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From waste/residual marine biomass to active biopolymer-based packaging film materials for food industry applications – a review

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Abstract:
Waste/residual marine biomass represents a vast and potentially underexplored source of biopolymers chitin/-chitosan and alginate. Their isolation and potential application in the development and production of bio-based food packaging are gaining in attractiveness due to a recent increment in plastic pollution awareness. Accordingly, a review of the latest research work was given to cover the pathway from biomass sources to biopolymers isolation and application in the development of active (antimicrobial/antioxidant) film materials intended for food packaging. Screening of the novel eco-friendly isolation processes was followed by an extensive overview of the most recent publications covering the chitosan- and alginate-based films with incorporated active agents.

Keywords: active food packaging materials, antimicrobial and antioxidant agents, biopolymers isolation, chitosan- and alginate-based active films, green processes, marine biomass

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1 Introduction

Our civilization is built on plastic, and according to The World Economic Forum, its amount is expected to triple by the year 2050 [1]. Yet, less than 15 % of it is currently being recycled. The highest use of plastic materials is intended for the packaging in the food industry, which represents up to 40 % of the total plastic consumption within the European Union [2]. Thus, the need for alternatives has recently got a lot of boost in the research of using bio-based or biodegradable materials.

Food processing and packaging are the most important parts of the food industry [3]. Due to increasing environmental burden, there is a growing effort to replace synthetic petroleum-based packaging materials with biodegradable and consumable materials synthesized from natural polymers. These changes are probably less related to any depletion of nonrenewable resources, but rather to increased interest in addressing sustainability aspects related to resource efficiency as well as waste disposal and treatment [4]. In this regard, governments, industries, and consumers are very much concerned about the impact of the products consumed. A recent review presents the valorization of abundant and available bio-wastes with high potential to manufacture value-added products, creating the first step to close the loop between waste and consumption in line to attain the main goal of the circular economy [5]. More processed and packaged food is consumed as a proportion of the total in better-off, urbanizing, and industrializing economies [6]. In the specific field of food packaging, there are clear trends with regard to the sourcing and use of raw materials.

Food is the main nutritional support for organism, hereby unsafe and contaminated food presents an unceasing health risk for billions of people all over the world. According to a comprehensive estimation of the global burden of foodborne diseases led by the World Health Organization (WHO), a consummation of contaminated food caused a hundred million cases of illnesses and thousands of deaths in 2010 [7]. Since microbial contamination can easily occur at every exposure of food to the external environment, conventional food preservation techniques (drying, fermentation, thermal processing, etc.) are often not enough to ensure high quality of food and efficient extension of food shelf life [8]. Referring to the aforementioned facts, it is obvious that new alternatives for limiting the microbial contamination and overall food deterioration are needed.

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Development of novel food packaging materials with antimicrobial and/or antioxidant activity is getting a broad research interest, whereby this kind of materials can be provided by the incorporation of various active agents (AAs) in the packaging formulations [9–12]. Since direct incorporation into food leads to a reduced antimicrobial activity over a short period of time, the incorporation into packaging matrix ensures greater agents stability. Providing slow but constant migration of AAs from the packaging material is another advantage of the active packaging systems since the control over microbial growth and antioxidant protection can be ensured for a prolonged period of time [8, 13–15].

The current review focuses on the biopolymers that are obtainable from the waste/residual marine biomass and that are potentially applicable in the preparation of active food packaging materials. In this regard, chitin (together with its derivative chitosan) and alginate are the most promising for this purpose due to their non-toxicity and good film-forming abilities leading to the production of mechanically stable films [16, 17]. Therefore, recent studies covering the most commonly used marine biomass as sources of the aforementioned biopolymers, extraction methods for their isolation, and consequent utilization in the preparation of active film materials are scrutinized.

2 Marine-based biomass as a source of chitin/chitosan and alginate

Chitin is known as the crucial structural polymer which constitutes a big portion of crustaceans' exoskeletons, whereby its content varies not only between different sources but also between different species [18]. In nature, there are three allomorphic forms of chitin: α-chitin (anti-parallel arrangements of polymer chains), β-chitin (parallel arrangements of polymer chains), and γ-chitin (with alternated arrangements of polymer chains; distinct, yet closer in structure to the previous two forms) [18, 19]. The most common α-chitin is found in crabs and shrimps (also in fungi, yeast, and insects), β-chitin is found in a combination with proteins (mostly in squid pens), while γ-chitin is found in the stomach of squids (and in the cocoon of moths and beetles) [19, 20]. Researchers have revealed the presence of chitin from other types of marine organisms as well (e.g. diatoms, corals, sponges) [21–24], further confirming its use in biological structures formations in nature. In terms of its availability, chitin is (next to cellulose) available to the extent of over 10 gigatons annually [25]. Besides, chitin is a precursor of chitosan, i.e. its N-deacetylated derivative whose chemical structure consists of D-glucosamine and N-acetyl-D-glucosamine sub-units linearly linked *via* β-1,4-glycosidic bonds [18, 26].

Alginates are naturally occurring, indigestible polysaccharides that are commonly produced by and refined from various brown seaweed (mainly from *Laminaria hyperborea*, *Macrocystis pyrifera*, *Ascophyllum nodosum*; in lesser extent from *Laminaria digitata*, *Laminaria japonica*, *Eclonia maxima*, *Lessonia nigrescens*, *Sargassum* sp.). The molecular structure of alginate is composed of unbranched, linear binary copolymers of α-D-mannuronic acid (M) and α-L-guluronic acid (G) residues linked *via* 1,4-glycosidic bonds. An algal-based alginate structure could be separated into three fractions (three uronic acid blocks): homopolymeric regions of M blocks, homopolymeric regions of G blocks, and alternating MG blocks containing both polyuronic acids [16]. The M:G ratio varies amongst brown seaweed taxonomic ranks (i.e. orders), and it is typically reported to be in the range between 0.8 and 2.2 [27]. Alginates isolated from *Laminaria hyperborea* generally have the highest guluronic acid content, whereas those extracted from *Laminaria japonica* and *Ascophyllum nodosum* are low in guluronic acid content [28–31]. Percentages of mannuronic and guluronic acids as well as M:G ratios of alginates from various commercial brown seaweeds are listed elsewhere in the literature (Table 2.1 in [32]).

2.1 Isolation of chitin/chitosan

Many different methods have been proposed for chitin (and hence chitosan) isolation, but no standard method has been adopted yet. Traditional methods are chemical-based and they rely on acidic demineralization and alkaline deproteinization as two major steps. Therefore, green technologies that are cost-effective and sustainable are being presented as a good choice [33]. A few novel alternative methods, such as those that are using enzymes and fermentation, deep eutectic solvents, ionic liquids, and plasma-based extraction, have been proposed as well (Figure 1).

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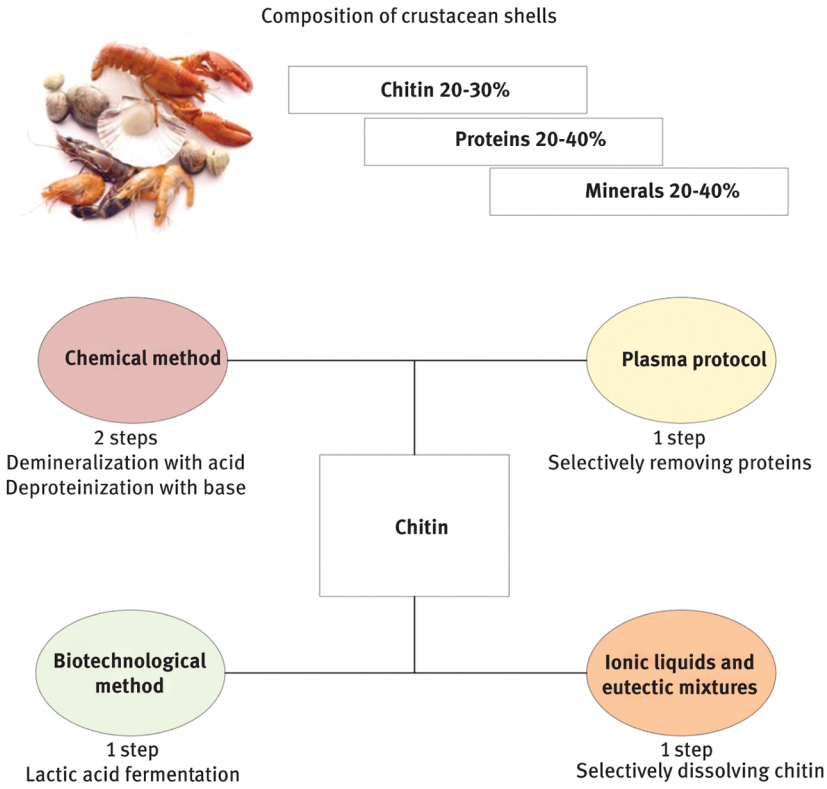

Figure 1: Schematic representation of available methods for the isolation of chitin from crustacean shells.

Chitin/chitosan have been successfully isolated from different marine organisms (e.g. shrimps [34–44], lobsters [43, 45], squid [46], crabs [38, 43, 47], crayfish [38], prawn and krill [43], etc.) by using methods summarized in Sections 2.1.1–2.1.4. Molecular weight (M_W) and degree of (de)acetylation of the final product(s) highly depend on the source, isolation methods, and deacetylation protocols, whereby more information on this topic can be found in other review articles dealing with chitin/chitosan extraction and characterization (e.g. Table 3 in [18] and Table 1 in [48]).

2.1.1 Chemical methods

The simplest and the most effective industrial method for the extraction of highly pure chitin is a chemical-based one, while other less efficient methods are more work- and time-consuming [49]. However, some chemical methods have several drawbacks: (i) large volume of corrosive acidic and basic wastewater hazardous to the environment, (ii) energy-consuming extraction and purification, and (iii) negative effect of strong acids on the physicochemical properties (lowering M_W). Although chemical methods are efficient, they do not grant full control over physical characteristics (crystallinity, purity, polymer chain arrangement, etc.), and besides other biomolecules (like proteins, lipids, carotenoids) are discarded [45, 50]. The chemical extraction of chitin followed by its derivatization into chitosan is conducted in three major steps: (i) demineralization, (ii) deproteinization, and (iii) deacetylation.

Demineralization: In this step calcium carbonate ($CaCO_3$) and other minerals are converted into water-soluble calcium salts (easily removed by washing) and carbon dioxide (CO_2) as a by-product. The most frequently used acids are hydrochloric (HCl), nitric (HNO_3), sulfuric (H_2SO_4), acetic (CH_3COOH), and formic (HCOOH), whereby HCl is being the most represented one. Parameters in this step (time, temperature, particle size, acid concentration, solid to liquid ratio) are determined empirically. Solid to liquid ratio is important since two molecules of HCl are needed for one molecule of $CaCO_3$, so acid intake should be equal or higher to the stoichiometric amount of minerals in order to achieve the complete reaction [50]. Usually up to 10 % of the acid is used with constant stirring at room temperature for about 2–3 h. To minimize depolymerization and deacetylation of chitin, HCl can be replaced with ethylenediaminetetraacetic acid (EDTA; $C_{10}H_{16}N_2O_8$), sulfurous acid (H_2SO_3), or CH_3COOH, but their usage increases the ash content [50]. Contrarily, the extraction of chitin from shrimp shells using mild conditions has been studied as well [34].

Deproteinization. This step is usually performed by chemical methods which assume the use of different deproteinization reagents such as sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), potassium hydroxide (KOH), calcium bisulfite ($CaHSO_3$), potassium carbonate (K_2CO_3), calcium hydroxide ($Ca(OH)_2$), sodium

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bicarbonate ($NaHCO_3$), sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), trisodium phosphate (Na_3PO_4), and sodium sulfide (Na_2S), among which NaOH is the most used one [50]. Instead of NaOH, cheaper calcium oxide (CaO) can be used to increase the ionic strength and to extract proteins. Anyhow, high ratios of solid to alkali (1:10 or 1:20) are suggested for the uniform reaction [34]. Alkali reagents can cause partial depolymerization and deacetylation of chitin due to continuous hydrolysis, therefore a change in the mechanical properties and lower M_W of chitin has been observed [50]. Reaction conditions vary considerably, and mean use of 0.125 M to 5 M NaOH with temperatures up to 160 °C and time from a few minutes up to several days. Longer times (up to 24 h) result only in a slight drop in the ash content, but on the other hand can cause polymer degradation [34]. It has been suggested that solid sodium chloride (NaCl) treatment followed by demineralization and deproteinization better preserves chitin structure [51]. Tolaimate et al. have proposed a new approach with successive baths with lower concentrations of HCl (0.55 M) and NaOH (0.3 M) for good preservation of the native chitin (100 % acetylated) [46]. Furthermore, a shorter alkaline process at room temperature has been suggested to avoid chitin depolymerization [52]. A simple fractionation method using hot water for deproteinization and carbonic acid (H_2CO_3) for demineralization with high efficiency and chitin purity in a short time (within hours) has been addressed as well [35]. After these steps, chitin can be still colored so the sample can be bleached, but it is neither really needed nor advised since it causes a decrease in the viscosity (i.e. M_W) of chitin [53].

Deacetylation: In the last step, acetyl groups are partially removed from chitin leaving behind chitosan with highly reactive amino groups. Acids or alkalis can be used, but the latter ones are preferred since the glycosidic bonds are sensitive to acids. Deacetylation can be divided into two categories: (i) heterogeneous (producing insoluble chitosan), and (ii) homogenous (producing soluble chitosan). Concentrated solutions of NaOH or KOH can be used, but the latter one is less effective [50]. In the methods that use highly concentrated NaOH (50–60 %) at high temperatures (130-150 °C), deacetylation is very fast (within 2 h) but a balance must be found between time and depolymerization [54]. The chemical deacetylation has some environmental disadvantages such as large energy input, large waste of concentrated alkaline solution as well as heterogeneous deacetylation range of soluble and insoluble products with different M_W [37, 50].

Several studies have been aiming to improve the chemical method yield and impact on the environment. One has been conducted to determine if the modifications in the production sequence have any effect on yield, physicochemical, and functional properties [39]. It was found that demineralization and deproteinization steps can be reversed, but for higher yields deacetylation is preferred to be performed the last one. The highest chitosan yield is obtainable with a sequence of demineralization, deproteinization, deacetylation, and decolorization [39]. Besides, chitin and chitosan can be modified into many products with desired novel attributes and functions suitable for different applications [18, 55, 56].

A new method using 3 % of sodium hypochlorite (NaClO) for 10 min before demineralization and deproteinization for time and energy saving has been proposed by Kaya et al. [38]. Furthermore, a soft alkaline treatment with much lower chemical use (and with possible NaOH and water recovery) has been suggested in order to improve the negative influence on the environment [57]. In addition to this, designers from the Royal College of Art (London, UK) and the Imperial College of London (London, UK) have developed a small-scale desktop chitin extractor from seafood waste called “Shelly”, which allows automated control over each parameter in order to obtain different grades of chitosan [58].

2.1.2 Biotechnological methods

Green isolation methods have been promoting the use of enzymes and microorganisms. Biotechnological-based extraction of chitin holds higher reproducibility, shorter processing time, lower solvent/energy consumption, and higher preservation of the native form [50]. Nevertheless, this method is still bound to the laboratory scale due to disadvantages such as low chitin yield, costly enzymes, challenging scale-up (entire process requires sterile conditions), and long cycles in the microbial fermentation [45, 50].

Chemical and biotechnological methods involve analog steps: (i) demineralization (using lactic acid bacteria in case of biotechnological method), (ii) deproteinization (with commercial enzymes or with proteolytic bacteria), and (iii) deacetylation (with chitin deacetylase or lactic acid bacteria), or by hydrolysis (using chitinolytic enzymes) [59]. A comparative study between chemical and biotechnological methods for chitin extraction has been performed by Khanafari et al. [41]. A biorefinery-based method, which means crustacean shells fractionation to the main components and their transformation into value-added materials, is still in the developing stage but it could create a new and profitable market with its multiple applications [60].

Enzymes can be used for deproteinization, therefore avoiding the application of strong alkaline treatments. Procedures with enzymes are fast, production conditions are mild, complicated equipment is not required, and lower deacetylation and depolymerization (in regard to the chemical method) have been reported [37, 61]. Due to lower efficiency, an additional NaOH step may be needed to achieve higher purity. Since minerals can limit

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proteases access and lower efficiency, demineralization should be performed first [50]. To enhance accessibility, a pre-treatment can be used with physical or chemical methods such as sonication, grinding, and heating [62]. A cheaper alternative to commercially purified enzymes is crude proteases, which are also more efficient and eco-friendly [50].

Sustainability assessment of chemical and enzymatic processes has been done by Lopes et al. [61]. It has been shown that even the production of enzymes and chemical reagents in small quantities requires more energy and raw materials. The energy and enzyme consumption is high due to a low yield, but the overall enzymatic process is in overall 20 % more favorable to the environment, as compared to the chemical one. The chemical process has high production costs and requires waste management, but a higher yield of chitin increases profit. However, a more homogenous biocatalytic production of chitosan with defined size and degree of acetylation (DA) has been conducted under mild conditions with recombinant chitin deacetylase [37].

The enzyme cost can be lowered if deproteinization is performed by a fermentation process. This can be achieved by endogenous microorganisms (auto-fermentation) or by the addition of selected microbial strains. In a microbial fermentation, deproteinization and demineralization steps are processed simultaneously [50]. Proteins and minerals are removed by a combination of enzymatic activity and mineral solubilization by organic acid produced during bacterial growth [42]. Fermentation process (deproteinization and demineralization) by protease and organic acid bacteria followed by deacetylation with chitin deacetylase is an example of the alternative and economical method [63]. For industrial requirements, a combined chemical and biotechnological (fermentation) methods with the application of seawater for chitin extraction could be used as well [43].

Fermentation of crustacean shells can be performed by bacterial strains that consume proteins and decompose $CaCO_3$, or with *Lactobacillus* strains, which produce lactic acid and proteases, whereby lactic acid reacts with $CaCO_3$ and forms a precipitate. Rao et al. have studied the effect of different fermentation parameters on deproteinization and demineralization [44]. In non-lactic acid fermentation, both bacteria and fungi can be used for crustacean shells fermentation. A one-pot fermentation has been reported for the production of chitin where fungi proteases hydrolyze proteins into amino acids that present a nitrogen source for fungal growth [36]. The biotechnological process can be also followed by mild chemical treatment to remove the residual protein and minerals [50].

2.1.3 Ionic liquids- and deep eutectic solvent-based methods

The use of ionic liquids (ILs) in chitin extraction is a relatively new approach, thus most of the studies are still at the laboratory scale. ILs are salts with unique properties, being composed of a wide range of raw and renewable materials such as organic salts, sugars, and amino acids. Their infinite anion/cation combinations give rise to the favorable designer solvent character, allowing them to be tailor-made according to the final applications [33].

Chitin extraction with ILs has many advantages: (i) less energy, time, and chemicals are used in comparison to the chemical methods, (ii) high M_W is achieved, (iii) direct chitin extraction from marine waste is possible, (iv) broad range of usage, (v) possibility for recycling/reuse, and (vi) more sustainable alternative to organic solvents due to higher thermic and chemical stability and low vapor pressure [64, 65]. Therefore, ILs can be recycled, which presents an important economic aspect. Nevertheless, they cannot be purified by distillation, so recycling with vacuum treatment, supercritical fluids, and soxhlet extraction can be used [64]. In contrast, IL extraction seems to be a promising method, but some disadvantages (moisture sensitivity, difficult recycling, high cost) challenge large-scale production [50, 60]. ILs are considered to be green solvents, although their effect on the environment has not been entirely understood yet [66].

The extraction process for chitin isolation requires only IL which dissolves chitin leaving the proteins and minerals undissolved, coagulation solvent (water or alcohols), and direct heating [33, 65]. For chitin extraction with ILs only a few studies exist, but ammonium-based and choline-based ILs with acetate and chloride are considered as the most promising and safe [64]. Series of ILs have been synthesized and their chitin-dissolution ability has been evaluated under mild condition [67]. Low-cost ILs with highly acidic and basic ions, such as $[NH_3OH][OAc]$, can be used to pulp shrimp shells with high chitin yield and purity. An aqueous solution of this IL was found to be effective solvent for chitosan at room temperature even in the presence of water [68]. A high DA of chitosan can be attained by a simple hydrothermal treatment in the 1-butyl-3-methylimidazolium acetate-chitosan-water system without alkali use, which also allows recovery and reuse of IL [69]. A pre-treatment with ionic liquids can also weaken chitin structure and decrease its crystallinity for better efficiency of double chitinase hydrolysis [47].

Deep eutectic solvents (DESs) are novel sustainable solvents that can replace organic solvents or ILs. DESs present a mixture of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) that self-associate

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through H-bonds and can be used to dissolve poorly soluble chitin. Chitin can be selectively isolated by breaking strong H-bonds in the reaction between chitin NH_2 and donors of substituents in a DES [70]. DESs are considered to be superior over ILs because they are biodegradable, but also have low toxicity and relatively low price. On the other hand, they share low volatility and wide polarity range with ILs [70–72]. A DES allowed a downstream protocol that enables multiple extractions in a sequence without the need to isolate minerals and proteins [73].

Natural deep eutectic solvents (NADESs) are able to produce chitin in a single, fast, and eco-friendly step to minimize water and toxic chemicals consumption in deproteinization and demineralization. The most promising commercially available NADESs are choline chloride lactic acid (CCLA), malonic acid (CCMA), urea (CCUR), citric acid (CCCA), thiourea (CCT), and glycerol (CCG) [45, 71, 73]. In a single step, NADESs have to play three roles: (i) demineralization − organic acid (HBD) must be used since $CaCO_3$ removal occurs under acidic conditions (in the same time minerals are partially degraded), (ii) deproteinization and chitin dissolution by breaking H-bonds with choline chloride (HBA) which is then precipitated with water. Bradić et al. have studied temperature and time influences on chitin extraction process for higher yield and purity [73]. In CCUR (alkaline pH) chitosan had the highest solubility, but that is not necessarily good, since proteins and minerals have to be removed first. By using CCMA, chitin can be divided into two parts (supernatant and precipitate) with different crystallinity and thermal stability. CCMA could successfully remove $CaCO_3$, so it could replace acid in chemical methods [45]. The alternative green approach to synthesize a permanently positively charged *N*-methylated chitosan for a better solubility has been introduced in order to avoid using organic solvents in alkaline conditions with non-selective methyl iodide (CH_3I) [74].

2.1.4 Plasma-based method

The first solvent-less protocol using atmospheric pressure dielectric barrier discharge plasma-based separation method as a pretreatment process for deproteinization in chitin production has been reported by Borić et al. [75]. Although the pre-treatment process was very fast (1.5 min – 6 min), proteins had been intensively removed while preserving the native structure of chitin. This method does not require any solvents or produces hazardous waste, and scale-up is possible due to operating at atmospheric pressure. This alternative method for chitin extraction uses plasma, which can break C–C or C–H bonds, but inorganic materials remain inert. The method is carried out by placing a whole shell body part in the gap between the electrode and the quartz tube. Therefore, plasma in combination with different gasses (N_2 and O_2) can be used for selective protein removal from the shrimp shells [75].

2.2 Isolation of alginate

Alginate is isolated from the cell walls of brown seaweed (about 40 % of dry weight), where it is responsible for the strength and flexibility. In the natural state, it is bonded with seawater ions, mainly Ca^{2+} and smaller amounts of Na^+, Mg^{2+}, Sr^{2+}, and Ba^{2+} [76]. Alginate is mostly used in a sodium form due to its better solubility in cold water. The aim of the extraction method is to turn water-insoluble alginate salts into water-soluble sodium salts, whereby cellulose remains undissolved [76]. Alginate is subsequently recovered as alginic acid or calcium alginate, and there are two isolation methods which start with similar extraction procedures but vary in the intermediates formed during precipitation. In the first one (which is also commercially used), calcium alginate and alginic acid are the main intermediates, and in the second one only alginic acid is formed [32]. The first method is usually preferred when alginic acid forms an insoluble gel that can plug filters. The chemical process can also be performed by a hot (50 °C) or cold (25 °C) method [77].

The chemical method usually has five relatively simple steps: (i) raw material fragmentation and ethanol treatment to remove pigments and lipids for easier processing, (ii) transforming alginate salts into insoluble alginic acid with acid pre-treatment (HCl or H_2SO_4) which also breaks the cell walls, (iii) transforming alginic acid into soluble sodium alginate (SA) with alkaline extraction (Na_2CO_3 or NaOH), (iv) precipitation (H_2SO_4, HCl, alcohol, or $CaCl_2$) followed by filtering, (v) and drying (if precipitated with alcohol) [32, 78]. Most of the unwanted substances (fucoidans, laminarins, and polyphenols) can be also removed by acid treatment. Polyphenols can oxidize into brown substances under alkaline conditions; therefore, a mild pre-treatment with formaldehyde is needed to make them insoluble by polymerization [32, 78]. Pre-treatments and alternative solvents (ethanol, methanol, acetone) that would allow alginate extraction and retrieve polyphenol-rich fraction have been investigated [79]. For pigment removal, formaldehyde can be avoided by using photobleaching, which was reported for agar but could also be tested on alginate [76]. The alkaline (or main extraction step) is time-, water-, and reactant-consuming, and is usually carried out as 2 % $CaCO_3$ with pH 10 at 80 °C, inde-

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pendently of species. On the other hand, acid treatment conditions vary greatly [80]. The alginate-influencing extraction parameters have been studied by Fertah et al. [81], while Davis et al. have shown that alginate yield is independent of the temperature or the extraction method employed [27]. Since alginate includes a lot of contaminants, it needs to be purified with ethanol, methanol, and acetone for medical use [81].

The chemical method for alginate isolation is not eco-friendly or cost-effective due to: (i) high energy, water, and solvent use, (ii) quite expensive alcohol, (iii) need for wastewater treatments, and (iv) lower yields caused by degradation (since alginate cannot be precipitated) [80, 82]. On the contrary, a study dealing with alkaline extraction kinetics has reported that alginate depolymerization in the alkaline step could reduce extraction time in order to obtain better rheological quality [83]. There has also been found a relation between extraction yield and algal destruction [83]. The chemical method has become traditional for industrial extraction, but still holds certain limitations such as efficiency and product consistency. On the contrary, some novel and greener extraction methods have been proposed, but many of them are still under development on the laboratory scale, so the most environmentally sustainable one has not been identified yet [76].

A continuous and green method for the industrial isolation of alginate might use reactive extrusion with a twin-screw extruder to avoid using the alkaline extraction step [83]. By using this method, yield can be increased by 15 %, time-scale shortened from hours to minutes, water and reactants use can be reduced two-fold, while the purity remains high in comparison to the chemical method. Besides, alginate of a high M_W and superior rheological properties can be obtained due to shorter processing time (which reduces depolymerization), while costly equipment could be a drawback of the method [83].

An alternative method might be a microwave-assisted extraction (MAE) since it could overcome drawbacks like alginate thermal instability, long processing time, cost-ineffectiveness, and low yield [84]. Although this isolation method is used for other compounds, hardly any reports have been published with MAE for alginate extraction. On the contrary to the chemical method, which only heats up the surfaces from where heat is conducted to the core of the particles, MAE works by heating up the system with microwave energy [85]. Acid pre-treatment with 0.1 M HCl for MAE has also been optimized for shorter times and lower solvent usage [80].

There are a few studies for ultrasound-assisted extraction (UAE) of alginate capable of replacing the alkaline step, and whose advantages encompass: (i) extraction in only minutes, (ii) high reproducibility, (iii) lower solvent consumption, (iv) high purity, (v) simple process, (vi) no wastewater treatment, (vii) very low energy use, and (viii) easy scale-up [86–88]. Ultrasound allows better solvent penetration into the sample, and hence increasing contact area and reducing extraction time without influencing the chemical structure or M_W [89]. Youssouf et al. have studied the effect of temperature, pH, and ultrasound power for optimal extraction [90]. UAE can be also coupled with microwave (UMAE), which is considered to be the most promising hybrid technique for fast and cost-effective extraction, but has not been applied by many authors yet. UAE could be also combined with supercritical fluid extraction or extrusion extraction [86].

For alginate extraction, complex algae cell walls need to be broken, therefore enzyme assisted extraction (EAE) method that applies enzymes such as proteases and carbohydrases, might be used [85]. EAE holds several advantages: (i) eco-friendliness, (ii) low cost, (iii) high yield, and (iv) ability to make water-soluble materials. A pre-treatment with cellulase or alcalase might as well be applied instead of the acidic step before the extraction with Na_2CO_3 [91, 92]. With cellulase, it is possible to achieve a high yield of highly pure alginate, which possesses immunostimulatory and weak antioxidant activity. Commercial enzymes might be used instead of the acid step [93], but also other compounds could be extracted after digesting the cell wall [94].

Compounds obtained with supercritical fluid extraction (SFE) show very high purity without any residual solvents. SFE method is (i) eco-friendly, (ii) low cost, (iii) non-flammable, and (iv) time-saving since the sample concentration is not needed [85]. Widely available, low cost, and eco-friendly water or CO_2 can be used as supercritical solvents. To the best of your knowledge, there is no report of the alginate extraction by this method. Nevertheless, a pressurized solvent extraction (PSE) in an extraction method that uses temperatures in the range from 50 °C to 200 °C and pressures in the range between 35 bar and 200 bar. A high temperature combined with increased pressure causes an increase of solubility and penetration of solvent into the sample and therefore enhancing the extraction process. This method is very similar to the soxhlet extraction, but the solvents employed are in subcritical state and thus have high extraction abilities. The advantages of PSE are high extraction efficiency, simple instruments, and relatively short extraction time [95, 96].

Finally, it is important to point out that biological and physicochemical (M:G ratio, M_W) properties of alginate are dependable on the extraction method. For instance, by applying different extraction methods it was possible to produce alginates from *Colpomenia peregrina* and *Sargassum angustifolium* with M_W ranging from ~247×10^3 g/mol to ~354×10^3 g/mol and from ~ 356×10^3 g/mol to ~557×10^3 g/mol, respectively [91, 92].

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3 Active chitosan- and alginate-based films

According to G.L. Robertson, active packaging can be defined as "packaging in which subsidiary constituents have been deliberately included in or on either the packaging material or the package headspace to enhance the performance of the package system" [97].

Most foods are susceptible to microbial contamination. A way to tackle this problem could be to add antimicrobial compounds directly in food products, but that in turn might lead to the reduction of the active compounds' efficiency and change foods' organoleptic properties. On the other hand, the application of antimicrobial films has shown to overcome these problems as well as preserve quality and increase the shelf life of various food products [9, 98–100]. The enhancement in the quality of food products is achieved throughout the inhibition of the target microorganisms. In addition to the chemical agents, a broad variety of natural antimicrobial components (essential oil, plant extracts, enzyme, bacteriocins, and probiotics) might be incorporated into packaging materials to boost their antimicrobial activity [12, 101–103]. Antioxidant protection of perishable foods also plays a crucial role [12, 104, 105], and therefore the improvements in the films' antioxidant activity after the incorporation of natural-based compounds is of paramount importance.

3.1 Antimicrobial activity of chitosan- and alginate-based films

Chitosan possesses antimicrobial activity against a wide range of bacteria, yeast, and fungi [106–108]. The most accepted hypothesis of its antimicrobial activity is based on the presence of positively charged amino groups (NH_3^+) of glucosamine (chitosan molecule becomes polycationic in the acidic environment, i.e. when pH is below the pKa of chitosan) which might react with negatively charged molecules on the microbial cell surface [109]. Such electrostatic interactions cause extensive alterations to the cell surface and leakage of intracellular components or inhibition of nutrient penetration into the cell, which eventually leads to cell death [109]. The charged amino groups interact either with lipopolysaccharides on the cell surface of Gram-negative (G–) bacteria or with teichoic acids on the cell surface of Gram-positive (G+) bacteria. A similar mechanism of action might be possessed against fungi, although chitosan's antifungal efficiency is shown to be low [110]. The key factors that affect chitosan's antimicrobial activity include environmental factors (pH, T), microbial factors (the type of microorganism and phase of the cell growth), and intrinsic factors (M_W, DA, derivate form, concentration, etc.) [111].

In spite of the fact that chitosan has inherent antimicrobial activity, chitosan-based films are usually incorporated by different AAs in order to boost it up (Sections 3.1.1 and 3.1.2). On the contrary to chitosan, alginate does not have inherent antimicrobial activity, but alginate-based films with incorporated AAs do have (Section 3.1.3).

3.1.1 Antibacterial activity of chitosan-based films with incorporated active agents

In all herein reviewed studies, the antibacterial activity of chitosan-based films was tested *in vitro*. The antibacterial efficiency is often expressed as a diameter of the inhibition zone using the disc diffusion method or by evaluating bacterial burden reduction through counting colony-forming units (CFU) or measuring the optical density of a sample. The tests were accomplished against the most common foodborne pathogens and representatives of (G+) and (G–) bacteria. Among (G–) bacteria, *Escherichia coli* and *Salmonella typhimurium* are reported as a leading cause of many severe and fatal foodborne outbreaks mostly related to meat and meat products [112]. In the majority of studies, *Staphylococcus aureus* was used as a representative of (G+) bacteria since it is a major public health concern worldwide as well as the most common cause of foodborne disease in the United States [113]. Another very concerning (G+) bacteria is *Listeria monocytogenes*, responsible for disease listeriosis associated with a high mortality rate [114].

Generally speaking, control chitosan-based films (i.e. without incorporated AAs) have showed certain antibacterial activity in the majority of overviewed studies (Table 1). However, in many cases a lack of the inhibition zone has been reported, whereby growth inhibition has been observed only in the area that is in direct contact with a film [131]. This is mostly on account of chitosan's solid-state possessed in the form of a film, which disallows efficient diffusion of chitosan into the agar medium and therefore to pathogenic microorganisms. Anyway, a quest for new methods and active components that could improve the antibacterial activity of chitosan-based films has appeared as a "hot topic" in recent times (Table 1).

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Table 1: Recent studies on the antibacterial activity of chitosan-based films with incorporated active agents.

Active agent (AA)	AA concentration in the film-forming solutions	Microorganism	Expression of antibacterial activity	CH[a]	CH-AA[b]	Ref.
Ziziphora clinopodioides EO (ZEO)	1 % (v/w)	*Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Bacillus subtilis*, *Bacillus cerus*	Inhibition zone, Log reduction of CFU/mL	–	+(i)	[115]
Grape seed extract (GSE)	1 % (v/w)			–	+(i)	
Turmeric extract (TEE)	1:2 (v/v) dilution of CH solution with TEE in ethanol (25 mg_{TEE}/mL)	*Staphylococcus aureus*, *Salmonella typhimurium*	Log reduction of CFU/g sample	+	+(i)	[116]
Zinc oxide (ZnO)	0.1–0.5 % (w/v)	*Escherichia coli*	Inhibition zone	+	+(i)	[117]
Neem oil	0.5 % (v/v)			+	+(i)	
Litsea cubeba oil (LEO)	4 – 16 %; total weight	*Escherichia coli*, *Staphylococcus aureus*	Inhibition zone	+	+(i)	[118]
Citrus extract	0.5 % (v/v)	*Listeria innocua*	Inhibition zone	–	+(i)	[119]
Naringin	0.05 – 1 % (w/v)			–	–(d)	
ε-Polylysine	ε-polylysine:chitosan mixtures (weight ratios 1:5–1:15) dissolved in distilled water	*Escherichia coli*, *Staphylococcus aureus*	Inhibition zone	–	+	[120]
β-Cyclodextrin (β-CD)/EO complex	0.25 – 1 % (v/v)	*Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium*	Log reduction based on optical density measurement	+	+(i)	[121]
Zinc oxide nanoparticles (Zn-NPs)	0.5 – 2 % (w/v)	*Escherichia coli*, *Staphylococcus aureus*	CFU counting	–	+(i)	[122]
Zinc oxide (ZnO)	2 – 8 %; total weight	*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cerus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*	Inhibition zone	–	+(i)	[123]
Montmorillonite-copper oxide (MMT-CuO)	1 – 5 % (w/w); based on CH mass	*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Bacillus cerus*	Mortality rate based on CFU counting	+	+	[124]
			Inhibition zone	+	+	
Spirulina extract (SE)	2.5 – 20 % (w/v)	*Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Bacillus subtilis*, *Bacillus cereus*	Inhibition zone	–	+(i)	[125]
Cinnamon bark oil (CBO)	1 – 3 % (w/w)	*Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*	Inhibition zone	–	+(i)	[126]
Citrus EOs	0.5 % (v/v)	*Listeria monocytogenes*	Log reduction of CFU/cm^2	+	+(i)	[127]
Eucalyptus globulus EO	1 – 4 % (v/v)	*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*	Inhibition zone	–	+(i)	[128]

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Caraway EO/beeswax	1 % (v/v) (caraway)/18 – 90 km/m^3 (beeswax)	*Escherichia coli*, *Staphylococcus aureus*	Reduction of CFU/mL	+	–	[129]
Hop extract (HE)	0.1–1.5 % (w/v)	*Escherichia coli*, *Bacillus subtilis*	Inhibition zone/Contact inhibition	+	+(i)	[130]
Oak extract (OE)/algal extract (AE)	0.1 % (w/v)	*Escherichia coli*, *Bacillus subtilis*	Inhibition zone/Contact inhibition	+	+	[131]
Propolis extract (PE)	2.5 – 20 % (w/w); based on CH mass	*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*	Inhibition zone/Contact inhibition	–	+(i)	[132]
Syringic acid	0.125–0.5 g in 50 mL of film-forming solution	*Escherichia coli*, *Staphylococcus aureus*	Inhibition zone	+	+(i)	[133]

[a] CH − chitosan-based films.
[b] CH-AA − chitosan-based films with incorporated AA: (–) no antibacterial activity; (+) antibacterial activity; (i) antibacterial activity increased after the incorporation of AA; (d) antibacterial activity decreased after the incorporation of AA.

Nanocomposites are based on natural polymer matrix incorporated with nanoparticles (NPs) [134]. Since some NPs have shown convincing antibacterial and antioxidant properties, nanotechnology has emerged as a good alternative for the improvement of chitosan-based films' antibacterial activity [135]. For instance, stable ZnO-NPs are classified as Generally Recognized as Safe (GRAS), and therefore represent one of the most frequently studied nano-based materials for the development of active food packagings. Consequently, ZnO-bionanocomposite-blended chitosan films have been used as pouches to study antimicrobial activity and effectiveness in extending the shelf life of meat, cheese, and carrots [117, 122, 123]. Next to it, montmorillonite-copper oxide (MMT-CuO) nanocomposite was incorporated into chitosan matrix as a reinforcement and antibacterial agent [124]. MMT also serves as a stabilizer of copper ions by preventing their uncontrolled leaching and toxicity. Since it has been shown that MMT-CuO nanocomposite significantly improves antibacterial activity against (G+) and (G–) bacteria, a chitosan-MMT-CuO nanocomposite film was considered as a promising novel active food packaging [124]. However, there is a growing concern related to the application of NPs because they might have different physicochemical properties than their larger counterparts, and therefore might cause health problems [134].

A growing awareness of food safety and increasing life standard have led to even higher public disapproval and negative perception of the application of synthetic additives as food preservatives. Essential oils (EOs), which are natural compounds, i.e. secondary metabolites of aromatic plants produced for their protection against pathogens and herbivores, present a good substitute [103, 136]. Nowadays, there are many studies that approve EOs' broad antibacterial activity against bacteria, yeast, and molds [136]. Although the exact mechanism of their action is still unknown, the most common explanation is related to hydrophobic nature of their main compounds which might contribute to a disruption of the cell membrane, cytoplasmatic leakage, cell lysis, and eventually cell death [136]. In general, (G+) bacteria are more susceptible than (G–) bacteria, which is attributed to differences in the cell wall structure of (G+) and (G–) bacteria. The latter is due to dense hydrophilic lipopolysaccharide covering, which prevents diffusion of hydrophobic compounds more resistant to the EOs [136]. *Ziziphora clinopodioides* EO (ZEO) and grape seed extract (GSE) [115], turmeric extract (TE) [116], *Litsea cubeba* oil (LEO) [118], *Eucalyptus globulus* EO [128], and caraway EO [129], present examples of a successful application of EOs as antibacterial agents in chitosan-based films (Table 1).

However, there are some drawbacks that limit the use of EOs as food preservatives. Low water solubility demands their incorporation in higher amounts in the film-forming formulations, which can negatively affect food organoleptic properties due to their intense aroma and potential toxicity. High extraction costs and a quick and significant decrease in their effectiveness due to relatively high volatility are just other restrictions to the extensive application of EOs. Sun et al. have developed β-cyclodextrin-EOs complexes that increase the water solubility of EOs and hence enable their use in lower concentrations [121]. Increased water solubility might also lead to the increased contact surface between pathogens and EOs, thus effectiveness is also improved [121]. EOs are also known to cause the formation of particular structures in the chitosan-based films that scatter visible light, whereby this problem was overcome by the incorporation of microemulsions of cinnamon bark oil and soybean oil [126].

The antibacterial activity of chitosan-based films can be also improved by the incorporation of ε-polylysine − a water-soluble, biodegradable, and non-toxic homo-poly-amino acid characterized by the peptide bond between the carboxyl and ε-amino groups of L-lysine [120]. Its antibacterial activity is related to the polycationic amino groups that are responsible for ε-polylysines electrostatic adsorption to the cell surface leading to the disruption of the outer cell membrane [120]. Besides, the extract from cyanobacterium *Spirulina* incorporated in chitosan-films has shown a positive antibacterial effect, because it is a good source of various active polyphenolic compounds [125].

3.1.2 Antifungal activity of chitosan-based films with incorporated active agents

Fungi present one of the major causes of post-harvest decay of various agricultural foods (such as cereal crops, fruits, vegetables), and are responsible for a big portion of food waste and thus large economic losses in agriculture [137, 138]. Besides, it could lead to serious life threats if fungi-contaminated food is consumed. By that, mycotoxin-producing fungi present the major health concern and a leading cause of acute poisoning. In general, *Aspergillus*, *Fusarium*, and *Penicillium* have been reported to be the most commonly responsible for mycotoxin food contamination [139]. The most recent publications of chitosan-based films with antifungal activity have been collected and presented in Table 2.

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Table 2: Recent studies on the antifungal activity of chitosan-based films with incorporated active agents.

Active agent (AA)	AA concentration in the film-forming solutions	Microorganism	Expression of antifungal activity	CH[a]	CH-AA[b]	Ref.
Anise, oregano, cinnamon EOs	250 ppm	*Penicillium* sp., *Rhizopus* sp.	Inhibition zone	–	+(i)	[140]
Quince juice, cranberry juice	lyophilised cranberry juice:water weight ratios: 1:19, 2:18, 3:17; lyophilised quince juice:water weight ratios: 1:16, 2:15, 3:14	*Penicillium expansum*	Inhibition zone	–	+	[141]
Cinnamon (CEO) and ginger (GEO) EOs	4.4–13.2 % (w/w) (CEO) and 3.5–10.6 % (w/w)(GEO); based on CH mass	*Aspergillus niger*	Inhibition zone	+	+(i)	[142]
Thyme-oregano; thyme-tea tree, or thyme-peppermint EOs mixtures	0.13 and 0.19 % (w/w)	*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Penicillium chrysogenum*	Log reduction of CFU/g sample	–	+(i)	[143]
Cinnamon leaf EO	0.25 – 1 % (w/w)	*Aspergillus niger*, *Botrytis cinerea*, *Rhizopus stolonifer*	Inhibition zone	–	+(i)	[144]
Eucalyptus globulus EO	1 – 4 % (v/v)	*Candida albicans*, *Candida parapsilosis*, *Botrytis cinerea*	Inhibition zone	–	+(i)	[128]

[a] CH − chitosan-based films.
[b] CH-AA − chitosan-based films with incorporated AA: (–) no antifungal activity; (+) antifungal activity; (i) antifungal activity increased after the incorporation of AA.

Incorporation of EOs has significantly improved the antifungal activity (usually increases with increasing concentration of EOs) of blended films, since pure chitosan films barely show any antifungal activity (Table 2). However, it highly depends both on fungi species and on the type of EO, whereby the following plant extracts have been tested so far: cinnamon [140, 142, 144], oregano and anise [140], ginger [142], quince and cranberry juice [141], thyme [143], and *Eucalyptus globulus* EOs [128]. For instance, cinnamon EO is more effective against *Aspergillus niger* than ginger EO [142]. Besides, it has been shown that antifungal activity could be improved by the incorporation of EOs mixtures instead of single sort of EOs [143]. A combination of EOs from thyme and oregano, tea tree, or peppermint has reduced fungal growth of *Aspergillus* and *Penicillium* species by 51 % – 77 % [143].

3.1.3 Antibacterial and antifungal activity of alginate-based films with incorporated active agents

Contrary to chitosan, SA has no inherent antimicrobial activity and thus fails to provide a barrier against microbial infections, which could restrict its application. However, it has been increasingly regarded as a promising food packaging material due to its water-solubility, non-toxicity, biocompatibility, biodegradability as well as capability of forming films with incorporated different AAs.

According to the recent publications related to the development of alginate-based films, SA is rarely used as a sole component. To improve mechanical and water-resistance properties, a formation of composite or nanocomposite films is preferred. Composite films are mostly gained through blending with other biopolymers, such as chitosan, carboxymethyl cellulose (CMC), or microfibrillated cellulose (MFC). Nanocomposites are formed through the incorporation of NPs, like nano-sized clay in SA matrix. Composites or nanocomposites also serve as a good matrix for the incorporation and stabilization of various antimicrobial agents [145]. The most recent studies dealing with the improvement of antimicrobial activity of alginate-based composites are summarized in Table 3.

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Table 3: Recent studies on the antimicrobial activity of alginate-based films with incorporated active agents.

Active agent (AA)	AA concentration in the film-forming solutions	Microorganism	Expression of antimicrobial activity	SA	SA-AA	Ref.
Ag-nanoparticles, grape seed extract (GSE)	10 % (w/w) of GSE; based on polymers mass	*Escherichia coli*, *Listeria monocytogenes*	Log reduction of CFU/ml	–	+(i)	[146]
Pyrogallic acid (PA)	0.01–0.04 % (w/w); based on polymers mass	*Escherichia coli*, *Staphylococcus aureus*	Inhibition zone	–	+(i)	[147]
Au-TiO_2-nanoparticles	up to 2.5 %; total weight	*Escherichia coli*, *Staphylococcus aureus*	Survival rate based on CFU counting	–	+(i)	[148]
Lactococcus lactis	0.5–2.5 % (w/w)	*Escherichia coli*, *Staphylococcus aureus*	Log reduction of CFU/cm^2	–	+(i)	[149]
Microfibrillated cellulose/chitosan-benzalkonium chloride complex (MFC/C–BC)	2 – 14 % (w/w); based on SA mass	*Escherichia coli*, *Staphylococcus aureus*	Inhibition zone	–	+(i)	[150]
Carboxymethyl chitosan-ZnO nanoparticles	0.005–0.05 % (w/w); based on SA mass	*Escherichia coli*, *Staphylococcus aureus*	Bactericidal ratio based on CFU counting	–	+(i)	[151]
Clove, coriander, caraway, marjoram, cinnamon, and cumin EOs	0.5–1.5 % (w/v)	*Listeria monocytogenes*	Log reduction of CFU/cm^2	–	+(i)	[152]
Lemongrass oil microcapsules (LMO)	1250 – 5000 ppm	*Escherichia coli*, *Listeria monocytogenes*	Growth inhibition based on optical density measurement	–	+(i)	[153]
Elicriso, chamomile blue, cinnamon, lavender, tea tree, peppermint, eucalyptus, lemongrass, lemon EOs	16 – 66 % (w/w); based on dry film mass	*Escherichia coli*, *Candida albicans*	Inhibition zone	–	+(i)	[154]

[a] SA: alginate-based film.
[b] SA-AA: alginate-based film with incorporated AA: (–) no antimicrobial activity; (+) antimicrobial activity; (i) antimicrobial activity increased after the incorporation of AA.

Regarding antibacterial activity, the majority of tests have been accomplished against *Staphylococcus aureus* as a representative of (G+) bacteria and as a representative of *Escherichia coli* as (G–) bacteria (Table 3). Antibacterial activity has been enhanced with the application of metal NPs (Ag, Au, ZnO, TiO_2) or organic salts complexes. For instance, it has been developed a TiO_2-nanocomposite-incorporated alginate-based film whose antibacterial activity stems from the photocatalytic activity of TiO_2 and reactive oxygen species (ROS) production upon illumination of the film with UV light [148]. In the same study, the antibacterial activity was further improved with the incorporation of plasmonic NPs such as Au in TiO_2 nanostructures what led to enhanced light absorption in the visible light region and more intensive ROS production [148]. A film that consists of a chitosan-based outer layer, an SA-based inner layer, and incorporated carboxymethyl chitosan-ZnO NPs has been developed, whereby the proposed mechanism of action was ROS production as well [151]. Moreover, the incorporation of biocomposite synthesized from chitosan-benzalkonium chloride (C-BC) complex and micro fibrillated cellulose (MFC) in SA formulation has shown improved antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [150].

Regarding increasing public demands for natural preservatives, some plant extracts and EOs have been also tried as the antibacterial agents in alginate-based composite films. For example, the antibacterial activity of pyrogallic acid (PA) was tested through its incorporation in a sodium alginate/carboxymethyl cellulose (SA/CMC) composite formulation [147]. Furthermore, Alboofetileh et al. have prepared a functional bionanocomposite film based on sodium alginate/montmorillonite (SA/MMT) formulation, whose antibacterial activity against *Listeria monocytogenes* was provided with the addition of either marjoram (MEO), cinnamon (CIEO), or clove (CEO) EOs [152]. All EOs have shown a significant reduction in the microbial count, whereby MEO has appeared as the most successful one [152]. The alginate-based films with microencapsulated lemongrass EO were able to inhibit the growth of *Escherichia coli* and *Listeria monocytogenes*, and therefore such films could also have a potential for the practical application in the food shelf life extension [153].

Composite hydrogel films containing Ag-NPs or GSE have been developed using three biopolymers: agar, SA, and collagen [146]. Ag-NPs-containing films and GSE-containing films have showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, respectively, whereby differences in the activity are explained by different cell wall characteristics of (G+) and (G–) bacteria [146]. *Lactococcus lactis*, a probiotic strain that inhibits pathogenic bacteria in the digestive tract by producing lactic acid and bacteriocin, has been also successfully incorporated in an SA/CMC composite film [149]. All films with added *Lactococcus lactis* have shown significant antibacterial activity, although it depends mostly on the amount of added bacteria, types of packaged food, and the initial amount of pathogens [149].

In the field of alginate-based films with antifungal activity, only one study has been found so far. Namely, nine different EOs (elicriso, chamomile blue, cinnamon, lavender, tea tree, peppermint, eucalyptus, lemongrass, lemon) were applied in increasing concentrations and the antifungal activity was tested against fungi *Candida albicans*, whereby the films with incorporated cinnamon, peppermint, and lemongrass EOs showed the highest inhibition zones [154].

3.2 Antioxidant activity of chitosan- and alginate-based films with incorporated active agents

Due to a bad image of the chemical-based additives, there is a growing interest in the application of natural antioxidant activity-enhancing components [155]. While blank chitosan-based films show some antioxidant activity itself, the antioxidant activity of alginate films is mostly due to the incorporation of AAs in the film matrix.

One of the oldest synthetic radicals used to test antioxidant activity is 2,2-diphenyl-1-picrylhydrazyl (DPPH) [156]. This (frequently used) method means that the films are soaked in methanol, ethanol, or water and allowed to interact with stable radical DPPH, whereby its disappearance is followed by measuring the absorption at 515 nm [157]. The antioxidant properties are quantified by the amount of antioxidant required to decrease initial DPPH concentration by 50 %, and by the time required to reach constant DPPH concentration [156, 157]. A potential drawback of the method is that DPPH interacts with other radicals (such as alkyl), and time needed to reach steady state of DPPH concentration is not linear with changing the antioxidant/DPPH ratios [103, 158]. In addition to this, the following antioxidant activity methods are also prevalent in the literature: reducing power assay [103], ferric reducing antioxidant power (FRAP) assay [103, 158], Trolox®-equivalent antioxidant capacity (TEAC) assay [159–162], ferrous ion chelating activity (FIC) assay [103, 163, 164], etc. The antioxidant activity of films is in a correlation with their total phenolic content (TPC) [128], which can be estimated by means of Folin-Ciocalteu (FC) reagent [130]. In this method, the reduction of reagent is associated with a colour change (from yellow to blue) detected spectrophotometrically, whereby gallic acid is used as a standard and the results are expressed as the mass of gallic acid equivalent (GAE) per mass of the film [130, 144, 165].

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The most frequently added class of bioactive antioxidants is polyphenols, which can be incorporated in biopolymer-based films in different ways. Table 4 summarizes the most recent studies on the antioxidant activity of chitosan- and alginate-based films with incorporated AAs.

Table 4: Recent studies on the antioxidant properties of chitosan- and alginate-based films with incorporated active agents.

Biopolymer	Active agent (AA)	Method	Ref.
Chitosan	Black and purple eggplant extract	DPPH	[166]
Chitosan	Gallic acid	DPPH	[167]
Chitosan	Black soybean seed coat extract	DPPH	[168]
Chitosan	Mango leaf extract	TPC, DPPH, TEAC, FRAP	[165]
Chitosan	Purple-fleshed sweet potato extract	DPPH	[169]
Chitosan/gelatin	Nanoemulsions encapsulating active compounds	DPPH, TEAC, FRAP	[170]
Chitosan	Grape seed extract	FC, DPPH	[171]
Chitosan	Kombucha tea	DPPH	[172]
Chitosan	Apple peel polyphenols	DPPH, TEAC	[173]
Chitosan	*Camelina sativa* seed EO	FRAP	[174]
Chitosan	*Nigella sativa* seed extract	FC, DPPH, FRAP	[175]
Chitosan/gelatin	Eugenol and ginger EOs	TEAC	[176]
Chitosan	Extracts of peanut skin/pink pepper residues	FC, DPPH, TEAC, ORAC, superoxide anione	[177]
Chitosan	Citric acid	H_2O_2 radical scavenging assay	[178]
Chitosan	Apricot kernel oil	DPPH, H_2O_2 radical scavenging assay	[179]
Chitosan	*Lepidium sativum* seedcake extract	FC, DPPH	[180]
Chitosan/starch	*Litsea cubeba* oil	DPPH	[118]
Chitosan	Clove essential oil, halloysite nanotubes	FC, DPPH, reducing power assay, migration studies	[181]
Chitosan	Capsaicin	DPPH	[182]
Chitosan	Oregano and thyme essential oils	DPPH	[183]
Chitosan	Olive pomace	DPPH	[184]
Chitosan/starch	Cranberry, blueberry, beetroot, pomegranate, oregano, pitaya/dragon fruit, resveratrol	FC	[185]
Chitosan	Blueberry and blackberry pomace extract	FRAP, TPTZ	[186]
Chitosan	Hop extract	FC (TPC)	[130]
Chitosan	Oak and algal extracts	FC (TPC)	[131]
Chitosan	Chestnut extract	FC (TPC)	[187]
Chitosan	Protocatechuic acid	FC, DPPH	[188]
Chitosan	Dimeric α,β-peptoids	DPPH	[189]
Chitosan	*Origanum vulgare* ssp. *gracile* EO	DPPH	[190]
Chitosan	*Carum copticum* EO	DPPH	[191]
Chitosan	Hydroxybenzoic acid	DPPH	[192]
Chitosan	Young apple polyphenols	DPPH	[193]
Chitosan/starch	Thyme extract	TEAC	[194]
Chitosan	*Eucalyptus globulus* EO	TPC, DPPH, NO-scavenging activity, H_2O_2 radical scavenging assay	[128]
Chitosan	Nettle (*Urtica dioica* L.) extract	DPPH	[195]
Chitosan	*Thymus* species EOs	DPPH, FRAP	[196]
Chitosan	Caraway EO/beeswax	DPPH	[129]
Chitosan	*Lycium barbarum* fruit extract	DPPH	[197]
Chitosan	Maqui berry	DPPH, FRAP, FIC	[198]
Chitosan	Carvacrol and pomegranate peel extract	TPC, FRAP	[199]
Chitosan	Cinnamon leaf oil or oleic acid	TEAC	[144]
Chitosan	Caraway EO	DPPH	[129]
Chitosan	Propolis extract	TPC, DPPH	[132]
Chitosan	*Pistacia terebinthus* extracts	DPPH	[200]
Alginate	Protein hydrolysates	TPC	[201]
Alginate /gelatin	Tea polyphenols	TPC	[202]
Alginate	Cinnamon leaf oil or cinnamon bark oil	DPPH	[203]
Alginate	Black chokeberry extract	TPC	[204]
Alginate	Green tea extract/grape seed extract	TEAC	[205]

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A good way of increasing the antioxidant activity *via* natural additives is by using extracts such as apple peel extract [173], *Nigella sativa* seed [175], thyme extract [194], peanut skin extract [177], *Lepidium sativum* seedcake extract [180], purple-fleshed sweet potato extract [169], tea extracts [172, 206], mango leaf extract [165], carvacrol and pomegranate peel extracts [199], thinned young apples polyphenolic extract [193], grape seed extract [171], hop extract [130], oak extract [131], chestnut extract [187], *Pistacia terebinthus* (stem, leaf, and seed) extracts [200], etc. Moreover, the antioxidant activity can be enhanced by the incorporation of EOs obtained from *Thymus* species [196], apricot kernel [179], oregano and thyme [183], *Origanum vulgare* ssp. *gracile* [190], clove [181], *Camelina sativa* [174], *Litsea cubeba* [118], *Eucalyptus globulus* [128], *Carum copticum* [191], black soybean seed coat extract [168], and ginger [176]. Furthermore, the antioxidant activity can be enhanced by the incorporation of berries, as reported in the case of maqui berry [198], and cranberry/blueberry [185]. It has been reported that agro-industrial residuals and olive pomace flour have enhanced the antioxidant activity of the films as well [177, 184].

Priyadarshi et al. have reported the incorporation of citric acid as an active ingredient for the extension of green chili shelf life [178]. Examples of grafting/incorporating chitosan-based films with hydroxybenzoic acid [192], protocatechuic acid [188, 207], gallic acid [167], or even capsaicin − an active substance isolated from chili peppers [182], have been also reported throughout the literature. However, the highest improvements of the antioxidant activity have been observed after the incorporation of AAs such as protein hydrolysates and propolis extract (Figure 2).

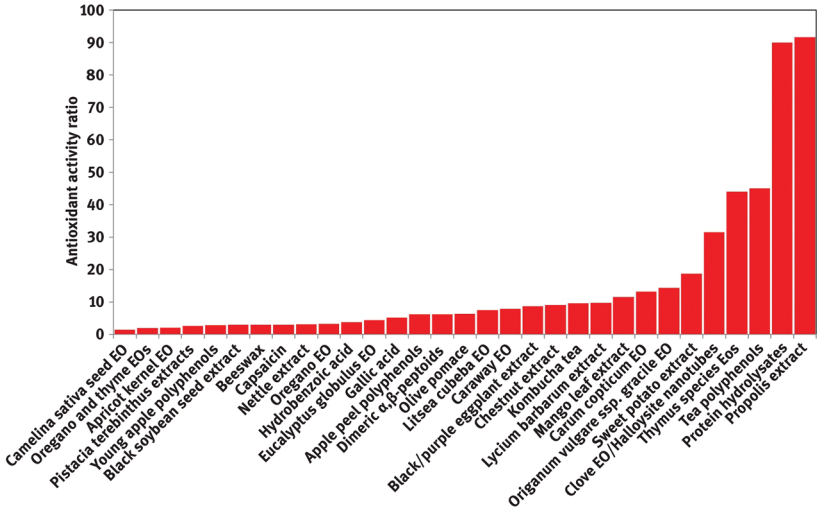

Figure 2: The effect of different active agents on the antioxidant activity of some chitosan- and alginate-based films. The effect was evaluated through the ratio between antioxidant activities of the film samples with and without the incorporated active agent.

4 Future perspectives and conclusions

Waste/residual marine biomass is a valuable source for the isolation of biopolymers such as chitin/chitosan and alginate. Their isolation can be followed by the development and production of advanced biopolymer-based packaging materials in order to create business for food industries, at the same time being aware of both the food quality (and safety) demanded by consumers and the environmental care demanded by the institutions and society. Therefore, this review aims to show that the food packaging films can be successfully prepared from biomass-derived chitosan and alginate as well as that the films' properties can be tailored in terms of antimicrobial and antioxidant activities by the incorporation of a wide variety of components.

Nevertheless, special attention should be devoted to the invention of advanced eco-friendly processes for both isolation of biopolymers and preparation of active agents in sufficient quantities at relatively acceptable costs and low ecological footprint. Besides, the preparation of film materials is a multi-task problem that should be carefully considered and planned. This is because the incorporation of active agents affects not only antimicrobial and antioxidant properties of the films, but simultaneously their mechanical (strengths, stiffness, elasticity) and barrier (against UV-vis light and gases) properties. The release of active agents from the films and their potential side effects on the organoleptic properties of food should be of paramount importance for further development of packaging materials with advanced properties as well. Last but not least, the film's

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biodegradability should be sufficient to strengthen the main concept of the circular economy and to make them competitive to other eco-unfriendly materials.

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