Chemical composition of sustainable Mediterranean macroalgae obtained from land-based and sea-based aquaculture systems

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ABSTRACT

Increased demand for macroalgae as human food may jeopardise the balance of macroalgae in the Mediterranean Sea. Aquaculture is a sustainable alternative source of macroalgae, which can be sea- or land-based. Much data on macroalgae composition can be found in the literature; however, no comparison between aquaculture types has yet been made. This paper compares the contents of two samples cultivated on land (*Ulva* sp. and *Ulva ohnoi*) and three on the sea, an *Ulva* sp. and two red macroalgae (*Gracilaria gracilis*, and *Geliduim* sp.). The fatty acid profile, iodine, and some heavy metals significantly differed in the samples grown in the tanks on land compared to those produced in the sea. In addition, a higher content of some essential amino acids was found in *U. ohnoi*. By cultivating macroalgae under controlled conditions, land-based aquaculture can help improve some macroalgae's nutritional value and reduce toxic components such as heavy metals.

1. Introduction

Asia has a long tradition of using seaweed as a culinary ingredient. However, human consumption of certain macroalgae in other continents is relatively recent. Global seaweed production has increased more than 60-fold since 1950, as macroalgae have many sustainable applications, such as fertilisers, biomass fuels (Bruhn et al., 2011), and bioremediation (Moreda-Piñeiro et al., 2011). Moreover, their use in Europe has awakened interest as they can be used as food supplements or ingredients with therapeutic and flavouring applications (Armeli Minicante et al., 2022; Francezon et al., 2021; Mouritsen et al., 2018). This interest stems from nutritional components such as minerals, vitamins, complex carbohydrates, proteins, antioxidants and polyunsaturated fatty acids (Cuchiaro & Laurens, 2019; Mandalka et al., 2022; Moreda-Piñeiro et al., 2011). Special care should be taken when using them as food, as they accumulate some inorganic elements, including heavy metals (Chen et al., 2018) or iodine (Biancarosa et al., 2018) and microbial contaminants (Mendes et al., 2022) when grown under uncontrolled conditions.

In contrast to Asia, most European seaweed production depends on harvesting wild stocks, with France, Ireland and Spain being the leading European producers of macroalgae (Araújo et al., 2021). However, the growing demand for certain types of macroalgae threatens the stability of the wild ecosystems they grow in (Campbell et al., 2019). For this reason, several European companies are developing macroalgae aquaculture in line with the current circular economy goals (European Commission, 2020). Across Europe, seaweed aquaculture is mainly carried out in nearshore systems (about 75% of total production). The remaining part is produced in land-based tanks under controlled growth conditions (Araújo et al., 2021). Due to structural limitations, land-based cultivation is still limited to certain species (Tabarsa et al., 2012). However, *Ulva* and *Gracilaria* spp. are increasingly used in land-based aquaculture due to their high growth capacity and stable nutritional composition.

Although land-based aquaculture has a lower productivity potential, it can produce stable quantities of macroalgae with a more reproducible nutrient composition. This characteristic and the possibility of utilising wastewater from fish aquaculture systems have encouraged the development of *Ulva* spp. in land-based aquaculture (Dominguez & Loret, 2019). The wastewater from some specific fish aquaculture systems has been optimal for *Ulva* sp. production, as water is rich in NH_4^+, an essential source of nitrogen (Sebök & Hanelt, 2023). Other studies have shown that crucial interactions between macroalgae and the environmental microbiome significantly influence algal growth and composition (Califano et al., 2020; Ghaderiardakani et al., 2019; Polikovsky

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et al., 2020; Wichard, 2023). Improving land-based macroalgae production by optimizing some specific variables remains possible.

The study of algal composition from a human nutritional point of view is not as widespread as other biological and aquaculture studies. Some studies have quantified specific nutrients from aquaculture-produced macroalgae (Biancarosa et al., 2018; Desideri et al., 2016; Peña-Rodríguez et al., 2011; Tibbetts et al., 2016), but studies involving a wide range of nutrients under controlled culture conditions are scarce.

Some researchers have examined the composition of red and green macroalgae in land-based aquaculture (Biancarosa et al., 2018; Desideri et al., 2016; Peña-Rodríguez et al., 2011; Roleda et al., 2021; Tibbetts et al., 2016). Gadberry et al., 2018, studied the seasonal variations of algae from Northwestern Pacific composition. However, no comparison was made with wild or any other type of macroalgae production. In another study that involved on-land cultivation of *Ulva clathrate* in Mexico, it was concluded that the nutritional properties of green macroalgae could be modified by changing cultivation methods. At the same time, no significant differences in the nutritional composition were observed between seasons within a year (Peña-Rodríguez et al., 2011). Another study comparing the composition of *Ulva* sp. from Integrated multi-trophic aquaculture (IMTA) during all seasons showed no significant differences in proximal composition, while differences in inorganic elements were registered (Laramore et al., 2022). Despite the knowledge that land-based cultivation can provide algae with stable composition throughout the year, there is a lack of specific studies comparing the chemical composition of the same type of seaweed cultivated in nearshore systems and land-based tanks. It is reasonable to hypothesize that some differences can be found between macroalgae cultivated nearshore, with no possibility of water composition modification, compared with in-land cultivation mode in which water can be enriched with nutrients and purified of heavy metals.

Therefore, the present study aims to compare the proximate composition and essential nutrients such as fatty acids, amino acids, antioxidants, and minerals to assess the presence of both beneficial and hazardous nutritional components such as heavy metals and antibiotics in *Ulva* sp. grown on land and in nearshore systems. The composition of the same variety of *Ulva* sp. cultivated on land and in the sea will be compared to see if land-based cultivation can help to produce safer macroalgae. In addition, the composition of the *Ulva* sp. samples will be compared with another land-grown species of the same genera, *Ulva ohnoi*, and two red macroalgae (*Gelidium* sp. and *Gracillaria gracilis*) from nearshore cultivation.

2. Materials and methods

2.1. Reactants

Sodium carbonate, sodium chloride, ferric chloride, potassium persulfate, glacial acetic acid, HPLC-grade ethanol, oxygenated water, and 99% GC-grade n-hexane were obtained from Panreac (Barcelona, Spain). Gallic acid, (±)-6-Hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid (Trolox), 99%, Folin & Ciocalteu reagent, 2.2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) 98%; 2.2-diphenyl-1-picrylhydrazyl (DPPH), 2.4.6-tris(2-pyridyl) S-Triazine (TPTZ), heptafluorobutyric acid (HFBA), sulfadiazine, chlortetracycline, flumequine, and oxytetracycline, formic acid (HPLC grade) and hepta-florobutytic acid (HFBA) were acquired from Sigma–Aldrich Co. (St. Louis, MO, USA). Besides, 37 component FAME mix certified reference material (CRM47885) and amino acid reference standard (AAS18) from Supelco (St. Louis, MO, USA). A multi-elemental standard stock solution (SCP33MS) from SCP Science (Clark Graha, Baie D'Urfé, Canada), a phosphorous single-element standard from Agilent (Santa Clara, CA, USA) and potassium iodine (99.5%) from Merck (Darmstadt, Germany) were used for elemental determination. A solution containing 10 ng mL^{-1} of Ge, Rh and Re was also used for the internal standardization of analyte signals. Milli-Q double-deionized water (18.2 MΩ/cm) was bought from Millipore (Molsheim, France).

2.2. Macroalgae samples

Mediterranean Algae, a start-up company dedicated to aquaculture macroalgae production in Alicante, Spain (Mediterranean Algae, 2022), provided three samples for the five macroalgae varieties harvested during the same period. Three macroalgae species were cultivated in the sea, one green (*Ulva* sp. 1) and two red (*Gracilaria gracilis* and *Gelidium* sp.). The other two samples grown on land were *Ulva* sp. (2) and *Ulva ohnoi*. *U. ohnoi* was genetically characterized and provided by the Departament d'Enginyeria Agroalimentària i Biotecnologia (Universitat Politècnica de Catalunya, Barcelona, Spain). The *Ulva* sp. from the nearshore system was grown on land, so the same variety was used for the study. No phylogenetic data was available for that sample; the denomination was *Ulva* sp.

The land-based aquaculture used filtered, UV-disinfected seawater with nutrient supplements, while the offshore cultivation was carried out in the Mediterranean Sea near North Africa. The producer did not provide further information, claiming it was confidential. Seaweed was harvested randomly, washed with tap water, then dried in the open air for 15 days and stored in bags in the dark to prevent degradation of macroalgal constituents. The samples were grounded using a Retsch cryo-mill (Düsseldorf, Germany). The results are the average of the three samples collected for each macroalgae species, with three replicates for each determination.

2.3. Antioxidant compounds extraction

A mixture of ethanol: water (70:30) was selected for the extraction as good recoveries were obtained, and ethanol is safer than methanol (Pappou et al., 2022). About 800 ± 1 mg of homogenized seaweeds were

accurately weighed in a tube, and 9 mL of the extractant was added. The mixture was shaken with a vortex for 1 min and let to stand for 30 min at room temperature. In another assay, the extraction was ultrasound-assisted in a bath at 25 °C during the 30 min period. The tubes with the mixture were homogenized again for 1 min. Then, the tubes were centrifuged for 10 min at 5000 rpm. The supernatant was collected with a Pasteur pipette and deposited in another tube. Three extracts of each sample were obtained and kept in the freezer until the analysis.

2.4. Total phenolic content (TPC)

Total phenolic content was determined by employing an adapted version of Folin & Ciocalteu method (Beltran Sanahuja et al., 2019). Briefly, 700 mg of macroalgae extract was mixed with 100 μL of Folin & Ciocalteu reactant solution in a 10 mL polyethylene tube and swirled for 10 s with a vortex. Subsequently, 500 μL of a Na_2CO_3 7% aqueous solution and 4 mL of distilled water were added. The mixture was left to react for 30 min in the dark. Afterwards, the absorbance of the samples and the gallic acid standard solutions were measured at 760 nm. Results were expressed in mg gallic acid equivalents (GAE) gram of macroalgae^{-1} dry weight (DW).

2.5. Antioxidant capacity determination

The antioxidant capacity was evaluated with a calibration graph using different standard concentrations. ABTS, FRAP, and DPPH results were expressed in mg of Trolox/100g DW of the sample as an average of triplicates.

The ferric-reducing antioxidant power assay (FRAP) is determined as an increase in absorbance due to the (Fe^{2+}-TPTZ) (Beltran Sanahuja et al., 2019; Karagecili, Yılmaz, et al., 2023). The procedure included mixing 200 μL of the sample extract with 3 mL of FRAP reactant. The solutions were shaken with a vortex for a few seconds and let stand for 30 min in the dark at room temperature. Afterwards, the absorbance of samples and standards was measured at 593 nm.

The DPPH Radical Scavenging Activity (Aytac et al., 2023; Munteanu et al., 2021) was determined by mixing 3 mL of DPPH reactant with 700 mg macroalgae extract. The absorbance was registered at 517 nm until a stable absorbance was reached (60 min), and the antiradical activity was calculated based on absorbance.

The ABTS$^{•+}$ is generated by reacting with a potent oxidizing agent (potassium persulfate) with the ABTS salt (Aytac et al., 2023; Karagecili, İzol, et al., 2023; Munteanu et al., 2021). For this reaction, 200 mg of the macroalgae extract reacted with 3 mL of the ABTS solution. The solutions were shaken with a vortex for a few seconds and held in darkness until stable absorbance was reached. Then, the absorbance at 734 nm of standards and sample extractants was obtained.

2.6. Proximate composition

Moisture and ash content were determined gravimetrically by weighing and drying the sample in an oven at 105 °C for 24 h and the ashes of 0.5 g of dry seaweed after incineration in a muffle furnace at 600 °C for 24 h. Total nitrogen was determined by the Dumas combustion method using a total carbon and nitrogen analyzer (TruSpec CN" LECO. St. Joseph. MI. USA). Total protein was calculated by multiplying the N% by a recommended conversion factor 4.92 instead of 6.25 (Lourenço et al., 2002).

Lipid extraction was conducted using the Folch method (Folch et al., 1957). For this purpose, 0.2 g of dry seaweed was weighed in a Pyrex tube, and 3 mL of chloroform: methanol (2:1) solution was added and then shaken with a vortex. Then, 4 mL of an aqueous 0.73% NaCl solution was added over the extracted solution to separate both phases. The organic phase was removed and filtered in a glass fibre with sodium sulfate to eliminate all solids and water traces. This operation was repeated twice. Nitrogen flow finally evaporated the solvent, and the fat percentage was gravimetrically determined. Total carbohydrates were computed as the difference between the sum of the other components (moisture, ash, protein and fat) and the total weight (Tibbetts et al., 2016).

Additionally, the concentration of soluble carbohydrates (SCH) was analyzed as an average of three successive extractions of 0.1 g of dried seaweeds with 10 mL distilled water in an ultrasound bath for 2 h at 70 °C. The three extracts were combined and mixed up to 50 mL with distilled water. Before analysis, all extracts were diluted 1:5 with the extractant solution (Mandalka et al., 2022). The value of SCH was calculated using the UV-sulfuric acid method (López-Legarda et al., 2017). Briefly, 0.6 mL of carbohydrate solution was mixed with 2 mL of concentrated sulfuric acid. The mix was cooled in an ice bath for 2 min, and the absorbance was measured at 315 nm. A standard glucose curve from 20 to 70 ppm was employed for quantification.

2.7. Amino acids composition

Ten milliliters of a 6 M hydrochloric acid solution was added to 100 mg of the dried sample in a closed vial and left in an oven at 110 °C for 24 h in a nitrogen atmosphere. The resultant hydroxylates were filtered and diluted with Milli-Q water to 50 mL. One milliliter of the hydroxylate was dried using a nitrogen flow and heating the tube at 40 °C. The dried samples were re-dissolved in 1 mL of a 20 mM hydrochloric acid solution and filtered through a 0.22 μm membrane (Jiménez-Prada et al., 2018). The amino acids were determined in an Agilent 1290 Infinity UHPLC System coupled to an Agilent 6490 triple quadrupole mass spectrometer (Waldron, Germany). Chromatographic conditions were adapted from an Agilent application (Pi et al., 2008). The column was an Agilent ZORBAX SB-C18 Rapid Resolution HB column, 2.1 × 50 mm, 1.8 μm. For the separation of the amino acids, a gradient of two mobile phases was employed at 25 °C and a mobile phase of 0.4 mL/min. Mobile phase A consisted of 0.05% formic acid and 0.03% HFBA in water, and mobile phase B was 0.05% formic acid and 0.03% HFBA in acetonitrile. The gradient employed was at 0 min 100% A, at 2.5 min 100% A, at 5.5 min 60% A, 60% A at 6 min and then back to 100% at 9 min. The sample injected was 1 μL. Detection was done in the Agilent jet stream ion source in positive ionization mode, as described in a previous study (Florencio-Ortiz et al., 2018). Details of the detection method are given in Supplemental Table 1.

2.8. Mineral composition

A microwave-assisted digestion method was used for the decomposition of the macroalgae. A sample of approx. 0.5 g of dry seaweed was placed in the PTFE digestion vessel with 6 mL of concentrated nitric acid (Suprapur Merck, Darmstadt, Germany) and 2 mL of H_2O_2. Digestions were done in a microwave digester Milestone Ethos (Sorisole, Italy) with an internal temperature sensor using the application note HPR-AG-02 of Milestones applications (Milestone Ethos One, 2011). The microwave digestion was performed at 200 °C and 45 bar. After cooling, acid digests were mixed up to 20 mL with Milli-Q water. The solutions were then analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using an Agilent Technologies 7700 series (Agilent Technologies, Palo Alto, CA). The instrumental conditions were a sample flow rate of 0.4 mL/min, a nebulizer gas flow rate of 0.7 L/min, and 15 L/min for the outer plasma gas. The intermediate plasma gas flow rate of 1.0 L/min, RF power was 1.6 KW, and the He flow rate of the collision cell gas was 4.3 mL/min. Depending on the atomic mass of the analyte signals of Ge (for masses up to 88), Rh (for masses between 95 and 137) or Re (for masses greater than 137) were used as an internal standard to correct for signal fluctuations. Multi-elemental standards were prepared from the SPCMS33 solution for all analytes but P and I. For the P determination, a stock solution from Agilent was used to prepare the standards; for I, a stock solution was prepared from potassium iodide. Besides, Hg

determination was conducted using a Direct Hg analyzer (Milestone DMA 80, Sorisole, BG, Italy).

2.9. Antibiotics determination

For the extraction of antibiotics, an adaptation of a previous method was employed (Chico et al., 2008). In brief, 0.5 g of DW seaweeds, 5 mL of MeOH: H_2O (70:30), 0.1 mL of an EDTA 0.1 M solution were mixed in a vortex for 30 s, left under mechanical shaking for 15 min, and then centrifuged at 5000 rpm for 5 min. Before the analysis, the organic phase was removed and filtered through a 0.22 μm syringe filter. Separation of analytes was performed on an Eclipse Plus C18 2.1 mm × 100 mm, 1.8 μm at 25 °C. The mobile phase consisted of solvent A (water + 0.1% Formic acid) and solvent B (Acetonitrile + 0.1% Formic acid) using the following gradient: 0 min, 50% A; 2 min, 30% A; 3 min, 25% A; 5 min, 0% A; 6.5 min, 50% A; at a constant flow rate of 0.45 mL/min. The injection volume was 1 μL.

Separation and detection of target analytes were done using a UHPLC 1290 Infinity LC System (Agilent Technologies, Palo Alto, CA), coupled to a triple quadrupole mass spectrometer (6490, Agilent 6490, Santa Clara, CA, USA). Supplemental Table 2 shows the specific MRM transitions and the optimized source parameters.

2.10. Fatty acid determination

The fatty acid profile was performed using a slightly modified method (Ichihara & Fukubayashi, 2010) by performing a mild methanolysis. 0.2 mL of toluene, 1.5 mL of methanol and 0.3 mL of 8% HCl solution were added to 10 mg of extracted fat (section 2.6). The solution was vortexed and kept at 45 °C in an oven for 16 h. After cooling at room temperature, 2 ml of hexane and 1 ml of water were added and vortexed. The hexane was extracted and analyzed using an Agilent 7890N gas chromatograph coupled to a 5977B mass spectrometer (Agilent Technologies, Palo Alto, CA). Mixtures of fatty acids standards at different concentrations were provided by (37 component FAME Mix Supelco, Sigma Aldrich, Spain).

The column selected for separating the methylated fatty acids was a BPX70 column (60 m × 0.25 mm × 0.25 μm). The helium flow rate was fixed at 1 mL/min, and samples were injected using a 1:10 split ratio. Ion source and GC–MS transfer line temperatures were 250 °C and 280 °C, respectively. FAMEs were identified using a National Institute of Standards and Technology (NIST) MS library matches. Quantitation was performed via the integration of the total ion current chromatogram. (Juan-Polo et al., 2022).

2.11. Statistical processing of data

All results were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was applied to test statistically significant differences among the means employing the program IBM SPSS statistics 28.0 (SPSS, IBM, Armonk, NY, USA). Significant differences between the means were tested using the Tukey-b post hoc test at a significant level of 0.05.

3. Results and discussion

3.1. Chemical composition

Macronutrient composition is essential to evaluate the quality of a food ingredient. The average moisture content was significantly different between all the samples, but moisture percentages encountered were in the same range of values as other data published before (Cavaco et al., 2021; Rodrigues et al., 2015; Tabarsa et al., 2012).

The protein percentage significantly differed among Ulva species (Cavaco et al., 2021; Pereira, 2011; Rodrigues et al., 2015). *Ulva* sp.1 presented a significantly lower protein content than its land-grown

counterpart, and *U. ohnoi* showed an even higher protein content (Fig. 1). However, the seaweed with the highest protein concentration was *Gelidium* sp. with 17,2% of the dry weight. Protein content depends on the nitrogen load of cultivation medium, reaching up to 40% with high N loads (Bruhn et al., 2011). This fact explains the wide range of values encountered in literature, even among seaweed from the same species. It may explain the differences in protein composition between *Ulva* sp. grown on land and in the sea (Tabarsa et al., 2012).

Concerning ash content (Fig. 1), *Ulva* sp. 1 and 2 did not show significant differences and had higher ash content than *U. ohnoi* and the red algae, which had a similar ash content. The ash content of the *Ulva* sp. was similar to other *Ulvae* washed in seawater characterized in the USA (Gadberry et al., 2018) and Indonesia (Poeloengasih et al., 2019) and was higher than in other works (Dominguez & Loret, 2019; Pappou et al., 2022). This fluctuation can be partly explained by the mineral load of the water used and the influence of the variety and season of cultivation. A previous study concluded that rinsing macroalgae with tap water reduced ash percentage from 35% to 13.6% (Poeloengasih et al., 2019). In the present study, the macroalgae were rinsed with tap water before drying, but it was not washed with abundant water, which can explain the high ash content.

The SCH content distinguished *G. gracilis* from the other samples, reaching more than twice the SCH content of Ulva samples. On the other hand, *Gelidium* sp. showed a higher non-soluble carbohydrates (NSCH) content than any other sample, and only a slight difference was found between land- and sea-grown *Ulva* sp. Carbohydrate content seems to be more influenced by the species of seaweed than the cultivation practice. The values obtained agree with bibliographic data (Ferreira et al., 2021; Rasyid et al., 2019; Rodrigues et al., 2015; Tabarsa et al., 2012).

Finally, the total fat% was statistically higher in the *Ulva* spp. than in the red macroalgae, but no significant difference was found between land- and sea-grown *Ulva* sp. This data are in accordance with previous studies (Farghl et al., 2021; Ferreira et al., 2021; Gadberry et al., 2018; Mandalka et al., 2022; Peña-Rodríguez et al., 2011).

3.2. Mineral composition

Inorganic elemental composition is an important variable from a nutritional point of view as some elements are essential for humans, and others may pose health risks to consumers if above recommended levels. It is known that macroalgae are rich in essential elements; in this study, the most abundant were Mg, K, Ca and Na, as previously reported (Roleda et al., 2021; Romarís-Hortas et al., 2010), followed by P and Fe. Na and K content in *Ulva* spp. were higher than other values (Table 1). This was explained by Queirós et al., who found that K and Na content in Ulva was higher for aquacultured than wild algae. Land-grown *Ulva* sp. nutritional contents significantly differed from those produced in the

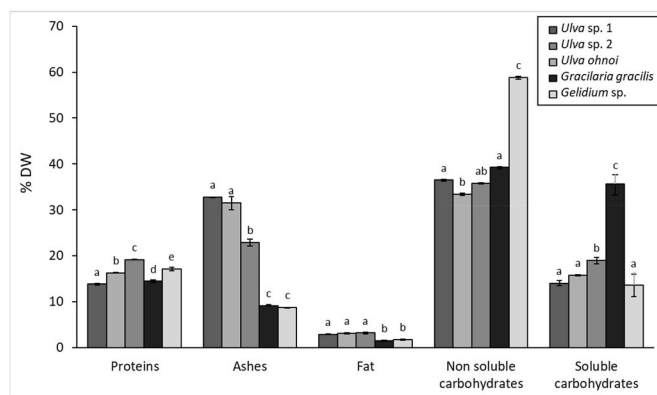
Fig. 1. Macronutrient mean values and the standard deviations for the five types of dry macroalgae. Different letters over each nutrient bar indicate statistically significant differences among the samples for each nutrient (n = 9).

Table 1

The average content of major inorganic elements in the macroalgae (mg 100/g DW) and comparison of values with some bibliographic data. The values given are the mean and SD (n = 9). Each mean macroalgae consisted of three samples collected in the same place (tank) in May 2022, repeated three times. 2 indicate land-based sample cultivation in Alicante, and 1 shows sea-based cultivation in the Mediterranean Sea near North Africa. Values in a column followed by a different letter are significantly different according to the Tukey b test at P < 0.05.

Sample Name	Na			Mg			K			Ca			Fe			P		
	Mean	SD	Biblio.[a]	Mean	SD	Biblio.[a]	Mean	SD	Biblio.[a]	Mean	SD	Biblio.[a]	Mean	SD	Biblio.[a]	Mean	SD	Biblio.[a]
Ulva sp. 1	1969b	147	364–4700	2343b	168	1590–3992	939c	69	467–3460	2393a	196	240–1830	519a	36	6.8–386	253b	8	128–181
Ulva ohnoi 2	2079b	134		3842a	249		2195b	161		689c	55		56c	3		300a	24	
Ulva sp. 2	3716a	74		3904a	81		3240a	73		568c	12		51c	1		210c	4	
G. gracilis 1	700c	35	3120	367c	18	175–431	1030c	50	5850–6510	536c	29	344–1290	333b	13	3–105	137d	4	177–235
Gelidium sp.1	632c	12	433	615c	12	127	860c	7	1238	1046b	7	74	62.9c	0.4		264b	4	

[a] Ranges of values obtained from the literature are also shown in the table (Bonanno & Orlando-Bonaca, 2018; Laramore et al., 2022; Paiva et al., 2016; Queirós et al., 2021; Rodrigues et al., 2015).

sea, with higher Mg and K contents but lower Ca and Fe contents. A lower Ca content could be due to the absence of invertebrates and calcareous particles typical in non-treated seawater (Roleda et al., 2021). Additionally, the red algae presented significantly lower Mg and Na content than *Ulva* spp. All values of Na, K, Ca, and Fe were comprised in or near the published data ranges(Table 1) (Bonanno & Orlando-Bonaca, 2018; Cavaco et al., 2021; Poeloengasih et al., 2019; Rey-Crespo et al., 2014; Rodrigues et al., 2015).

The ratio Na/K has nutritional importance due to its impact on human blood pressure. A ratio near one or lower is nutritionally favourable (Peña-Rodríguez et al., 2011). In the case of the *Ulva* sp. cultivated on land, the Na/K relation was near one, and in the *Ulva* sp. developed in the sea, this ratio was near 2. For the red algae, this ratio was lower (0.7). The Na/K ratio highlights the possibility of obtaining healthier macroalgae in land-based aquaculture as some elemental content can be modified.

Concerning macroalgae trace elements, *Ulva* sp. *1* presented the highest Al content, higher than bibliographic data (Monteiro, Sloth & Abstractsk, 2019) (Table 2). *Ulva* spp. grown on land showed lower Al content, which was not significantly different to *Gelidium* sp., while *G. gracilis* presented an intermediate value. A recent review revealed that more information on Al content in macroalgae is needed due to its possible toxicological effect (Bonanno & Orlando-Bonaca, 2018). Land-based aquaculture could contribute to producing macroalgae with lower Al content and, thus, safer food (Gadberry et al., 2018). Ga, Mn, Cu, Zn and Sr content were also higher in *Ulva* sp. 1 than the other *Ulva* spp. cultivated on land. Although the red algae samples were grown the same way as *Ulva* sp. 1, they had lower levels of these minerals but were in line with previously reported values (Bonanno & Orlando-Bonaca, 2018; Laramore et al., 2022; Paiva et al., 2016; Queirós et al., 2021; Rodrigues et al., 2015).

Ultra-trace elements (Table 3) Sn, Ba, V, Cr, Co, Mo, Sn, Li and Ni in Ulva spp. cultivated on land were significantly lower than in *Ulva* sp.1 grown in the sea. On the contrary, the macroalgae that grew on land presented significantly higher contents in Sb and Ag. Additionally, sea-grown algae showed higher content in V and Se. It is known that the type of aquaculture influences the mineral composition of macroalgae, as well as the species, among other variables (Cavaco et al., 2021; Moreda-Piñeiro et al., 2011).

There is still no regulation in the EU about the tolerable limits of macroalgae ingestion linked to the presence of heavy metals. However, the European Regulation 1881/2006 for authorized supplements established the maximum levels of toxic contaminants in macroalgae-based supplements at 3.0 mg/kg for Cd and Pb and 0.1 mg/kg for Hg (Commission Regulation, 2006)). In the case of Cd and Hg, the content found in all the samples was under the regulation-established limits (Table 4). However, the health risk for Pb consumption could not be considered negligible, mainly for *Ulva* sp. 1 and *G. gracilis*, which presented 7.8 and 19.2 mg/kg DW, respectively. *Ulva* spp. grown on land presented significantly lower content in Hg, As, Pb and I than sea-based *Ulva* sp. This exciting observation confirms the interest in land cultivation by controlling the crop medium, temperature, and microbiota (Califano et al., 2020; Roleda et al., 2021) for increased productivity and possible improvement in the nutritional value and health risk. Values obtained for As, Hg, and I were lower or similar to the average value obtained in the report by AESAN for *Ulva* sp. (Ministerio de Consumo, & Agencia Española de Seguridad Alimentaria y Nutrición, 2019) and by Monteiro et al., 2019, but Pb and Cd were higher. Pb content in *Ulva* was similar to another study in Morocco in which *Ulva lactuca* was found to be a good accumulator of Cd and Pb (Green et al., 2023).

Taking into consideration the mineral nutrient reference values (NRV) given in the (European Council, 2011) regulation **1169/2011** concerning food labelling, the mineral NRV limits provided by an algae portion (Regulation (EU), 2011) were computed (Fig. 2). The macroalgae portion considered, 10.4 g, was the average consumption of macroalgae by Spanish consumers (Ministerio de Consumo, & Agencia

Table 2

The average content of traces of inorganic elements (mg 100 g^{-1} DW) and comparison of values with some bibliographic data. The values given are the mean and SD (n = 9). Each mean macroalgae consisted of three samples collected in the same place (tank) in May 2022, repeated three times. 2 indicates land-based sample cultivation in Alicante and 1 indicates sea-based cultivation in the Mediterranean Sea near North Africa. Values in a column followed by a different letter are significantly different according to the Tukey b test at P < 0.05.

Sample Name	B Mean	B SD	B Biblio.[a]	Ga Mean	Ga SD	Ga Biblio.[a]	Mn Mean	Mn SD	Mn Biblio.[a]	Cu Mean	Cu SD	Cu Biblio.[a]	Zn Mean	Zn SD	Zn Biblio.[a]	Sr Mean	Sr SD	Sr Biblio.[a]	Al Mean	Al SD	Al Biblio.[a]
Ulva sp. 1	10.4c	0.8	21.6–30	12.4a	0.7	n	10.0a	0.7	0.5–11.4	2.0c	0.1	0.3–3.0	2.9b	0.2	0.8–16.6	23.6a	1.8	6.5	490a	27	0.53–33.1
Ulva ohnoi 2	19.4b	1.4		1.5c	0.2		3.5b	0.2		1.72b	0.09		2.2c	0.1		7.8b	0.5		55c	8	0.17–450
Ulva sp. 2	7.3d	0.2		1.24c	0.03	n	1.79b	0.03		1.74b	0.03		2.17c	0.03		6.3b	0.1		45.5c	0.4	
G. gracilis 1	22.3a	0.9	33–53.7	4.1b	0.1	50	7.3a	4	2–104	5.2a	0.2	0.2–1.4	3.3b	0.1	2.5–10	4.8c	0.2	n	164b	5	1.9–19.6
Gelidium sp.1	22.4a	0.6	n	1.7c	0.01		6.96a	0.06	n	1.06c	0.03	n	9.1a	0.2	n	6.40b	0.05	n	67.5c	0.5	n

n: No bibliography values have been found.

[a] Range of values obtained from the literature is also shown in the table (Bonanno & Orlando-Bonaca, 2018; Laramore et al., 2022; Paiva et al., 2016; Queirós et al., 2021; Rodrigues et al., 2015).

Table 3

The average content of ultratraces of inorganic elements (mg 100 g^{-1} DW) and comparison of values with some bibliographic data. The values given are the mean and SD (n = 9). Each mean macroalgae consisted of three samples collected in the same place (tank) in May 2022, repeated three times. 2 indicates land-based sample cultivation in Alicante and 1 indicates sea-based cultivation in the Mediterranean Sea near North Africa. Values in a column followed by a different letter are significantly different according to the Tukey b test at P < 0.05.

Sample Name	Sn Mean	Sn SD	Sn Biblio.[a]	Ba Mean	Ba SD	Ba Biblio.[a]	V Mean	V SD	V Biblio.[a]	Cr Mean	Cr SD	Cr Biblio.[a]	Co Mean	Co SD	Co Biblio.[a]
Ulva sp. 1	731b	95	n	4920a	213	n	1394b	85	444–1570	2813a	159	54–1700	200b	13	12–658
U. ohnoi 2	168c	25		680c	82		196d	13		244d	18		33c	2	
Ulva sp. 2	251c	17		332d	6		89.5e	0.8		300d	8		38.1c	0.9	
G. gracilis1	1077a	76	4080–18,100	1439b	26		1501a	56	817–3809	470c	8	17–568	959a	47	3–267
Gelidium sp.1	68d	18	n	410d	3	n	432c	3	a	731b	8	a	62c	1	a

Sample Name	Sb Mean	Sb SD	Sb Biblio.[a]	Ni Mean	Ni SD	Ni Biblio.[a]	Se Mean	Se SD	Se Biblio.[a]	Mo Mean	Mo SD	Mo Biblio.[a]	Li Mean	Li SD	Li Biblio.[a]	Ag Mean	Ag SD	Ag Biblio.[a]
Ulva sp. 1	6.1c	0.4	3–184	856a	58	n	59a	6	14.9–483	121b	6	21.6–30	371a	21	50–1380	18d	1	12–658
U. ohnoi 2	183b	14		247d	14		14.5cd	0.7		17d	1		71c	10		265b	11	
Ulva sp. 2	242a	16		326c	5		9d	1		23.8d	0.2		92bc	2		493a	2	
G. gracilis1	8.2c	0.8	n	275d	5		27.7b	0.7	6.41–852	274a	9	33–53.7	114b	3	110–1481	8.1d	0.4	3–267
Gelidium sp.1	3.0c	0.8	n	489b	7	50	18.4c	0.9	n	47.5c	0.6	n	77c	1	n	43.6c	0.9	a

n: No bibliography values have been found.

[a] Range of values obtained from the literature are also shown in the table (Bonanno & Orlando-Bonaca, 2018; Cabrita et al., 2016; Q. Chen et al., 2018; Chico et al., 2008; Filippini et al., 2021; Paiva et al., 2016; Rodrigues et al., 2015).

Table 4

The average content of As, Cd, Pb, Hg and I (mg/kg DW), comparison of values with some bibliographic data and retention factor obtained after boiling the macroalgae. The values given are the mean and SD (n = 9). Each mean macroalgae consisted of three samples collected in the same place, (tank) in May 2022, repeated three times. 2 indicates land-based sample cultivation in Alicante and 1 indicates sea-based cultivation in the Mediterranean Sea near North Africa. Values in a column followed by a different letter are significantly different according to the Tukey b test at P < 0.05.

	As mg/kg	SD	Cd mg/kg	SD	I mg/kg	SD	Pb mg/kg	SD.	Hg mg/kg	SD
Ulva sp. *1*	3.90c	0.28	0.18b	0.02	34.4c	12.3	7.83b	0.37	0.00639a	0.00003
U. ohnoi 2	0.59d	0.03	0.41a	0.03	6.12e	0.91	1.53d	0.18	0.00083c	0.00002
Ulva sp. *2*	0.24e	0.01	0.19b	0.01	27.32d	4.43	1.01d	0.01	0.00082c	0.00001
G. gracilis1	12.8a	0.6	0.12c	0.01	254b	26.8	19.24a	0.66	0.00308b	0.00004
Gelidium sp.*1*	3.70c	0.07	0.50a	0.01	623a	114	2.11c	0.66	0.00284b	0.00006
Ulva spp. *(AESAN, 2019)*[a]	4.49		0.09		64.77		0.58	0.02	0.01	
Bibliographic data[b]	21.4–56.3		0.0168–0.059		17.2–20.8		2.7–4.8[c]		0.005–0.008	
Retention factor %[c]	71.5%	3.5	70,8%	4.8			79,1%	4.3		

[a] *Ulva* spp. average values obtained in the study done by AESAN on metals and iodine content (AESAN, 2019) are shown for easy comparison with the values of this study.

[b] Range of values obtained in the literature (Green et al., 2023; Monteiro et al., 2019).

[c] Retention factor % is the term obtained from the element content after cooking concerning the element content present in the raw macroalgae.

Española de Seguridad Alimentaria y Nutrición, 2019), which is higher than 5 g, the value used in the EFSA report (Monteiro M., Sloth et al., 2019). Zn, Na, K, Ca, P and Se were present in most samples in amounts lower than 15% of NRV limits per portion. However, Cu and Mo were higher in sea-grown algae than in land-based crops. Mg, Fe, Mn, Cr and I were present in significant amounts in all seaweeds. Macroalgae have the greatest iodine level of any vegetable (Moreda-Pieiro et al., 2011); hence extreme caution should be exercised when consuming I to avoid health problems caused by a rise in thyroid volume. (Ministerio de Consumo & Agencia Española de Seguridad Alimentaria y Nutrición (AESAN), 2019). In this study, *Ulva* sp. 1 and mainly *U. ohnoi* have shown lower iodine NRV % than other algae, *G. gracilis* and *Gelidium* sp. In this sense, it seems that the macroalgae genera has more impact on the iodine content than the style of production (Moreda-Piñeiro et al., 2011). Moreover, *U. ohnoi*, shows a lower NRV% for I (Fig. 2), indicating that an adequate selection of algae genera can be interesting for human consumption. However, after inspection of the data, the iodine is the element that should limit the macroalgae ingestion per day. In this sense, land-based aquaculture could contribute to the reduction of iodine content, as well as other variables such as the species of *Ulva*, temperature (Queirós et al., 2021), cultivation medium (Sebök & Hanelt, 2023; Zemah-Shamir et al., 2021) and microbiota (Ghaderiardakani et al., 2019; Polikovsky et al., 2020; Wichard, 2023). Additionally, the retention factor% (heavy metal content in cooked seaweed *100/heavy metal content in raw seaweed) was determined by boiling 5 g of the dried U. ohnoi for 10 min in 200 mL of tap water. Significant losses of Cd, As and Pb in the thermal procedure were observed (Table 4). This preliminary result highlights that culinary techniques could reduce the excessive content of some elements by leaching (Nielsen et al., 2020).

3.3. Amino acids content

The protein quality depends on the proportion of amino acids; for that reason, the amino acid profile was determined after the acid hydrolysis. Fig. 3A compares the FAO/WHO reference essential amino acids (EAA) requirements for adults (WHO/FAO/UNU, 2007) with the EAA macroalgae content. The limiting amino acids were Met + Cys and His in all the samples, as the other EAA were present in higher proportions than the requirements, in agreement with previous studies (Siddique et al., 2013; Tabarsa et al., 2012). No significant differences were found among the macroalgae except with *U. ohnoi* content of Ile, Lys and Phe + Cys at p < 0.05.

The most significant non-essential amino acids (NEAA) were Ala, Glu, Gly, Asp, and Ser, as shown in a previous study (Rasyid et al., 2019) (Fig. 3B). No significant differences in the macroalgae average were found for Gly, Ser, Asn and Hpro; however, *Ulva* sp. grown in the sea

significantly differed from the one produced on the land for Ala. Moreover, *U. ohnoi* significantly differed from *Ulva* sp. in Pro and Gln. Additionally, NEAA content was higher than EAA, being Leu the most abundant EAA; and Asp, Ala, Gln, and Gly NEAA as observed previously (Roleda et al., 2021; Tibbetts et al., 2015). However, Gly contents were slightly higher in our study and similar to Tabarsa et al. data (Tabarsa et al., 2012). The same variability in the proportion of some amino acids, such as Tyr and His, Phe, Thr, Val and Ser have been related to the symbiosis of some Ulva spp. with specific beneficial bacteria (Califano et al., 2020). In this sense, better control of macroalgae EAA content requires a better understanding of the interaction with certain selected bacteria, which should be done in future studies (Ghaderiardakani et al., 2019; Polikovsky et al., 2020; Wichard, 2023).

3.4. Fatty acid profile

It was found that the fatty acid profile was significantly more saturated for the macroalgae cultivated in the sea (p < 0.05), with *G. gracilis* having the highest percentage of saturated fatty acids (SFA) (Table 5), meanwhile the *Ulva* spp. grown on land presented the lowest SFA. As previous work stated, the main SFA were C16:0, followed by C18:0 (Roleda et al., 2021). Regarding monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA)%, *G. gracilis* showed a lower proportion, whereas *Ulva* sp. 1 and *Gelidium* sp. presented intermediate ratios. PUFA % in both *Ulva* grown on land was significantly higher than in the other samples, mainly due to some omega 3 (C18:3n3 & C16:4n) and omega 6 (C18:2n6c) fatty acids (Peña-Rodríguez et al., 2011). The samples of this study were all collected in the same month, so the season and temperature are not discernible factors. The macroalgae cultivated on land were supplied with some nutrients, so the higher PUFA content of *Ulva ohnoi* and *Ulva* sp. 2 could be related to the N supplements in the water. Moreover, some health-related indices (Table 5) were calculated, such as n6/n3, ∑PUFA/∑SFA, Atherogenic Index (AI) and Hypocholesterolemia/hypercholesterolemia index (h/H) (Chen & Liu, 2020). In all cases, the land-cropped *Ulva* spp. presented the most convenient values from a cardiovascular perspective Chen & Liu, 2020).

3.5. Antibiotics residuals

Many antibiotics are frequently used in aquaculture systems, such as flumequine, oxytetracycline, chlortetracycline, and sulfadiazine (Burridge et al., 2010; Yipel et al., 2017). As seaweeds are usually used in the bioremediation of polluted seawater and wastewater, these antibiotics' possible accumulation or transformation must be controlled, especially when macroalgae application is for human or animal food. No residues of the targeted antibiotics were detected in any samples independently of their origin (land- or sea-based systems). Similarly, in a recent study
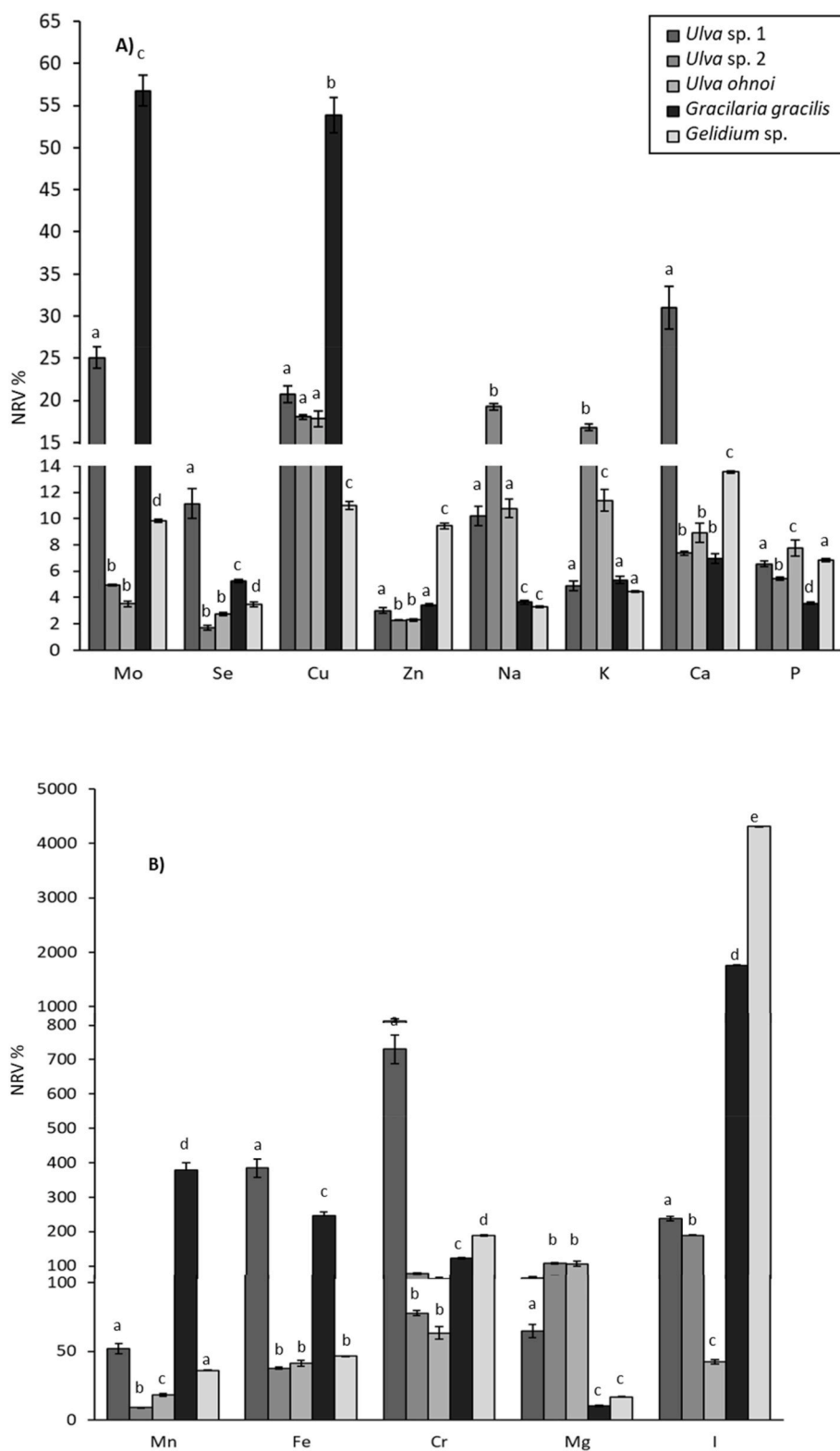
Fig. 2. The percentage of minerals provided by an algae portion of 10.4 g respect the reference values given by EU regulation **1169/2011** concerning food labelling (Regulation (EU), 2011). Different letters over bars indicate significant differences among the five macroalgae for each component with Tukey's tests ($p < 0.05$). Values over 15% of nutrient daily reference values are present in substantial amounts in the algae portion.

in which toxicity and removal of micropollutants in *Ulva* crops was measured where all variables were controlled, no quantifiable amount of antibiotics appeared in *Ulva* sp. (Hardegen et al., 2023). The authors concluded that antibiotics do not contaminate *Ulva* and other algae. In this sense, both studies are consistent, and *Ulva* and its associated bacteria do not assimilate antibiotics such as oxytetracycline from the culture medium.
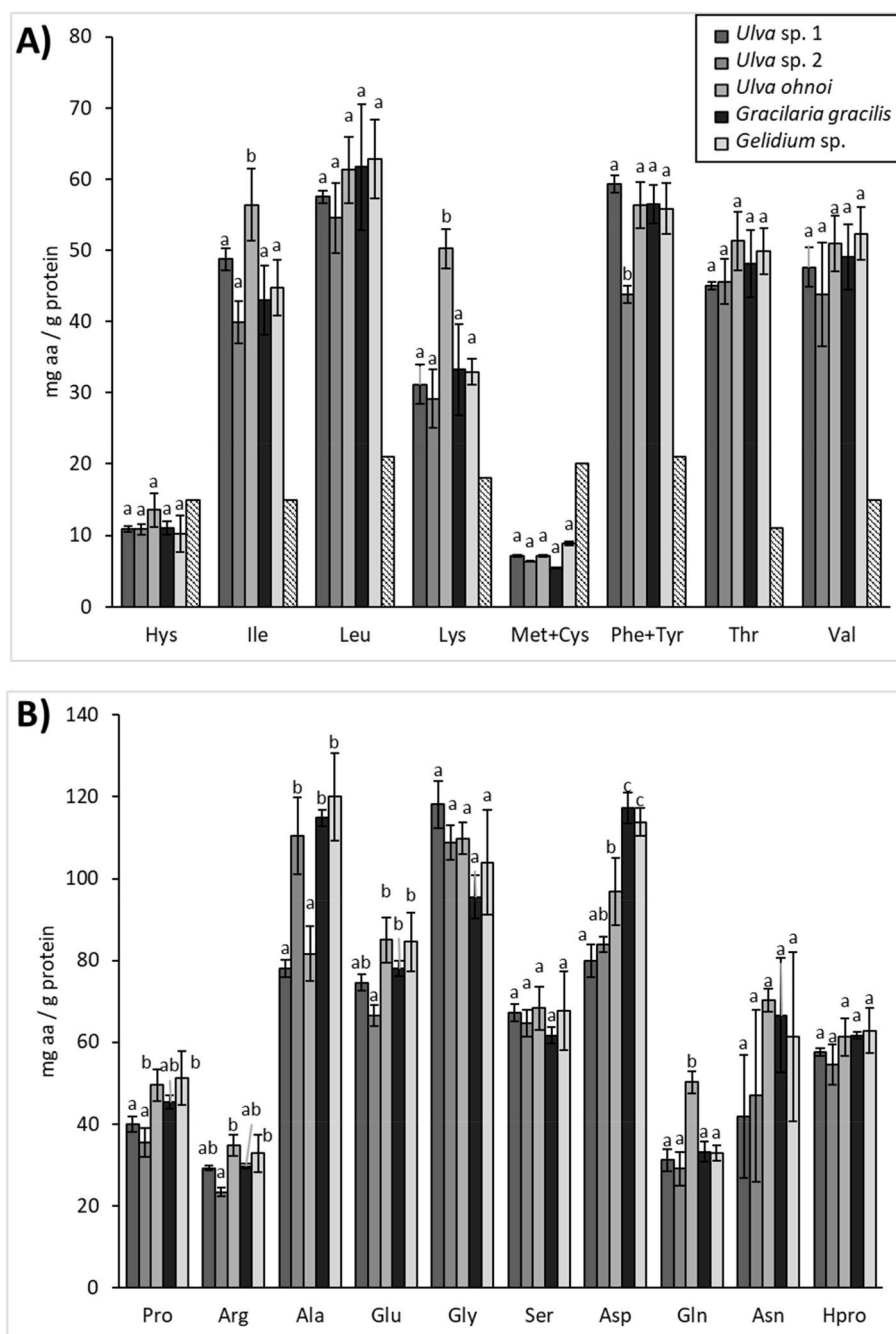
Fig. 3. Comparison content among the five types of macroalgae in mg of amino acids per gram of total protein (A) the essential amino acids average values respect the FAO/WHO daily reference requirement for adults (B) the non-essential amino acids average values. Different letters over bars for each amino acid represent significant differences among the five macroalgae with Tukey's tests (p < 0.05). The error bars are the standard deviation.

3.6. Antioxidant capacity and total polyphenols content

Antioxidant capacity is a crucial nutritional property of macroalgae. A second study compared vortex with ultrasound-assisted extraction of antioxidants (Fig. 4A). It was verified that no significant differences (P < 0.05) were found between the extraction techniques for any of the macroalgae and assay employed (FRAP, ABTS, DPPH, and TPC), so it was decided to choose non-ultrasound-assisted extraction for ulterior extracts with a preservation time in the freezer of no longer than 21 days.

Among the green macroalgae, according to TPC, the *Ulva* spp. grown on land showed significantly higher content than *Ulva* sp. 1. In contrast, for FRAP, ABTS and DPPH, *U. ohnoi* showed higher significant values

than the two other *Ulva* sp. samples, independently of the system in which they were cultivated (Fig. 4B). For every seaweed, the ABTS values were much higher than FRAP or DPPH values. This behaviour could be attributed to the different active compounds measured with the diverse antioxidant methods. Concerning the red algae, *Gelicium* sp. and *G. Gracielis* presented lower FRAP and ABTS antioxidant capacity than *Ulva* spp. At the same time, the DPPH and TPC values were not significantly different than the *Ulva* sp. cultivated in the sea. Comparison with bibliographic data was impossible, as the extractants and the extraction procedure differed.

Table 5
Comparison of the main fatty acid percentages (n = 9), % saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) for the five dry macroalgea. Additionally, some health-related indices are shown such as n6/n3, ∑PUFA/∑SFA, Atherogenic Index (AI) and Hypocholesterolaemia/hypercholesterolemia index (h/H) (Chen & Liu, 2020). The values given are the mean and SD (n = 9). Each mean macroalgae consisted of three samples collected in the same place, (tank) in May 2022, repeated three times. 2 indicates land-based sample cultivation in Alicante and 1 indicates sea-based cultivation in the Mediterranean Sea near North Africa. Values in the same raw followed by a different letter are significantly different according to the Tukey b test at P < 0.05

	Ulva sp. 1		Ulva ohnoi		*Ulva* sp.2		G. gracilis 1		*Gelidium* sp. 1	
	%	SD	%	SD	%	SD	%	SD	%	SD
C14:0	2.44	0.05	1.6	0.1	1.4	0.5	7.4	0.9	6.5	0.4
C15:0	0.8	0.1	0.59	0.04	0.55	0.05	0.45	0.03	0.47	0.03
C16:0	47.5	0.4	31.7	1.6	35.4	1.8	59.7	5.2	45.7	3.2
C17:0	0.6	0.1	0.8	0.0	1.0	0.1	0.6	0.1	0.34	0.02
C18:0	14.6	0.6	8.6	1.0	9.8	0.8	20.9	1.4	13.7	1.1
C20:0	0.37	0.00	0.22	0.02	0.26	0.03	0.23	0.02	0.19	0.01
∑SFA %	**66.3a**		**43.5c**		**48.4c**		**89.2b**		**66.9a**	
C16:1n7	5.2	0.1	5.0	0.4	2.0	0.1	2.0	0.2	1.5	0.1
C17:1n7	0.9	0.1	1.8	0.1	0.82	0.05	<LOD		<LOD	
C18:1n9t	0.21	0.00	0.3	0.0	0.3	0.0	1.8	2.6	0.3	0.0
C18:1n9c	14.5	0.7	17.4	1.3	10.9	0.6	3.8	1.5	14.0	0.6
C20:1n9	0.28	0.04	0.22	0.01	<LOD	0.00	<LOD		0.44	0.02
C22:1n9	<LOD		0.28	0.02	0.44	0.04	1.0	0.1	0.16	0.02
C24:1n9	<LOD		<LOD		0.34	0.02	0.2	0.1	5.2	0.2
∑MUFA %	**21.03a**		**25.01a**		**14.68b**		**8.75c**		**21.67a**	
C16:2n4	0.7	0.1	0.9	0.1	1.1	0.1	0.6	0.1	0.39	0.03
C16:3n3	0.28	0.01	0.64	0.03	1.04	0.06	0.44	0.09	0.26	0.04
C16:4n3	1.29	0.04	5.4	0.5	6.4	0.4	<LOD		<LOD	
C18:2n6c	2.4	0.1	4.4	0.3	7.2	0.4	1.2	0.1	1.05	0.04
C18:3n6	0.16	0.01	0.31	0.03	0.96	0.06	0.27	0.02	0.09	0.01
C18:3n3 ALA	3.0	0.1	8.4	0.7	10.4	0.6	0.32	0.03	0.18	0.01
C18:4n3	4.1	0.1	9.1	0.7	8.1	0.5	<LOD		<LOD	
C20:3n6	<LOD		0.13	0.01	0.31	0.03	<LOD		0.13	0.02
C20:4n6	0.21	0.01	0.14	0.00	0.35	0.02	0.23	0.06	5.23	0.19
C20:4n3	0.27	0.02	0.7	0.1	0.4	0.1	0.12	0.01	0.17	0.03
C20:5n3 EPA	0.18	0.03	0.13	0.01	0.27	0.03	<LOD		4.31	0.19
C22:5n3 DPA	0.39	0.02	1.8	0.2	1.2	0.1	<LOD		<LOD	
∑PUFA %	**13.00b**		**32.00a**		**37.62a**		**3.23c**		**11.80b**	
n3	9.5		26.1		27.7		0.9		4.92	
n6	2.6		4.6		7.8		1.4		6.41	
n6/n3	0.3		0.2		0.3		1.6		1.30	
∑PUFA/∑SFA*	0.2		0.7		0.8		0.0		0.18	
IA[a]	1.7		0.7		0.8		7.5		2.14	
HH[b]	0.6		1.5		1.3		0.1		0.50	

[a] IA= ([C12:0 + (4 × C14:0) + C16:0]/∑MUFA.

[b] HH= ((*cis*-C18:1 + ∑PUFA)/(C12:0 + C14:0 C16:0).

4. Conclusions

When comparing both types of aquacultures, *Ulva* spp. cultivated on land presented significantly higher content in total protein, Mg, K, Sb and Ag compared to *Ulva* sp. of nearshore aquaculture; the concentration of the rest of the elements were lower except for Na and Cd, for which no differences were found. Interestingly, Ulva sp. grown in land-based aquaculture systems presented significantly lower levels of As, Pb, Al and Hg and SFA, but higher levels of PUFA% and TPC, implying better food safety and health benefits. Another finding was that *U. ohnoi* showed compositional differences compared to the *Ulva* sp. cultivated under the same conditions. This result confirms the hypothesis that controlling the cultivation variables could help maintain the seaweed chemical composition in safe values. Specifically, it differs in having higher protein, Ile, Lys and Gln concentration and antioxidant capacity, but a lower iodine content, having a slightly better nutritional profile than *Ulva* sp. In addition, *Ulva* spp. showed a higher protein and fat content and a lower iodine content than the red algae tested, regardless of the type of cultivation, making them more suitable for nutritional and cosmetic purposes.

It is therefore possible to reduce the risk components and produce safer and healthier macroalgae than those grown at sea. Appropriate selection of algae cultivars and microbiomes in the cultivation systems could also positively influence the nutritional composition of the algae. However, the application of macroalgae as new food has still some uncertainties in relation with their safety that aims the authors of the present document to continue researching in this field. New studies should be carried out with higher number of samples grown under different growing conditions to find the ones with the lower content in toxic substances.

Authors contribution statement

Soledad Prats Moya and Salvador Maestre Pérez: Conceptualization; Data curation; Formal analysis; Funding acquisition; Project administration; Supervision; Resources. **Soledad Prats Moya**: Writing - original draft **Tiphaine Benoist and Victor Arcos Limiñana**: Investigation; Methodology; Software; Validation; Visualization; **Silvia Anton Sempere**: Methodology. **Soledad Prats Moya, Salvador Maestre Pérez, Tiphaine Benoist, Victor Arcos Limiñana, Silvia Antón Sempere**: Writing - review & editing.

Declaration of competing interest

The authors confirm that they have no conflicts of interest in the work described in this manuscript.
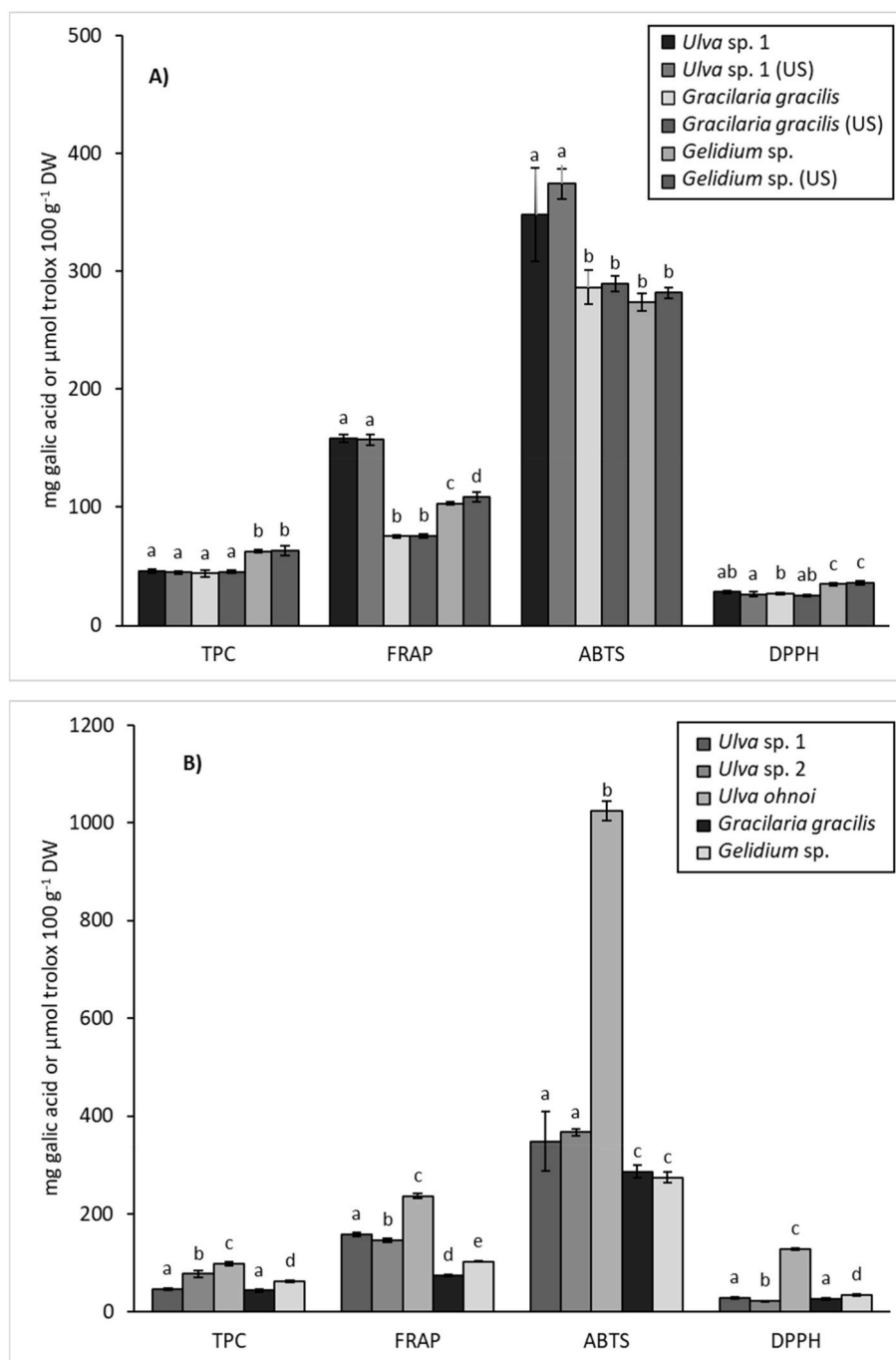
Fig. 4. Total phenol content, DPPH, ABTS, and FRAP antioxidant capacity average values (n = 9), (A) comparison of different antioxidant extraction modes, vortex assisted and ultrasound-assisted with ethanol: water (7:3), and (B) compositional comparison for the five macroalgae samples. Different letters over bars for each antioxidant capacity method represent significant differences among the macroalgae samples with Tukey's tests (p < 0.05).

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2023.102902.

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