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Review

Antimicrobial potential of macro and microalgae against pathogenic and spoilage microorganisms in food



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ABSTRACT

Algae are a valuable and never-failing source of bioactive compounds. The increasing efforts to use ingredients that are as natural as possible in the formulation of innovative products has given rise to the introduction of macro and microalgae in food industry. To date, scarce information has been published about algae ingredients as antimicrobials in food. The antimicrobial potential of algae is highly dependent on: (i) type, brown algae being the most effective against foodborne bacteria; (ii) the solvent used in the extraction of bioactive compounds, ethanolic and methanolic extracts being highly effective against Gram-positive and Gram-negative bacteria; and (iii) the concentration of the extract. The present paper reviews the main antimicrobial potential of algal species and their bioactive compounds in reference and real food matrices. The validation of the algae antimicrobial potential in real food matrices is still a research niche, being meat and bakery products the most studied substrates.

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1. Introduction

Research on the production of algae under controlled conditions started after the Second World War, with the United States, Japan

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and Germany as pioneers in research and investment in this area (Guedes, Barbosa, Amaro, Pereira, & Malcata, 2011). Furthermore, the study of natural bioactive compounds obtained from marine organisms is a relatively new field of research (since the '90s), with enormous scientific potential (Cakmak, Kaya, & Asan-Ozusaglam, 2014; Mehadi et al., 2015). Given that oceans cover three quarters of the Earth's surface, these marine organisms are of potential interest not only as ingredients for the production of food but also as practical and necessary metabolites with medical and technological properties, such as lipids, enzymes, biomass, polymers, toxins, pigments, and clean fuel, which can be produced, concentrated, and successfully isolated from these small but efficient "marine bioreactors". These organisms are a viable and economical source for the production of these substances, and are in great demand in the nutraceutical, pharmaceutical, chemical, food, and cosmetic industries because of their moisturizing, antioxidant, and regenerative properties (Batista, Gouveia, Bandarra, Franco, & Raymundo, 2013; Devi, Suganthy, Kesika, & Pandian, 2008; Rani, Singh, & Maheshwari, 2013). The possibility of an efficient and sustainable use of marine resources offers an important way of providing staple food, animal feed, pharmacological products, functional ingredients, and medical solutions for a global population that is rapidly increasing.

The term "algae" comprises a complex and heterogeneous group of photosynthetic organisms characterized by their photosynthetic nature and their simple reproductive structures. The algae group is divided into multicellular organisms, MACROALGAE or seaweed, and unicellular organisms, known as MICROALGAE (measuring from 1 μm to several cm). Macroalgae are often fast-growing, reaching sizes of up to 60 m in length. A standard classification has been established, dividing seaweed organisms into three groups on the basis of their pigmentation: i) brown seaweed (*Phaeophyceae*); ii) red seaweed (*Rhodophyceae*) and iii) green seaweed (*Chlorophyceae*). Seaweeds are mainly used for the production of food and the extraction of hydrocolloids. On the other hand, microalgae are microscopic bodies that normally grow in suspension, some with features characteristic of bacteria. These algae grow in seawater, in the region where light penetrates (photic zone), basically up to a depth of 200 meters. Diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*), and golden algae (*Chrysophyceae*) are the most important microalgae in terms of abundance, but blue-green algae (*Cyanophyceae*) are also classified as microalgae (e.g. *Spirulina*) (Guedes et al., 2011).

More than ten million algal species are estimated to exist nowadays, most of them microalgae, representing a virtually unexplored plant material. So far, only about 50 species have been studied in detail from a physiological and biochemical point of view, in the discipline of "phycology".

The remarkable biodiversity of algae, ranging from species found in the coldest regions of Antarctica to those that grow in the hottest deserts, opens up a new research niche, mainly in the baseline of the "functionality of these marine vegetables".

Among the various research fields in which macro- and microalgae are appearing, gastronomy and food technology are two of the most important areas. New trends in cooking are emphasizing the use of algae as healthy, tasty, colorful ingredients to accompany the most innovative dishes. France, Ireland, Canada, and the United States are particularly active in introducing seaweed into local cuisine, and the movement is spreading to cookery books that include recipes with algae as the main ingredient (FAO, 2012). Consumers have a good perception of algae as "natural products", like lettuce, chard, or broccoli, with well-known, accepted health benefits (Honkanen, 2009). Humans have certainly been consuming algae for years: pet foods, baby foods, dairy products, instant soups, meat coatings (cooked ham), and many others commodities are clear examples of the presence of seaweed in our daily diet. Nowadays,

most of these algae ingredients correspond to EU labeled additives E-406, E-401, E-402, E-403, and E-404 (Regulation (EC) No 1333/2008).

Exciting organoleptic properties (color, flavor, aroma, and taste) are being developed by introducing algae into the formulation of novel products with additional technological functions, including preservative functions (antibacterial, antifungal, antiviral, bacteriostatic) (El Shoubaky & El Rahman-Salem, 2014; Sanmukh et al., 2014; Tüney, Çadirci, Ünal, & Sukatar, 2006), structural functions (emulsifying, gelling, and thickening properties attributed to algae) (Ursu et al., 2014), and nutritional properties (vitamins, proteins, polyunsaturated fatty acids) (Bishop & Zubeck, 2012; El-Baky, El Baz, & El-Baroty, 2008). *Spirulina maxima*, *Chlorella vulgaris*, *Haematococcus pluvialis*, *Diacronema vlkianum*, and *Isochrysis galbana* are some of the most interesting algae with potential bioactive properties (Batista et al., 2013). All of them are able to accumulate high amounts of bioactive compounds with functional and technological properties. Several products, such as pasta, bread, and snacks, are being developed with the incorporation of algae extracts in their formulation, and expectations about this practice are promising for the food industry.

In view of the development of antibiotic resistance in bacteria and international trade pressure to achieve a high level of consumer protection, new alternatives to traditional preservatives should be developed and introduced by the food industry, even in products with limited shelf life. The new line of preservatives should also accomplish two further objectives, (i) to preserve the quality and organoleptic value of the product, and (ii) to satisfy consumer demand for natural, functional, ready-to-eat meals. In this connection, marine algae are emerging as a new generation of potential preservatives, macro- and microalgae extracts, or pure ingredients with health benefits and demonstrated antibacterial, antifungal, and antiviral activities (Dai & Mumper, 2010; Devi et al., 2008).

Although intensive study of the antimicrobial potential of algae has begun [2005–2016], most of the studies that have been published are about therapeutic and antibacterial/antiviral capabilities of algae compounds, their ability to inhibit or kill clinical bacteria (Mehadi et al., 2015; Rajeshkumar et al., 2014; Rhimou, Hassane, José, & Nathalie, 2010), but not about the effect of these bioactive molecules against foodborne pathogens and spoilage microorganisms commonly found in food matrices.

2. General view of algae compounds with antimicrobial potential

Among the major bioactive constituents of algae with demonstrated antimicrobial potential, proteins, polysaccharides, polyunsaturated fatty acids (PUFAs), especially EPA and DHA, amino acids, and antioxidants (polyphenols, flavonoids, and carotenoids) are the most important ones (Al-Saif, Abdel-Raouf, El-Wazanani, & Ibrahim, 2014; Senthilkumar & Sudha, 2012). However, the identification of compounds directly responsible for the antimicrobial potential of algae is still a relatively incipient field of research, mainly owing to the new kinds of compounds found in recent years (Amaro, Guedes, & Malcata, 2011).

2.1. Protein and peptides

The recent year 2014 was designated as "Protein Year", underlining the importance of finding alternative sources of these valuable molecules, proteins of animal and vegetable origin. In this field, algae take a high position among the raw materials proposed as alternative protein sources, together with soy, beans, grains,

and, recently, insects (FAO, 2016). Algae protein quality has been considered superior to that of other plant sources, such as wheat, rice, or beans, but poorer than that of animal protein sources, such as milk or meat (Mendes, Lopes da Silva, & Reis, 2007). However, interest in marine proteins might be directly correlated not only with intact protein but also with the possibility of generating bioactive peptides. Small peptides are generally considered to be the most ancient antimicrobial agents because of their ubiquity and simple molecular structure. In general, bioactive peptides comprise relatively small molecules (<10 kDa, or 12–50 amino acids) that do not present any bioactivity prior to being released from the intact parent protein. However, in various processes such as digestion or *in vivo* hydrolysis, or as a result of application of technological treatments such as high pressure processing, these peptides demonstrate many physiological functions, including antioxidant, antihypertensive or ACE inhibition, anticoagulant, and antimicrobial properties (Ngo, Wijesekara, Vo, Ta, & Kim, 2011). Antimicrobial peptides are recognized as being divided into three groups: (i) linear α -helical peptides; (ii) cysteine-rich peptides; (iii) certain amino-acid-enriched peptides. Critical factors affecting the antibacterial activity and modes of action of antimicrobial peptides are size, charge, conformation/secondary structure, hydrophobicity, and origin (animal/plant or marine) (Aneiros & Garateix, 2004).

According to the studies of Al-Saif et al. (2014), the higher antimicrobial potential of several algae strains against *Escherichia coli* (ATCC 25322), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), and *Enterococcus faecalis* (ATCC 29212) was directly related to the greater protein content detected in them. According to those studies, the most effective marine alga against the bacteria that were tested was *G. dendroides*, with a protein content of 13.4%, followed by *U. reticulata* (5.8%), *Cladophora socialis* (2.3%), and *C. occidentalis* (1.7%).

Lectins are a class of carbohydrate-recognizing proteins that bind to cells, promoting hemagglutination and an antimicrobial effect. Smith, Desbois, and Dyrzynda (2010) reported the antimicrobial potential of lectins obtained from algae, with two red algal species, *Euclima serra* and *Galaxaura marginata*, being responsible for an inhibiting potential against *Vibrio vulnificus* and *V. parvulus*. The antimicrobial activity of lectins obtained from red algae against some clinical microorganisms was also observed by Alves-Vasconcelos et al. (2014). Although the way in which lectins act against bacteria is not well defined, Paiva et al. (2010) reported that the antibacterial activity of these compounds against Gram-positive and Gram-negative microorganisms is related to the interactions that occur between lectins and other bacterial cell wall components, including teichoic acids, peptidoglycans, and lipopolysaccharides (Table 1).

Table 1
Most effective antimicrobial extracts from algae against foodborne pathogenic bacteria.

Microorganism	Solvent extract	Algae	References
Gram (+) bacteria			
<i>Staphylococcus aureus</i>	Ethanol extract	<i>Scenedesmus obliquus</i>	Najdenski et al. (2013)
	Ethanol extract	<i>Chlorella vulgaris</i>	Danyal et al. (2013)
	Methanol extract	<i>Nostoc sp.</i> ; <i>Microcystis</i> sp.; <i>Scenedesmus</i> sp.; <i>Oscillatoria geminata</i> ;	Salem et al. (2014)
	Methanol and Ethylacetate extracts	<i>Chlorella vulgaris</i>	
	Methanol and ethanol extracts	<i>Turbinaria conoides</i>	Manivannan et al., 2011
Most effective (38 mm inhibition zone)	Diethyl ether extract	<i>Dunaliella salina</i>	Cakmak et al. (2014)
		<i>Enteromorpha linza</i>	Tüney et al. (2006)
<i>Bacillus cereus</i>	Dichloromethane: methanol extract	<i>Spirulina maxima</i>	El-Baky et al. (2008)
	Methanol extract	<i>Laurencia okamurae</i> ; <i>Dictyopteris undulata</i>	Jang and Lee (2015)
Most effective (27 mm inhibition zone)		<i>Chaetomorpha linum</i>	Senthilkumar and Sudha (2012)
<i>Listeria monocytogenes</i>	Methanol extract	<i>Ecklonia cava</i>	Nshimiyumukiza et al. (2015)
	Ethanol extract	<i>Dunaliella salina</i>	Cakmak et al. (2014)
	Ethanol extract	<i>Myagropsis myagroides</i>	Lee et al. (2014)
	Diethyl ether, <i>n</i> -hexane, and chloroform	<i>Himantalia elongata</i>	Rajauria and Abu-Ghannam (2013)
Most effective (10 mm inhibition zone)			
Gram (–) bacteria			
<i>Escherichia coli</i> O157:H7	Phlorotannins (PT)	<i>Ascophyllum nodosum</i>	Wang et al. (2009)
	Methanol extract	<i>Chaetomorpha linum</i>	Ngo et al. (2011)
	Diethyl ether extract	<i>Enteromorpha linza</i> ; <i>Ulva rigida</i>	Senthilkumar and Sudha (2012)
	Ethanol extract	<i>Dunaliella salina</i>	Tüney et al. (2006)
	Diethyl ether extract	<i>Padina gymnospora</i>	Cakmak et al. (2014)
Most effective (22 mm inhibition zone)			Manivannan et al. (2011)
<i>Samonella spp.</i>	Diethyl ether extract	<i>Dictyota dichotoma</i>	Thirumaran and Anantharaman (2006)
	Diethyl ether extract	<i>Turbinaria conoides</i>	Manivannan et al. (2011)
	Chloroform and Methanol extracts	<i>Padina gymnospora</i>	Manivannan et al. (2011)
	Ethanol extract	<i>Pithophora oedogonium</i>	Sanmukh et al., 2014
	Ethanol and methanol extracts	<i>Dunaliella salina</i>	Cakmak et al. (2014)
Most effective (17 mm inhibition zone)			

2.2. Carbohydrates

Macroalgae are rich sources of dietary fiber (25–75%), of which water-soluble fiber constitutes approximately 50–85% (wet basis). Polysaccharides are some of the most important constituents of seaweed (Kraan, 2012). *Phaeophyta*, or brown algae, are specifically rich in polysaccharides, including alginates, laminarins, fucans, and cellulose. *Chlorophyta*, or green algae, are mainly composed of ulvan. The principal polysaccharides found in *Rhodophyta*, or red algae, are agars and carrageenans. These polysaccharides obtained from algae have been related to positive effects on gut microbiota, acting as prebiotics, a prebiotic being a “selectively fermented ingredient that allows specific changes, both in the composition and/or in the activity of the gastrointestinal microflora, that confers benefits upon host well-being and health”. Reduction of enteric infections in pigs and cattle is possible by means of administration of prebiotic compounds in the diet which promote the activity and proliferation of beneficial gastrointestinal microflora to the detriment of pathogenic bacteria (Uyeno, Shigemori, & Shimosato, 2015). The use of marine algal prebiotics as sources to control and reduce pathogenic bacteria and to improve animal and human health is another possibility that this line of research offers (Promya & Chitmanat, 2011). The polysaccharide ulvan is easily extracted from *Ulva rigida*, and could be hydrolyzed to produce bioactive oligosaccharides (Mišurcová, Škrovánková, Samek, Ambrožová, & Machů, 2012). Also, oligosaccharides from brown algae have demonstrated antimicrobial potential *in vivo* inhibiting *Salmonella enteritidis* colonization in broiler chickens (Asthon Acton, 2012).

Another very interesting group of polysaccharides obtained from algae with a demonstrated antimicrobial potential is Fucoindans (polysaccharides from *Phaeophyta*). Among the most important properties of these polysaccharides are the following: anticoagulant, antithrombotic, antiviral, antitumor, immunomodulatory, antioxidant, and anti-inflammatory (Li, Lu, Wei, & Zhao, 2008; Marudhupandi & Kumar, 2013). According to the studies of De Jesus Raposo, Bernardo de Moraes, and Santos Costa de Moraes (2015), sulphated polysaccharides from seaweeds (among them alginates, fucoidans and laminaran) have demonstrated to be effective against *E. coli* and *Staphylococcus aureus* (*Chaetomorpha aerea*, 50 mg/mL of extract). Moreover, also carrageenans and the sulphated exopolysaccharide (sEPS) from the red microalga *Porphyridium cruentum* are specifically effective inhibiting one of the most relevant foodborne pathogens, *Salmonella enteritidis* (Pierre et al., 2011).

Recent studies have also demonstrated the potential of fucoidans for preventing *Helicobacter pylori* infection, one of the most concerning emergent foodborne pathogens affecting 50–80% of the worldwide population. The effect of fucoidan from brown algae

as a potential antimicrobial against *H. pylori* thereby reduces the risk of associated gastric cancers (Marudhupandi, Kumar, Senthil, & Devi 2014). *Laminaria* spp. extract containing either laminarin or fucoidan, or a combination of both, resulted in a reduction of fecal *E. coli* populations in piglets fed with 0.3 and 0.24 g/kg, respectively (O'Doherty, McDonnell, & Figat, 2010), consequently reducing the initial bacterial load in derived raw meat products (Table 2).

These studies point out the potential of prebiotics from algae origin not only as potential antimicrobial ingredients in food, but also such as antimicrobial agents *in vivo* to be regularly added to diet and inhibiting the pathogenic bacterial proliferation in intestine (e.g. in both animal and human fed).

2.3. Polyphenols and other antioxidants

One of the most valuable nutritional properties of algae is related to their high content of polyphenols, carotenoids, and flavonoids, referred to as antioxidants. Antioxidants act to protect the human body against damage by reactive oxygen species (ROS), which can lead to health disorders such as cancer, diabetes mellitus, neurodegenerative diseases, and inflammatory diseases with severe tissue injuries (Rani et al., 2013).

Among the different metabolites in algae, antioxidants are the most extensively studied (Manivannan, Karthikai, Anantharaman, & Balasubramanian, 2011; Senthilkumar & Sudha, 2012). Antioxidants are very powerful tools with which to fight oxidative stress and thus improve the health status of the general population (Rani et al., 2013). Phenols constitute the largest group of secondary metabolites identified in algal species. In recent studies, a broad spectrum of *in vitro* antibacterial activity has been associated not only with plant phenols but also with phenols from algae, specifically against *Staphylococcus aureus* and *Bacillus* spp. Other antimicrobial phenolic compounds isolated from the marine environment include anthraquinones, coumarins, and flavonoids (Amaro et al., 2011).

Rutin, quercetin, and kaempferol flavonoids have been identified in all the algal species with antimicrobial potential, being present in different ratios in different species. According to the studies of Al-Saif et al. (2014), the alga *G. dendroides* showed the highest concentration of these three flavonoids (rutin, 10.5 mg/kg; quercetin 7.5 mg/kg; kaempferol 15.2 mg/kg), and was also the most effective of the flavonoids studied in inhibiting bacterial growth (*E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis*).

2.4. Fatty acids (FAs)

The first antibacterial compound isolated from a microalga, *Chlorella* spp., was a mixture of fatty acids. This compound was

Table 2
Some antimicrobial applications of algae in real food matrices.

Product	Algae	Effect	Microorganisms	References
Meat/fish and derived products				
Frozen meat products	3% (w/w) dried <i>Ascophyllum nodosum</i>	Reduction 1-log ₁₀ cycle	Gram (–) bacteria	Brownlee et al. (2012)
Frozen meat products	Methanolic extracts <i>Ascophyllum nodosum</i>	Reduction growth kinetics	<i>Bacillus cereus</i> and <i>Staphylococcus aureus</i>	Brownlee et al. (2012)
Half smoked sausages	Brown algae [10–15]%	Bacteriostatic effect	<i>S. aureus</i> <i>E. coli</i>	Brownlee et al. (2012)
Bacon	Red algae edible films	0.45 log ₁₀ CFU/g 0.76 log ₁₀ CFU/g	<i>E. coli</i> O157:H7L. <i>monocytogenes</i>	Shin et al. (2012)
Cold smoked salmon	Alginate and carrageenan edible films	3–4 log ₁₀ CFU/g	<i>L. monocytogenes</i>	Neetoo et al. (2010)
Bakery products				
Bread	<i>Ascophyllum nodosum</i> 50% (w/w)	Equivalent to 5 g sodium chloride	Mould growth inhibition	Brownlee et al. (2012)

effective against both Gram-positive and Gram-negative bacteria (Vello et al., 2014). Membrane-derived FAs from macro- and microalga species have been associated with microbicidal activity as a mechanism of defense against viruses, protozoans, and bacteria (Leflaive & Ten-Hage, 2009).

The characteristic saturated and unsaturated fatty acids profile in algae, with a predominance of myristic, palmitic, oleic, and eicosapentaenoic acids (EPA), is a specific feature associated with the antimicrobial potential of algal species (El Shoubaky & El Rahman-Salem, 2014). Furthermore, palmitic acid has been assumed to be primarily responsible for the antibacterial activity of algae (Al-Saif et al., 2014; Bazes et al., 2009). According to Al-Saif et al. (2014), the algae extracts richest in palmitic acid > oleic acid > linoleic acid > myristic acid in concentration ranges of [43.7–75.5]% > [3.53–17.24]% > [0.6–16.56]% > [2.13–11.2]%, respectively, were associated with the highest antimicrobial potential, and the alga with the highest percentage of palmitic acid (75.5%), *G. dendroides*, was the most effective one against the bacteria studied. According to Guedes et al. (2011), the mechanism of action of fatty acids as antimicrobials may be due to cell leakage derived from membrane damage.

Some efforts are now being focused on identifying the compounds directly responsible for the antimicrobial capability of macro- and microalgae, taking into account that this is a relatively unexplored field of study (Sanmukh et al., 2014). In general, it could be said that the most antimicrobial compounds in algae are mainly polyphenols and polysaccharides that act by inhibiting microbial growth, or directly by destroying the living structures of microorganisms (Bajpai, 2016).

3. Techniques for extracting ANTIMICROBIALS from algae

Novel extraction technologies (Araujo et al., 2013; Esquivel-Hernández et al., 2016) and chemical extraction procedures (Adam, Abert-Vian, Peltier, & Chemat, 2012; Hamed et al., 2013), are both applied to obtain selective extracts of algae rich in desired functional/antimicrobial compounds (Dai & Mumper, 2010).

Since the late '70s, various processes for obtaining extracts from seaweed have been developed, based on solid–liquid extraction, mainly by combining organic solvents, for example, [chloroform: methanol] mixtures, or acetone. Additionally, some operations such as ultrafiltration processes are added to enhance selectivity. Other methods use ethanol in a first step to precipitate protein, followed by a second stage extraction with hexane, butanol, or ethyl acetate. Recent studies show that it is possible to use ternary mixtures of solvents (ethanol–hexane–water [77:17:6]) to form a homogeneous solution, with the advantage of significantly increasing the extraction yield and purity of the compounds extracted by a single stage, based on the high solubility of the compounds in the extractant mixture (Parniakov et al., 2015). The antimicrobial potential of algae extracts is dependent on the capability of the solvent to extract certain bioactive compounds, and also dependent on the sensitiveness of bacteria or fungi to these selective extracted compounds (Al-Saif et al., 2014; Cakmak et al., 2014). In general, these traditional extraction techniques, such as Soxhlet, solid–liquid extraction (SLE), or liquid–liquid extraction (LLE), are time-consuming procedures that use high volumes of solvents and obtain low extraction yields.

Growing concern about the use of clean technologies to extract bioactive compounds from algae has led the international scientific community and R & D engineers to invest in technologies such as supercritical fluid extraction, extraction using high intensity

pulsed electric fields (PEFs), ultrasonically assisted extraction (USE) (Parniakov et al., 2015), microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE), using pressure and temperature (Esquivel-Hernández et al. 2016) to preserve as much as possible of the quality and bioactivity of the extracted compounds. Degradation of cell walls by an enzymatic pathway has also been used in microalgal treatment (e.g. in *Chlorella vulgaris*), but it is still too expensive to be applied widely in industry (Hamed et al., 2013).

The process of supercritical fluid extraction using CO₂ is one of the most advanced techniques for obtaining bioactive compounds from algae. It is a highly efficient and fast way of obtaining extracts with high purity and rich in the desired functional compounds, but it remains relatively expensive as an industrial-scale method (Mendiola et al., 2007).

Ultrasonically assisted extraction (USE) is based on mechanical acoustic cavitation effects exerted on algae cell walls. Among its main advantages are the effectiveness of the extraction process at room temperature, and therefore minimal loss of bioactive compounds. In microalgae, the use of ultrasound is already beginning to be widely used, with good results (Adam et al., 2012; Araujo et al., 2013).

The use of high intensity electrical pulses (PEFs) is a method in which the application of high voltage pulses disrupts cellular material, facilitating the release of components such as proteins, chlorophylls, and carotenoids, among others. It has been successfully applied in bioactive extracts from *Spirulina* and *Chlorella* species. The method is based on the theory of electroporation, as the conductivity and permeability changes that occur in the cell membrane favor the formation of small pores that allow release of intracellular components to the environment (Parniakov et al., 2015).

Various *Dunaliella* genus microalgal species have been treated by MAE to favor extraction of carotenoids. Microwave-assisted extraction promotes extraction by ohmic heating and homogeneous temperature distribution. The use of this technology in *Dunaliella tertiolecta* and *Cylindrotheca closterium* species has resulted in a process that combined rapid extraction, reproducibility, and a high yield (Pasquet et al., 2011), and other antimicrobial compounds (Kadam, Tiwari, & O'Donnell, 2013).

4. Antimicrobial potential of algae against foodborne pathogens and spoilage microorganisms

Until now, the antimicrobial potential of algae has generally been tested *in vitro*, using the agar diffusion method (Cakmak et al., 2014; Manivannan et al., 2011; Qiao, 2010). The broth dilution method (Gupta, Rajauria, & Abu-Ghannam, 2010) has also been used, providing a robust quantitative estimation of minimum inhibitory concentration (MIC) values in a large number of samples.

In the context of the present *state of the art*, in response to the food industry's demand for novel and alternative ingredients with high technological potential, the possibility of using seaweed and microalgae as natural preservatives to be added in the formulation of “clean labeled foods” will soon become a reality. Nowadays, the antimicrobial potential of algae against the main foodborne pathogens and spoilage microorganisms is one of the most interesting fields of research regarding the use of marine vegetables as sources of staple food and bioactives for human nutrition. The scientific advances and research focusing on this field that have been published to date are detailed below in order to provide the scientific community with a useful overview to help to guide future research trends/needs of research and the establishment of innovative projects.

4.1. *Staphylococcus aureus*

According to Najdenski et al. (2013), *Scenedesmus obliquus*, *Chlorella* sp., and *Nostoc* sp. ethanolic extracts showed antibacterial potential against *S. aureus*. Similarly, according to the studies of Danyal, Mubeen, and Malik (2013), ethanolic extract of *Pithophora oedogonium* was effective in inhibiting the *S. aureus* growth. Methanol and acetone extracts of *Scenedesmus* spp. also exhibited antibacterial activity against *S. aureus* according to Guedes et al. (2011). Furthermore, Ishaq, Matias-Peralta, and Basri (2016) found significant inhibitory activity of *Scenedesmus* spp. acetone extract (0.35 mg/mL–3.48 mg/mL) against *Staphylococcus aureus* (ATCC 25923).

Methanolic extracts from *Nostoc* spp., *Microcystis* spp., *Scenedesmus* spp., *Oscillatoria geminata*, and *Chlorella vulgaris* exerted a high antimicrobial potential against *S. aureus* and *B. subtilis*, with inhibition zone diameters between 16 and 18 mm (Salem, Hoballah, Ghazi, & Hanna, 2014). These results are in agreement with those obtained by Prakash, Marimuthu, and Jeeva (2011) regarding the antimicrobial potential of *Oscillatoria sancta* and *Lyngbya birgei* against this foodborne pathogen.

Manivannan et al. (2011) tested the brown seaweeds *Turbinaria conoides* and *Padina gymnospora* for their antimicrobial potential against *S. aureus*. *S. aureus* proved to be more resistant to *T. conoides* extracts (3–15 mm inhibition zone), but was more sensitive to *P. gymnospora* (10–16 mm inhibition zone). Methanol and ethyl acetate extracts of *T. conoides* were the two most inhibitory ones against *S. aureus*. However, when growing *S. aureus* cells were exposed to *P. gymnospora*, diethyl ether and acetone were the extracts that inhibited cellular proliferation most effectively. Methanolic and ethanolic extracts of *Dunaliella salina* showed the lowest MBC against *S. aureus* (1.25 mg/mL) compared with other solvent extracts, such as hexane (MBC = 10 mg/mL) (Cakmak et al., 2014).

The greatest antimicrobial potential of algae extracts against *S. aureus* was reported by Tüney et al. (2006), who observed an inhibitory diameter zone > 50 mm due to exposure of *S. aureus* to diethyl ether extract (0.5 g/mL) of fresh *Enteromorpha linza* (0.5 g/mL), and a 38 mm inhibition zone in the case of fresh *Ulva rigida*. This antimicrobial capability was not detected when the same fresh alga material was extracted with ethanol solvent. This led the researchers to the conclusion that some active compounds responsible for the antimicrobial potential against *S. aureus* are effectively extracted in diethyl ether, which produces larger halo zones than methanol, acetone, and ethanol, in which antimicrobial compounds are not completely dissolved and extracted. Among the most effective antimicrobial compounds found in these algal species are terpenes, (e.g. usneoidone E, zosterdiol A, zosterdiol B, zosteronol, and zosteronediol) responsible for the antimicrobial and antiviral activity attributed to them (Plaza del Moral & Rodríguez-Meizoso, 2013).

The antimicrobial potential of algae extracts is frequently compared with the potential of other currently used preservatives (at food industry level) or antibiotics (at clinical level). For example, Devi et al. (2008) observed the effectiveness of *Haligra* spp. seaweed extract (50 mg/mL) against *S. aureus* (MTCC 96), and found that the inhibitory potential of this alga extract was higher than the antimicrobial capability of sodium benzoate applied at a higher concentration (200 mg/mL).

To date, most of the antimicrobial *in vitro* studies regarding the potential of algae against *S. aureus* have not been validated in food matrices. However, the promising results obtained against *S. aureus* were validated by the Devi et al. (2008) research group by including *Haligra* spp. extract at low concentrations in skimmed milk, demonstrating the effectiveness of 5 mg/mL as the minimum inhibitory concentration (MIC) against *S. aureus* growth in dairy products.

4.2. *Escherichia coli*

Ascophyllum nodosum (brown *Phaeophyceae* alga, in the *Fucales* family) has been described by several authors as being effective in reducing the prevalence of *E. coli* O157:H7 in cattle before harvest (Wang, Xu, Bach, & McAllister, 2009). Moreover, according to the studies of Wang et al. (2009), antimicrobial activity from *A. nodosum* phlorotannins (PTs) against various rumen microbes was observed, and it affected ruminal fermentation. In 2009 they published a new research work in which the bacteriostatic and bactericidal effects of PTs from *A. nodosum* were evaluated against *E. coli* O157:H7, and were then compared with the antimicrobial potential of other tannins from terrestrial plant sources. An MIC of 25 µg/mL was required to inhibit growth of *E. coli* O157:H7 strains (EDL933 and E318 N) for 24 h at 37 °C. PT bacteriostatic concentrations of 25 µg/mL or higher, and a bactericidal concentration ≥ 50 µg/mL, were required against the *E. coli* O157:H7 strains studied. According to Ngo et al. (2011), the potential biological activities of PTs can protect the quality of food products against oxidative degradation by means of their antioxidant properties, and they can improve the safety of non-sterilized products as a control measure against microbial proliferation in the food chain.

The antimicrobial potential exerted by PTs from *A. nodosum* was explained by Wang et al. (2009). The technique of transmission electron microscopy was used to reveal the mechanism of action of PTs against *E. coli* cells. The results of Wang et al. (2009) indicated that tannins acted primarily on the bacterial cell wall. It seems that the patterns of structural disorganization by means of PT intervention were higher than the pattern observed as a result of the effect of terrestrial tannins *E. coli* O157:H7 cells.

The antimicrobial potential of *Chaetomorpha linum*, a green seaweed from the southeast coast of India, was tested against *Escherichia coli* (MTCC No. 443), *Salmonella* Typhimurium (MTCC No. 98), and *Bacillus cereus* (MTCC No. 430). Methanolic extracts (90% w/v) of algae were tested by means of the agar diffusion method, assessing not only their antimicrobial capability but also their antioxidant potential, using the 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) method. Bacterial suspensions containing 1.5×10^8 CFU/mL were inoculated in Mueller–Hinton agar plates. Algal extracts were prepared at concentrations of 100, 300, and 500 mg/mL. The Petri dishes were incubated for 16 h at 37 °C and the inhibition zone was examined. A high correlation was found between the DPPH antioxidant potential of methanolic *C. linum* extracts and the microbial inhibition capability exerted. The highest phenolic content of *C. linum* extract was 672.3 mg/GAE/100 g/extract. *E. coli* was the most resistant bacterial strain to the effect of *C. linum* methanolic extract (Senthilkumar & Sudha, 2012).

The antimicrobial potential of *T. conoides* and *P. gymnospora* (Kütz) Vicker was tested by Manivannan et al. (2011) against several microorganisms, including *E. coli*. The extraction procedure was carried out successively during a period of 10 h, using the following solvents: methanol, acetone, petroleum ether, ethanol, ethyl acetate, chloroform, and diethyl ether. The study conducted by Manivannan et al. (2011) revealed that *P. gymnospora* extracts were significantly more effective against *E. coli* (8–17 mm inhibition zone diameter) than *T. conoides* extracts (2–8 mm inhibition zone diameter). When the bacterial growth was exposed to *P. gymnospora* extracts, the diethyl ether extract showed the greatest potential for inhibiting bacterial growth in agar plates, with a 17 mm inhibition zone diameter.

The antimicrobial effectiveness of methanol, acetone, diethyl ether, and ethanol extracts from eleven seaweed species, fresh extract and dried extract, against growth of *E. coli* was assessed by Tüney et al. (2006). The results revealed that diethyl ether extracts of fresh *C. mediterranea*, *E. linza*, *U. rigida*, *G. gracilis*, and *E. siliculosus* (0.5 g/mL) showed effective results against

Gram-positive and Gram-negative bacteria. The best inhibition results obtained with fresh and dried diethyl ether seaweed extracts were assayed against various pathogenic microorganisms. *U. rigida* and *E. linza* diethyl ether extracts showed the highest inhibition potential against *E. coli* (22 mm inhibition zone diameter), being *C. mediterranea* and *G. gracilis* also effective, with inhibition zone values between 16 and 20 mm. All of the most effective inhibition results were obtained with fresh materials.

With regard to microalgae, the antimicrobial potential of *D. salina* against *E. coli* has also been tested. Among the extracts studied, methanol and ethanol extracts of *D. salina* showed the highest effectiveness, achieving the lowest MBC against *E. coli*, 2.50 mg/mL, compared with other solvent extracts such as hexane, which had an MBC of 10 mg/mL (Cakmak et al., 2014).

4.3. *Salmonella* spp

According to Manivannan et al. (2011), *Salmonella* Typhimurium was slightly affected by *T. conoides* extracts, and was particularly sensitive to diethyl ether and petroleum ether extracts, with inhibition zone diameters in the range [8–11] mm. These results are in agreement with those obtained by Thirumaran and Anantharaman (2006) regarding the antimicrobial potential of *Dictyota dichotoma* against *Salmonella* spp., concerning which they remarked that diethyl ether extracts showed the greatest inhibitory potential against *Salmonella* Paratyphi.

On the other hand, *P. gymnospora* extracts showed higher antimicrobial potential against *S. Typhimurium*, with inhibition zones of [7–24] mm. Among the *P. gymnospora* extracts, chloroform and methanol were the most effective ones against this foodborne pathogen. *T. conoides* ethyl acetate extract showed the lowest inhibitory potential against *Salmonella* spp. (≈ 3 mm inhibition zone). The same inhibitory potential was detected for *T. conoides* acetone extract against *Salmonella* spp. (≈ 2 mm inhibition zone). The ethanolic extract of *Pithophora oedogonium* was particularly effective against *Salmonella* spp. (Sanmukh et al., 2014).

Similarly, ethanol extract of *D. salina* showed the lowest MBC against *S. enteritidis* (1.25 mg/mL), followed by methanolic extract with an MBC of 2.50 mg/mL. Hexane extract was the least effective against *S. enteritidis*, with a required MBC of 10 mg/mL (Cakmak et al., 2014). High inhibition potential against *S. Typhimurium* was exerted by *Chaetomorpha linum* methanolic extract with concentrations in the range [100–500] $\mu\text{g/mL}$, producing inhibition zone diameters in the range [15–17] mm (Senthilkumar & Sudha, 2012).

Ethanol extracts of *P. oedogonium* and *Botrydiopsis arhiz* algal species were tested to assess their antimicrobial capability against two *Salmonella* species isolated food samples, egg and meat, and named *Salmonella* 1 and *Salmonella* 4, respectively. The results revealed that *P. oedogonium* ethanolic extract was effective in inhibiting *Salmonella* 1 growth, with an MIC of 4 mg/mL (Danyal et al., 2013).

4.4. *Bacillus cereus*

According to the studies of El-Baky et al. (2008), several extracts of *Spirulina maxima* were obtained from cells grown under different nitrogen levels [0.625–2.5 g/L NaNO_3], and their antimicrobial potential was assayed against various strains of bacteria. The *S. maxima* extracts were effective against *B. cereus*, with an antibacterial activity that was dose-dependent. MICs in the range [30–50] $\mu\text{g/mL}$ were obtained, depending on the *S. maxima* extract applied. *S. maxima* extracts from cells grown at higher N levels were the most potent against all bacteria, with MIC values of 30 $\mu\text{g/mL}$. The antibacterial activity of *S. maxima* was attributed by El-Baky

et al. (2008) to the presence of certain active components in all organic extracts, such as lipophilic and phenol compounds.

According to the studies of Cakmak et al. (2014), different extracts (ethanol, hexane, dichloromethane, and methanol) of the microalga *D. salina* Teodoresco (*Dunaliellaceae*) were tested against various microorganisms, including *Bacillus cereus* RSKK 863. A minimum bactericidal concentration of 0.65 mg/mL of ethanol extract was required against *B. cereus*, corresponding to 2.50 mg/mL of *D. salina* fatty acids (palmitic (C16:0) > linolenic (C18:3 $\omega 3$) > oleic (C18:1 $\omega 9$)).

The studies of Jang and Lee (2015) revealed the high antimicrobial potential of two Korean domestic algae, *Laurencia okamurae* Yamada and *Dictyopteris undulata* Holmes, against foodborne pathogens. *B. cereus*, *S. aureus*, and *L. monocytogenes* were particularly sensitive to *Laurencia okamurae* Yamada and *Dictyopteris undulata* Holmes extracts, and the antibacterial potential of both algae extracts was higher than that of streptomycin. *Dictyopteris undulata* Holmes was slightly more effective against *B. cereus* than *Laurencia okamurae* Yamada, with inhibition zones of 5.0 ± 0.2 mm and 4.0 ± 0.2 mm, respectively.

The most effective inhibition potential against *B. cereus* has been reported by Senthilkumar and Sudha (2012). *C. linum* methanolic 500 $\mu\text{g/mL}$ extract resulted in a 27 mm inhibition zone against this foodborne pathogen. The total phenolic content (TPC) of the methanolic *C. linum* extract was determined by means of the Folin–Ciocalteu method, yielding a TPC value equal to 672.3 mg GAE/100 g extract, related to significant antioxidant activity (as ascorbic acid equivalents). The higher scavenging activity of *C. linum* may be attributed to the structure of phenolic compounds, specifically to hydroxyl groups. Accordingly, it could be said that the antimicrobial potential exerted by this methanolic *C. linum* extract is closely related to its antioxidant activity as measured by the DPPH method, with the antioxidant potential of the extract being dose-dependent, with an IC_{50} value of 9.8 $\mu\text{g/mL}$.

4.5. *Listeria monocytogenes*

The antimicrobial potential of *Ecklonia cava* (*Laminariaceae* family) was tested by Nshimiyumukiza et al. (2015) against *Listeria monocytogenes*. Although the biological activities of *E. cava* (including antioxidant, antimicrobial, and immunomodulatory properties) have been previously reported, the study conducted by Nshimiyumukiza et al. (2015) is the first to reveal the potential that this algae specie has against this relevant foodborne pathogen. Several solvents were assayed in the process of extraction (methanolic extract, dichloromethane fraction, ethyl acetate fraction, butanol extract, and water fraction) of bioactive antimicrobial compounds. The methanolic extract of *Ecklonia cava* exhibited the highest antibacterial activity against *L. monocytogenes* KCTC 3710, with the ethyl acetate (EtOAc) soluble fraction having an MIC value of 256 $\mu\text{g/mL}$ and an MBC value of 512 $\mu\text{g/mL}$.

Methanolic and ethanolic extracts of the microalga *D. salina* Teodoresco (*Dunaliellaceae*) were particularly effective against *Listeria monocytogenes* ATCC 7644, with a MBC of 2.5 mg/mL compared with 5 mg/mL for algal fatty acids (FAs) (Cakmak et al., 2014). The antimicrobial potential of this alga has been attributed to its very valuable FA content, with an $\omega 3/\omega 6$ ratio equal to 2.15, higher than the proportion found in some fish (Cakmak et al., 2014). This is in agreement with the studies of Ohta et al. (1994), which emphasize the antimicrobial potential of FA-rich oil extracted from the microalga *Chlorococcum* HS-101 against another important methicillin-resistant Gram-positive bacterium, *S. aureus*.

The antimicrobial potential of *Myagropsis myagroides* (*Sargassaceae* family in Phaeophyta) against *L. monocytogenes* KCTC 3569 was assessed by Lee, Kim, Lim, and Ahn (2014). This macroalga revealed antimicrobial activity against Gram-positive bacteria,

and the ethanolic extract was effective with an MIC of 0.013 mg/mL against *L. monocytogenes*. A liquid–liquid technique was used to obtain five fractions, and the chloroform (CH₄) fraction exerted a strong antimicrobial activity against *L. monocytogenes*, with an MIC of 0.031 mg/mL. The effects of these fractions were analyzed by means of transmission electron microscopy (TEM), which detected serious damage to the cell envelope, leading to leakage of cytoplasmic material. A significant release of adenosine triphosphate (ATP) ($>10^{-6}$ M ATP) was also observed in the CH₄-treated bacterial population, and this was defined as one of the main causes of bacterial death.

4.6. Antifungal effects

With regard to the antifungal effects of macro- and microalgae, there is a particularly important study by Indira, Balakrishnan, Srinivasan, Bragadeeswaran, and Balasubramanian (2013) that assesses the antimicrobial potential of a seaweed (*Halimeda tuna*) against a wide range of foodborne pathogens, including nine fungi: *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternaria*, *Candida albicans*, *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Penicillium* spp., and *Rhizopus* spp. According to that study, the minimum fungicidal concentration (MFC) of all the seaweed extracts studied (ethanolic, methanolic, and chloroform extracts) was 500 µg/mL, while aqueous extracts had an MFC value ranging between 250 and 500 µg/mL. It can be concluded that *H. tuna* extracts were very effective against *A. niger*, *A. flavus*, *A. alternaria*, *C. albicans*, and *E. floccosum*, and the methanol extract was the most effective one against fungi.

Salem et al. (2014) studied the antimicrobial potential of *Nostoc* spp., *Microcystis* spp., *Scenedesmus* spp., *Oscillatoria geminata*, and *Chlorella vulgaris* algal strains against several food bacteria, including *Aspergillus niger*. Dried algal biomass was sonicated and extracted with 95% methanol and 95% acetone. Extracts were prepared in DMSO for evaluation of antimicrobial activity. Algal extracts from *Nostoc* spp., *Microcystis* spp., *Scenedesmus* spp., and *Oscillatoria geminata* revealed antifungal activity, and the methanol extract of *Nostoc* (50 mg/mL) was the most effective one against *A. niger*, with an inhibition zone diameter of 22 mm. Acetone extract was also effective against *A. niger* (30 mg/mL), with a 21 mm inhibition zone diameter. An acetone extract of *T. conoides* also exerted a mild antimicrobial capability against *A. niger*, with an inhibition zone diameter close to 3 mm (Manivannan et al., 2011). Extracts of *Trichodesmium erythraeum* (hexane and ethyl acetate 5 mg/disc) were also effective against *A. niger* and *A. flavus*. In a comparison of the hexane and ethyl acetate extracts, the latter had a higher antifungal potential than the hexane algal extract. Ethyl acetate extract of *T. erythraeum* inhibited growth of *A. niger* and *A. flavus* at 1000 µg/mL (Thillairajasekar, Durairajandiyar, Perumal, & Ignacimuthu, 2009). Both extracts are rich in myristic, oleic, linoleic, and linolenic acids. There is an established relationship between the richness in polyunsaturated fatty acids (lauric, palmitic, linoleic, linolenic, stearic, and myristic) of algal extracts and the antibacterial, antiviral and antifungal potential exerted by them (El Shoubaky & El Rahman-Salem, 2014).

The antifungal potential of two brown seaweeds was also tested against *A. niger* by Manivannan et al. (2011). According to their results, *Padina gymnospora* chloroform, ethanol, and ethyl acetate extracts were extremely effective in inhibiting *A. niger* growth (inhibition zone [15–17] mm). On the other hand, a smaller antifungal potential was attributed to *T. conoides* extracts (3–11 mm), the only noteworthy result being the inhibition observed after exposure of *A. niger* cells to diethyl ether *T. conoides* extract, with an inhibition zone diameter equal to 11 mm.

The antifungal activity of seaweed extracts has also been related to the presence of phenolic compounds and their impact

on spore germination. Some algal extracts have also been shown to inhibit fungal enzyme activity owing to the presence of bioactive metabolites (Salem, Galal, & El-deen, 2011).

4.7. Antiviral effects

There is a serious lack of information about the antiviral potential of algae against foodborne viruses. Mainly, Noroviruses (NoVs) and other commonly viral contaminants in food (Hepatitis A (HAV), Human Rotavirus (HRV), Hepatitis E virus (HEV), Nipah virus, Highly Pathogenic Avian Influenza (HPAI) virus, SARS-causing Coronavirus) are of high concern for the scientific community (Koopmans, 2012).

The main substances from algae that have been related to antiviral potential are sulfated polysaccharides, including fucoidan, sulfoglycolipids, carrageenans, sesquiterpene hydroquinones, etc. (Elizondo-Gonzalez et al., 2012). Suppression of DNA replication and inhibition of host cell colonization by the virus are some of the effects of algal polysaccharides (e.g. fucan, laminaran, and naviculan) as natural antiviral compounds (Ahmadi, Moghadamtousi, Abubakar, & Zandi, 2015).

Focused on foodborne viruses inhibition by marine algae it is remarkable the research work of Wang, Wang, and Guan (2012). The study of Wang et al. (2012) reviewed the anti-viral potential of polysaccharides from brown seaweeds, mainly alginates and fucans, revealing a significant inhibiting activity against hepatitis B virus (HBV) DNA polymerase, and consequently affecting its replication. Algae polysaccharides have exerted also anti-viral potential against rotaviruses. These viral agents are the most important causing gastroenteritis, mainly in children, with fatal consequences. The effect of sulfated polysaccharides interfering with the adsorption process of enveloped viruses has been considered in the invention developed by Anderson, Schaller, Mazer, and Kirchner (1997) to demonstrate that carrageenan, and particularly λ-carrageenan, is an effective inhibitor of rotavirus infection in animal cells. Crude methanol extract (70% v/v) of *Spirulina platensis* also showed antiviral activity with 56.7%, inhibition rate against rotavirus Wa strain (Hetta et al., 2014). According to the studies of Hetta et al. (2014), free fatty acids (FFA) – induced endoplasmic reticulum (ER) stress and suppressor of cytokine signaling protein (SOCS3) levels are the two main factors responsible for the signaling reduction and impairing the antiviral response.

According to the studies of Eom et al. (2015) phlorotannins from *Eisenia bicyclis* showed a strong antiviral potential against norovirus (murine norovirus, MNV), with 50% effective concentration (EC₅₀) of 0.9 µM. The inhibition of oxidative phosphorylation, and the ability of Phlorotannins to bind with proteins, such as enzymes and cell membranes, are the main causes explaining the antiviral activity of these compounds, that finally act causing cell lysis (Shannon & Abu-Ghannam, 2016).

Although the high anti-viral potential of algae compounds (FFA and polysaccharides, mainly) against clinical and foodborne viruses, scarce information has been reported up to date (Shannon, 2016; Wang et al., 2012). More research is needed to cover the self-evident gap in knowledge regarding the anti-viral applications of algae compounds from both approaches, pharmaceutical and food safety.

5. Antimicrobial potential of algae validated in REAL FOOD MATRICES

A wide variety of algae have been introduced in food formulations for various purposes. Meat, dairy, and bakery products are some of the best-known examples of innovative products that include these healthy vegetable ingredients. In Europe, for a long time interest in algae has focused on the extraction of phycocol-

loids with gelling, thickening, and stabilizing properties for use in many applications in the food industry (Enzing, Ploeg, Barbosa, & Sijtsma, 2014).

The main limitations affecting the application of algae in food products are related to sensory (residual flavor and aroma) and toxicological aspects (high iodine levels, or accumulation of arsenic, heavy metals, and contaminants) (Bouga & Combet, 2015). These risks must be minimized in order to maximize the technological, functional, and nutritional advantages that are associated with macro- and microalgae materials and bioactive compounds. The introduction of microalgae in animal feed has been a reality since the '70 s, and is a good way to improve animal health and the subsequent quality of meat-derived products. In aquaculture feed, algae supplements are also being used, with good prospects (Yaakob, Ali, Zainal, Mohamad, & Takriff, 2014).

In food for human consumption, seaweed has been introduced in breads, pizza bases, and cheese (e.g. *Ascophyllum nodosum*) (Hall, Baxter, Fryirs, & Johnson, 2010), and has also been added to pasta (e.g. *Sargassum marginatum*) (Prabhasankar et al., 2009) and meat products (Cofrades, López-López, Ruiz-Capillas, Triki, & Jiménez-Colmenero, 2011). The development of algae-based lipid powders and flours is also one of the hot topics that has been included in novel cuisine, and they are even being used instead of eggs, with really promising results for the vegan market.

However, with regard to preservation there is scarce literature about validating the functionality of these ingredients as antimicrobials in real food matrices (Gupta et al., 2010; Sălăgean, Pop, Catrinoi, & Nagy, 2015). Although some researchers are making efforts to introduce raw algae and processed materials in the formulation of innovative meat-derived products (e.g. sausages and hamburgers), some aspects such as quality and organoleptic acceptance of the final formulation still need to be analyzed in depth. With regard to the validation of the antimicrobial potential of algae in meat products, a noteworthy study has recently been published by Sălăgean et al. (2015). Among the main advantages that the introduction of algae in meat-formulated products aims at are the following: (a) the introduction of a protein source of vegetable origin, (b) lower content of cholesterol, calories and fat in the finished products, and the associated (c) beneficial bioactive compounds present in algae-derived ingredients. According to the results obtained by Sălăgean et al. (2015), in the formulation of half-smoked sausages (75% first-quality beef, [10–15]% animal fat, and brown algae [10–15]%) the addition of algae in the formulation led to a final product with higher quality (physicochemical and organoleptic), with improved functionality resulting from the antimicrobial potential of brown algae against *S. aureus* and *E. coli* (storage 7 days, 10–12 °C). Furthermore, a recent industrial patent has been obtained for the introduction of algae in meat-derived products. According to Cofrades et al. (2011), a new method for the formulation of healthier meat products has been developed, incorporating less than 5 wt.% of *Himantalia elongata* brown alga. Brown algae are more suitable for meat formulations than red or green algae, not only because of their nutritional profile but also because of the color balance between animal tissue (cattle, pigs, sheep, goats, horses, poultry) and these pigmented algae. The result shows a reduction in sodium levels and an improvement in the lipid profile of the final product (Cofrades et al., 2011).

The antimicrobial capability of macroalgae has been reported to reduce the need for the addition of salt, especially against Gram-negative bacteria. A reduction of nearly 1 log₁₀ cycle was observed against coliforms during the shelf life of frozen processed meat products (Brownlee, Fairlough, Hall, & Paxman, 2012). Similarly, in bread products the antifungal potential of green seaweed added to bakery products suppressed mold growth for up to 9 days in preservative-free bread, equivalent to the control bread containing 5 g of sodium chloride (Brownlee et al., 2012).

In the research line of novel meat products with longer shelf life, edible films and coatings (EFC) are a promising preservation technology that provides a good barrier against spoilage and pathogenic microorganisms when natural antimicrobials are added. According to Sánchez-Ortega et al. (2014), the addition of edible algae films and coatings on meat products had the following main advantages: reduction of lipid oxidation, increase in stability of red meat color, prevention of moisture loss, reduction of spoilage and pathogenic microorganism load, and partial inactivation of deteriorative proteolytic enzymes on the surface of the coated meat. The development of red algae films applied to bacon impacted positively on the microbiological quality of the product reducing *E. coli* O157:H7 by 0.45 log CFU/g and *L. monocytogenes* by 0.76 CFU/g with respect to the controls. The application of edible coatings from algae to cheese also reduced *E. coli* O157:H7 and *L. monocytogenes* populations by 1.21 and 0.85 log CFU/g, respectively, compared with the control after 15 days of storage (Shin, Song, Seo, & Song, 2012; Sánchez-Ortega et al., 2014). Other algae derivatives such as alginate and κ-carrageenan have also been used in the formulation of edible films with antimicrobial properties against *L. monocytogenes*, e.g. in cold smoked salmon (Neetoo, Ye, & Chen, 2010).

6. Conclusion

Effective antimicrobial potential has been demonstrated against foodborne pathogens by both macro- and microalgae compounds. However, some contradictions can be found in the literature as a result of the different strains used in the determination of antimicrobial activity, different methods of extraction applied, and different ranges of alga material concentration used in the assays. The promising results of bacterial growth inhibition and inactivation by raw and purified algal compounds, and the lack of studies carried out in real food matrices open a research niche with high application at food industrial level. A valuable relationship could be established between the antioxidant potential of algae and their antimicrobial capability. Consequently, the twofold use of algae extracts as antioxidants and antimicrobials in food products has good prospects in response to the Horizon 2020 call for white label developments and innovation in sustainable food products that are as natural as possible.

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