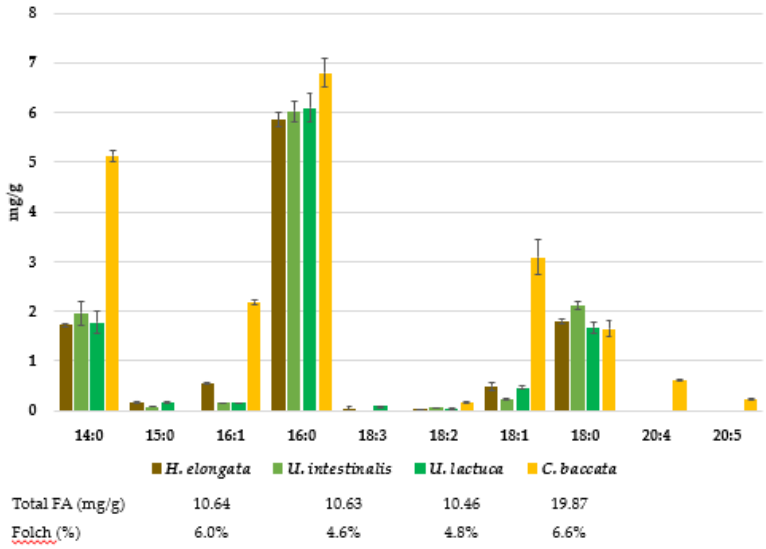

Figure 2. Fatty acid (FA) profile of Ulva intestinalis, Ulva lactuca, Cystoseira baccata and Himanthalia elongata.

Then, ASE was used to obtain crude extracts of these algae which were tested for antioxidant and antimicrobial activities. The accelerated solvent extractor was set at already optimized conditions for algae (pressure of 100 bar, 120 °C and a duration of 10 min) and using ethanol as solvent. Not only FA, but also carotenoids and phenols which are also reported for antioxidant and antimicrobial properties are susceptible to be extracted by this technique. The extracts were obtained in triplicate and then they were used to screen bioactivities. The antioxidant activity by the DPPH test showed that *H. elongata* and *C. baccata* displayed the higher antioxidant properties at dose-response rates under concentrations of 64 and 28 μg/mL, respectively (Figure 3).

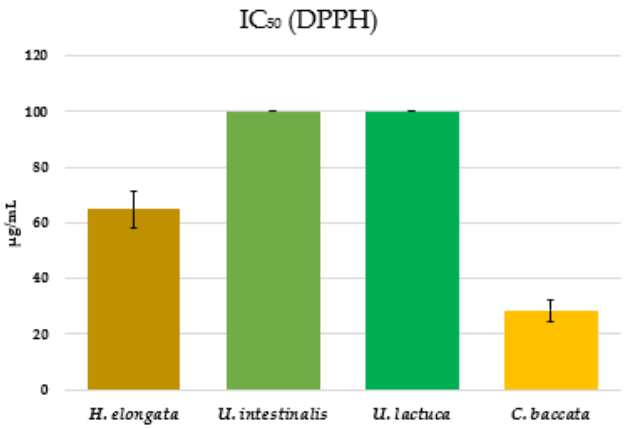

Figure 3. DPPH assay of *Ulva intestinalis*, *Ulva lactuca*, *Cystoseira baccata* and *Himanthalia elongata* ethanolic PLE extracts (100 bar, 120 °C, 10 min).

In addtion, antibacterial bioactivities were determined targeting two species representative of Gram + and Gram– bacteria, as well as common food pathogens, namely *Staphylococcus aureus* and *Escherichia coli*. Using serialized dilutions concentrations, the minimum inhibitory concentrations was evaluated. It was used 2.5 mg/mL of algae extract for 24 h and measured absorbance at 0 and 24 h. The four macroalgae species showed a slight bacterial inhibition (Figure 4), nonetheless, *C. baccata* exerteded the higher inhibition (Figure 4D), reaching 60% inhibition for *E. coli* and 40% for *S. aureus*.

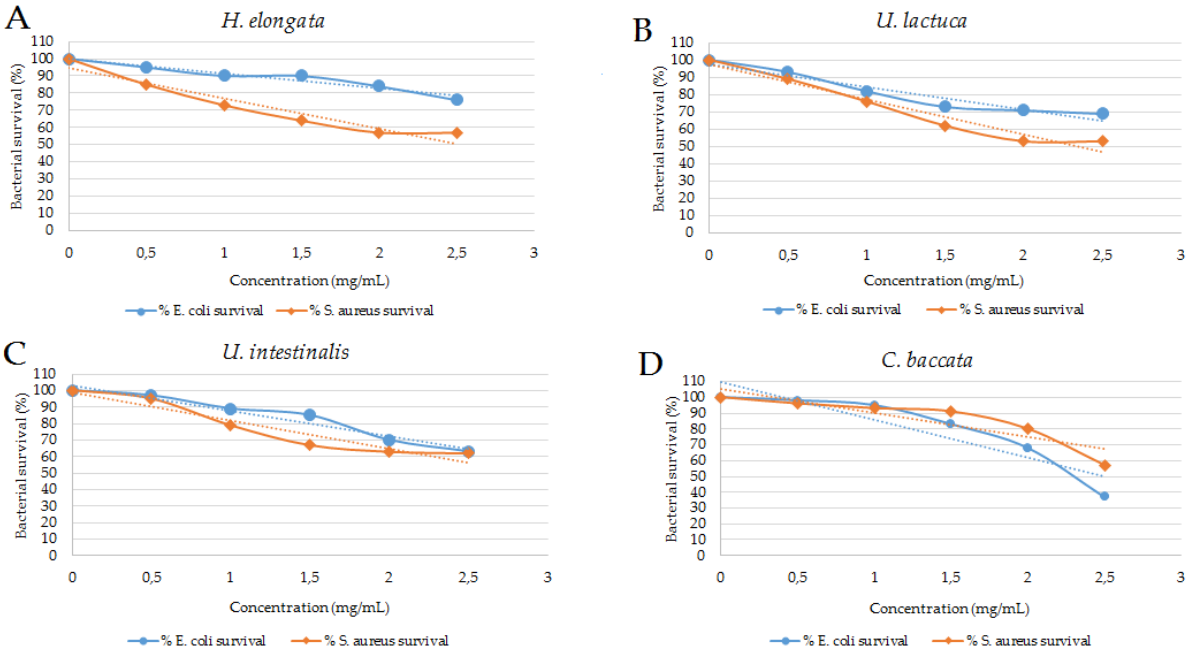

Figure 3. Antimicrobial activity of the ASE ethanoic extracts seaweeds against the two pathogenic bacterial strains. The activity is reported as % of bacterial survival against the controls and each graph correspond to *Himanthalia elongata* (A), *Ulva lactuca* (B), *Ulva intestinalis* (C) and *Cystoseira baccata* (B) ASE extracts. Results show mean ± standard error of the mean (SEM) of three experiments.

4. Conclusions

Algae have been postulated as promising antidiabetic, antibacterial and antioxidant agents. From 4 seaweeds studied, *C. baccata* extracts showed the higher antioxidant capacity of inhibiting 50% of DPPH (1,1-diphenyl-2-picryl hydrazyl) in comparison with the other species studied (IC_{50} of 28 µg/mL). The potential antimicrobial activity tested on *Escherichia coli* and *Staphylococcus aureus* shows that *C. baccata* extract exerted the highest inhibition: 40% and 60%, respectively. In addition, ASE is an efficient alternative technique for obtaining high-quality lipids, while avoiding the use of hazardous solvents. Therefore, the large number of bioactive compounds present in *C. baccata* makes them a powerful row material for developing functional foods which could reduce the risks of acute and chronic diabetic complications, cancer, and coronary diseases and enhance the immune systems by increasing heathier diets.

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References

1. Galanakis, C.M.; Aldawoud, T.M.S.; Rizou, M.; Rowan, N.J.; Ibrahim, S.A. Food Ingredients and Active Compounds against the Coronavirus Disease (COVID-19) Pandemic: A Comprehensive Review. *Foods* **2020**, *9*, 1701; 1–16, doi:10.3390/foods9111701.
2. Crisis, C.-P. The Food Systems in the Era of the Coronavirus. *Foods* **2020**, 9, 523; 1–10. doi:10.3390/foods9040523.
3. Galanakis, C.M.; Rizou, M.; Aldawoud, T.M.S.; Ucak, I.; Rowan, N.J. Trends in Food Science & Technology Innovations and technology disruptions in the food sector within the COVID-19 pandemic and post-lockdown era. *Trends Food Sci. Technol.* **2021**, *110*, 193–200, doi:10.1016/j.tifs.2021.02.002.
4. Moreira, A.S.P.; da Costa, E.; Melo, T.; Lopes, D.; Pais, A.C.S.; Santos, S.A.O.; Pitarma, B.; Mendes, M.; Abreu, M.H.; Collén, P.N.; et al. Polar lipids of commercial ulva spp. Of different origins: Profiling and relevance for seaweed valorization. *Foods* **2021**, *10*, doi:10.3390/foods10050914.
5. Otero, P.; López-martínez, M.I.; García-risco, M.R. Journal of Pharmaceutical and Biomedical Analysis Application of pressurized liquid extraction (PLE) to obtain bioactive fatty acids and phenols from Laminaria ochroleuca collected in Galicia. *J. Pharm. Biomed. Anal.* **2019**, *164*, 86–92, doi:10.1016/j.jpba.2018.09.057.
6. Pinteus, S.; Silva, J.; Alves, C.; Horta, A.; Fino, N.; Rodrigues, A.I.; Mendes, S.; Pedrosa, R. Cytoprotective effect of seaweeds with high antioxidant activity from the Peniche coast (Portugal). *Food Chem.* **2017**, *218*, 591–599, doi:10.1016/j.foodchem.2016.09.067.
7. García-Fernández, A.; Bárbara, I. Studies of Cystoseira assemblages in Northern Atlantic Iberia. *An. del Jard. Bot. Madrid* **2016**, *73*, 1–21, doi:10.3989/ajbm.2403.
8. Otero, P.; Saha, S.K.; Gushin, J.M.; Moane, S. Identification of optimum fatty acid extraction methods for two different microalgae *Phaeodactylum tricornutum* and *Haematococcus pluvialis* for food and bi. **2017**, doi:10.1007/s00216-017-0412-9.
9. European Commision. Directive 2009/32/EC of the European Parliament and of the Council of 23 April 2009 on the Approximation of the Laws of the Member States on Extraction Solvents Used in the Production of Foodstuffs and Food Ingredients; Official Journal of the European Union: Brussels, Belgum, 2009
10. Otero, P.; López-Martínez, M.I.; García-Risco, M.R. Application of pressurized liquid extraction (PLE) to obtain bioactive fatty acids and phenols from *Laminaria ochroleuca* collected in Galicia (NW Spain). *J. Pharm. Biomed. Anal.* **2019**, *164*, 86–92, doi:10.1016/j.jpba.2018.09.057.
11. Folch, J.; Lees, M.; Sloane-Stanley, G.H. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509, doi:10.1016/s0021-9258(18)64849-5.
12. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.* **1995**, *28*, 25–30, doi:10.1016/S0023-6438(95)80008-5.