Food Research International 137 (2020) 109676

Contents lists available at ScienceDirect

Food Research International

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

Review

Seaweed-based natural ingredients: Stability of phlorotannins during extraction, storage, passage through the gastrointestinal tract and potential incorporation into functional foods

Lucia Cassani [a,b,*], Andrea Gomez-Zavaglia [b,c], Cecilia Jimenez-Lopez [d,e], Catarina Lourenço-Lopes [d], Miguel A. Prieto [d], Jesus Simal-Gandara [d]

[a] Research Group of Food Engineering, Faculty of Engineering, National University of Mar del Plata, RA7600 Mar del Plata, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[c] Center for Research and Development in Food Cryotechnology (CIDCA, CCT-CONICET La Plata), RA1900 La Plata, Argentina
[d] Nutrition and Bromatology Group, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, E32004 Ourense, Spain
[e] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolonia, 5300-253 Bragança, Portugal

ARTICLE INFO

Keywords:
Health-related compounds
Renewable resources
Bioavailability
Extraction and stabilization approaches
Natural plant-derived ingredients

ABSTRACT

Adding value to seaweed by extracting their different bioactive compounds and incorporating them into foods represent an interesting and strategic approach to diversify the functional foods offer. However, once harvested, fresh seaweed must overcome a sequence of crucial steps to confer their biological activity. Pre-processing operations and extraction processes, as well as long-term storage, play important roles in improving or decreasing the phlorotannins content. In their way to the gut (biological target), phlorotannins are exposed to the human gastrointestinal tract (GIT), where the physiological pH and digestive enzymes can significantly affect the phlorotannins' stability and thus, alter their biological activity. Besides, the subsequent incorporation into foodstuffs could be limited due to sensory issues, as tannins have been associated with astringency and bitter taste, and thus effective phlorotannins doses may negatively affect the sensory attributes of foods. These drawbacks expose the need of applying smart strategies to develop a final product providing the necessary protective mechanisms to maintain the active molecular form of phlorotannins up to the consumption time, also controlling their release upon arrival to the gut. In this context, the impact of these technological processes (from pre-processing to the passage through the GIT) on phlorotannins stability, as well as the innovative developed approaches to overcome these issues will be deeply discussed in this review. Besides, recent findings related to the phlorotannins' health benefits will be pointed out. Special attention on the potential incorporation of phlorotannins into functional foods will be also put it on.

1. Introduction

The concept of nutrition has progressively evolved over the last few years. Indeed, food is not only considered as a nutritional source of compounds to prevent energetic deficiencies but also as a rich source of bioactive compounds that have demonstrated to play an important role in health promotion and prevention of chronic diseases (Gupta & Abu-Ghannam, 2011). In particular, algae have gained attention as rich sources of under-exploited bioactive compounds, potentially useful as novel functional ingredients (Li & Kim, 2011; Plaza, Cifuentes, & Ibáñez, 2008).

According to Ferdouse, Holdt, Smith, Murua, and Yang (2018), the global seaweed market is continuously growing in terms of value and volume because of the increasing demand of the food industry. Considering that the annual growth rate is about 10%, it is expected that the total value would increase up to USD 26 million by 2025 (Ferdouse et al., 2018). Despite being a millionaire industry, the global seaweed production is still limited to the cultivation of edible species [e.g., more than 90% of Japanese farms cultivate nori (*Porphyra spp.*), kombu (*Saccharina japonica*) and wakame (*Undaria spp.*)] and to the phycocolloids industry (e.g., agar-agar, carrageenan, and alginate extracts) (Ferdouse et al., 2018; Kadam, Tiwari, & O'Donnell, 2013). For this

* Corresponding author at: Research Group of Food Engineering, Faculty of Engineering, National University of Mar del Plata, RA7600 Mar del Plata, Argentina.
E-mail address: lcassani@fi.mdp.edu.ar (L. Cassani).

https://doi.org/10.1016/j.foodres.2020.109676
Received 21 April 2020; Received in revised form 3 August 2020; Accepted 6 September 2020
Available online 17 September 2020

reason, increasing attention has been paid to exploit novel biologically active compounds (e.g., phlorotannins, sulfated polysaccharides, carotenoid pigments, phytosterols, dietary fiber, omega-3 fatty acids, and bioactive peptides) from algae with health benefits (Kadam et al., 2013; Magnusson et al., 2017; Yuan et al., 2018).

Among bioactive compounds, phytochemicals are biologically active secondary metabolites produced by algae to protect themselves against hostile environmental conditions (e.g., temperature, UV–vis radiation, herbivores, salinity concentration, lack of nutrients, etc) (Li, Wijesekara, Li, & Kim, 2011; Plaza et al., 2008). Phlorotannins represent an important group of phytochemicals derived from brown algae. They have been the focus of several studies in the last years because of the important biological activities (e.g., antimicrobial, antioxidant, anti-inflammatory, anti-allergic, anti-diabetic and anticancer) (Li et al., 2011).

A systematic search in several databases (Scopus, ScienceDirect and PubMed) and free-access repositories (Google Scholar) was carried out based on the keyword 'phlorotannins'. This extensive information was further sub-classified into different sections as presented in this review namely, chemical and analytical aspects (related to pre-processing and extraction operations), health benefits, bioavailability and interaction with food matrix. According to the Scopus database (consulted in June 2020), 656 articles with the keyword 'phlorotannins' have been published. Among such documents, research articles have been mainly published (77.9%), followed by reviews (10.4%), book chapters (8.7%), conference papers (2.1%) and short surveys in minor proportion. When analyzing the distribution of documents by subject area, more than 52% of the articles correspond to agricultural, biological and biochemistry sciences. This clearly indicates the potential of seaweed-based polyphenols to be explored by the food and pharmaceutical industries. When analyzing the patents, the number of patents related to phlorotannins is much lesser, 207, but more than 75% of total were registered in the last nine years. Thus, the scientific research on this topic has experienced a systematic and continuous growth still observed up to now.

What makes the seaweed harvesting attractive for industrial exploitation is the easy cultivation, rapid growth, and simple technology requirement. Such advantages are translated into low initial capital investment and easy management of cultivation conditions, leading to a controlled and standardized production of bioactive compounds (Ferdouse et al., 2018).

In spite of these advantages, once harvested, fresh seaweed must overcome a sequence of crucial steps to confer their biological activity. Pre-processing operations, extraction processes, as well as long-term storage, food manufacturing, and digestion conditions, play important roles in improving or reducing the phlorotannins content which may affect their health benefits. Taking this into account, the impact of technological processes (from pre-processing to the passage through the gastrointestinal tract) on phlorotannins stability and the innovative approaches developed to overcome them will be deeply discussed in this review. Besides, recent findings related to the phlorotannins' bioavailability and health benefits will be pointed out. Special attention to the potential incorporation of phlorotannins into functional foods will be also put it on.

2. Phlorotannins: Structure and chemical characterization

Phlorotannins are polyphenolic compounds biosynthesized in brown algae (Phaeophyceae) (Singh & Sidana, 2013). From a structural point of view, they are a heterogeneous group of compounds in terms of size and composition, absent in terrestrial plants, resulting from the polymerization of phloroglucinol units (Montero, Herrero, Ibáñez, & Cifuentes, 2014). According to the structural bonding between the phloroglucinol monomeric units and the number and distribution of hydroxyl (—OH) groups, phlorotannins can be classified into four main categories (Fig. 1):

(i) *phlorethols and fuhalols*, which possess an ether bond. The main difference among them is that fuhalols show additional —OH groups in every third ring;
(ii) *fucols*, characterized by phenyl linkages;
(iii) *fucophlorethols*, having both ether and phenyl bonds;
(iv) *eckols and carmalols*, which have a 1,4-dibenzodioxin bond. The main difference among them is that carmalols have the characteristic bond at positions 3 and 7 while eckols possess the 1,4-

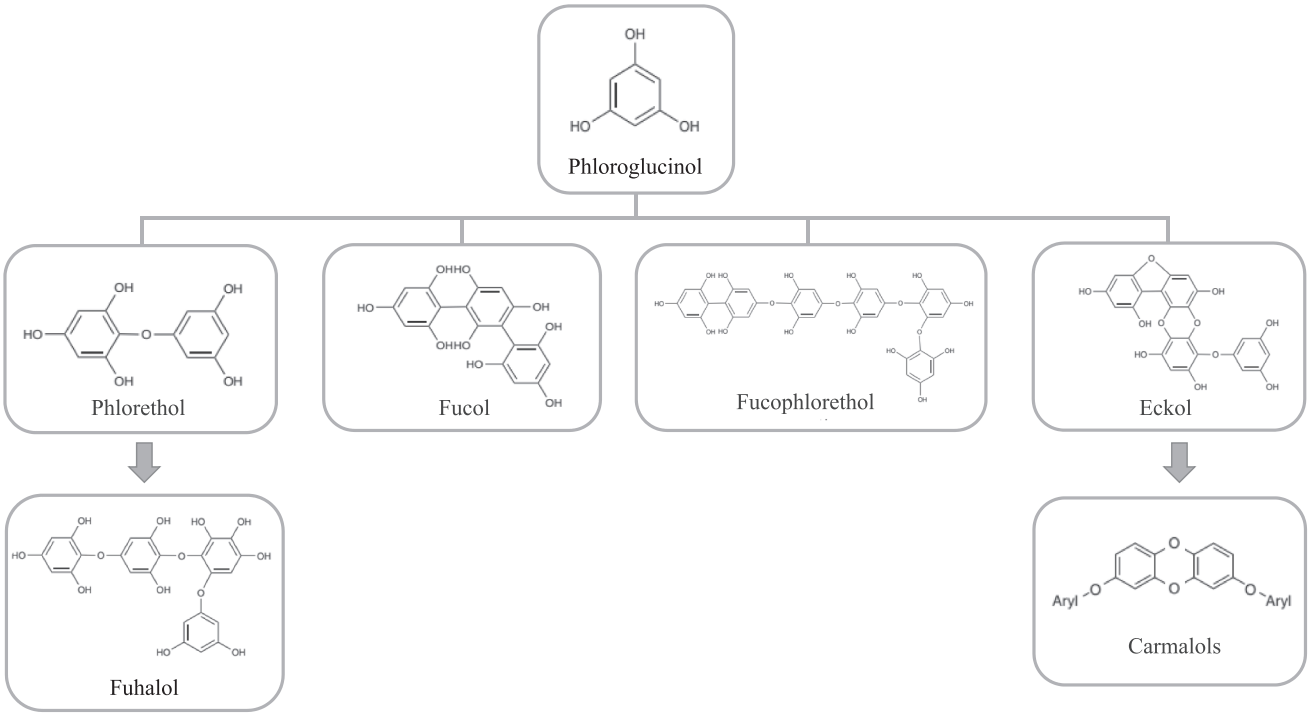

Fig. 1. The primary classification of phlorotannins derived from brown seaweed according to the bonding type between phloroglucinol units.

dibenzodioxin bond at positions 4 and 8 (Fig. 1) (Li et al., 2017; Montero et al., 2014).

Considering that structural and conformational isomers are also present within each class of compounds, the complexity and variability of these compounds can increase even more. Low, intermediate and high molecular weight phlorotannins (ranging from 126 Da to 650 kDa) have been found as soluble and cell wall-bound forms in brown seaweed. Soluble phlorotannins are stored in physodes (cell organelles) located in the cell cytoplasm and represent the most abundant phlorotannins in algae (up to 25% of dried alga). On the other hand, the cell wall-bound phlorotannins are forming complexes with different cell wall components, such as alginic acid (Barbosa, Lopes, Andrade, & Valentão, 2019). The phlorotannins content is variable even within the same algae class because its production is strongly influenced by intrinsic and environmental factors.

Several authors have determined the phlorotannins content of crude extracts by using spectrophotometric-based methods, such as Folin-Ciocalteu and 2,4-dimethoxybenzaldehyde (DMBA) (Anaëlle et al., 2013; Belda et al., 2016; Magnusson et al., 2017). These colorimetric methodologies are simple to use and allow rapid screening of the content of polyphenols present in the crude extract. However, none of them is specific because the presence of interfering substances can lead to overestimations (Lopes et al., 2018). In this sense, purification of the crude extract is an important and favorable step to enhance the chromatographic resolution of the target compounds. These procedures are focused on retaining the phlorotannins-rich fraction by removing other co-extracted compounds namely, carbohydrates which are also present in the crude extract (Lopes et al., 2018). Nuclear magnetic resonance (NMR) spectroscopic methods have been reported for characterizing phlorotannins from brown algae, as well as, for testing the efficiency of semi-purification methods in a rapid way since the obtaining spectra can provide useful information about the phenols abundance in contaminated samples (Gall, Lelchat, Hupel, Jégou, & Stiger-Pouvreau, 2015; Kang, Eom, & Kim, 2013; Le Lann et al., 2016). These spectroscopic methods are generally combined with high-performance liquid chromatography (HPLC), leading to a quick and reliable structural analysis and identification of compounds with almost no preparation of sample (Koivikko, Loponen, Pihlaja, & Jormalainen, 2007). However, the identification of phlorotannins with a high degree of polymerization (DP) is still a challenging task because of the absence of commercial standards (Melanson & MacKinnon, 2015). For that reason, technologically-advanced analytical methods, such as mass spectrometry, provide accurate information about the chemical structure of the target compounds (Melanson & MacKinnon, 2015). In a recent study, Lopes et al. (2018) identified twenty-two phlorotannins in purified extracts from *Fucus* species by using HPLC diode array detection coupled to tandem electrospray ionization mass spectrometry (HPLC-DAD-ESI/MSn) and ultra-performance liquid chromatography-electrospray ionization coupled to quadrupole time-of-flight high-definition mass spectrometry (UPLC-ESI-QTOF/MS). The use of these modern techniques allowed confirming the presence of both low molecular weight phlorotannins (within 370 and 746 Da) and phlorotannins with low DP (3 to 6 phloroglucinol monomeric units). UPLC-TQD/MS analysis has also shown to be useful in the study of the isomeric complexity of phlorotannins. Similarly, Montero et al. (2016) optimized a two-dimensional liquid chromatography (LC × LC-ESI-MS/MS) method to separate and tentatively identify a pool of phlorotannins with different DPs (from 3 to 11) and structures from *Sargassum muticum*.

3. Stability of phlorotannins during pre-processing, extraction and storage

Despite being part of the staple diet of Asian countries for centuries, adding value to seaweed by extracting their different bioactive compounds and incorporating them into foods represent an interesting and strategic approach to diversify the functional foods offer. This also contributes to satisfying the increasing demands of vegetarian and vegan consumers worldwide for natural plant-based ingredients. However, fresh seaweed must overcome a sequence of crucial steps to confer their biological activity (Fig. 2). In this way, ensuring a high phlorotannins content with desirable bioactivity and high extraction yields has been the main objective of many optimization approaches, particularly focused on the use of updated extraction protocols, reducing the use of organic solvents, the extraction times and the energy consumption, following the concept of green chemistry. Nevertheless, the stabilization of phlorotannins is also a crucial issue since storage-related factors could be detrimental to these sensitive molecules. Besides, the subsequent incorporation into foods and the passage through the GIT represent additional challenges. In this context, many factors could affect the phlorotannins concentration during their production (intrinsic and environmental factors), pre-processing, extraction, storage, and also their transit through the GIT. Therefore, preservation strategies should be appropriately selected to ensure the phlorotannins stability with health benefits (Fig. 3).

3.1. Factors affecting the content and concentration of phlorotannins

3.1.1. Pre-processing

Once algae are harvested from the sea, they are exposed to some pre-processing operations before being used in any nutritional assay, industrial process or storage. Among these operations, drying is the widely applied technique for extending the algae shelf-life by reducing the moisture content and thus, minimizing the algae spoilage before extraction. Besides, the seaweed volume is reduced thereby packaging, storage and transportation cost are significantly minimized (Fig. 3) (Leyton et al., 2016). Air-drying is an economical and rapid technique, chosen by industrial producers because of the high production rates are yielded. However, the air-drying process leads to a loss of phlorotannins content as a result of exposure to high temperatures and oxidative processes. On the other hand, freeze-drying maintains the phlorotannins content at stable levels but in an expensive and time-consuming way (Cassani, Gomez-Zavaglia, & Simal-Gandara, 2020; Chowdhury et al., 2011). Leyton et al. (2016) evaluated the application of hot air-drying at different temperatures (30, 40, 50 and 60 °C) on the phlorotannins' content of *Macrocystis pyrifera*. These authors found that at the optimal drying temperature (40 °C), the content of phlorotannins was lower than that of fresh algae, possibly due to the degradation of the compounds caused by heat treatments. Chowdhury et al. (2011) compared different drying pre-treatments (sun-drying, shadow-drying, oven-drying, and freeze-drying) on the phlorotannins extraction from *Ecklonia cava* and found that drying process significantly changed the phlorotannins content in the crude extract as the freeze-dried tissue yielded the highest amount of compounds whereas sun-dried tissues showed the lowest ones. Shadow- and oven-dried tissues were extracted at almost 80% of the freeze-dried sample. Besides, these authors reported that algae washed with tap water before drying showed lower crude phlorotannins than non-washed tissue. According to these findings, the lesser pre-treatments the algae receive, the better the phlorotannins content maintenance.

After drying, algae are usually ground into a powder and homogenized. In this way, the seaweed particle size is mechanically reduced and the specific surface area for extraction is increased, aiding the solvent penetration into the matrix. Consequently, the extraction rate is increased while the extraction time is reduced (Leyton et al., 2016).

Another pre-processing method proposed by Chowdhury et al. (2011) consists of boiling or steaming the seaweed tissue before drying to denature enzymes responsible for decomposing bioactive compounds. However, these procedures significantly reduce the phlorotannins' content to half in comparison to freeze-dried tissue since considerable amounts of phlorotannins remain in the water after boiling.

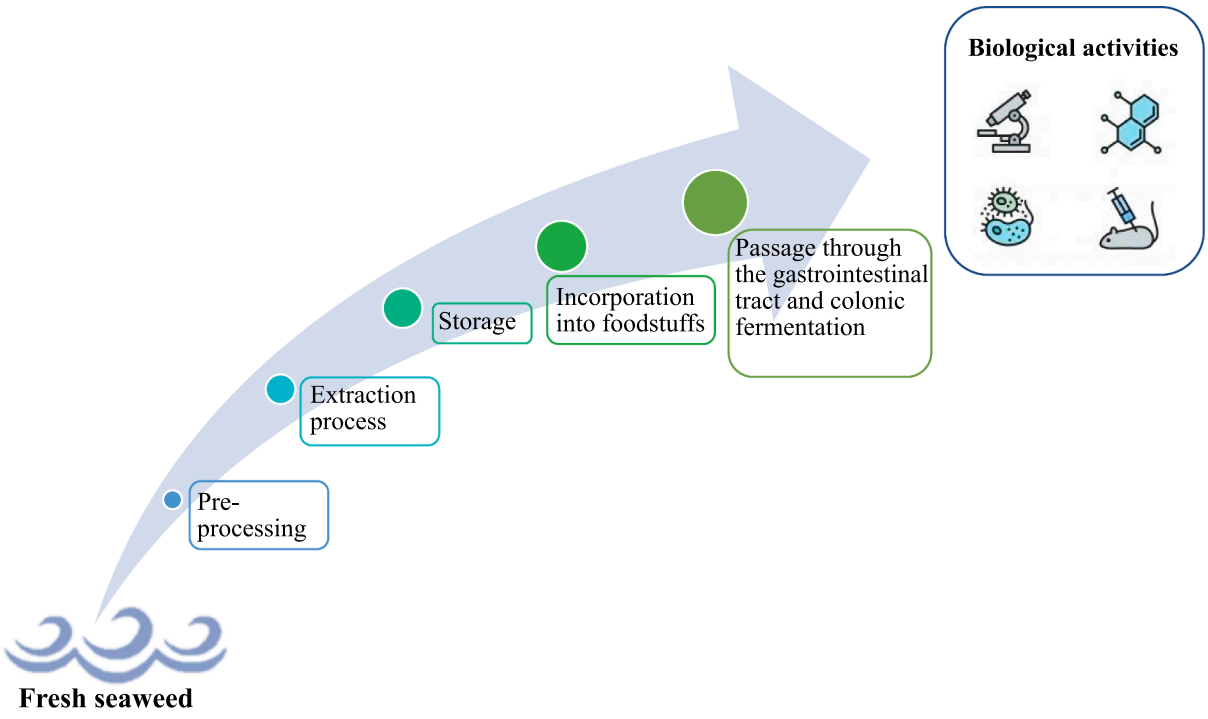

Fig. 2. Crucial steps the fresh seaweed must overcome to confer their biological activity.

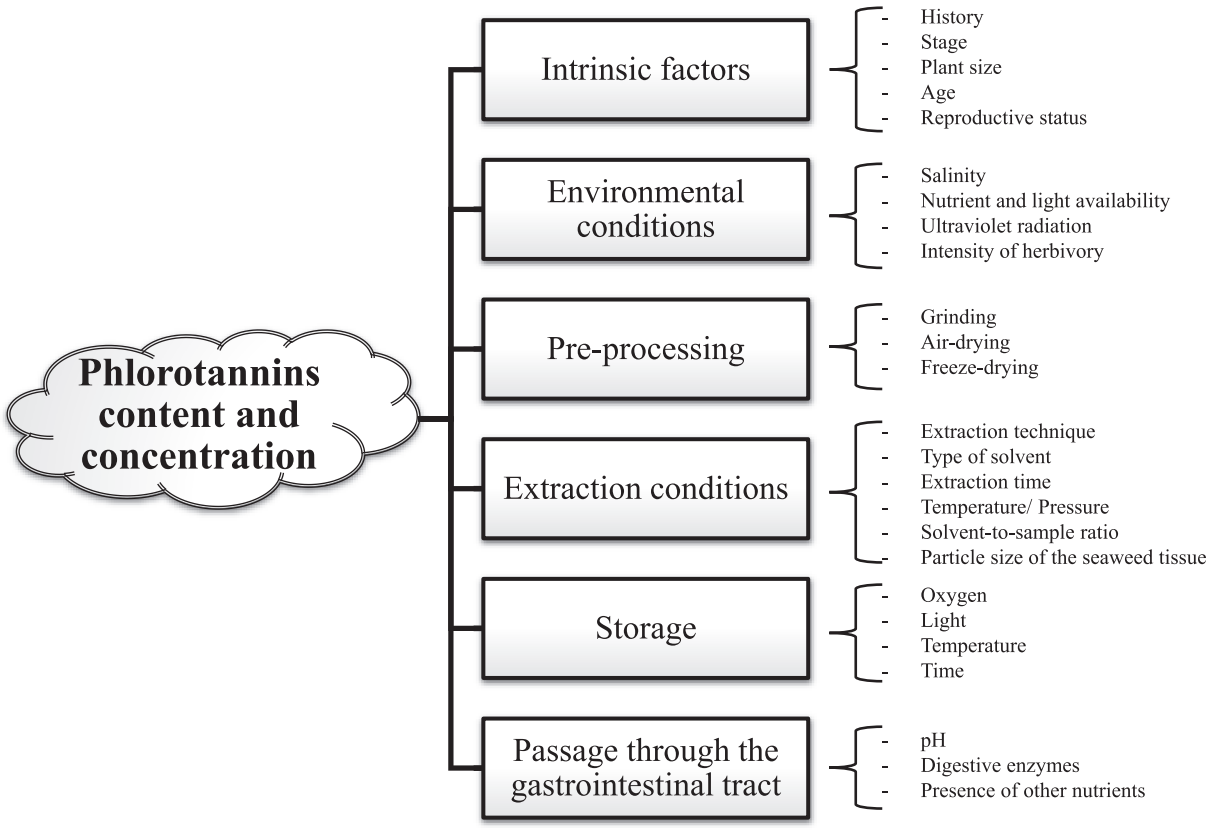

Fig. 3. Important factors affecting the concentration and content of phlorotannins.

3.1.2. Extraction proceeding

The extraction procedures represent another important factor that significantly affects the phlorotannins content and concentration (Fig. 3). Conventional extraction techniques, such as Soxhlet and maceration have been widely used for extracting valuable bioactive compounds from plant-based sources and are still considered as reference methods when compared to the extraction yield of a novel developed methodology. However, traditional methods are time and energy-

consuming and require the use of large amounts of solvents and the application of heat. Considering that some bioactive compounds are thermosensitive, there is a growing interest in developing novel, efficient and environmentally friendly extraction techniques, focused on improving the extraction yield without affecting the bioactivity of compounds. In this way, different non-conventional methods, such as pressurized liquid, ultrasound, microwave, supercritical fluid, subcritical water extraction, and enzyme-assisted extraction have been proposed to fill this gap (Table 1). For example, Anaëlle et al. (2013) found that pressurized liquid extraction allowed obtaining a rich fraction of phenolic compounds from *S. muticum* with good efficiency in comparison to classical methods. Similarly, He et al. (2013) compared the extraction yield obtained after applying microwave and conventional extraction methods on phlorotannins content from *Saccharina japonica* and found that the alternative green technology led to a higher extraction yield. It is well-known that microwave-assisted extraction allows a better solvent diffusion into the seaweed tissue by altering the cell wall and producing migration of dissolved ions and increasing its porosity, which facilitates the extraction of the desired compounds (Kadam, Álvarez, Tiwari, & O'Donnell, 2015). The application of ultrasound-assisted extraction has also shown to enhance the extraction of phenolic compounds with high molecular weight from *Ascophyllum nodosum* (Kadam, Tiwari, Smyth, & O'Donnell, 2015). These authors concluded that the ultrasound efficiency could be attributed to two main mechanisms: (i) the improved mass transfer between solutes from the algae tissue and solvent by diffusion and osmotic processes and (ii) dissolution of soluble components on the surfaces of the seaweed matrix.

Depending on the alternative technology selected, the influence of some process parameters on the extraction yield and bioactivity should be thoroughly studied (Fig. 3). In this context, the solvent type used for

Table 1
Green approaches employed in phlorotannins extraction.

Brown algae species	Green technology	Operational conditions	Quantification and/or separation method	Reference
Sargassum muticum	supercritical fluid extraction (SFE)	- Solvent: CO_2:EtOH (88:12) - Pressure: 15.2 MPa - Temperature: 60 °C Time: 90 min	Folin–Ciocalteu assay	(Anaëlle et al., 2013)
	pressurized liquid extraction (PLE)	- Solvent: EtOH:water (75:25; 25:75) - Pressure:10.3 MPa - Time: 20 min - Solid/liquid ratio: 378.78 g/L	Folin–Ciocalteu assay	(Anaëlle et al., 2013)
		- Solvent: EtOH:water (95:5) - Pressure: 10.3 MPa - Time: 20 min - Temperature: 160 °C	- 2,4-dimethoxybenzaldehyde (DMBA) assay - The purification method involved a sample clean-up with dichloromethane followed by precipitations using acetone and - ethanol finishing with an ethyl acetate extraction - Separation approach using LC × LC-ESI-MS/MS	(Montero et al., 2016)
	enzyme-assisted extraction (EAE)	- Enzymes: viscozyme L, cellulose, alcalase, flavourzyme - Temperature: 90–100 °C - Time: 10 min	Folin–Ciocalteu assay	(Rodrigues et al., 2015)
	EAE combined with PLE	- EAE as pre-treatment (alcalase 2 and 4 h, viscozyme 2 and 4 h, alkaline hydrolysis) - PLE treatment (EtOH:water, 25:75, 120 °C, 20 min)	DMBA assay	(Sánchez-Camargo et al., 2016)
Sargassum vestitum	microwave-assistedextraction (MAE)	- Solvent: EtOH:water (70:30) - Time: 75 s - Power: 960 W - Solid/liquid ratio: 20 g/L	Folin–Ciocalteu assay	(Dang et al., 2018a)
Saccharina japonica	MAE	- Solvent: EtOH:water (55:45) - Time: 25 min - Power: 400 W - Temperature: 60 °C - Solid/liquid ratio: 40 g/L	Folin–Ciocalteu assay	(He et al., 2013)
	SFE	- Solvent: CO_2:Water (98:2) - Pressure: 300 bar - Temperature: 48.98 °C	Folin–Ciocalteu assay	(Saravana et al., 2018)
Ascophyllum nodosum	ultrasound assisted extraction (UAE)	- Solvent: HCl (0.03 M) - Time: 25 min - Solid/liquid ratio: 100 g/L - Ultrasonic amplitude: 114 μm - Power: 750 W	- Molecular weight cut-off (MWCO) dialysis - Quadrupole time-of-flight-mass spectrometry (Q-TOF-MS)	(Kadam, Tiwari, et al., 2015)
	MAE	- Solvent: EtOH:water (70:30) - Time: 15 min - Temperature: 110 °C - Frecuencia: 2.45 GHz - Solid/liquid ratio: 100 g/L	Folin–Ciocalteu assay	(Yuan et al., 2018)
Lobophora variegata	Surfactant-mediated extraction (SME)	- Surfactant: guaiacol and triacetin as individual or binary mixtures - Solvent: buffer solution - Temperature: 60 °C - Time: 3.5 h - Solid/liquid ratio: 40 g/L	DMBA assay	(Gümüş Yılmaz, Gómez Pinchetti, Cifuentes, Herrero, & Ibáñez, 2019)
Carpophyllum flexuosum	MAE	- Solvent: water - Temperature: 160 °C - Time: 3 min - Solid/liquid ratio: 33.33 g/L	Folin–Ciocalteu assay	(Magnusson et al., 2017)

extraction has shown to significantly influence the extraction efficiency and selectivity of phlorotannins. The solvent polarity and the solubility of the target compound are both the main factors affecting the phlorotannins yield. Thereby, the extraction of a pool of phenolic compounds from different plant tissues needs different polarities (Koivikko, Loponen, Honkanen, & Jormalainen, 2005). According to the similarity and intermiscibility theory, which states that polar molecules of solutes can be easily dissolved in polar solvents, phlorotannins are better extracted with polar solvents than the non-polar ones (He et al., 2013).

A suitable solvent should meet some characteristics, such as low toxicity, low boiling point (to be easy to evaporate), good extraction power, environmentally friendly, etc. Taking into account that solvents less polar than water are usually the most efficient extractants (because of their better capacity to solubilize phenolic compounds) (Wang et al., 2012), the most commonly used organic solvents are ethanol, methanol, acetone and ethyl acetate (Koivikko et al., 2005; Wang et al., 2012). However, it was stated that a solvent system composed of two substances (a mixture of water and organic solvent) has a major extraction power than a mono-component solvent system (just water or organic extractant) for the phenolic compounds' extraction due to the synergistic effect of its components (Koivikko et al., 2005; Li et al., 2017). Water would swell plant cells while ethanol could aid to disrupt the bonds between solutes and plant tissues (Liu, Luo, Wang, & Yuan, 2019). Thus, the mixture of water and organic solvents is usually preferred (Rajauria, Jaiswal, Abu-Gannam, & Gupta, 2013). For example, Wang et al. (2012) studied the effect of different solvents (water, 50% ethanol, 80% methanol, 80% ethanol, 70% acetone, and 80% ethyl acetate) on the extraction of phlorotannins from *F. vesiculosus*. They observed that the extraction yield decreased as the solvent polarity diminished, 70% aqueous acetone being the extractant leading to the highest recovery. Several studies have proposed the use of aqueous mixtures of ethanol over acetone and methanol for the extraction of food-grade natural antioxidants (Belda et al., 2016). For example, Li et al. (2017) reported that the phlorotannins content significantly increased as the ethanol proportion raised to a certain extent (30% ethanol). Above this value, no further improvement in phlorotannins extraction was observed. Similarly, He et al. (2013) reported that the phlorotannins content increased when the ethanol concentration was between 40 and 50%, followed by a significant reduction at higher concentrations. A recent study conducted by Magnusson et al. (2017) proposed the use of water as a single solvent over ethanol, acetone, propan-1-ol and ethyl acetate for the microwave-assisted extraction of polyphenols from *Carpophyllum flexuosum*. However, these authors only used the non-specific spectrophotometric Folin-Ciocalteu (FC) assay to quantify the polyphenol content of the extracts, without taking into account some substances (e.g., reducing sugars which can also reduce the FC reagent), thus giving an over-estimated phlorotannins content. In such cases, the use of chromatographic techniques for characterizing these algal constituents has demonstrated to be a more powerful and reliable tool.

In general, serial consecutive extractions with the same or different solvent polarity have been successfully applied to extract a wide pool of phenolic compounds from both terrestrial and marine plants (Abu-Ghannam & Rajauria, 2013). Koivikko et al. (2005) reported that when applying a single extraction, most of the soluble phlorotannins (78% of the total) were extracted, but after applying four consecutive extractions, the extraction yield increased up to 93.5–95% of the total amount. Possibly, for industrial purposes, the application of one or two serial extractions would be enough to obtain a significant phlorotannins content at lower costs.

Other important factors affecting the phlorotannins content and concentration are extraction time, temperature/pressure, solvent to solid ratio and the particle size of the seaweed tissue (Fig. 3). Li et al. (2017) observed that during the traditional extraction procedure, the phlorotannins content increased as the extraction time raised to a maximum value (30 min), and then decreased. Probably, at larger times phlorotannins are exposed to oxidative processes affecting their stability. On the other hand, He et al. (2013) studied the effect of time (as a single factor) on phlorotannins content after microwave-assisted extraction from *S. japonica* and found that over the evaluated extraction period (5–25 min), the phlorotannins content significantly increased. Therefore, a proper selection of extraction time is needed for both ensuring an effective bioactive compound extraction and reducing energy-related costs (Catarino, Silva, Mateus, & Cardoso, 2019).

It was stated that an appropriate selection of extraction temperature could increase the phlorotannins content by enhancing their solubility (He et al., 2013). In addition, high temperatures could disrupt the algae cell wall structure, softening their tissue and thus facilitating the phlorotannins release (Dong et al., 2019). The application of heat treatments also reduces the viscosity and surface tension of extractants, enhancing the mass transfer (Abu-Ghannam & Rajauria, 2013). However, degradation of phlorotannins can also occur when high temperatures are applied. Catarino et al. (2019) reported the phlorotannins recovery from *F. vesiculosus* increased as extraction temperature raised from 17 to 25 °C. Above this value, a gradual decrease was observed. Similarly, Li et al. (2017) found that the phlorotannins content was increased when temperature ranged between 15 and 25 °C, followed by a decrease in the extraction yield at higher temperatures.

Solvent to solid ratio has also been reported as an influencing factor on phlorotannins content. According to the mass transfer principle, increasing the solvent volume leads to a higher concentration gradient allowing the solutes transference from the cell matrix to the external solvent (Catarino et al., 2019). Several works have shown that the recovery of polyphenols from seaweed increased as solvent-to-solid ratio raised until reaching an equilibrium (maximum value), from which no further improvement on the extraction of these bioactive compounds was observed (Catarino et al., 2019; Li et al., 2017; Topuz, Gokoglu, Yerlikaya, Ucak, & Gumus, 2016).

Taking into account that several factors and their interactions affect the complex phlorotannins structure and their properties, it is difficult to establish a unique extraction procedure that allows obtaining high yield and desirable biological activity and being useful for most of algae, even within the same class. For that reason, many optimization approaches based on response surface methodology (RSM) have been proposed for extracting bioactive compounds from seaweed (Catarino et al., 2019; Dang, Bowyer, Van Altena, & Scarlett, 2018a; Kadam, Tiwari, et al., 2015; Topuz et al., 2016). RSM is a mathematical tool that allows to simultaneously analyze the effect of many influencing factors (independent variables) and their interactions on several responses of interest (i.e., the extraction yield of a process) with the main advantage of minimizing the experimental runs and thus, reducing labor and time (Liu et al., 2019). To carry this out, a statistical experimental design (Box-Behnken, Placket-Burman, and central composite design, are the most common) is selected and the number and level of independent variables are established. Then, the response-surface is modeled by regression analysis and validation experiments are carried out to test the reliability of the optimized process (Cassani, Tomadoni, Moreira, Ponce, & Agüero, 2017). In this context, Catarino et al. (2019) successfully applied RSM with a three-level, three-variable Box–Behnken design, obtaining the optimal conditions of acetone concentration (67% v/v), solvent-solid ratio (70 mL/g) and extraction temperature (25 °C) that maximized the total phlorotannin content (2.92 ± 0.05 mg PGE/g) of *F. vesiculosus*. Similarly, Dang et al. (2018a) found that the optimal microwave-assisted extraction conditions using RSM with Box-Behnken design for total phenolic compound and antioxidant activities of *S. vestitum* were: ethanol concentration of 70%, radiation time of 75 s and power of 80%. Under these optimal conditions, the phenolic compounds' recovery was higher than that recorded using conventional and ultrasonic methods. Liu et al. (2019) optimized the antioxidant extraction process (extraction temperature, solvent-to-solid ratio, and ethanol concentration) from *A. nodosum* using RSM with three-level, three-factor Box-Behnken design. These authors reported that the extraction conditions that optimized each variable (phenolic compounds and antioxidant

activity) were quite different from each other and thus, they proposed two sets of conditions that individually maximized each variable. However, when the extraction process is intended to be scale-up, a compromise solution should be reached to simultaneously optimize all variables at the same time. In this sense, the desirability function has demonstrated to be a complementary tool to solve this issue, as allows finding the optimal experimental conditions to successfully satisfy the optimization of all responses (Cassani et al., 2017; Cassani, Tomadoni, Moreira, & Agüero, 2018; Tomadoni, Cassani, Ponce, Moreira, & Agüero, 2016).

3.1.3. Storage

Once extracted, phlorotannins, as most of the polyphenols from terrestrial plants have demonstrated to be very sensitive to storage-related factors, such as dissolved oxygen, light, temperature and time (Fig. 3) (Mourtzinos & Goula, 2019). In this way, Cuong, Boi, and Van (2016) evaluated the phlorotannins content of six dried *Sargassum* species (19% of final moisture content) during two years of storage in polyethylene bags at 30 °C. These authors reported a significant decrease of phlorotannins content as the storage time increased varying between the studied species.

Stabilization of phlorotannins is a crucial issue, as processing and then, gastrointestinal conditions can cause damage to these sensitive molecules. Thus, applying smart strategies to develop a stable product providing the necessary protective mechanisms to maintain the active molecular form of phlorotannins during storage up to the consumption time are highly needed (Fang & Bhandari, 2010; Mourtzinos & Goula, 2019). In this sense, encapsulation involves the entrapment of an active ingredient (in this case polyphenols) in micro or nanocapsules composed of another solid or liquid immiscible substance, often called, coating, wall material, carrier, shell, etc. Such coating acts as a physical permeability barrier, limiting the molecular oxygen diffusion and thus, extending the encapsulated product shelf-life (Mourtzinos & Goula, 2019).

Several biomaterials approved for food use and "generally recognized as safe" (GRAS) have been proposed to encapsulate polyphenols from terrestrial plants, including carbohydrate polymers, proteins, and lipids. In this sense, Vinceković et al. (2017) presented a complete list of suitable food-grade materials to be used for encapsulating bioactive compounds. Regarding the encapsulation techniques, it can be roughly classified in mechanical processes (spray-drying, freeze-drying, electrospinning, electrospraying, fluid bed coating, extrusion, etc.), physicochemical methods (including coacervation, liposome entrapment, ionic gelation, emulsification, etc.) and chemical methods (interfacial polycondensation, polymerization and crosslinking, and *in situ* polymerization) (Munin & Edwards-Lévy, 2011; Vinceković et al., 2017).

Many efforts have been made to develop innovative encapsulation methods and wall materials to the stabilization, solubilization, and delivery of phenolic compounds from terrestrial plants. However, research focusing on encapsulating polyphenols from seaweed remains relatively scarce. In a recent study, Surendhiran, Cui, and Lin (2019) successfully encapsulated phlorotannins in nanofibers made of biopolymers (sodium alginate and poly(ethyleneoxide)) applying electrospinning technology, to be used as a natural antimicrobial agent for food packaging purposes. These authors reported that the phlorotannins' nanofibers demonstrated to be physicochemically and mechanically stable, ensuring a firm encapsulation of phlorotannins within the nanofibers. Also, these authors concluded that the phlorotannins' nanofibers were able to preserve the microbiological quality and safety of stored chicken. In another study, Hermund et al. (2019) developed electrosprayed capsules of fish oil with an antioxidant-rich extract from the brown alga *F. vesiculosus* using different wall materials (dextran and glucose syrup). These authors found that the addition of phlorotannins improved the oxidative stability of the fish oil capsule when dextran was used as a biopolymer. Besides, these authors reported a prooxidant effect of the seaweed extract in the glucose syrup capsules (which also exhibited a significantly lower encapsulation efficiency than that reported for dextran capsules), attributed to some interactions between metal ions and non-encapsulated oil and different distribution of phlorotannins inside the capsules.

4. Bioavailability

The term 'bioavailability' refers to the integration of various processes in which a fraction of ingested nutrient is available for digestion, absorption, distribution, metabolism and elimination (Chiou et al., 2014). In this regard, the biological activity of polyphenols depends on their bioavailability, and several studies have been carried out to understand the transformations that plant-derived polyphenols suffer during their passage through the GIT, as well as, the relevance of their interaction with gut microbiota (Duda-Chodak, Tarko, Satora, & Sroka, 2015; Selma, Espin, & Tomas-Barberan, 2009). The interest in seaweed-derived polyphenols is increasing, but information about their bioavailability is still scarce, and this is a limitation to understand their bioactivity and mechanism of action *in vivo*. Thus, in the absence of specific information about phlorotannins' bioavailability and interaction with intestinal bacteria, considering the absorption' mechanisms of plant-derived polyphenols constitute a very useful starting point.

Most polyphenols from terrestrial plants, often called dietary polyphenols, are not digested in the upper intestinal tract and reach the colon almost intact. There, dietary polyphenols act as substrates for several enzymes secreted by the gut microbiota and are biotransformed by different metabolic pathways including, hydrolysis, reduction, decarboxylation, demethylation, dehydroxylation, isomerization, and fission (Cherry et al., 2019; Selma et al., 2009). As a result, small bioactive metabolites are produced, which in turn, are potentially more absorbable than the polyphenol parent compounds (Tomás-Barberán, Selma, & Espín, 2016). Some studies have demonstrated that unabsorbed dietary polyphenols and their bioactive metabolites had a significant effect on modulating the intestinal microbiota composition. In particular, hydrolysable and condensed tannins have shown a 'prebiotic-like' effect by positively promoting the growth of lactobacilli and bifidobacteria and thus, conferring benefits to the host (Duda-Chodak et al., 2015; Tomás-Barberán et al., 2016). Considering that the gut microbiota composition and the expression of metabolizing enzymes vary among the human population, different phenolic metabolic profiles are produced and thus, their physiologic response is highly variable between different individuals (Tomás-Barberán & Espín, 2019).

Regarding brown seaweed-derived compounds with 'prebiotic-like' effect, the *in vitro* evidence suggests that the complex polysaccharides (alginate, laminarin and fucoidan) are metabolized by the fecal microbiota and thus, stimulating the growth of certain beneficial populations (namely, *Bifidobacterium, Bacteroides, Lactobacillus*, etc) with the respective increment in the short chain fatty acids (SCFA) metabolites composition (Charoensiddhi, Conlon, Vuaran, Franco, & Zhang, 2017; Cherry et al., 2019; Lopez-Santamarina et al., 2020; Shang et al., 2016). However, little is known about the fate of polyphenols from seaweed, in particular, phlorotannins during their passage through the GIT and their interaction with gut microbiota. In a study conducted by Corona et al. (2016), phlorotannins with high molecular weight from *A. nodosum* showed poor absorption in the small intestinal resulting in the production of phase II conjugate metabolites (glucuronides, sulphates). Then, the unabsorbed conjugated compounds reached the colon where they were further fermented by gut microbiota resulting in the formation of lower-molecular-weight metabolites. Some phlorotannin oligomers (*e.g.*, hydroxytrifuhalol A, 7-hydroxyeckol and the C-O-C dimer of phloroglucinol) and seven non-identified metabolites were detected in plasma and then excreted in urine (Corona et al., 2016). Considering that phlorotannins are a structurally heterogeneous group of compounds in terms of size and composition, the absence of available commercial standards make their characterization further complex in particular, for those analyses of plasma, urine and digested materials.

In another study, Corona et al. (2017) reported that both the phlorotannins content (obtained from *A. nodosum*) and the *in vitro* antioxidant capacity were significantly reduced when exposed to *in vitro* gastrointestinal digestion conditions and colonic fermentation (CF). However, phlorotannins in the GIT fraction were able to inhibit HT-29 cell growth (human colorectal adenocarcinoma cells), while those present in the CF extracts positively counteracted H_2O_2 induced DNA damage, showing a promising anti-genotoxic effect.

On the other hand, Charoensiddhi et al. (2017) studied the potential prebiotic effect of some *E. radiata* extracts, in particular those enriched with phlorotannins and polysaccharides (low and high molecular weight) using an *in vitro* anaerobic 24 h-fermentation method containing human fecal inoculum. These authors reported that the colonic fermentation of phlorotannin-rich fractions produced a significantly lower content of SCFA metabolites in comparison to the polysaccharides fermentation. In addition, phlorotannins positively served as substrates for the growth of *Bacteroidetes*, *Clostridium coccoides*, *Escherichia coli*, and *Fecalibacterium prausnitzii* populations but inhibited the growth of *Enterococcus* and *Lactobacillus* bacteria after 24 h fermentation. Probably, the antimicrobial activity of phlorotannins could explain the significant reduction on microbial counts of certain populations and the low SCFA production (Charoensiddhi et al., 2017).

The application of biotechnological treatments by using enzymes (*e.g.* fungal and bacterial glycosidases) and bacteria (*e.g.* probiotics which be able to modulate gut microbiota) could be an interesting strategy to induce changes in the phlorotannins structure which may favor their interaction with gut community (Tomás-Barberán & Espín, 2019).

In light of the above, more studies are highly required to fully understand how phlorotannins are absorbed and metabolized during gastrointestinal digestion and interact with gut microbiota. Other questions that should be addressed to fill this gap include, which bacterial strains are capable of fermenting phlorotannins and their respective catabolic mechanisms bearing in mind that the interindividual variation of the gut microbiota composition leads to different polyphenols metabolic profiles. When a phlorotannins-rich food formulation is carried out, how food matrix and technological processes (*i.e.*, dehydration, grinding, high concentrations of salt or sugars, thermal treatment, etc.) can affect the phlorotannins composition, their stability during their passage through the GIT and the final interaction with colonic microbiota? Clearly, it is a hot topic which represents a research opportunity.

5. Health beneficial effects of phlorotannins

As was mentioned before, the increasing interest in using phlorotannins as functional ingredients has been directly related to their desired health beneficial effects. This section is focused on pointing out the well-recognized biological activities of these phytochemicals, their mechanism of action and the most common assays used to determine them (Fig. 4).

In vitro antioxidant activity of different brown algae has been widely studied, being phlorotannins the main contributors to that property (Honold, Jacobsen, Jónsdóttir, Kristinsson, & Hermund, 2016). The complex chemical structure and the molecular weight of these bioactive compounds could explain their high antioxidant activity. In this sense, the presence of several interconnected aromatic rings and the proximity of many hydroxyl groups (acting as hydrogen donors) lead to improve the free radical scavenging capacity of phlorotannins, which could be associated with the number of sites available in the bioactive compound structure (Heffernan, Brunton, Fitzgerald, & Smyth, 2015; Honold et al.,

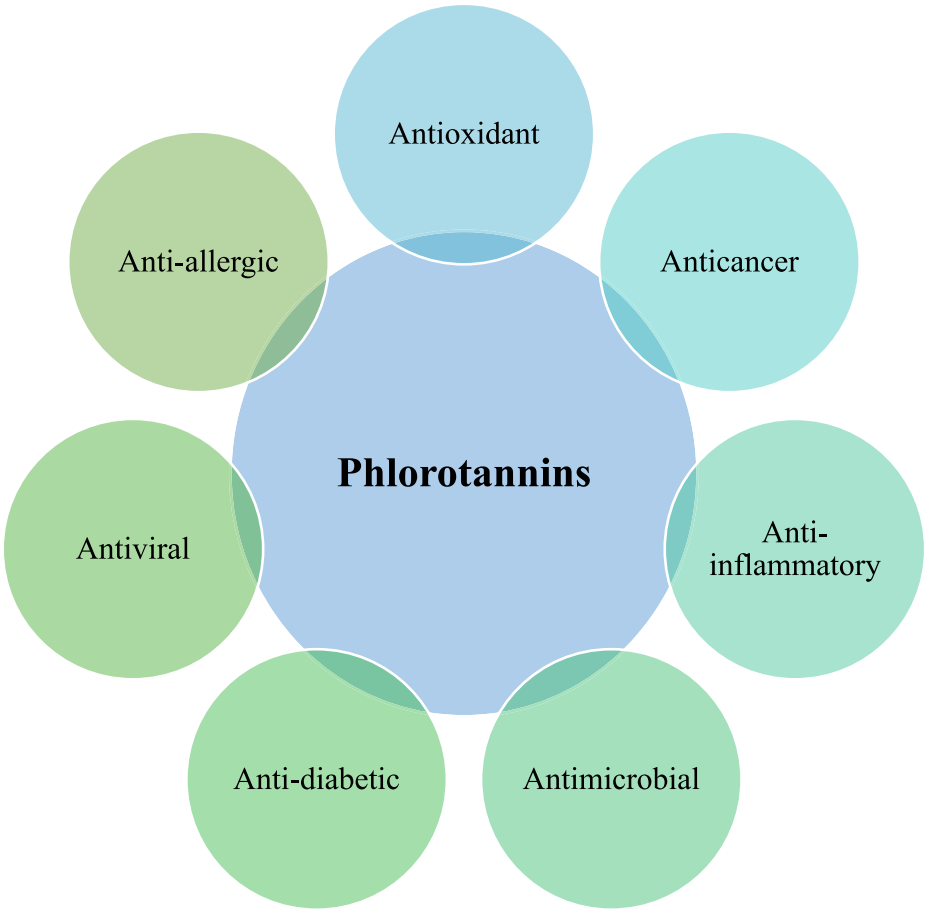

Fig. 4. Biological activities ascribed to phlorotannins.

2016). According to these findings, the higher the molecular weight of phlorotannins, the stronger their antioxidant capacity. However, in a recent study, Hermund et al. (2018) reported that low molecular weight phlorotannins from *F. vesiculosus* exhibited greater antioxidant activity than that of larger polymers, and that capacity decreased as increasing the degree of polymerization. The results of their study suggested that the availability of free hydroxyl groups had a more important impact on the phlorotannins' antioxidant activity than polymerization. A possible explanation of this finding could be those large phlorotannin polymers would enfold the hydroxyl groups inside the structure and thus reducing their scavenging capacity (Hermund et al., 2018). Similarly, Heffernan et al. (2015) observed that phlorotannins from *F. vesiculosus* with DP between 4 and 7 showed a significantly higher antioxidant capacity than that reported for other brown species (*F. serratus*, *Himanthalia elongata,* and *Cystoseira nodicaulis*) mainly composed of phlorotannins with DP ranging from 9 to 12. These authors concluded that phlorotannins with high DP have a lesser number of available –OH groups than shorter phlorotannins, as many of them are involved in monomer linkages. The most common methods used to determine the *in vitro* antioxidant activity of phlorotannins include 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH) (Liu et al., 2019), ferric reducing antioxidant power (FRAP) (Heffernan et al., 2015) and Trolox equivalent antioxidant capacity (TEAC) based on the scavenging ability of antioxidants to the radical anion $ABTS^{*+}$ (Dang, Bowyer, Van Altena, & Scarlett, 2018b). These methods are based on the single-electron mechanism (SET) which detects the antioxidant capacity to transfer one electron to reduce any compound, including metals, carbonyls, and radicals (Prior, Wu, & Schaich, 2005). However, as antioxidants may respond in a different way to different sources of radical or oxidant, adding complementary assays namely, those based on the hydrogen atom transfer (HAT) mechanism (namely, oxygen radical absorbance capacity (ORAC) and crocin or β-carotene bleaching by peroxyl radicals) is highly recommended to reflect the total antioxidant activity of a compound (Prior et al., 2005).

Phlorotannins have also shown to possess antimicrobial activity and thus, having the potential for replacing synthetic preservatives in foodstuffs. Although the exact antimicrobial mechanism of phlorotannins has not been completely clarified, it is thought that the interaction between phlorotannins and bacterial proteins could play an important role in their antimicrobial activity (Abu-Ghannam & Rajauria, 2013). As many polyphenols from terrestrial plants, the damages on the bacterial cell membrane are the main targets of phlorotannins (Hierholtzer, Chatellard, Kierans, Akunna, & Collier, 2013; Wang, Xu, Bach, & McAllister, 2009). Electron microscopic observations support these findings by showing the morphological and physical changes of the bacterial cell membrane when exposed to phlorotannin treatments (Hierholtzer et al., 2013; Surendhiran et al., 2019; Wang et al., 2009). When the cytoplasmic membrane is damaged, the cell lysis occurs, resulting in the release of cell constituents including potassium, phosphates and those materials which can be spectrophotometrically measured and are related to nucleic acids (Hierholtzer et al., 2013). The complex phlorotannin structure, having up to eight interconnected rings makes algae polyphenols stronger antimicrobial agents than those extracted from terrestrial plants which show three or four rings (Abu-Ghannam & Rajauria, 2013). In addition, it was stated that the antibacterial activity of phlorotannins depends on their degree of polymerization and hence, the more polymerized the phloroglucinol monomer is, the higher bacterial inhibition is obtained (Hierholtzer et al., 2013; Nagayama, Iwamura, Shibata, Hirayama, & Nakamura, 2002). Some works have reported that the effectiveness of the phlorotannins antimicrobial activity also depends on the bacteria cell wall structure since gram-positive microorganisms have shown to be more susceptible to the phlorotannins action than the gram-negative ones (Lopes et al., 2012). The latter group of microorganisms possesses a more complex external membrane with high lipopolysaccharide content which confers resistance against different antibiotics including natural antimicrobials. *In vitro* antimicrobial activity assays, such as microdilution methods, have been employed to determine the minimum inhibitory concentration (MIC, that is, the minimum required concentration to reduce the 90% of the microbial growth) (Tomadoni, Cassani, Moreira, & Ponce, 2015), as well as the minimum bactericidal concentration (MBC, which means the lowest concentration able to destroy the 99.9% of the initial inoculum) (Pellegrini et al., 2014) of phlorotannins extracts against a wide spectrum of gram-positive and gram-negative bacteria (Eom et al., 2015; Nagayama et al., 2002; Surendhiran et al., 2019). In this regard, Lopes et al. (2012) found the lowest MIC and MBC values in both gram-positive (*Staphylococcus aureus, S. epidermidis, Micrococcus luteus, Enterococcus faecalis* and *Bacillus cereus*) and gram-negative bacteria (*Proteus mirabilis, E. coli, Pseudomonas aeruginosa* and *Salmonella typhimurium*) when treated with phlorotannins' extracts from *F. spiralis* and *C. nodicaulis,* and these results were related to the high content of phlorotannins presented in the studied algae.

The antifungal activity of phlorotannins was also studied by Lopes, Pinto, Andrade, and Valentao (2013) providing insights about the mechanism of action of these algae phytochemicals against *Candida albicans*. In this regard, phlorotannins seem to inhibit the yeast dimorphic transition allowing decreasing the microorganism's adhesion to target epithelial cells and thus, reduce its virulence.

Another important biological activity associated with phlorotannins is their anti-inflammatory capacity. Briefly, the methodology used for analyzing the anti-inflammatory activity of phlorotannins consists in inducing an extracellular stimulus with the bacterial lipopolysaccharide (LPS) to activate the *in vitro* model of RAW 264.7 macrophages. Once activated, macrophages elicit the production of pro-inflammatory cytokines (*e.g.* interleukin (IL)-1β and IL-6, and tumor necrosis factor (TNF)-α) and mediators (*e.g.* nitric oxide (NO) and prostaglandin (PG) E_2) (Barbosa et al., 2019). Over-production of these pro-inflammatory components is related to inflammatory and neurodegenerative diseases. In response to stimuli, free NF-κB (nuclear transcription factor) is translocated into the nucleus and induces the transcriptional expression of inflammation-related protein genes, including inducible nitric oxide synthases (iNOS) and cyclooxygenase-2 (COX-2) which catalyze the synthesis of NO and PG E_2 (Kim et al., 2016; Wei et al., 2016). Therefore, several works have studied the ability of phlorotannins to inhibit the NF-κB activation and thus, the production of inflammatory mediators. In this way, Wei et al. (2016) demonstrated that phlorotannins from *E. stolonifera* (e.g., 2-phloroeckol, 6,6′-bieckol, phlorofucofuroeckol A, phlorofucofuroeckol B, and 974-B) were able to inhibit the production of pro-inflammatory cytokines and suppress the NF-κB activation which led to the NO and PG E_2 inhibition in LPS-treated RAW 264.7 cells. Similarly, Yang et al. (2016) demonstrated that dieckol, the major compound in the *E. cava* extract, significantly inhibited the production of NO, PG E_2 and HMGB-1 (high-mobility group box-1, a key cytokine of local inflammation) in LPS-induced septic mouse model. These authors concluded that the anti-inflammatory activity of phlorotannins from *E. cava* could be associated with the negative regulation of pro-inflammatory factors via the Nrf2/HO-1 and NFκB pathways. Another study conducted by Casas et al. (2016) showed that phlorotannins from *S. muticum* inhibited the PG E_2 production in human blood by suppressing the COX-2 expression.

Anti-diabetic (Lee & Jeon, 2013), anti-allergic (Barbosa et al., 2019), anticancer (Kumar, Yuvakkumar, Vijayakumar, & Vaseeharan, 2018) and antiviral (Kwon et al., 2013) activities are other interesting biological activities that have also been ascribed to phlorotannins-rich extracts. However, further *in vivo* studies are required to properly understand the mechanisms proposed in the numerous works based on *in vitro* assays.

6. The potential incorporation of phlorotannins into functional foods

The development of functional food products is a growing sector of

the food industry concerned at fulfilling the demand of increasingly exigent consumers. In particular, the systematically growing number of vegans, having especial difficulties to achieve the daily requirements of certain nutrients (*e.g.*, vitamins, minerals), underlines the need for formulating healthy innovative food products. In this context, phlorotannins constitute natural antioxidants and antimicrobials ingredients with well-demonstrated health beneficial properties, valuable for the formulation of functional foods.

Using whole algae extracts allows the simultaneous incorporation of different functional ingredients (*e.g.*, dietary fiber, polyphenols, minerals, vitamins, carotenoids, etc). Although it represents a simple procedure to incorporate functional ingredients, the concentration of phlorotannins in the extracts is much lower than that of pure phlorotannins' extracts. However, extraction and purification of phlorotannins contained in the extracts can increase the production costs. Therefore, a balance between concentration/availability of phlorotannins and extraction costs must be considered when phlorotannins are to be incorporated into food products. In this regard, Charoensiddhi et al. (2018) performed a case-study for the simulated industrial-scale production of high-value functional food products from the brown seaweed *E. radiata*. They analyzed the economic feasibility of different scenarios at a batch processing scale of 2000 kg seaweed and underlined the importance of assessing a balance between the desired purity of the extract and the operative costs of the process (Charoensiddhi et al., 2018).

All these considerations explain the reason why whole algae extracts are commonly employed as functional ingredients containing phlorotannins. This way, brown seaweed powders have been used in the formulation of pork meat, beef, and chicken products, fish in oil-enriched mayonnaise (Honold et al., 2016), milk (Hermund et al., 2015) and granola bars (Hermund et al., 2016), bakery products, among the most important ones (Roohinejad et al., 2017). Besides the antioxidant properties (mainly arising from phlorotannins), different technological properties (*e.g.*, texture, viscosity, gelification), ascribed to polysaccharides, are responsible for the benefits of using whole algae extracts.

Using phlorotannins' extracts in the formulation of functional foods is not extended so far. The main limitation of producing phlorotannins-rich extracts is related to the difficulty of producing them at an industrial scale and affordable costs and the lack of comprehensive knowledge of its chemical characterization. This explains why their application is still limited to specific technological purposes, and commercial phlorotannins are mostly employed (instead of phlorotannins extracted and purified from algae). The high cost of producing phlorotannins rich formulations may also justify their commercialization as nutraceuticals or their incorporation into premium products (also including nutraceuticals or cosmeceuticals), and opens up different markets for their commercialization. Several customers concerned about the incorporation of antioxidants would be willed to pay the costs of such premium products. In this sense, incorporating phlorotannins represents a great added value to diversify the offer of antioxidants containing products or shelf-stable products with natural antimicrobials and contributing to the development of innovative functional products.

However, the incorporation of these bioactive compounds into food formulations is a challenging task since the interaction between phlorotannins and the food matrix could significantly affect their stability. As the impact of food structure and technological processing on phlorotannins properties has not been addressed so far, it is useful to consider the interaction of plant-derived tannins (*e.g.* the condensed ones) with food macromolecules as a guide. It is well-known the affinity of proanthocyanidins for proteins, indeed, high molecular weight tannins are more expected to precipitate the saliva proteins than the smaller ones leading to a higher astringency sensation of certain plant-based foods (Cheynier, 2005). In addition, the acidic pH favors the formation of protein-tannin complexes. Briefly, proanthocyanidin link to proteins through hydrogen bonds and hydrophobic interactions resulting in soluble protein-proanthocyanidin complexes. Then, aggregates are formed by self-association which finally precipitate forming compacted colloids (Renard, Watrelot, & Le Bourvellec, 2017). On the other hand, condensed tannins also interact spontaneously with polysaccharides (mainly, those forming the cell-wall structure) during mechanical processing via hydrogen bonds and hydrophobic interactions, forming non-covalent complexes (Le Bourvellec et al., 2019). In addition, it was reported that during pasteurization of apple sauces which involved low pH conditions the procyanidin depolymerization may occur, forming covalent bonds by reacting with the nucleophilic groups of the cell wall. Considering these aspects, those fruits and vegetables-based products having low pH, organic acids, dissolved oxygen and other polyphenols compounds, as well as, demanding many processing steps may not be an adequate carrier of free phlorotannins.

Regarding bioavailability, the scientific evidence suggests that the complexes formed between tannins and carbohydrates or proteins are poorly digested in the upper intestinal tract because of steric hindrance, reaching almost unaltered the colon where they serve as substrates for the microbial community, resulting in readily absorbable metabolites. However, further research is required to better understand possible interactions between polyphenols and other constituents of the food matrix and how these complexes could interfere in the polyphenols-gut microbiota interactions.

Considering that these interactions between tannins and food constituents could be similar to those occurring between phlorotannins and food matrix during processing, strategies to enhance the stability and solubility of seaweed-derived polyphenols are thoroughly required. In this sense, the development of active food packaging materials containing phlorotannins may be a smart strategy to effectively preserve the overall quality of different food products and hence, extending their shelf-life without using synthetic additives (Surendhiran et al., 2019). Encapsulated phlorotannins' nanofibers have been proposed to wrap chicken meat and preserve it from eventual *Salmonella* contaminations (Surendhiran et al., 2019). These authors reported that encapsulated phlorotannins treatment was effective in reducing *S. enteritidis* counts of chicken inoculated since significant differences in comparison to non-treated samples during storage at 4 and 25 °C were observed. Also, they found that samples treated with encapsulated phlorotannins' fibers showed an enhanced sensory quality in comparison to non-treated ones and those samples treated with sodium nitrite (synthetic preservative). In this study, there is no mention of the safe arrival of phlorotannins-containing nanocapsules to the gut. However, Khan et al. (2019) reported that the administration of nanoencapsulated flavonoids improved their solubility, the gastrointestinal biodegradation and absorption when compared to free compounds. Considering these aspects, nanoencapsulation appears as a promising technology to ensure phlorotannins stability during food processing and their passage through the GIT.

Phlorotannins from *S. tenerimum* have demonstrated to be efficient for the ice preservation of white shrimps as inhibitory effects against polyphenol oxidase activity and melanosis formation during ice storage were observed (Sharifian, Shabanpour, Taheri, & Kordjazi, 2019). These authors concluded that the presence of resorcinol moieties in the phlorotannins composition could be associated with the inhibitory polyphenol oxidase activity. Also, those shrimps immersed in 5% phlorotannins solution showed higher scores on overall sensory acceptability in comparison to the control ones, and thus the sensory shelf-life of treated samples was extended for four more days.

The addition of phlorotannin-extracts into milk as functional ingredients was also investigated. In this regard, O'Sullivan et al. (2014) studied the incorporation of 0.25 and 0.5% (w/w) phlorotannins extracted from *A. nodosum* and *F. vesiculosus* into raw-homogenized milk. Then, enriched-milk was pasteurized (63 °C, 30 min), aseptically packaged and stored for 11 days at 4 °C. The authors reported that the addition of free phlorotannins significantly affected sensory attributes of milk by increasing the greenness and yellowness values and off-flavors

scores which led to a decrease in the product acceptability. Authors attributed these changes to the seaweeds pigments but no mention regarding the impact of pasteurization on phlorotannins structure and their interaction with other milk components which could have led to sensory changes was done. Possibly, these negative effects on sensory quality could have been avoided by adding phlorotannins in the encapsulated form. Another interesting result arising from this study is that the antioxidant activity of phlorotannins-rich milk samples (determined by the DPPH radical scavenging assay) remained stable after a simulated *in vitro* digestion, suggesting that the passage of phlorotannins through the GIT would not be affected by interaction with other milk components (O'Sullivan et al., 2014). Probably, the buffering capacity of milk (attributed to their whey proteins, caseins and fat) could have offered good storage protection of phlorotannins during their delivery to the GIT.

7. Conclusion and future prospects

The algae industry is highly committed to find natural sources of functional rich ingredients (*e.g.* phlorotannin, fucoidans, laminarin, fucoxanthin, carrageenan, alginate, etc.) from sustainable and cost-effective raw materials useful for innovation in the food and cosmetic industries. Although algae constitute a highly widespread renewable resource in fact, most brown seaweeds are underexploited and processed primarily into fertilizers and animal feeds, more studies are mandatory to better understand the effects of seaweed cultivation and exploitation on safety, toxicity and environmental issues.

The development and commercialization of phlorotannins-containing products are currently limited by the difficulty of producing them at an industrial scale and affordable costs and the lack of comprehensive knowledge of its full chemical characterization, which is necessary to support the potential biological activities reported in the documented *in vitro* assays. In this sense, more efforts are required on running advanced analytical techniques such as MALDI-ToF and HRMS, capable of unequivocally identifying complex phlorotannins mixtures (in terms of isomerization degree and different molecular weights) and resolving the metabolite profiles.

Pre-processing operations, extraction processes, as well as long-term storage, food manufacturing, and digestion conditions, play important roles in improving or reducing the phlorotannins content, thus altering their health benefits. This review showed the most recent applied approaches to overcome the effects of technological processes (from pre-processing to the passage through the gastrointestinal tract) on phlorotannins stability.

Concerning the stability of phlorotannins during pre-processing, extraction and storage, the most interesting observations drawn from this review are:

- Immediately after harvesting, freeze-drying algae material (free of impurities) and storage in vacuum-packed packages at freezing temperature can extend the algae shelf-life by reducing the moisture content and minimizing spoilage before extraction. Indeed, the lesser pre-treatments the algae receive, the better the phlorotannins content maintenance.
- As several parameters can affect the phlorotannins extraction efficiency including, solvent type, extraction time, temperature/pressure, solvent to solid ratio and the particle size of dry seaweed, optimization approaches focused on analyzing the effects of these influencing factors and their interactions on the extraction yield are highly required.
- Encapsulation can be a promising strategy to develop a stable product providing the necessary protective mechanisms to maintain the active molecular form of phlorotannins during storage up to the consumption time.
- Further research is needed to develop sustainable processing approaches of seaweed that ensure cleaner exploitation, easier industrial scale-up, improved resource efficiency, and production of co-products instead of waste (supported on the Green Chemistry principles).

Regarding health benefits, bioavailability and incorporation of phlorotannins into food, the most interesting conclusions are:

- Further *in vivo* scientific studies are required to support the health claims associated with the use of seaweed to prevent certain diseases, since *in vitro* fermentation studies only simulate static microbial metabolism without considering the dynamic colonic fermentation in which metabolites are continuously generated, absorbed and excreted.
- Few studies have shown that phlorotannins are poorly absorbed in the small bowel and further fermented by gut microbiota, resulting in the formation of lower-molecular-weight metabolites. However, scientific evidence supporting the metabolites' bioavailability is very limited (or even nonexistent), mainly because of the absence of available commercial standards which make the phlorotannins' characterization further complex.
- Comprehensive knowledge to elucidate the bacterial strains and enzymes responsible of fermenting phlorotannins, as well as, a more detailed understanding of their catabolic mechanisms is needed.
- Improved understanding is required from the impact of food matrix and technological processes (*i.e.*, dehydration, grinding, high concentrations of salt or sugars, thermal treatment, etc.) on phlorotannins stability during their passage through the GIT and the final interaction with colonic microbiota.

In light of the above explained, there is still a large gap with a great place for innovative developments and research opportunities to further explore the potential of seaweed-based polyphenols by the food and pharmaceutical industries.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work has received funding from CYTED (Ibero-American Program for the Development of Science and Technology) for the AQUACIBUS network (Strengthening aquaculture in Iberoamerica: quality, competitiveness and sustainability; Ref. 318RT0549), from the Argentinean Agency for the Scientific and Technological Promotion [PICT start-up (2016)/4808 and PICT(2017)/1344]. L.C. acknowledges the Secretaria General Iberoamericana (SEGIB) - Fundacion Carolina for a grant. L.C. and A.G.-Z are members of the research career of the Argentinean Research Council (CONICET). The research was also funded by FEDER under the program Interreg V Spain-Portugal (POPTEC, ref. 0377-Iberphenol-6-E); by MICINN supporting the Ramón&Cajal grant for M. A. Prieto (RYC-2017-22891); by Axudas Conecta Peme (Xunta de Galicia) supporting the IN852A 2018/58 NeuroFood Project supporting the pre-doctoral grant for Catarina Santos Pires Lourenço Lopes.

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