POLITÉCNICO DE LEIRIA | ESCOLA SUPERIOR DE TURISMO E TECNOLOGIA DO MAR

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Master's degree in Marine Resources Biotechnology

Comparative study of biological activities of macroalgae after decoction and infusion

Miguel Forte Caetano

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Comparative study of biological activities of macroalgae after decoction and infusion

Miguel Forte Caetano

Dissertation submitted to obtain the master's degree in Marine Resources Biotechnology

Masters dissertation conducted under the supervision of Doctor Maria Joaquina da Cunha Pinheiro and Doctor Rui Manuel Maneta Ganhão

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Publications

The present dissertation for obtaining the master's degree in Marine Resources Biotechnology at ESTM-Politécnico de Leiria resulted in scientific works, subjected to peer review, and presented as oral communication at the following International Conference:

(1) Caetano, M., Gomes, M., Pinheiro, J., Ganhão, R. (2023). Infusion of macroalgae: a promise methodology for obtaining a healthy food ingredient. *Oral communication* at International Conference on Water Energy Food and Sustainability - ICoWEFS 2023, 10-12 may, Leiria, Portugal.

(2) Caetano, M., Gomes, M., Ganhão, R., Pinheiro, J. (2023). Effects of decoction treatment on bioactivities of seaweed. Oral communication at the S4Agro International Congress 2023, 2-3 march, Castelo Branco, Portugal.

Resumo

Nas últimas décadas, as macroalgas têm vindo a despertar o interesse na comunidade científica devido à sua riqueza em termos nutricionais bem como ao seu elevado teor em compostos bioativos com potencial de aplicação na área alimentar.

O presente trabalho teve como objetivo avaliar o efeito do processo de infusão e decocção em duas espécies de macroalgas, verde e castanha, *Ulva* sp. e *Fucus vesiculosus*, respetivamente. Para tal, foram testadas duas proporções de macroalga e água (1:16 e 1:50 g/mL), e tempos de tratamento de 5 e 15 minutos no caso da infusão, e de 15 e 30 minutos na decocção. Posteriormente, atributos de qualidade como o teor de sólidos solúveis (TSS, % ºBrix), o pH e a cor (CIE L*a*b*), bem como o teor de fenólicos totais (TPC) e a capacidade antioxidante expressa por diferentes metodologias como captura do radical livre DPPH e capacidade de redução do Fe (III) foram avaliados nos extratos sem tratamento (CTR) e após infusão (INF) e decocção (DEC).

A qualidade dos extratos das macroalgas verde e castanha manteve-se em termos maioritários de forma similar nos atributos avaliados. No entanto, a decocção aplicada na proporção de macroalga *Fucus vesiculosus* e água de 1:50 (g/mL) durante 30 minutos conduziu a alterações significativas de cor, nomeadamente no parâmetro de cor b*, que reflete a coloração amarela.

De uma forma geral, também se verificou que a decocção conduziu a uma maior extração de compostos fenólicos, bem como ao aumento da capacidade antioxidante, quando comparado com os resultados obtidos nas respetivas amostras controlo. Neste sentido, conclui-se que processos como a infusão e a decocção permitem a extração de compostos de interesse presentes nas macroalgas estudadas e, em particular, na *Fucus vesiculosus*. Este fato, combinado com a composição nutricional das macroalgas, potenciam a sua inclusão quer na sua forma natural quer como ingrediente natural a ser adicionado a formulações de alimentos da Dieta Mediterrânea.

Palavras-chave: Antioxidantes, Decocção, Infusão, Indústria alimentar, Macroalgas

Abstract

In the last decades, macroalgae have been attracting a great interest in the scientific community, due to their richness in nutritional terms and their high content of bioactive compounds with potential applications in the food sector.

The aim of this study was to evaluate the effect of the infusion and decoction processes on two species of macroalgae, green and brown, *Ulva* sp. and *Fucus vesiculosus*, respectively. For this purpose, two proportions of macroalgae and water were tested (1:16 and 1:50, g/mL), with treatment times of 5 and 15 minutes for the infusion and 15 and 30 minutes for the decoction. After these processes, the quality attributes, such as soluble solids content (TSS, % ºBrix), pH and color (CIE L*a*b*), as well as the total phenolic content (TPC) and antioxidant capacity expressed by different methodologies such as DPPH free radical scavenging activity and Fe (III) reduction capacity, were evaluated in the extracts without treatment (CTR) and after infusion (INF) and decoction (DEC).

The quality of the green and brown macroalgae extracts remained similar in most of the attributes evaluated. However, the decoction applied in the ratio of macroalgae *Fucus vesiculosus*:water of 1:50 (g/mL) for 30 minutes led to significant color changes, particularly in the color parameter b*, which reflects yellow coloration.

In general, it was also found that the decoction led to a greater extraction of phenolic compounds and higher antioxidant capacity when compared to the results obtained with the respective control samples. In this sense, it can be concluded that processes such as infusion and decoction allow the extraction of compounds of interest present in the macroalgae studied, in particular in *Fucus vesiculosus*. This fact, combined with the nutritional composition of macroalgae, makes them ideal for inclusion both in their natural form and as a natural ingredient to be added to food formulations for the Mediterranean Diet.

Keywords: Antioxidants, Decoction, Infusion, Food Industry, Macroalgae

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List of abbreviations

(m:w)_macroalgae:water

DEC_ Decoction

DPPH_1,1-Diphenyl-2-picrylhydrazyl free radical

FRAP_ Ferric ion reducing antioxidant power

INF_ Infusion

ND_ No data

SSC_ Soluble Solids Content

TPC_ Total phenolic content

1 Introduction

1.1 Food Industry

The world population is growing exponentially so the current food production system will not be able to provide food for a growing population that is expected to exceed 10 billion in the coming decades. Intensive agriculture, as well as reduced access to fresh water, has led to the search for and development of new and sustainable food sources.

In recent years there has been an increase in demand for new foods with sustainable development to try to cope with population growth and reduce the effect of climate change. Sustainable food development aims to promote the sustainability of food production, food security and the improvement of the diet and well-being of the entire population (Leandro et al., 2020). Nowadays, consumers are increasingly looking for foods of natural origin that may bring health benefits and improve their quality of life. In response to this issue, the food industry has been looking at (and currently using) a wide range of natural compounds that can bring nutritional improvements that benefit human health. Some examples of these compounds are carotenoids, fatty acids, polyphenols, antioxidants. Aside from bringing nutritional improvements and health benefits, some of these compounds can effectively increase the shelf life of food products helping in their preservation, as is the case of antioxidants (Pereira et al., 2021).

The ocean contains several marine organisms such as algae, mollusks, sponges, among many others. Regarding marine resources, their exploitation and commercialization in the food industry has been growing because many of these organisms contain bioactive compounds with beneficial properties such as antioxidant, anti-inflammatory and anti-aging effects. Despite the various studies on the beneficial properties and the existence of products based on marine resources, they continue to be little investigated and exploited in view of all its untapped capacity (Leandro et al., 2020).

1.2 The importance of macroalgae

The worldwide exploration of the oceans is rapidly growing, and seaweeds are one of the growing resources in the market. Macroalgae are an easily accessible resource, have great potential, and their exploration does not entail high costs (Pereira et al., 2021).

Algae arouse great interest in the scientific community today because they contain numerous relevant compounds, like polyphenols and dietary fibers, with potential biological activity (Sanz-Pintos et al., 2017).

For thousands of years, algae have been used for various activities, like cooking and agriculture. In recent decades, macroalgae have been a viable resource in food supplements and animal feed production. In Asia, several species of macroalgae, such as *Undaria* (Wakame), *Laminaria* (Kombu), and *Porphyra* (Nori), are used for human consumption. Increased demand and practice of healthy eating have led to a growth in scientific research on the nutritional value of seaweed and its application in food products. In European and North American countries, the use of macroalgae for human nutrition is growing, which has resulted in an expanding market in the last decades (Bayomy, 2022).

Macroalgae are very rich in vitamins, fibers, minerals, and proteins (Soares et al., 2021). These characteristics make algae's use in food very appealing and help to promote food products with added qualities, of natural origin, for the functioning of the human organism and helping to improve human health. The replacement of synthetic chemical components macroalgae in food for human consumption has become a factor of great importance, both in terms of the planet's sustainability, given the exponential increase in the world's population, and of the increased pollution seen in recent decades (Leandro et al., 2020).

1.3 Macroalgae

Macroalgae are organisms of macroscopic size and can easily be found in coastal areas at low tide. These present a diversity of colors, shapes and sizes and can be classified as follows: *Rhodophyceae* (red algae), *Chlorophyceae* (green algae) and *Phaeophyceae* (brown algae) (Roleda et al., 2021).

1.3.1 Rhodophyceae

Red algae, so called because of their characteristic coloring, belong to the *phylum Rhodophyta*. There are about 10,000 species of red algae, most of which are marine species. Geographically, they grow in tropical and temperate climate zones (Seo et al., 2010).

It is estimated that humans have been consuming red algae for at least 2800 years. The most cultivated red algae are *Porphyra*, *Eucheuma*, *Kappapycus*, and *Gracilaria*. Red algae are composed mainly of small amounts of polysaccharides, inorganic matter, and small amounts of proteins and lipids. They are relevant producers of bioactive compounds with antiviral, antimicrobial and anti-tumor properties, usable by cosmetics, pharmaceuticals, and food industries. However, there is still a lot of agronomic and biotechnological potential to be explored and researched (Aziz et al., 2021).

1.3.2 Chlorophyceae

Green algae are a large and important group of seaweed species belonging to the *Phylum Chlorophyta*. This group contains a great diversity in terms of species and morphology, with about 500 genders and 15,000 species. With a fundamental ecological role, they absorb a large amount of carbon and release oxygen (Leliaert, 2019).

Among green algae species, there is a diversity and alternation in characteristics such as nutrient absorbance, sunlight uptake, and geographical distribution. Green algae are geographically distributed all over the globe, from colder areas such as the poles to tropical zones close to the Equator line. The diversity within this group allows them to adapt and survive in different environments.

Green algae have chlorophyll, are rich in proteins, mineral salts, and vitamins, and have antioxidant and antiviral properties. The interest of many studies in these compounds has caused their growth and increasing appreciation in the market (Bayomy, 2022).

1.3.2.1 *Ulva* sp.

Ulva sp., a species of green algae (Figure 1) also known as sea lettuce, can be found on rocky coastlines anywhere in the world. This species is one of the most present in human food nowadays (Roleda et al., 2021).

.

Figure 1- Specie of *Ulva* sp. (Source: Algaplus)

Ulva species have been on the market for many years all over the world. They are usually consumed as sea vegetables or used as biostimulants. In recent years, their commercial importance has been growing. Due to their accelerated reproduction process, these algae are very appealing to the aquaculture sector (Monteiro et al., 2022).

The *Ulva* sp. is an opportunistic and resilient species causing ecological disturbances, for example, the so-called green tides. The valorization of this species is of enormous importance for the sustainability of ecosystems (Dominguez & Loret, 2019).

1.3.3 Phaeophyceae

Brown algae, belonging to the *Phylum Phaeophyceae*, include more than 1500 species, all multicellular. Brown algae are distributed in various coastal regions, dominating preferentially in cold and temperate zones (Li et al., 2021).

An increase in the consumption of macroalgae has been observed over the last decades, with brown algae representing a significant majority of this consumption and having various applications.

The composition of brown macroalgae has been much studied due to its great nutritional value. Brown seaweed are especially rich in secondary metabolites with antioxidant, anti-inflammatory and antimicrobial properties. Polysaccharides, like alginates, are present in brown algae in high quantities (Francisco et al., 2020).

This macroalgae is also a source of polyunsaturated fatty acids (PUFAs), such as omega-3 and omega-6, essential to human nutrition. Despite containing lower amounts of lipids than many fish species, brown seaweed are a source of interest due to its high bioavailability (Francisco et al., 2018).

1.3.3.1 *Fucus vesiculosus*

Fucus vesiculosus, a species of brown algae (Figure 2), belongs to the genus *Fucus* which comprises 66 species with varying morphologies, which has been increasingly studied in

recent years. This group of comestible algae is found mainly in the northern hemisphere, in areas with a cold climate (Catarino et al., 2018).

Figure 2 - Specie of *Fucus vesiculosus.* (Source: Algaplus)

Brown algae belonging to the genus *Fucus* have been used in human nutrition for many years, mainly in Asia. This group of macroalgae has high fibre, vitamins, and mineral content and is low-fat, making them interesting on a nutritional level (Catarino et al., 2018).

1.4 Macroalgae on the Portuguese coast

The Portuguese coast has a total coastline of 830 km, consisting of 350 beaches. The Portuguese coast is relatively linear, but morphologically diverse containing extensive sandy and rocky areas, many of them rich in seaweed with huge cliffs. The Portuguese shore presents a thermal variation from the North to the South of the Portuguese coast, with the coast bathed by the Atlantic Ocean presenting colder waters, and the further South with more temperate waters bathed by the Mediterranean Sea (Pereira, n.d.).

The first studies of the Portuguese algal flora were published at the end of the 18th century, followed by several other studies on the algal flora. In the late 1960s, Ardré (1971) made an exhaustive study of the Portuguese algal flora having identified and described 246 species of Rhodophyceae, 98 Phaeophyceae, and 60 Chlorophyceae. The Portuguese coast shows a marked gradient in the distribution of the algal flora, with the flora in the north of the country like that found in central Europe, and the algal flora of the south of the country with an influence from the Mediterranean and the northern part of the West African coast. Another characteristic of the Portuguese coast is that it presents an increase in the number of red macroalgae and a decrease in the number of brown macroalgae from north to south (Pereira, n.d.).

Currently, there has not been a significant difference in the number of known algal species.

The invasion of species from other regions of the world has been another characteristic observed in the algal flora of the Portuguese coast (Pereira, n.d.). The appearance of invasive species is due to factors such as the increased maritime mobility of humans and climate change, among others. These invasive species upset the balance of ecosystems, thus altering their biodiversity. The use of these species in their application in products promotes the balance and sustainability of the ecosystems of the Portuguese coast.

1.5 Mediterranean diet

The Mediterranean diet has its origins in the countries bordering the Mediterranean Sea. The concept of this diet was originally conceived by Ancel Keys, in 1970 (Yannakoulia et al., 2015). Foods such as bread, wine, and olive oil were the staple foods of Roman and Greek ancient civilizations. Today, olive oil is the main fat used in the Mediterranean diet (Davis et al., 2015).

In this 25-year study investigating of the Mediterranean Diet the risk of developing coronary heart disease, it was concluded that there is a lower risk of the disease in

countries where this diet is practiced compared to Northern European countries and the United States of America (Dominguez et al., 2021).

The Mediterranean Diet is defined by a lifestyle based on diversity and a diet characterized by a high consumption of plant-based products. Using olive oil as the main fat, eating vegetables and fruits, and drinking plenty of water are some factors of a healthy diet with health benefits. The increase in the average life expectancy observed in recent times across the globe has consequently increased the risk of age-related diseases, such as cardiovascular and neurodegenerative diseases. Many scientific studies point to the benefits of the Mediterranean diet in improving health over time. Healthy eating promotes an improved quality of life and the prevention of associated diseases (Morris & Bhatnagar, 2016).

The preference for traditional products, respecting their seasonality, is an important feature of the diet. In fact, this promotes a healthier and more sustainable diet based on low-processed products. The demand for natural and low-processed products is increasing and thus becoming an emergent market. This diet is also characterized by low consumption of saturated fats and simple carbohydrates, which are harmful to health and are associated with an increased risk of diseases as atherosclerosis and diabetes (Urquiaga & Rigotti, n.d.).

1.6 Bioactive compounds in food

The incorporation and consumption of seaweed in a varied and healthy diet brings health benefits, resulting from the consumption of foods rich in fiber, vitamins, and bioactive compounds, associated with the prevention of diseases such as diabetes, cardiovascular diseases, and degenerative diseases. These properties are attributed to foods that have antioxidants such as carotenoids and phenolic compounds (Santos-Buelga et al., 2019). These compounds are directly implicated in the development, growth, or reproduction conditions to perform physiological functions. Nowadays, the interest in the cultivation and exploitation of macroalgae in the most varied forms has increased. The macroalgae

are already used in many countries for very different purposes, like extraction of compounds with antiviral, antibacterial, or antitumor activity (Leandro & Gonçalves, 2019).

Oxidative damage at the cellular level is a major factor in premature aging, as well as in the development of neurodegenerative, cancer, and cardiovascular diseases. Free radical scavenging is important for decreasing oxidative damage through bioactive compounds with antioxidant properties.

Phenolic compounds are secondary metabolites, and about 8,000 phenolic compounds with varying biological activities are known. The main sources of phenolic compounds in the human diet are plant foods. These compounds possess antioxidant properties with the ability to neutralize free radicals and inhibit the formation of reactive species. Phenolics also participate in the processes responsible for the color and aroma of various foods (Anantharaju et al., 2016).

1.7 Methods for antioxidant compounds detection

Antioxidant capacity assays may be broadly classified into two types: assays based on Hydrogen Atom Transfer reactions and assays based on Single Electron Transfer.

Hydrogen atom transfer (HAT) - based methods measure the classical ability of an antioxidant to quench free radicals by H-atom donation. Single Electron Transfer (SET) − based assays measure the capacity of an antioxidant in the reduction of an oxidant, whose color changes when reduced. The degree of color change (either an increase or decrease of absorbance at a given wavelength) is correlated to the concentration of antioxidants in the sample. SET assays include the ABTS/TEAC, CUPRAC, DPPH, Folin-Ciocalteu, and FRAP methods, each using different chromogenic redox reagents with different standard potentials (Moharram & Youssef, 2014).

1.8 Methods for antioxidant compounds extraction

Extraction is the scientific term used for the processes of separating active compounds from animal or plant tissues. For thousands of years, the active components present in medicinal plants have been extracted by simple extraction processes, such as infusion (Majekodunmi, 2015).

Recently, extraction processes have been used to extract bioactive compounds present in compounds, using simple techniques such as infusions, decoctions, and ultrasound. The extracted bioactive compounds have been studied for application in different areas, such as pharmaceuticals and food (Pandey & Tripathi, 2013).

The solvent to be used in the extraction process must be chosen depending on the type of ingredient and the nature of the bioactive compounds to be extracted. Normally, polar solvents such as water and ethanol are widely used in the extraction of polar compounds. In simple processes such as infusion and decoction, water is the most used solvent for extracting compounds (Abubakar & Haque, 2020).

The extraction process by decoction and infusion usually has a fixed ratio of 1:4 or 1:16, the ratio of seaweed:water. The volume is reduced to a quarter of its original volume by boiling (Handa S et al., 2008).

1.8.1 Infusion

Infusion is the process of simple extraction of bioactive compounds widely used in domestic life in the preparation of teas.

In the infusion process, using a solvent such as water, the solvent is heated to boiling point. When it reaches boiling, the biomass is added, left for a certain period, and then filtered.

In previous studies the process of infusion of plants took place under the following conditions 1:200 (m/v, g/mL) for 5 minutes (Martins N., 2014; Dias M.I., 2015). Other studied the conditions of process with herbal preparations are 1:30 (m/v, g/mL) at time 15 minutes (Fotakis et al., 2016).

1.8.2 Decoction

Decoction involves a continuous heat extraction, whereby the biomass and solvent are heated together. Once the boiling temperature is reached, the process continues for a set time, and is then filtered.

The process of decoction, like the infusion process, is commonly used in the extraction of compounds from medicinal plants. In literature the decoction process of aromatics, plants, according to the previous studies (Majekodunmi, S. O., 2015; Handa S., 2008), occurred according to the ratio 1:4 or 1:16 (m/v, g/mL). In other studies, the process of decoction of plants 1:200 (m/v, g/mL) for 5 minutes (Martins et al., 2014; Dias et al., 2015) and 1:30 (m/v, g/mL) during 15 minutes (Fotakis et al., 2016).

OBJECTIVES

The main objective of this study was to compare the effect of decoction and infusion, namely in their biological activities of green and brown macroalgae, *Ulva* sp. and *Fucus vesiculosus*, respectively. Also, evaluation of the effect of decoction and infusion process on the bioactive and antioxidant components of macroalgae, along with characterization of the aqueous extracts of algae with respect to the components of interest according to the type of algae under study, like antioxidants, phenolic compounds. The figure 3 shows a representative diagram of the work stages.

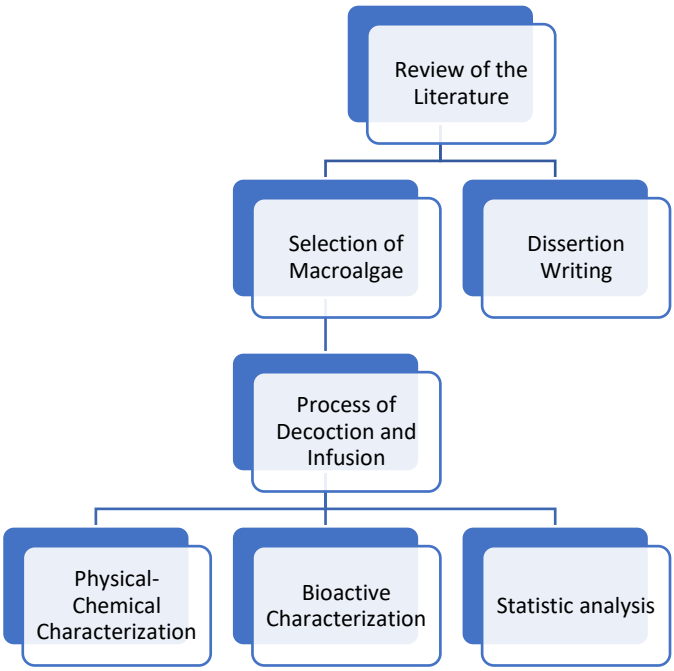

Figure 3- Representative scheme of the work steps.

2 Materials and methods

2.1 Materials

The dried macroalgae used in the present study, *Fucus vesiculosus* and *Ulva* sp. were acquired from the Portuguese company Algaplus (Ílhavo, Portugal). After packing at proper conditions were transported to the Research Laboratory of CETEMARES at Peniche (Portugal). Figure 4 shows the dried macroalgae *Fucus vesiculosus* and *Ulva* sp. used in the experimental procedure with different granulometry and nutritional value (see Table 1 in Appendix).

Figure 4 – Dried macroalgae before the process of infusion/decoction, *Fucus vesiculosus* (a) *and Ulva* sp, (b).

All chemicals and reagents used in the present work were AAS grade. The reagents used in all applied methodologies, were: Folin-Ciocalteu's phenol reagent (Merck, Germany); gallic acid - analytical reagent (BIOCHEM Chemopharma, France); sodium carbonate anhydrous (BIOCHEM Chemopharma, France); 1,1-diphenyl-2-picrylhydrazyl Free radical, DPPH (TCI, Japan); 2,4,6-Tris(2-pyridyl)-s-triazine (for spectrophotometric det. Fe > 99%, HPLC (Sigma-Aldrich, Switzerland); iron (III) chloride hexahydrate ISSO (Carlo Erba, France); sodium acetate 3-hydrate for analysis ACS, ISSO (Panreac Applichem, Germany); iron (II), sulfate 7-hydrate (Reag. USP), ACS, (Panreac Applichem, Germany); acetic acid (VWR, France); hydrochloric acid (ANALAR NORMAPUR, VWR, France).

2.2 Methods

In this study, the control samples are the extracts of macroalgae that have not undergone heat treatment. These samples were extracted at room temperature for 5 and 15 minutes for infusion treatment and 15 and 30 minutes for decoction treatment.

After the processes of infusion and decoction, the liquid phase was separated from the solid phase by filtration. The process of filtration took place through recourse a sifter.

2.2.1 Infusion treatment

In this study, the infusion process takes place over a defined period (5 and 15 minutes), after the initial water boiling moment. In previous studies, such in Abubakar (2020), the ratio of macroalgae and solvent were: 1:4 and 1:16 (m/v, g:mL) and the value of ratio worked in this work were 1:16 and 1:50 (m:v, g:mL). To evaluate the effect, *Fucus vesiculosus* and *Ulva* sp. extracts, were considered the samples of control without process of infusion at room temperature (CTR) (Figure 5 and Figure 6).

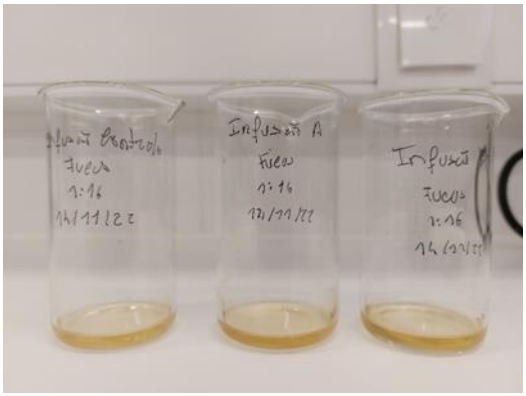

Figure 5 - Macroalgae *Fucus vesiculosus* after the infusion extraction process, with a ratio of 1:16 (g/mL) and time at 15 minutes.

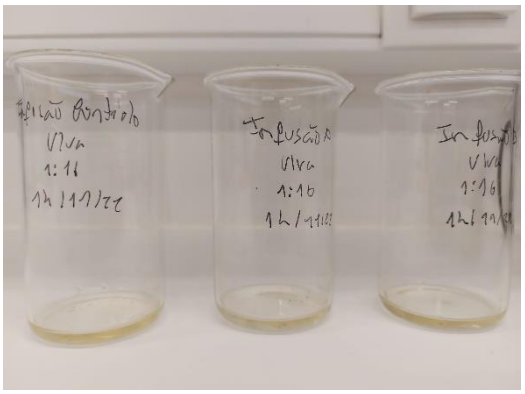

Figure 6 - Macroalgae *Ulva* sp. after the infusion extraction process, with a ratio of 1:16 (g/mL) and time at 15 minutes.

2.2.2 Decoction treatment

In the decoction process, the macroalgae is added to the water and then the heat process is applied. The process takes place over a defined period (in this study 15 and 30 minutes), after the initial water boiling moment. Although in the work developed by Abubakar (2020), the ratio of macroalgae to water is 1:4 or 1:16 (w/v, g/mL), in this experimental work, the ratios of 1:16 and 1:50 (w/v, g/mL) were used.

To evaluate the effect of the process of decoction in macroalgae, *Fucus vesiculosus* and *Ulva* sp., respectively, were considered: control without decoction at room temperature (CTR). Figures 7 represents the macroalgae *Fucus vesiculosus* and *Ulva* sp., respectively, after the decoction process of both experimental studies.

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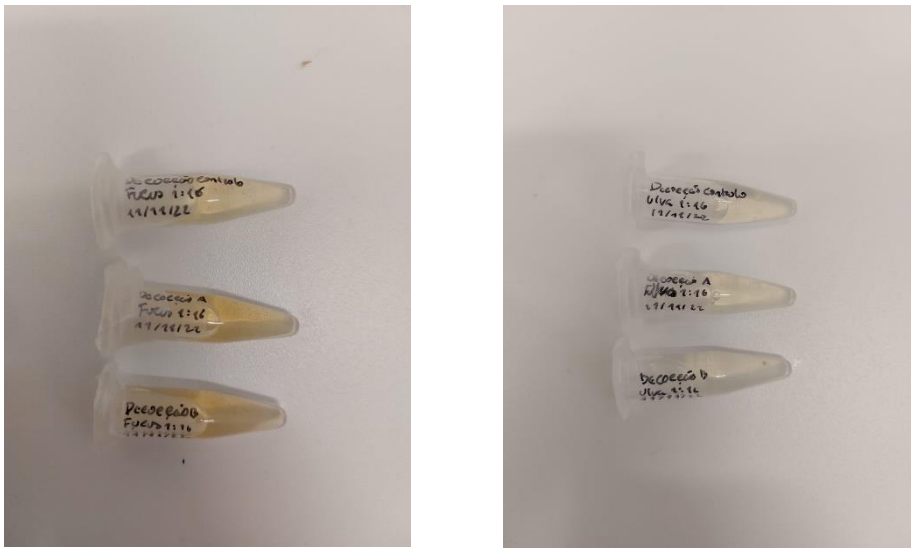


Figure 7 - Images of the macroalgae *Fucus vesiculosus* and *Ulva* sp, respectively, after the decoction extraction process, with a ratio of 1:16 (g/mL) and time at 15 minutes.

2.2.3 pH-value, soluble solids content (SSC) and colour CIELab

The pH value of the macroalgae extracts obtained after decoction and infusion treatment was determined using a pH meter (SP70P, SympHony, Radnor, PA, USA). After calibration according to the process recommended by the manufacturer, samples were measured at room temperature (21 ± 1 °C). The results were expressed as an average of three determinations of infusion/decoction samples.

The soluble solids content (SSC) of the samples was measured in a refractometer (BST, RFM340+, Kent, UK) and expressed in ºBrix. The results were expressed as an average of three determinations of infusion/decoction sample.

The color of the samples was evaluated by a tristimulus colorimeter (Minolta chroma Meter, CR-400, Osaka, Japan) after calibration of the apparatus with a standard blank, using illuminant D65 and observer 2º. The obtained color parameters L* a* b*, represent luminosity, green to red variation and blue to yellow variation, respectively. The instrument was calibrated using a standard white tile (L* = 97.10, a* = 0.19, b* = 1.95),

and the results of the color parameters (L*a*b*) were expressed as the average of three determinations per samples.

2.2.4 Determination of total phenolic content

The total phenolic content (TPC) of the samples was determined after the infusion and decoction process, which were then stored in a refrigerated environment. The TPC of the samples was determined by the Folin-Ciocalteau method (Singleton & Rossi, 1965) with some modifications as follows. Briefly, in a 96-well microplate, 20 µL of sample/standard was added followed by 100 µL of Folin-Ciocalteu (1/10; v/v). After 4 minutes of reaction, 80 µL of Na_2CO_3 (7.5%, w/v) was mixed. After 2 hours in the dark at room temperature, the reaction was performed and analysis of results was realized in a microplate reader (Bioteck, SynergyH1, Thermo-Scientific, Winooski, USA) at 750 nm. Gallic acid was used as standard for the curve of calibration line using the concentration between 0.05 and 0.25 mg. mL-1 ($y = 6{,}2845x + 0{,}0696$; $R^2 = 0{,}9948$). The results obtained were expressed as mg of gallic acid equivalents per 100 g of seaweed (mg GAE/100 g). The results were expressed as the average of three determinations per sample.

2.2.5 Determination of antioxidant capacity expressed by the DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was determined according to the modified method of Brand-Williams (1995). In a 96-well microplate, 50 µL of the sample was added to 150 µL of previously prepared DPPH solution with 80 % of methanol. The reaction was left to stand for 30 min in the dark at room temperature, and then the absorbance was measured at a wavelength of 517 nm in an Eppoch microplate reader (Bioteck, SynergyH1, Thermo-Scientific, Winooski, USA). Finally, the antioxidant capacity of the samples was calculated using the following equation:

$$Antioxidant\ capacity\ (RSA, \%) = \frac{Abs\ DPPH\ inicial - Abs\ sample}{Abs\ DPPH\ inicial} \times 100 \qquad \textbf{Equation (1)}$$

The percentage inhibition was expressed as the DPPH radical scavenging activity (% RSA). The results were expressed as the average of three determinations per sample.

2.2.6 Determination of antioxidant capacity expressed by the ferric ion reducing antioxidant power (FRAP)

The iron ion reduction method was performed according to Ganhão et al. (2019). The FRAP reagent was prepared with the mixture of 3 solutions: 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) making up with HCl, the ferric solution using $FeCl_3$ and an acetate buffer solution. 2.7 mL FRAP plus 90 µL of extract and 270 µL of water were added and reaction occurred during 30 minutes at 37 °C. Then the absorbance was measured at 593 nm and $FeSO_4$ was used as a standard. The results were expressed as the average of three determinations per sample.

2.2.7 Statistical analysis

The analysis of variance (ANOVA) of results was carried out using the Statistica™v.8.0 Software from Statsoft (StatSoft Inc., Tulsa, OK, USA, 2007). For each infusion and decoction treatment conditions studied three replicates were carried out.

The obtained results were considered statistically significant at a level of significance of 5% (P-value < 0.05), following the Tukey HSD (*Honestly Significant Difference*) test. Data were presented as mean ± standard deviation. To evaluate the differences for the mean values of pH, SSC (ºBrix) and color, either from the different methods (TPC, DPPH and FRAP), the analysis of Variance (ANOVA) was used. Whenever significant differences were detected, the respective multiple comparison tests were performed, namely the Tukey HSD test (Jerrold, 2009). As such, letters were used to represent the significant differences.

3 Results and Discussion

3.1 Impact of infusion on its physical properties

The effect of infusion on physical properties, as pH, soluble solids content (SCC) and color parameters (L*, a* and b*), of both macroalgae in study (*Ulva* sp. and *Fucus vesiculosus*), in the different treatments, as compared to the control samples (CTR), can be observed in Table 2.

The pH value of green macroalgae *Ulva* sp. ranged from 5.49 and 6.00, both obtained in extracts without treatment in a ratio of 1:16 and 1:50 (g/mL), respectively. Despite the similar value of pH after 5 min and 15 min of infusion, in each proportion of macroalgae and water studied, when the pH value was compared, a significant difference ($P < 0.05$, Tukey test) between them was registered. The same behavior was observed in the brown macroalgae, *Fucus vesiculosus*. The proportion of macroalgae and water of 1:50 (g/mL) allowed to obtain a slightly but significantly ($P < 0.05$, Tukey test) pH value (5.60 – 5.67) compared to observe in the 1:16 ratio (g/mL): 5.30 – 5.40. Mohammed et al. (2021) reported a similar pH value on brown macroalgae, 5.55 ± 0.03 and 6.29 ± 0.01, in *Himantalia elongata* (sea spaghetti) and *Alaria esculenta* (Irish wakame), respectively.

The soluble solids content (SSC) indicates the presence of water-soluble compounds as organic sugars such as glucose, sucrose and fructose among others (Liu et al., 2010). Comparing the SSC of *Ulva* sp. extracts without infusion (CTR) at ratio a 1:16 and 1:50 (g/mL), during 5 min, an identical value was obtained, 2.35 % ($P > 0.05$, Tukey test). By increasing the treatment time to 15 min at a proportion of macroalgae:water of 1:16 (g/mL) a significant ($P < 0.05$, Tukey test) increase of 60% was observed. The brown macroalgae *Fucus vesiculosus*, demonstrated the highest SSC in proportion of macroalgae:water of 1:16 (g/mL) with the increase of infusion, demonstrating the influence of heat and time on the extraction of the water-soluble compounds. On the other hand, the results obtained in the ratio of 1:50 (g/mL), in both studied macroalgae, showed

that the maximum extraction was achieved in the first minutes of infusion, and after a decrease of soluble solids was occurred.

The color of green macroalgae, *Ulva* sp., after the applied four infusion treatments, denotes a similar value ($P > 0.05$, Tukey test) of luminosity of samples. This colour parameters represents the clarity (higher value) and darkness (low value of L*) of macroalgae, and based on our results, the impact of heated water did not contribute to a perceptible change. However, the b* color parameter decreased to near zero when the macroalgae was subjected to infusion treatment during 15 min. This behavior reflects a yellowish color weak, as can be observed in Figure 8.

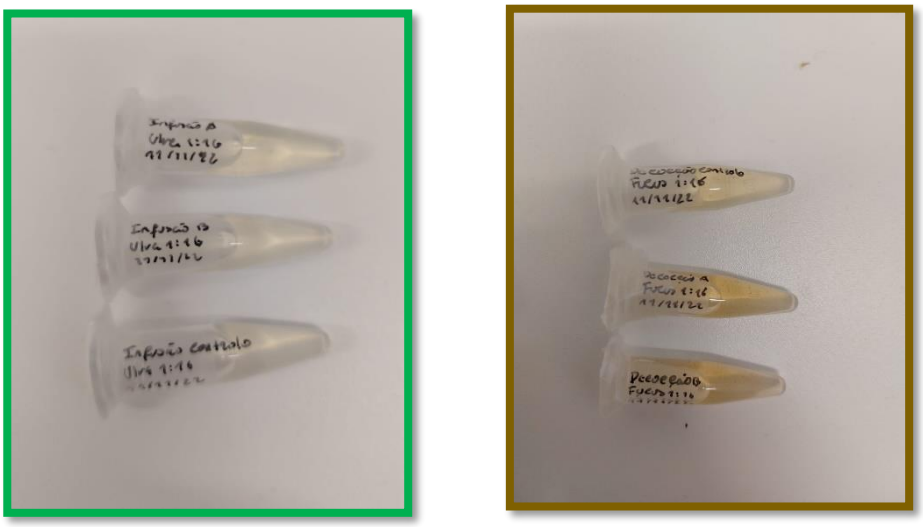


Figure 8 - *Ulva* sp. and *Fucus vesiculosus* (right and left, respectively), after the infusion treatment.

Regarding the color of the brown macroalgae *Fucus vesiculosus*, a similar value for the different parameters measured (L*, a*, b*) was detected, which shows that the proportion of macroalgae and water studied, as well as the treatment time, had no influence on this quality parameter.

Table 2 - Physical properties (pH, soluble solids content (SSC, % ºBrix) and color parameters) of macroalgae extracts, without (CTR) and after infusion (INF) at different treatment conditions (mean ± standard deviation).

	***Ulva* sp**							
	1:16 (m:w; g/mL)				**1:50 (m:w; g/mL)**			
	5 min		**15 min**		**5 min**		**15 min**	
Quality	**CTR**	**INF**	**CTR**	**INF**	**CTR**	**INF**	**CTR**	**INF**
pH	5.49 ± 0.03 a	5.53 ± 0.02 a	5.54 ± 0.01 a	5.6 ± 0.006 a	6.0 ± 0.02 b	5.95 ± 0.04 b	5.96 ± 0.02 b	5.9 ± 0.01 b
SSC (% ºBrix)	2.35 ± 0.02 a	2.08 ± 0.01 a	2.45 ± 0.02 a	3.92 ± 0,006 b	2.35 ± 0.03 a	2.39 ± 0.1 a	0.7 ± 0.01 c	0.72 ± 0.02 c
L*	31.4 ± 0.005 a	31.42 ± 0.06 a	31.68 ± 0.03 a	31.77 ± 0.04 a	31.56 ± 0.06 a	31.54 ± 0.06 a	31.64 ± 0.04 a	31.6 ± 0.06 a
a*	1.6 ± 0.006 a	1.6 ± 0.05 a	1.69 ± 0.01 a	1.71 ± 0.04 a	1.63 ± 0.03 a	1.6 ± 0.03 a	1.71 ± 0.03 a	1.49 ± 0.04 b
b*	1.64 ± 0.03 a	1.42 ± 0.03 a	0.37 ± 0.01 b	0.15 ± 0.06 c	0.7 ± 0.01 b	0.84 ± 0.03 b	0.5 ± 0.02 b	1.28 ± 0.02 a
	***Fucus vesiculosus***							
	1:16 (m:w; g/mL)				**1:50 (m:w; g/mL)**			
	5 min		**15 min**		**5 min**		**15 min**	
Quality	**CTR**	**INF**	**CTR**	**INF**	**CTR**	**INF**	**CTR**	**INF**
pH	5.32 ± 0.03 a	5.3 ± 0.03 a	5.4 ± 0.02 a	5.38 ± 0.006 a	5.6 ± 0.02 b	5.62 ± 0.006 b	5.65 ± 0.01 b	5.67 ± 0.02 b
SSC (ºBrix)	1.69 ± 0.01 a	2.1 ± 0.02 a	2.27 ± 0.02 a	2.75 ± 0.04 b	2.02 ± 0.03 a	3.0 ± 0.1 b	0.45 ± 0.02 c	0.57 ± 0.02 c
L*	31.1 ± 0.006 a	30.2 ± 0.23 b	31.61 ± 0.01 a	31.15 ± 0.02 a	31.49 ± 0.01 a	30.9 ± 0.03 c	31.07 ± 0.02 a	30.98 ± 0.03 c
a*	1.62 ± 0.06 a	1.62 ± 0.03 a	1.64 ± 0.03 a	1.51 ± 0.04 b	1.69 ± 0.02 a	1.64 ± 0.07 a	1.65 ± 0.05 a	1.64 ± 0.07 a
b*	2.03 ± 0.03 a	4.08 ± 0.05 b	1.24 ± 0.04 c	2.89 ± 0.06 a	0.85 ± 0.02 c	3.04 ± 0.01 a	1.62 ± 0.01 a	3.43 ± 0.03 b

Different letters in the same line indicate significant differences at P-value <0.05 (Tukey test). **Source** of macroalgae image: Henriques, B. (2021).

3.2 Impact of infusion on its bioactive composition

The effect of infusion on green and brown macroalgae, *Ulva* sp. and *Fucus vesiculosus*, performed at different conditions of treatment (time and proportion of macroalgae and water) on total phenolic content (TPC) can be observed in Figure 9.

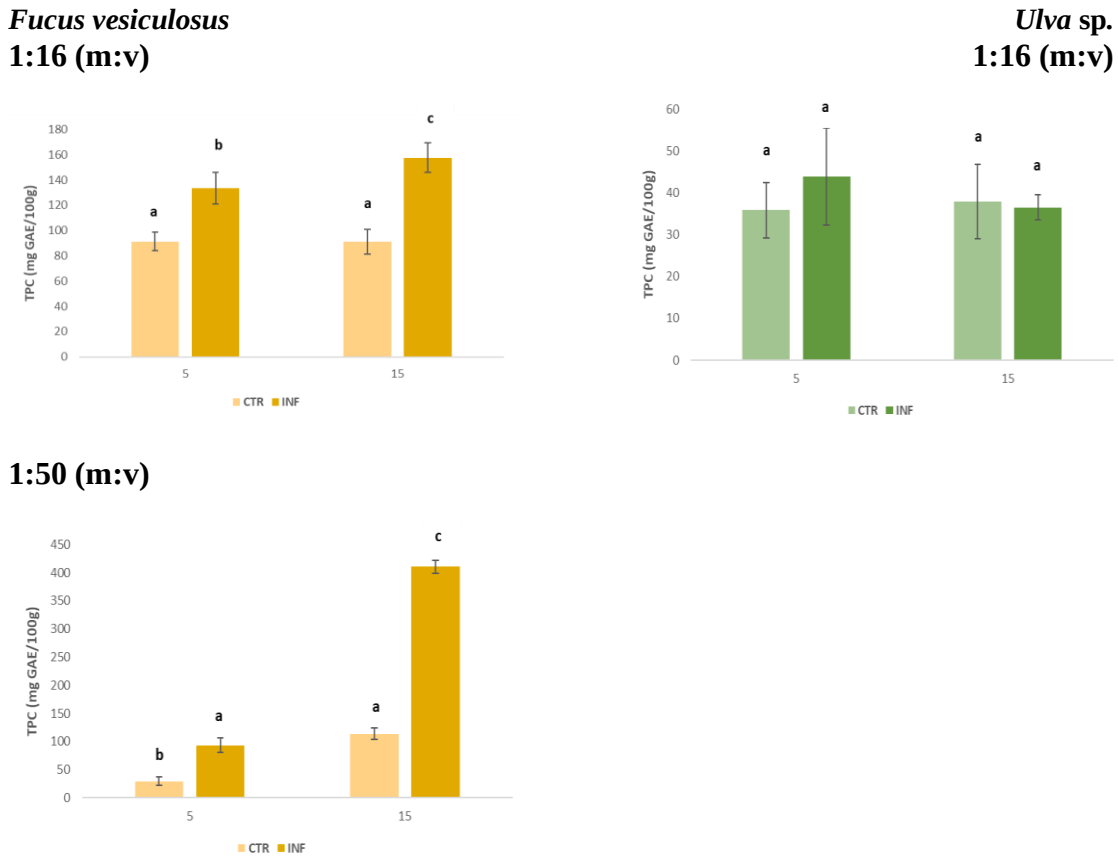


Figure 9 – Total phenolic content (TPC, mg GAE/100 g) of *Ulva* sp. and *Fucus vesiculosus* extracts without (CTR) and with infusion (INF). The vertical lines represent the standard deviation and different letters indicate significant differences at P-value <0.05 (Tukey test).

In the present study, the green macroalgae, *Ulva* sp. had an interesting content of phenolic compounds, 42.55 mg GAE/100 g. The impact of infusion on the ratio of macroalgae and water of 1:16 (g/mL) during 5 min and 15 min did not contribute to an effective extraction of these compounds, since no significant difference (P > 0.05, Tukey test) was obtained. Also, when the proportion of macroalgae and water increase to 1:50 (g/mL), no positive value was achieved (TPC < 0 mg GAE/100g). This fact could be due several factors such

as, the low content of these compounds in green macroalgae, the low extraction efficacy by using water as solvent, and the heat treatment in infusion (Pappou et al., 2022). On the other hand, the extracts obtained with the brown macroalgae, *Fucus vesiculosus*, revealed a significant ($P < 0.05$, Tukey test) increase of TPC after the infusion treatment compared to CTR samples (Figure 9). The *Fucus vesiculosus* extracts in a proportion of macroalgae and water of 1:50 (g/mL) denotes the highest phenolic content (410.55 mg GAE/100 g, $P < 0.05$, Tukey test). Comparing the two macroalgae extracts, the *Fucus vesiculosus* denotes a superior value of phenolic content than *Ulva sp*. This demonstrates the impact of macroalgae selection on the infusion success as treatment for extraction of interesting bioactive compounds such as phenolics. In another study, the phenolic content obtained from the macroalga *Fucus vesiculosus* after extraction with water at temperature of 90 ºC for 30 minutes and a ratio of 1:20 (g/mL), exhibited the lowest values, about ten and twenty times than obtained in the present study, during 15 minutes at both proportion of macroalgae and water studied, respectively (Neto et al., 2018). In another study, the green macroalgae *Ulva australis*, after being subjected to infusion for 5 minutes showed a phenolic content high compared to our study, 69.98 mg GAE/100 g and 38.09 mg GAE/100 g. This difference could be due to the different species of *Ulva* sp. and their geographical location (Trentin et al., 2020).

The antioxidant capacity expressed by the DPPH radical scavenging activity of *Ulva* sp. and *Fucus vesiculosus* extracts without and with infusion at different conditions can be observed in Figure 10.

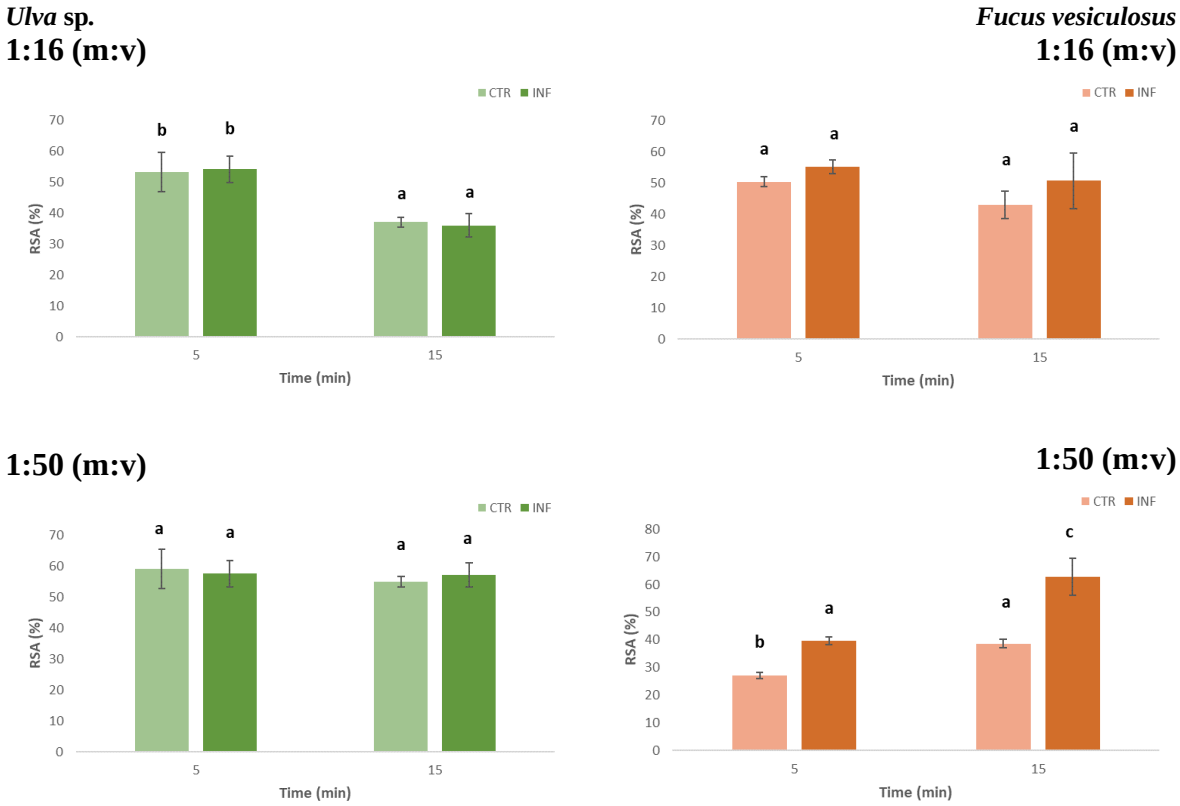


Figure 10 - Antioxidant capacity expressed by the DPPH radical scavenging activity (RSA, %) of *Ulva* sp. and *Fucus vesiculosus* extracts without (CTR) and with infusion (INF). The vertical lines represent the standard deviation and different letters indicate significant differences at P-value < 0.05 (Tukey test).

By observation of the antioxidant capacity of *Ulva* sp. extract, no significant difference ($P > 0.05$, Tukey test) were denoted between the treatment conditions as the macroalgae and water ratio and the infusion time. The highest value of DPPH radical scavenging capacity on *Ulva* sp. extracts was observed after 5 min of infusion ($P < 0.05$, Tukey test). This was higher than 50 %, which was not observed for the infusion time of 15 min with values lower than 50 %. This indicates that the antioxidant capacity expressed by the DPPH during the infusion time decreases.

The antioxidant capacity of *Ulva* sp. extracts without and with infusion was similar in the ratio of 1:50 (g/mL) ($P > 0.05$, Tukey test) ranging from 54 % to 58 %.

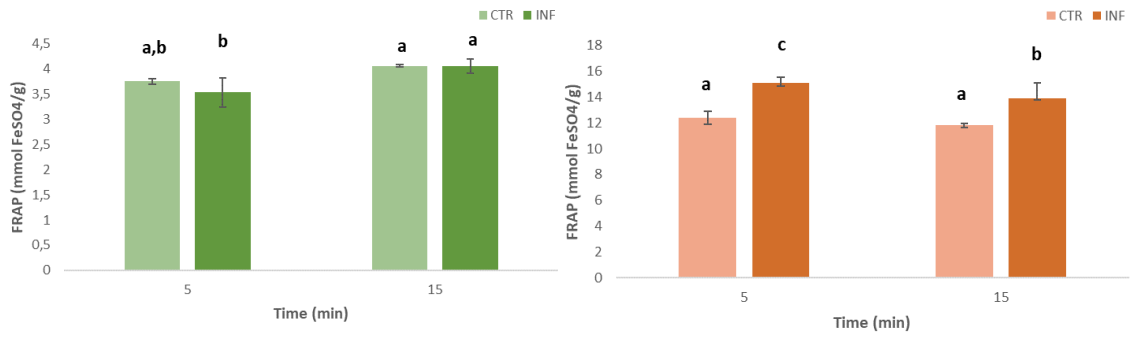
In the case of *Fucus vesiculosus* extracts the DPPH radical scavenging activity of more than 50 % in both treatment times, 5 and 15 minutes, was observed. Slightly higher values were found for the extracts of 15 minutes, which may indicate that the reduction capacity increases over the infusion time, contrary to the observed in *Ulva* sp. extracts.

Fucus vesiculosus extracts demonstrated only a greater than 50 % capacity for a treatment time of 15 minutes, the highest value recorded in this study. Comparing both proportion of macroalgae and water, the lowest and highest value of antioxidant activity was achieved on a ratio of 1:50 (g/mL) for 5 minutes, and for 15 minutes, respectively. Our study is in line with others authors, which highlighted the antioxidant richness of the macroalgae *Fucus vesiculosus* (Corsetto et al., 2020).

The impact of infusion treatment on the antioxidant capacity expressed by the ferric ion reducing antioxidant power (FRAP) of *Ulv*a sp. and *Fucus vesiculosus* macroalgae, is expressed at Figure 11.

***Ulva* sp.**
1:16 (m:v)

Fucus vesiculosus
1:16 (m:v)



1:50 (m:v)

1:50 (m:v)

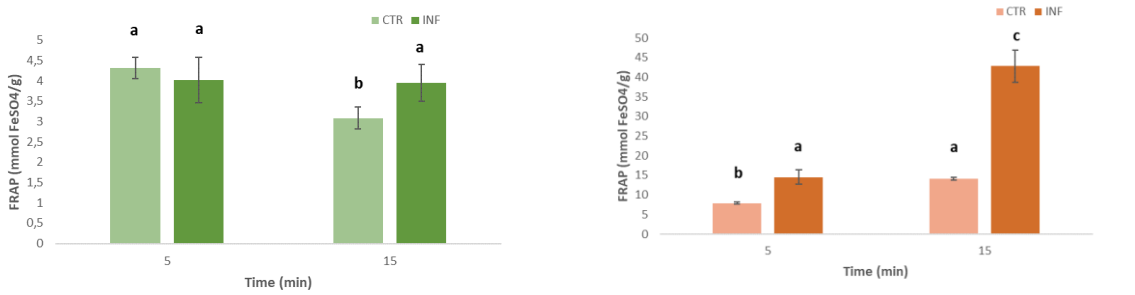


Figure 11 – Antioxidant capacity expressed by the ferric ion reducing antioxidant power (FRAP, mM FeSO4/g) of *Ulva* sp. and *Fucus vesiculosus* extracts without (CTR) and with infusion (INF). The vertical lines represent the standard deviation and different letters indicate significant differences at P-value <0.05 (Tukey test).

In green macroalgae extracts, no significant differences (P > 0.05, Tukey test) between the studied ratio (g/mL) were registered. In the brown macroalgae extracts an increase of the antioxidant capacity from 14.10 to 41.77 mM FeSO4/g was detected after the infusion during 15 min at a ratio of 1:50 (w:v). This fact indicates that *Fucus vesiculosus* demonstrates an interesting antioxidant capacity when the applied infusion treatment and the ratio of macroalgae:water were 1:50 (g/mL). Through the FRAP methodology it is possible to observe higher values in both macroalgae extracts, this demonstrates the great potential of the antioxidant capacity of both extracts which is in accordance with others authors (Silva et al., 2019).

Comparing the antioxidant capacity of both macroalgae extracts using the FRAP methodology, higher values were attained with *Fucus vesiculosus* compared to *Ulva* sp.. High phenolic content is associated with higher antioxidant capacity (Magalhães &

Santos, 2021). A similar behavior of antioxidant capacity by the DPPH and FRAP methodology was observed in all macroalgae extracts, which demonstrate the antioxidant potential of both extracts of macroalgae to be used in food industry. In other studies, the process of herbal infusions, in the conditions of 1:30 (w:v) at 15 minutes, showed higher values of the antioxidant capacity of the plants over the decoctions process determined by the FRAP method, comparing with decoction process at 2 minutes (Fotakis et al., 2016).

3.3 Impact of decoction on its physical properties

The decoction process with two proportions of macroalgae and water (1:16 and 1:50, g/mL) during 15 and 30 minutes, compared to the extracts without decoction (control samples - CTR) can be observed at Table 3.

Table 3 - Physical properties (pH, soluble solids content (SSC, % ºBrix) and color parameters) of macroalgae extracts, without (CTR) and after decoction (DEC) at different treatment conditions (mean ± standard deviation).

	***Ulva* sp.**							
	1:16 (m:w; g/mL)				**1:50 (m:w; g/mL)**			
	15 min		**30 min**		**15 min**		**30 min**	
Quality	**CTR**	**DEC**	**CTR**	**DEC**	**CTR**	**DEC**	**CTR**	**DEC**
pH	5.56 ± 0.05 a	5.52 ± 0.02 a	5.52 ± 0.01 a	5.51 ± 0.03 a	5,94 ± 0.04 b	5.64 ± 0.03 a	5.94 ± 0.02 b	5.93 ± 0.03 b
SSC (%ºBrix)	0.62 ± 0.01 a	4.79 ± 0.09 c	3.14 ± 0.03 b	2.01 ± 0.04 b	0.55 ± 0.03 a	1.18 ± 0.06 a	0.53 ± 0.02 a	0.74 ± 0.01 a
L*	31.25 ± 0.03 a	30,93 ± 0.01 b	30.6 ± 0.02 b	31.23 ± 0.04 a	31.54 ± 0.03 a	31.59 ± 0.01 a	31.54 ± 0.02 a	31.4 ± 0.01 a
a*	1.47 ± 0.03 a	1.54 ± 0.03 a	1.63 ± 0.04 a	1.47 ± 0.07 a	1.67 ± 0.01 a	1.37 ± 0.02 b	1.68 ± 0.01 a	1.46 ± 0.04 a
b*	1.76 ± 0.02 a	1.67 ± 0.04 a	0.53 ± 0.01 b	1.55 ± 0.01 b	0.58 ± 0.03 b	3,2 ± 0.02 c	0.6 ± 0.03 b	2.51 ± 0.05 c
	***Fucus vesiculosus***							
	1:16 (m:w; g/mL)				**1:50 (m:w; g/mL)**			
	15 min		**30 min**		**15 min**		**30 min**	
Quality	**CTR**	**DEC**	**CTR**	**DEC**	**CTR**	**DEC**	**CTR**	**DEC**
pH	5.21 ± 0.03 a	5.42 ± 0.02 b	5.43 ± 0.02 b	5.32 ± 0.03 a	5,59 ± 0.01 b	5.45 ± 0.02 b	5.59 ± 0.02 b	5.45 ± 0.04 b
SSC (ºBrix)	0.67 ± 0.02 a	3.52 ± 0.05 c	2.38 ± 0.01 b	2.1 ± 0.03 b	0.73 ± 0.02 a	1.35 ± 0.04 a	0.6 ± 0.006 a	0.59 ± 0.01 a
L*	31,6 ± 0.01 a	30.58 ± 0.04 b	31.59 ± 0.02 a	27.95 ± 0.01 c	31,22 ± 0.03 a	27.5 ± 0.04 c	31.23 ± 0.03 a	27.57 ± 0.02 c
a*	1,6 ± 0.01 a	1.69 ± 0.02 a	1.6 ± 0.03 a	2.95 ± 0.05 b	1,71 ± 0.01 a	3.02 ± 0.05 b	1.71 ± 0.02 a	3.2 ± 0.04 b
b*	1,19 ± 0.03 a	3.94 ± 0.03 b	1.21 ± 0.03 a	7.85 ± 0.07 c	1,42 ± 0.01 a	7.09 ± 0.12 c	1.41 ± 0.02 a	7.55 ± 0.14 c

Different letters in the same line indicate significant differences at P-value <0.05 (Tukey test). **Source** of macroalgae images: Henriques, B. (2021).

The pH values of *Ulva* sp. and *Fucus vesiculosus* decoctions are associated with the low acidity due the range from 5.51 to 5.94 and 5.21 to 5.59, respectively.

An identical behavior of soluble solids content (SSC) in both macroalgae decoctions was achieved. There is an identical behavior for both macroalgae, in that for both proportions of macroalgae and water, as the heat applied during the decoction process, there is a decrease in the extraction of compounds. Therefore, to achieve greater efficiency and extraction of the SSC, it is necessary to use heat in the first few minutes of the decoction process.

The decoction treatment induces a significant ($P < 0.05$, Tukey test) change in luminosity at proportion of macroalgae and water (1:16; g/mL) with values between 30.93 and 31.59 in decoction after 5 min and 15 min, respectively.

The b* colour parameter, which represents the yellowish color of the product, denotes higher values for a ratio of 1:50, and with time this value decrease ($P < 0.05$, Tukey test). The extracts of macroalgae *Fucus vesiculosus* after decoction processes presented low luminosity values, with values between 27.5 and 30.58 ($P < 0.05$, Tukey test). Positive values were observed for the b* parameter, with higher values compared with the macroalgae *Ulva* sp. Color is an important attribute of product quality that influences consumer´s choice. It is therefore essential to develop a product whose appearance meets the consumer's intentions (Pinheiro et al., 2022). In terms of color parameters, the extracts of both macroalgae showed low luminosity, a color close to red (a* values) and yellow (b* values).

The effect of hydrothermal processing on color in brown macroalgae (Rajauria et al., 2010), shows that the heat treatment is directly related with the color change of the macroalgae, as well the correlation with color change with antioxidant and phenolic content.

3.4 Impact of decoction on its bioactive composition

Figure 12 allows to observe the impact of the different algae/water ratios at decoction process in the total phenolic content.

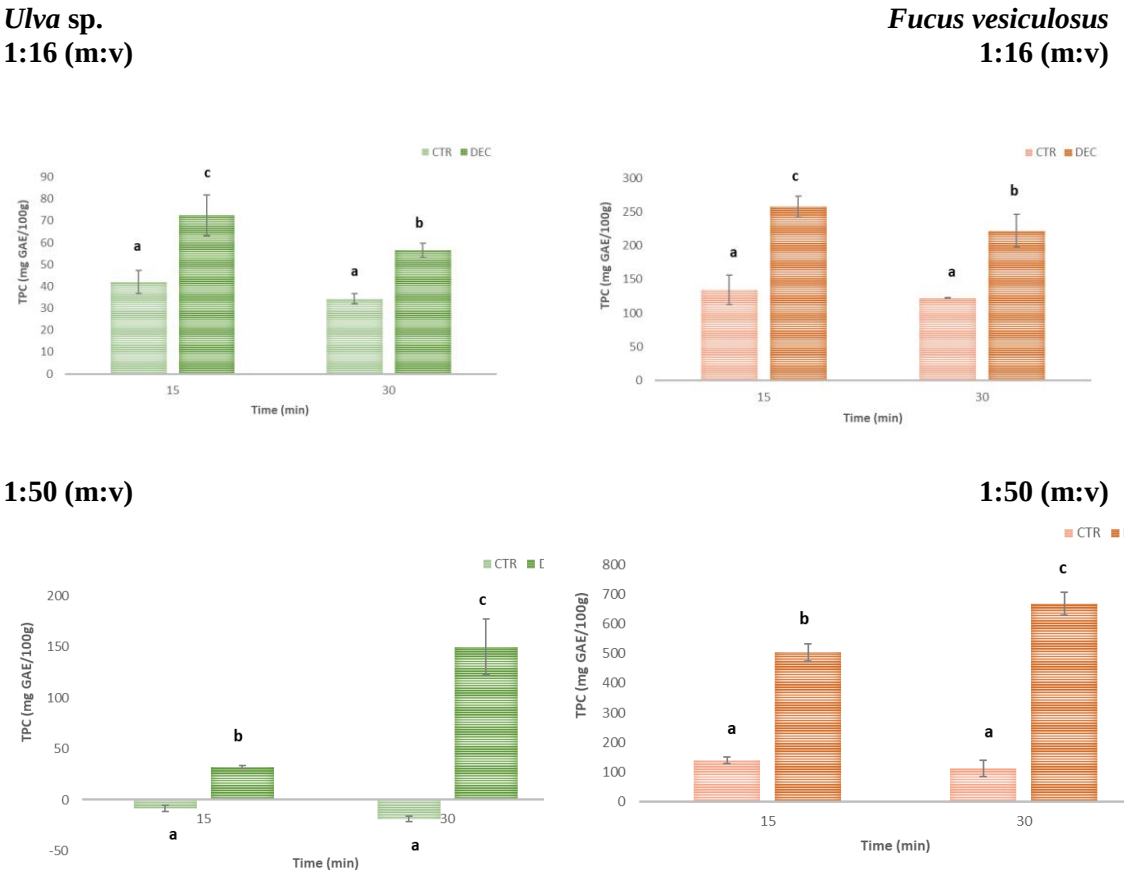


Figure 12 - Total phenolic content (TPC, mg GAE/100g) of *Ulva* sp. and *Fucus vesiculosus* extracts without (CTR) and after decoction (DEC). The vertical lines represent the standard deviation and different letters indicate significant differences at P-value <0.05 (Tukey test).

Comparing the total phenolic content (TPC) of CTR and DEC of *Ulva* sp. extracts in the ratio of 1:16 (g/mL), an increase of 30.54 and 22.16 mg GAE/100g was observed after 15 min and 30 min, respectively. In the macroalgae subjected to decoction in the proportion of macroalgae and water of 1:50 (g/mL), the highest value of TPC was achieved, 23.99 and 149.66 mg GAE/100g, after 15 min and 30 min, respectively. Despite the low value in the CTR extracts of *Ulva* sp. in the proportion of macroalgae and water

of 1:50 (g/mL), when this mixture was exposed to heat treatment, an increase of 32.42 and 164.66 mg GAE/100 g, after 15 min and 30 min was accomplished, respectively.

In the brown macroalgae, *Fucus vesiculosus,* and comparing both ratio of macroalgae and water, the highest and significant ($P < 0.05$, Tukey test) TPC was attained at 1:50 (g/mL). As constated in green macroalgae, the decoction treatment is efficient to extract the phenolic compounds, since when applied during 15 min and 30 min, 71 % and 65 % in proportion of 1:16 (g/mL) and 24 % and 150 % in proportion of 1:50 (g/mL) were reached.

In the study developed by Catarino et al., (2017), the aromatic plants treated with decoction in the ratio of 1:40 (g/mL) during 15 minutes, demonstrated the highest phenolic content 649.2 mg/g of extract. In a study by Trentin et al. (2020) the values of phenolic content were higher for infusion of *Ulva australis* comparing with decoction process. However, in our study the extracts of *Ulva* sp. achieved better results after decoction process compared with the infusion process.

The antioxidant capacity expressed by the methodology DPPH scavenging activity in control (CTR) samples and after decoction (DEC) of *Ulva sp.* and *Fucus vesiculosus,* are illustrated in Figure 13.

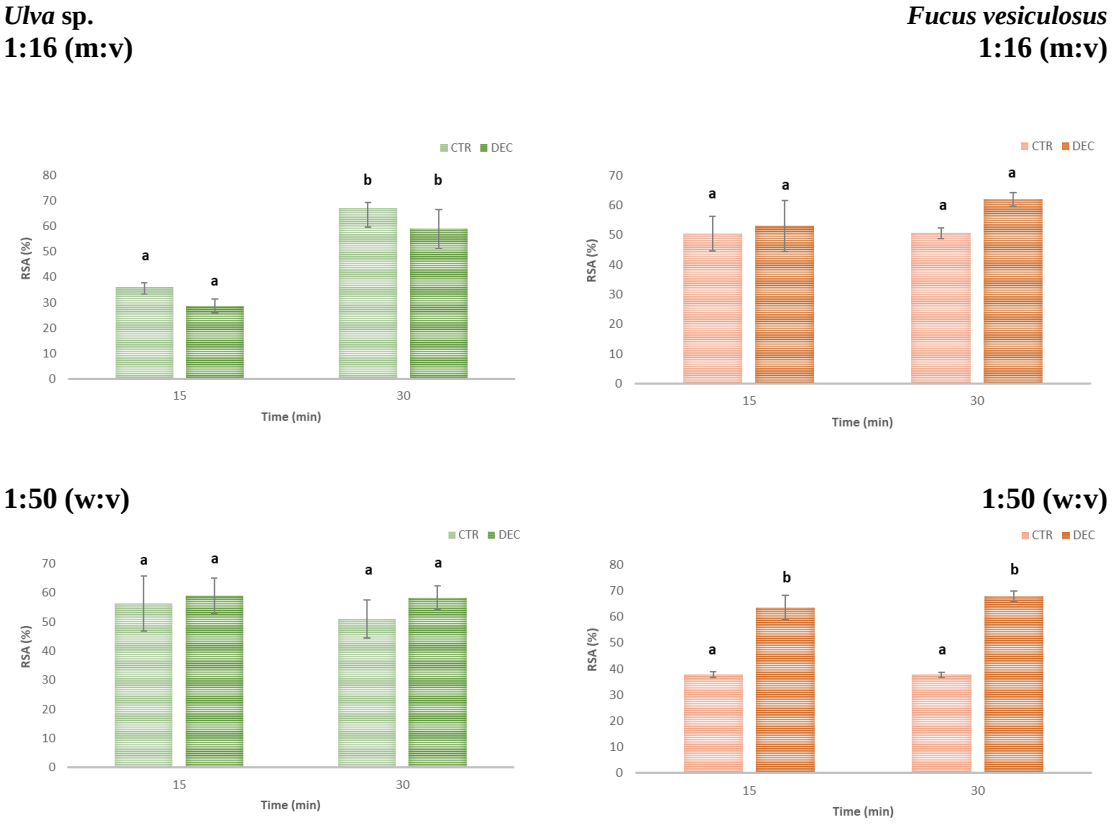


Figure 13 - Antioxidant capacity expressed by the DPPH radical scavenging activity (RSA, %) of *Ulva* sp. and *Fucus vesiculosus* extracts without (CTR) and after decoction (DEC). The vertical lines represent the standard deviation and different letters indicate significant differences at P-value < 0.05 (Tukey test).

In green macroalgae, the antioxidant capacity expressed as DPPH radical scavenging activity (RSA) was affected by the decoction treatment during 15 and 30 minutes. At both time treatment a decrease was observed in *Ulva* sp. extract, 7% and 8%, after 15 and 30 minutes, respectively. This reduction was not significant different between the antioxidant capacity expressed in control samples. However, a significant difference ($P < 0.05$, Tukey test) between the treatment time at a proportion of macroalgae and water of 1:16 (g/mL), was observed. Nonetheless, the scientific literature that relates the impact of decoction treatment on antioxidant capacity of the macroalgae under study, *Ulva* sp. and *Fucus vesiculosus*, is limited.

Regarding the impact of decoction treatment on *Fucus vesiculosus*, at macroalgae and water proportion of 1:16 (g/mL), an identical antioxidant capacity (DPPH) between the CTR and DEC extracts at both treatment time ($P > 0.05$, Tukey test), was attained. At a

proportion of macroalgae and water 1:50 (g/mL) a significant impact of the DPPH radical scavenging activity (RSA) between CTR and DEC and after 15 and 30 minutes, was observed ($P < 0.05$, Tukey test). Moreover, an increase of 26% and 30%, after 15 and 30 min, was achieved. Comparing the green and brown macroalgae, both species have different behavior at the same conditions.

The DPPH radical scavenging capacity of *Ulva sp.* and *Fucus vesiculosus* extracts, demonstrates the influence of factors like treatment time and ratio in the behavior of both macroalgae, where it has been evidenced a higher capacity for extract of *Ulva* sp. for a ratio 1:50 (g/mL) for 15 minutes, and for *Fucus vesiculosus* extract at ratio of 1:50 with a time of 30 minutes, 59% and 68%, respectively.

In a study reported by Wang et al., (2009), the obtained results, in proportions of 1:20 (w:v), for the macroalgae *Fucus vesiculosus* are in agreement with those obtained in the present study. In the literature (Wang et al., 2009), the results obtained showed a weak antioxidant power of the macroalgae, which in the present study showed an enriched antioxidant power, which can be explained by the difference in the preparation method for extraction).

The results of antioxidant assessment expressed by ferric ion reducing antioxidant power (FRAP) for both seaweeds after decoction process can be observed in Figure 14.

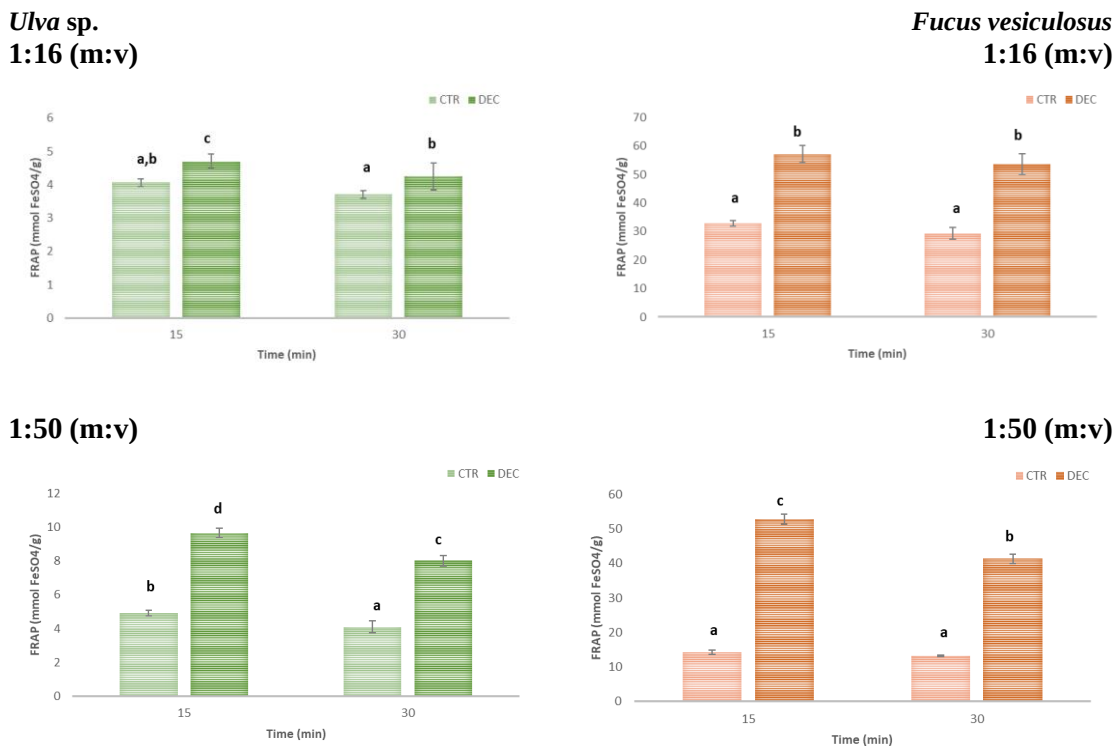


Figure 14 – Antioxidant capacity expressed by the ferric ion reducing antioxidant power (FRAP, mM $FeSO_4$/g) of *Ulva sp*. and *Fucus vesiculosus* extracts, without (CTR) and after decoction (DEC). The vertical lines represent the standard deviation and different letters indicate significant differences at P-value <0.05 (Tukey test).

The macroalgae *Ulva* sp. treated with the proportion of 1:16 (g/mL) presented a slight but significant ($P < 0.05$, Tukey test) increase of antioxidant capacity expressed as FRAP, 4.25 and 4.7 mM $FeSO_4$/g after 15 and 30 minutes, respectively. The increase of antioxidant capacity was more evident with the proportion of 1:50, where 8.0 and 9.6 mM $FeSO_4$/g, were achieved after decoction at 15 min and 30 min, respectively.

In the FRAP assay, *Fucus vesiculosus*, exhibit the highest antioxidant FRAP in the proportion of 1.16 (g/mL) compared to 1:50 (g/mL). In both macroalgae and water ratios, the value of treated extracts was much higher than the extracts without treatment.

Regarding both treatment times, the *Fucus vesiculosus* shows a better result obtained after 15 minutes, despite with a proportion of 1.16, the value was like obtained after 30 minutes

(53.6 mM $FeSO_4$/g). This suggests that as the decoction time increases the antioxidant properties of *Fucus vesiculosus* slowly decrease. The influence of the decoction process in this seaweed, induces the increase of antioxidant properties verified in both DPPH and FRAP methodologies. Comparing the treatment conditions, the influence of ratio and time on the antioxidant capacity of the macroalgae extracts, the better was the 1:50 (g/mL) and 15 minutes.

In other studies, the phenolic content and antioxidant activity were evaluated (using the TPC, DPPH and FRAP methods) of four macroalgae *Ulva rigida*, *Gracilaria* sp., *Fucus vesiculosus* and *Saccharina latissima*, and the *Fucus vesiculosus* showed better results than the other macroalgae (Neto et al., 2018). Similar results were obtained were *Fucus vesiculosus* that showed better results than *Ulva sp*. A study (Wang et al., 2010) showed the antioxidant potential of the extracts of *Fucus vesiculosus* in the inhibition of lipid oxidation in fish model systems. As the mentioned studies our results demonstrated that the macroalgae, *Fucus vesiculosus* and *Ulva* sp., frequently found in Europe, can be used in the food industry as substitutes of synthetic antioxidants.

4 Conclusions

The macroalgae *Ulva* sp. and *Fucus vesiculosus* showed a high content of phenolic compounds and good antioxidant activity using both infusion and decoction as extraction methods. This fact, combined with the nutritional composition of macroalgae, makes it possible to include them both in their natural form and as a natural ingredient to be added to food formulations for the Mediterranean Diet.

Regarding the impact of decoction and infusion processes on the bioactive activity of macroalgae, the extract with better performance was obtained from the brown macroalgae, *Fucus vesiculosus*, at proportion of 1:50 (g/mL) during 30 minutes. It would also be interesting to carry out a microbiological analysis of the product/macroalgae extracts, to assess its microbial stability, for use in food processing in the future.

5 Proposals for future work

The Mediterranean Diet, awarded by UNESCO as a cultural and intangible heritage of humanity, is characterized by a healthy and diverse diet with many benefits for human health, as well as a high consumption of vegetables.

The present study consisted of a first approach to evaluate the effect of two aqueous extraction treatments on a natural marine resource, which has shown potential to be included in the Mediterranean Diet, thus emerging as innovative ingredients with benefits for the well-being of society. In this sense, future research studies will be proposed, such as the addition of infusion and decoction extracts of green and brown macroalgae in a new food product, for example an enriched pumpkin soup.

Soup is an easily digestible meal with high nutritional value, containing large amounts of vitamins and minerals. In addition, the choice of pumpkin soup will be considered as the next steps because pumpkin is an ingredient rich in antioxidants, vitamins, minerals, and fiber, with cardiovascular properties. Pumpkin is one of the main agricultural products in Portugal, and in recent years there has been an increase in its production, mainly in the Western Region of Portugal. When formulating the soup, the macroalgae must first be decocted and then the ingredients cooked in the extract resulting from the decoction process. After optimizing the formulation of the enriched pumpkin soup, quality assessment will be carried out, such as pH value, CIE Lab color, antioxidant capacity methodology (DPPH, FRAP), total phenolic content (TPC), microbiological stability, shelf-life assessment, other important quality attributes focused on pumpkin soup. In addition, a sensory analysis of all the food samples will be carried out to assess consumer acceptance and quality control tests on a panel of trained tasters.

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7 Appendices

Table 1 – Nutritional value of dried macroalgae *Fucus vesiculosus and Ulva* sp.

Macronutrients	***Fucus vesiculosus* (per 100 g of seaweed)**	***Ulva sp.* (per 100 g of seaweed)**
Energy (kCal)/(kJ)	209 / 865	206 / 861
Total fat (g)	2,40	3,53
Saturated FA (g)	0,46	0,36
Polyunsaturated FA (g)	0,38	0,57
Carbohydrates (g)	10,8	13,8
Sugar (g)	0,20	0,20
Protein (g)	14,50	15,90
Dietary fiber (g)	43,10	34,40
Micronutrients – Vitamins and minerals	**(per 100g of seaweed)**	**(per 100g of seaweed)**
Potassium (mg)	3272	1952
Calcium (mg)	1167	1198
Magnesium (mg)	885	2776
Iron (mg)	14,70	78,90
Zinc (mg)	8,20	3,70
Cooper	0,40	1,30
Salt (g)	7,60	(--)
Moisture (%)	12	(--)

Source: Nutritional composition of *Ulva* sp. and *Fucus vesiculosus*, Ceva (December, 2021).

(--) Not evaluated.