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# Seaweeds polysaccharides in active food packaging: A review of recent progress

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## ABSTRACT

**Background:** Due to its short lifetime food packaging leads to a rapid accumulation of plastic in our surroundings and thereby also has a huge impact on environmental pollution. To reduce these effects and create a more sustainable approach towards food packaging, biodegradable and biobased polymers have been developed and are emerging on the market.

**Scope and approach:** This review provides the current state of research regarding active packaging and the incorporation of seaweed into food packaging. Further, it summarises the resulting consequences of the seaweed incorporation on mechanical, physical, thermal, antioxidant, antimicrobial and chemical properties, as well as the release of active compounds to show the advantages of the polysaccharides as well as possible shortcomings in current research.

**Key findings and conclusions:** To improve these polymers regarding their mechanical, thermal and antimicrobial properties etc. a variety of polysaccharides such as seaweeds can be used. They not only lead to an increase in hydrophilicity and improved mechanical properties such as tensile strength and elongation at break, but also create the possibility of using it as active packaging. This can be achieved due to the naturally occurring antioxidant properties in seaweed, which can minimise lipid oxidation and thereby increase the shelf life and nutritional value of food as well as reduce free radicals which might have a carcinogenic, mutagenic or cytotoxic effect. Some seaweeds such as *H. elongate* have also proven to inhibit the growth of gram-positive and gram-negative bacteria, meaning that they could possibly be used as antimicrobial packaging.

## 1. Introduction

Packaging is an essential part of the modern food industry. It is needed to preserve food and assures the safety and the integrity of the food (Nešić et al., 2019). These advantages make packaging an essential part of our supply chain, however, it also poses challenges such as migration of contaminant residues, cost and energy efficiency as well as sustainability (Karmaus et al., 2018; Marsh & Bugusu, 2007). Glass, paper, various metals and plastic are commonly used materials for food packaging, though, plastics are the most frequently used material after paper (Marsh & Bugusu, 2007). According to PlasticsEurope, about 51.2 Mt of plastics was used in Europe, with 39.9% was estimated to be used for packaging (PlasticsEurope, 2019). Plastic that is mostly used in food

packaging is thermoplastics, which soften under heat exposure and then returns to its former condition when cooled down (Matthews, Moran, & Jaiswal, 2020).

The most commonly used thermoplastics are high-density polyethylene, low-density polyethylene, polypropylene and polyethylene terephthalate, which usually have a lifetime of less than a year before being discarded (Geyer et al., 2017; Marsh & Bugusu, 2007). This short lifetime leads to a rapid accumulation of plastic waste in the environment. About 10–20 Mt of plastic ends up in the ocean each year, which is due to currents and wind, spread throughout the marine environment thereby damaging its ecosystem and wildlife. It also leads to the transfer of microplastic, containing toxic additives, into the food chain (Gourmelon, 2015). In recent years, numerous studies were carried out in

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search of viable, environment friendly alternatives such as biodegradable and/or biobased polymers and significant advancement has been made in this direction (de Oliveira et al., 2020; Latos-Brozio & Masek, 2020; Sharma et al. 2020a, 2020b, 2020c).

Biodegradable polymers such as polylactic acid (PLA), polyhydroxy butyrate (PHB) etc. are made from natural and fossil resources. These biodegradable polymers could be metabolised by the microorganisms and subsequently return to the nature in a short period of time. Whereas bio-based plastics are purely made from bio-based resources like protein/polysaccharides/microbial polyesters which are extracted from plants, marine organisms or produced by microorganisms through fermentation process. The polysaccharides used in bio-based polymers are biologically degradable and non-toxic (Nešić et al., 2019). These biological materials are also known to be biocompatible, which describes 'the ability of a material to perform with an appropriate host response in a specific application' (Williams, 1987). These characteristics, thereby, offer significant advantages in food packaging, particularly in edible coatings and films. Due to inherent antimicrobial, antioxidant and numerous other biological properties polysaccharides also provide a sustainable option for active packaging (Nešić et al., 2019). Seaweeds are rich source of polysaccharides and therefore is a promising raw material for active packaging, especially when combined with biodegradable polymers, it offers a sustainable alternative superior to traditional materials.

This review provides the current state of research regarding active packaging and the incorporation of seaweed into food packaging. Furthermore, it summarises the resulting significances of the seaweed incorporation on mechanical, physical, thermal, antioxidant, antimicrobial and chemical properties of packaging materials. This article also provides overview on release of active compounds resulting from the addition of seaweed polysaccharides into food packaging. Furthermore, legal aspects of the use of seaweed polysaccharides in food packaging and future trend were discussed.

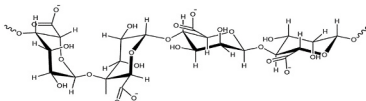
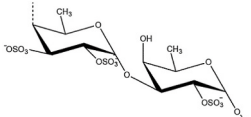
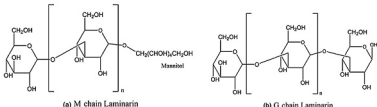
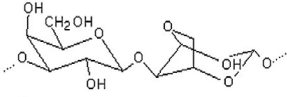
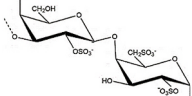
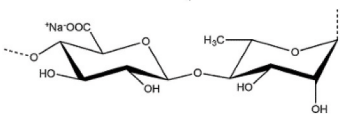
## 2. Seaweeds

Seaweeds are macroscopic, multicellular, benthic algae, which, compared to terrestrial plants, are more efficient when it comes to photosynthesis causing a rapid accumulation of biomass due to a faster growth rate. It is a major component of the aquatic biomass and represents around 50% of earth's primary productivity and commonly used as food, feed and in numerous other applications (Salehi et al., 2019; Khatri, Rathore, Agrawal, & Jha, 2019). Due to its high abundance the rise in seaweed demand over the last decade led to a spread of seaweed cultivation. This results in a considerable quantity of seaweed waste from various industrial processes such as food industry where the seaweed needs to meet certain quality standards. These wastes can be incorporated into a circular economy, where they can be used as a third generation of biomass for feed, agriculture, biofuel or in pharmaceutical applications. The reuse of the waste material is crucial, since the release of these products into nature could have a huge impact on the marine environment. It further indicates a great potential for the use of algae as a renewable source of energy and other high value products (Khatri, Rathore, Agrawal, & Jha, 2019; Torres, Kraan, & Domínguez, 2019).

There are more than 10,000 different species of seaweed worldwide, which grow in up to 180 m depth on solid substrates such as rock. It is one of the most important living resources in the ocean for marine biodiversity. Seaweeds can be categorized into 3 groups based on pigmentation type, namely: red (Rhodophyta), brown (Ochrophyta) and green (Chlorophyta). These seaweeds are a source of numerous bioactive compounds with diverse applications in different fields such as the pharmaceutical, agricultural and the medical industry (Salehi et al., 2019). Table 1 shows different types of seaweeds and associated polysaccharides and their chemical structure.

Red seaweeds differ from brown and green seaweeds due to the presence of red and blue pigment, phycocyanin and phycoerythrin as well as chlorophyll *a*. Due to a diverse range of plant forms and a variety of life cycles, red seaweed has a high diversity of species, for example, the most common species are *Poryphyra capensis*, *Aeodes orbitosa* and *Notogenia stiriata* (Pal et al., 2014). These seaweeds contain sulphated

**Table 1**  
Important seaweed polysaccharides and their chemical structure.

Seaweed	Polysaccharide	Chemical structure	Reference
Brown seaweed (Phaeophyta) e.g. <i>Laminaria pallida</i> , <i>Fucus</i> and <i>Zonaria</i> species	Alginate		Venkatesan et al. (2015)
	Fucoidan		Venkatesan et al. (2015)
	Laminarin		Kadam et al. (2015)
Red seaweed (Rhodophyta) e.g. <i>Poryphyra capensis</i> , <i>Aeodes orbitosa</i> , <i>Notogenia stiriata</i>	Agar		(El-hefian et al., 2012)
	Carrageenan		Venkatesan et al. (2015)
Green seaweed (Chlorophyta) e.g. <i>Cladophora</i> , <i>Ulva</i> and <i>Monostroma</i> species	Ulvan		Venkatesan et al. (2015)

galactans such as carrageenan's and agar, which are widely used biopolymers for food industrial applications (Delattre et al., 2011). Agar and carrageenan are considered non-digestible oligosaccharides which are non-cariogenic in humans, possess prebiotic, anti-tumour, antioxidant and has immune-modulatory properties (Cheong et al., 2018). The main components of agar are agarose and agaropectin which are similar to carrageenan in structure and function properties. It contains gelling, emulsifying and thickening properties which makes it useful in the scientific field as well as for commercial use in food, medical applications etc. The chemical structure of agar consists of  $\alpha$  (1–4)-3, 6-anhydro-L-galactose and  $\beta$  9(1–3)-D-galactose residues with esterified sulphate in small amounts up to 6% (w/w) (Pal et al., 2014).

Carrageenan's consist of an alternating backbone of  $\alpha$  (1–4)-3, 6-anhydro-D-galactose and  $\beta$  (1–3)-D-galactose with varying degrees of sulphation (Pal et al., 2014). It has hydrophilic and anionic properties and its solubility depends on the degree of sulphate ester, meaning the higher the amount of sulphate ester the higher the solubility. They are divided into three classes which vary in degrees of sulphation, the highest sulphated carrageenan class is the Lambda type with 40% (w/w). Unlike the other carrageenan's they cannot form gels but possess thickening properties (Delattre et al., 2011; Pal et al., 2014). Iota carrageenan's are less sulphated than the Lambda type; hence, they could form soft gels in the presence of calcium ions. The carrageenan with the lowest level of sulphate ester is the kappa family with a sulphate level of 20% (w/w) (Pal et al., 2014). These carrageenan's from strong gels in the presence of potassium ions and are used for cohesive, transparent films (Abdul Khalil et al., 2017).

Green algae are common in both fresh and saltwater and are categorized in that way due to the presence of chlorophyll *a* and *b* which gives them a green colour. Common green seaweeds are *Chaetomorpha* and *Cladophora* species as well as sea lettuce, which consists of the *Ulva* and *Monostroma* species. Ulvan is a cell wall polysaccharide which contributes up to 36% of the dry weight of green seaweed, with various potential uses in agriculture, pharmaceutical and food applications (Cunha & Grenha, 2016; Pal et al., 2014). It is made up from  $\alpha$ - and  $\beta$ -(1, 4)-linked monosaccharides such as rhamnose, xylose, glucuronic acid and iduronic acid with characteristic repeating disaccharide units consisting of aldobiuronic acid and minor ulvanbioses (Kidgell et al., 2019). As with other seaweed polysaccharides, the properties of ulvan strongly depend on the eco-physiology, the source species, and extraction methods (Cunha & Grenha, 2016; Kidgell et al., 2019; Pal et al., 2014). However, the recent research showed that the gels formed by ulvan are thermo-reversible and that the ulvan conformation and thereby also the gel formation, is influenced by a high or low ion concentration, as well as pH variations. The bioactivity of ulvan also depends on the molecular mass (Cunha & Grenha, 2016). Polysaccharides with lower molecular weight have proven to have a higher antioxidant activity overall and a higher antilipidemic effect on triglycerides and HDL-cholesterol, whereas fractions with high molecular weight show an increased antilipidemic effect on total serum and LDL-cholesterol. The polysaccharide has also proven to have an inhibitory effect on different viruses depending on the administered dose and the virus strain (Cunha & Grenha, 2016).

Brown seaweeds vary highly when it comes to size, species and overall morphology, however, they usually have a brown colour due to a photosynthetic pigment. Widespread types of brown seaweeds are kelp such as *Laminaria pallida*, *Fucus* species and the *Zonaria* species (Pal et al., 2014). Polysaccharides, which can be found in brown seaweed are alginate, laminarians and fucoidans (FUC). Alginates are components of the cell walls and the intracellular matrix of brown seaweed consisting of alternating blocks of  $\beta$ -D-mannuronic acid and  $\alpha$ -L-guluronic acid. The properties of alginates depend on the monomer sequence, which makes them a versatile material and can be used as film due to interaction with divalent and trivalent cations or as a disintegrating agent in tablets due to swelling in water (Abdul Khalil et al., 2017; Pérez et al., 2016). Laminarin is the main polysaccharide used for glucose storage in brown

seaweed and has a versatile range of reported bioactivities such as anti-tumour activity, antioxidant activity etc. The main industrial use, however, is as a ligand for pattern recognition receptors in the innate immune system (Smith et al., 2018). Another polysaccharide which can be extracted from brown seaweed is FUC consisting of complex, heterogeneous sulphated polysaccharides composed of L-fucose and sulphate ester groups with small amounts of different molecules such as (acidic-)/monosaccharides, proteins and acetyl groups. The composition and the properties of the polysaccharide are highly dependent on species, season, geographical origin as well as the extraction method (Cunha & Grenha, 2016). Some of the possible properties, however, are antitumor, antiviral, anticoagulant and anti-inflammatory activities, therefore, it is mainly used in biomedical related fields (Cunha & Grenha, 2016). Further components of seaweed consist of lipids, fatty acids, sterols, phenolic compounds, pigments, alkaloids, terpenes and halogenated compounds (Pérez et al., 2016).

As mentioned above, there are several possible applications in which seaweed could be used as a source for sustainable biomass. The Sea-BioPlas project coordinated by the "Bantry Marine Research Station Limited" showed, that sustainably grown seaweed can be used to produce the biopolymer PLA in an attempt to reduce the use of fossil-based plastics. The resulting waste can then be further processed as part of the circular economy (CORDIS, 2016). Research shows that big potential for the use of seaweed as well as a demand for research to assure a successful use of this potential in various field such as active food packaging.

### 3. Active packaging

Active packaging is a system in which product, packaging and environment interact in a positive way to extend the shelf life, improve the safety as well as the sensory properties and maintain the quality of the product. It incorporates components which enable the exchange of substances from the product into the environment, the environment to the product or the packaging to the product. The active packaging could be monolayered or multi-layered (Fig. 1) (Domínguez et al., 2018). In the monolayer system the active compound is incorporated with the polymer whereas in multilayer the active compound is entrapped between the layers of polymer to control its release. This process relies on strategies like temperature control and the addition of chemical. Currently, there are numerous active food packaging systems developed such as oxygen scavengers, carbon-dioxide generating systems, antimicrobial active packaging, moisture control packaging, ethylene scavengers and flavour and odour absorbent packaging (Biji et al., 2015; Sharma et al., 2020d). Also, the chemicals used as an active agent in these systems are shown in Fig. 2.

Oxygen scavengers (OS) are the most widely used forms of active packaging with the purpose of absorbing oxygen within the environment thereby preventing oxygen from reacting with the product. This would otherwise cause the growth of aerobic microbes, colour change, the reduction of shelf life and the survival of insects (Vilela et al., 2018). In general OS are defined as "materials incorporated into the package structure which form a chemical bond with, and thus effectively remove, oxygen from the inner package environment" (Brody et al., 2001). Packaging techniques such as vacuum packaging and modified atmosphere packaging are based on the same goal, however, with these methods a complete oxygen removal is not assured (Table 2). The operating principle behind O<sub>2</sub> scavengers varies depending on the active compounds in use such as iron, catechol, ascorbic acid, oxidative enzymes or unsaturated hydrocarbons and polyamides etc. (Vilela et al., 2018). A common and commercially used system is iron powder which is stored in a permeable sachet and enters an oxidation-reduction reaction with oxygen thereby removing it from the atmosphere (Cooksey, 2014).

The use of enzymes as the OS can be achieved by incorporating the immobilized enzyme into the package structure. Although there are many possibilities of enzymes, which can be used only a few have been

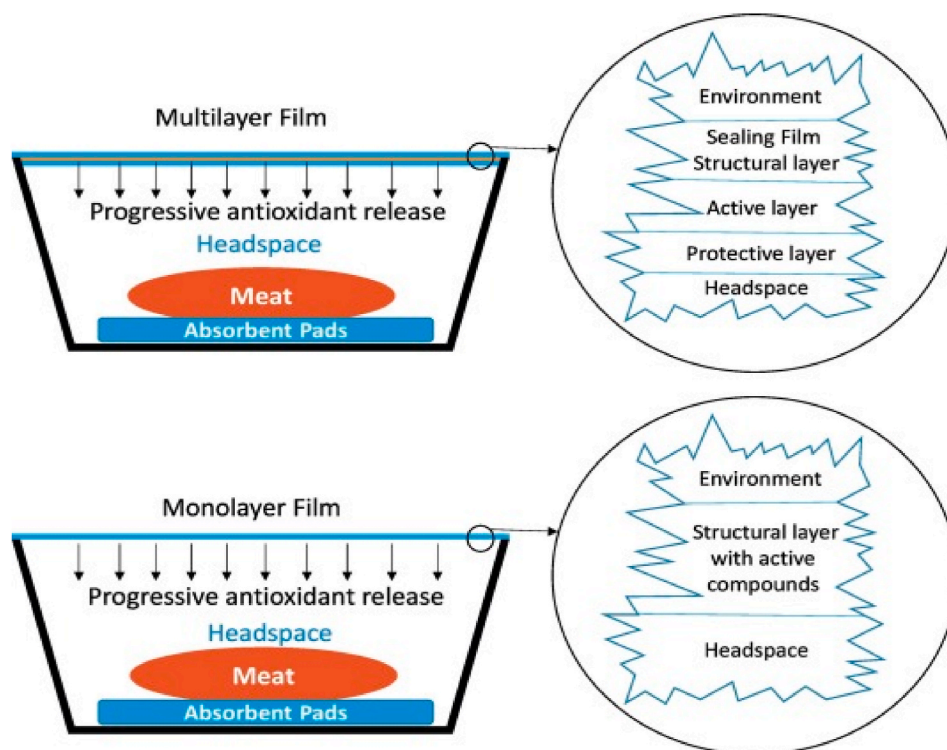
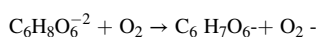


Fig. 1. Graphic representation of active packaging (multilayer and monolayer) (Domínguez et al., 2018).

further investigated with the most important ones being the addition of glucose oxidase and catalase. The glucose oxidase catalyses a reaction transforming glucose, oxygen and water to glucono-delta-lactone and hydrogen peroxide. To remove the hydrogen peroxide, which is also a very good oxidising agent, catalase has to be added as well, which then turns the  $H_2O_2$  into  $H_2O$  and  $O_2$  thereby binding half the oxygen present (Brody et al., 2001). A recent study by (Lee et al., 2018) investigated the use of non-ferrous OS consisting of activated carbon and sodium L-ascorbate in raw meatloaf packaging. The results showed lower thio-barbituric acid reactive substances and less microbiological changes, thus proving the efficacy of the OS in the food packaging industry. This method was based on ascorbic acid oxidation which follows this reaction equation:



In which  $C_6H_8O_6^{2-}$  is the sodium L-ascorbate and  $C_6H_7O_6^-$  is the L-ascorbate radical. Similarly, to the oxygen scavengers carbon dioxide generating system suppresses the microbial growth in the meat products as well as reduce the respiration of fresh produce (Biji et al., 2015). This effect is highly dependent on the amount of dissolved  $CO_2$  in the product and the overall solubility rate, which increases with decreasing temperature and depends on the properties of the food.  $CO_2$  emitters in general allow for a higher filling degree and reduced package size, which also leads to less environmental impact and better transport efficiency. Due to the release of carbon dioxide the  $CO_2$  absorption of the food product is compensated, which means no negative pressure will develop within the food packaging and the possibility of packaging deformation is lowered (Yildirim et al., 2018). The documentation of the technology behind  $CO_2$  emitters and their active compounds is often insufficient, but in general they are based on ferrous carbonate or a combination of ascorbic acid and sodium bicarbonate (Fang et al., 2017; Vilela et al., 2018). Current applications for  $CO_2$  emitters are  $CO_2$ ® Fresh Pads, Ultra Zap® Xtenda Pak pads and SUPERFRESH, which are  $CO_2$  emitting pads,  $CO_2$  emitting and antimicrobial pads and a box system with  $CO_2$  emitter respectively (Fang et al., 2017).

Antimicrobial packaging tends to reduce, inhibit or retard the growth of microorganisms by incorporating or coating antimicrobial agents in/on food packaging and thereby extending the lag phase and reducing the growth of microorganisms (Biji et al., 2015). There are four categories in which antimicrobial packaging can be classified, they are, antimicrobial sachet or pad, direct integration in polymer, antimicrobial coating and inherently antimicrobial polymer. In antimicrobial sachet or pad antimicrobial substance is incorporated in a sachet and added to the packaging. Another class is the direct incorporation of the antimicrobial agent into the polymer which will then gradually release the agent to the packaging headspace or the surface of the food (Fig. 1). The third category is the coating of the packaging with a matrix acting as a carrier for the antimicrobial substance which then would either evaporate into the headspace or migrate into the food through diffusion. Using polymers which are inherently antimicrobial is the last category to achieve antimicrobial properties (Fang et al., 2017). A few examples of antimicrobials which are being used are ethanol, which finds its application in bakery products, cheese and fish and is emitted from sachets as vapour (Fig. 2). Another antimicrobial agent effective against viruses, fungi and bacteria is chlorine dioxide. In this method sodium chlorite and acid precursors are stored in a hydrophobic and hydrophilic copolymer and are released to form chlorine dioxide after moisture from the product comes in contact with the hydrophobic phase (Biji et al., 2015). An example for antimicrobial polymers would be Nisin-loaded chitosan/poly L-lactic acid, which showed potential as a novel active food packaging film due to its antibacterial behaviour against *Staphylococcus aureus* (Wang et al., 2015). Other antimicrobial agents could be enzymes (e.g. lysozyme), essential oils (e.g. cinnamon essential oil), organic acids (e.g. citric acid) and nanoparticles (e.g. silver) (Yildirim et al., 2018).

The moisture content of a product is a critical factor which needs to be closely controlled in order to ensure the safety and quality of various foods. Dry products might deteriorate even at lower relative humidity (RH) levels, that means the product is more perceptible to microbial spoilage and loses its appearance or texture, thereby reducing the shelf life. When it comes to products such as fish or meat the RH should be

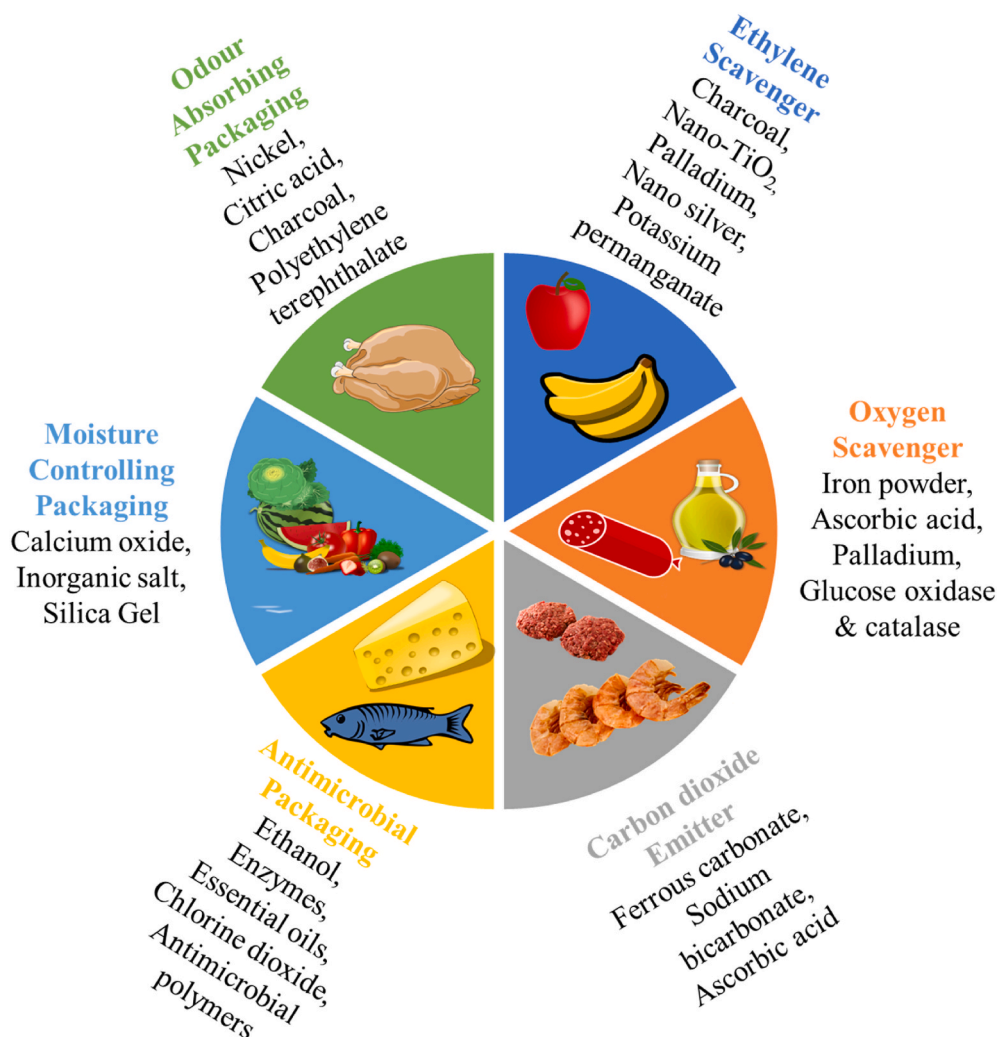


Fig. 2. Chemicals used in active food packaging.

high, this however, leads to the accumulation of liquid inside the package which makes the product less desirable for customers. Moisture controlling packaging can be divided into two categories, such as, RH controllers which remove the humidity in the headspace and the other being moisture removers which absorb liquids inside the packaging (Yildirim et al., 2018). The former usually are desiccants like silica gel, calcium oxide as well as pads of inorganic salt and are used in dry foods like nuts, chips, candy etc. Moisture absorbers come in the form of pads, sheets and blankets consisting of two layers, one being a micro porous polymer like polyethylene and the other being a super absorbent polymer like free-flowing granules. They are used in fish, meat, poultry and produce and controlling the liquid inside the packaging (Biji et al., 2015).

Ethylene is a small molecule which acts as a growth-stimulating hormone and thus accelerates the ripening process in fruit and vegetables. This leads to a reduction in shelf life during postharvest storage, enhanced softening of the food and a higher degradation rate of chlorophyll in leafy greens (Biji et al., 2015). A way of reducing the amount of ethylene around the product would be a sachet or blanket containing, on inert minerals immobilized, potassium permanganate which oxidises/inactivates the ethylene (Table 2). This method, however, is never used in direct contact with food due to the toxicity of potassium permanganate. Alternative systems can consist of nanoparticles such as Nano-TiO<sub>2</sub> which can oxidise ethylene into H<sub>2</sub>O and CO<sub>2</sub>, further possibilities are minerals such as charcoal with palladium and nano silver etc. (Vilela et al., 2018; Yıldirim et al., 2018).

During the storage of products like fish and poultry the breakdown of protein and other organic components can produce amines, aldehydes and sulphides. These volatile compounds can be selectively scavenged to prevent the mix of odours from different products during transportation. The odour resilient packaging relies on polyethylene terephthalate, an incorporated part which allows the passage of gases and a sachet consisting of nickel and charcoal to scavenge the odour. Amines which are generated in fish packaging due to the breakdown of protein can be removed through the incorporation of citric acid or other acidic compounds into the polymer film (Biji et al., 2015).

#### 4. Applications of seaweed in food packaging

Use of chemicals as an active agent in packaging could be toxic due to the migration of the chemical from polymer to the food. Therefore, the research focus is shifting for the use of natural compound as an active agent instead of any chemical. Seaweeds are considered to be a potential active agent or raw material as they are the rich source of polysaccharides. They are the better alternatives to the traditional material when combined with any biodegradable polymer as it enhances the sustainability, functionality and the sensory property of the product (Fig. 3). Seaweeds could be used to develop sustainable packaging, biodegradable plastics, active packaging, edible packaging and sachets.

Currently there are broad variety of possible applications for packaging containing seaweed and other composites (Table 3). In a study conducted by Shahbazi et al. (2016) the mechanical and structural

**Table 2**  
Current applications of active packaging.

Type of packaging	Principle/reagents	Application	Reference
Antimicrobial packaging	Bacterial cellulose modified by polypyrrole – Zinc oxide nanocomposite	Decrease the growth of both mesophilic and psychrophilic bacteria in chicken thigh	Pirsa and Shamusi (2019)
	Polyvinyl alcohol/cinnamon essential oil/ $\beta$ -cyclodextrin	Prolonging the shelf life of strawberries through antimicrobial activity	Wen et al. (2016)
	Phenolic acids/extracts, e.g. gallic acid, vanillic acid, thymol, cinnamic acid, carvacrol	Fruit and seedling coating applications to control bacterial contamination/spoilage	Alkan and Yemenicioğlu (2016)
Oxygen scavengers	Modified atmosphere packaging with iron-based oxygen scavengers	Reduction of microbial and oxidative changes in chicken thigh meats	Demirhan and Candoğan (2017)
	Rosemary active packaging	Reduction of lipid oxidation after high pressure processing of pork patties	Bolumar et al. (2016)
	Alginate-based edible coating containing natural antioxidants (rosemary and oregano essential oils)	Decreased colour losses, water losses and shear force, increase in odour, flavour and overall acceptance of beef steaks	Vital et al. (2016)
Ethylene scavenger	Potassium permanganate-based ethylene scavenger supported by sepiolite	Delay the ripening process of fresh apricots, and probably other $C_2H_4$ sensitive fruit, prolonging their postharvest storability and improving consumer acceptability	Álvarez-Hernández et al. (2020)
Oxygen and volatile organoleptic compound scavenger	Zeolite formulation and barrier layers	Entrapment of volatile organoleptic compounds	McAllister et al. (2019)

properties of a chitosan/ $\kappa$ -carrageenan ( $\kappa$ -CG) blend film were determined. The results showed that through the incorporation of  $\kappa$ -CG in the chitosan film had enhanced the flexibility as well as led to a smooth and uniform surface. Further, it was determined, that the water solubility, the water vapour permeability and the elongation at break decreased after the addition of  $\kappa$ -CG. It also led to a higher moisture affinity at higher relative humidity, an increase in tensile strength and to improved surface hydrophobicity. A  $\kappa$ -CG/chitosan-based film containing allyl isothiocyanate was investigated by Olaimat et al. (2014) showed that the oppositely charged polysaccharides film had good coating properties and showed improvement regarding the gas barrier (Olaimat et al., 2014). It also led to a delayed release of bioactive compounds which were incorporated into the film. Improved antimicrobial properties against *B. subtilis* and *B. cereus* was observed by Dima et al. (2014) regarding a film consisting of a Chitosan/ $\kappa$ -CG complex. It also showed a slower release rate of essential oil and stayed transparent throughout the whole pH range.

Rhim et al. (2013) developed nanocomposites film using PLA and

laminated agar/ $\kappa$ -carrageenan/clay bio-nanocomposite film and observed higher tensile strength, increased water vapour permeability, water uptake ratio and water solubility. The newly developed composite also showed improvement in thermal stability and water resistance. For application in the food and pharmaceutical industry Paramita et al. (2015) developed a high-solid matrix consisting of  $\kappa$ -CG and polydextrose which is amorphous in nature, determines the stability as well as the quality control and exhibits diffusional mobility of  $\alpha$ -lionic acid. Moreover, the film composed of polylysine,  $\kappa$ -CG and pectin had showed the electrostatic attraction of the respective components and led to a stronger complex forming an antimicrobial delivery system for foods and beverages (Lopez-Pena & McClements, 2014).

Huq et al. (2012) had studied the reinforcement of alginate based biodegradable nanocomposites with nanocrystalline cellulose and observed an increase in tensile strength, elastic modulus, thermal stability, mechanical and barrier properties. The study also reported a decrease in swelling properties, water vapour permeability and elongation at break (Huq et al., 2012). However, an increase in tensile strength and swelling ratio was noticed by Rhim et al. (2011) in a film containing agar and nanoclay for biodegradable food packaging (Rhim et al., 2011). It also showed a decrease in water vapour permeability, contact angle of water and water solubility. Azarakhsh et al. (2014) had used a combination of alginate and lemongrass essential oil film as packaging for fresh cut fruit and observed a decrease in the respiration rate, weight loss and change of colour. Further, the low microbial activity, less changes in sensory and morphology attributes were noted (Azarakhsh et al., 2014). Additionally, while using a film consisting of salty alginate in microwaveable packaging the results showed a better heat distribution throughout the product and the film was acting as a susceptor during the heating process (Albert et al., 2012). In order to achieve shelf life extension of food Martin et al. (2013) had studied a blend of carrageenan, locust bean gum and organically modified nano-clay which showed a bactericidal effect on *L. monocytogenes*. It also led to higher tensile strength, elongation at break, aggregation degree and delaying thermal degradation (Martins et al., 2013). Another study by Kanmani and Rhim (2014) showed that a blend made up from carrageenan and grapefruit seed extract had increased UV barrier properties, moisture content, water vapour permeability as well as elongation at break. The results also showed antimicrobial activity, especially against gram-positive bacteria and a reduction in tensile strength, elastic modulus and water contact angle.

With the aim of creating antimicrobial food packaging Shojaee-Aliabadi, Mohammadifar, et al. (2014) produced a  $\kappa$ -CG, montmorillonite, *zataria multiflora* boiss essential oil, which showed an inhibitory effect of *S. aureus*, *B. cereus*, *E. coli*, *S. typhimurium* and *P. aeruginosa*. In addition, the film properties were improved elongation at break, lower moisture content, decrease in water solubility, water vapour permeability and tensile strength (Shojaee-Aliabadi, Mohammadifar, et al., 2014). Atef et al. (2015) found that a mix of agar, nanocrystalline cellulose and savoury essential oil led to a decrease in water solubility, water vapour permeability, tensile strength and elastic modulus. The packaging improved the safety and shelf life of foods and, also showed an increase in water contact angle, swelling ratio, viscosity and elongation at break.

## 5. Effect of seaweed incorporation on the film microstructure

The incorporation of seaweed into other polymers changes the mechanical, thermal, optical and chemical properties of the materials. These effects are dependent on the seaweed polysaccharides, which was added and need to be thoroughly evaluated to create a safe and effective packaging. The effects depend not only on the kind of seaweed polysaccharide which is being used for the incorporation but also on the polymer in which it will be incorporated. A brief overview of results published over the past five years is given in Table 4.









Seaweed	Characteristics	Applications
<b>Red Seaweed</b> <i>Poryphyra capensis</i> <i>Aeodes orbitosa</i> Agar Carrageen 	<b>Sustainable Source</b> Eco-friendly and Biodegradable Reduces carbon emission  <b>Active Functions</b> Antioxidant Antimicrobial agent	 → Sustainable Packaging   → Bioactive Plastic
<b>Green Seaweed</b> <i>Ulva</i> <i>Monostroma</i> <i>Cladophora rupestris</i> <i>Codium tomentosum</i> 	<b>Shelf Life</b> Enhanced low water content Increased stability  <b>Health</b> Nutraceutical Nutritious	 → Active Packaging   → Edible Packaging
<b>Brown Seaweed</b> Laminaria Kelp Fucus, <i>Sargassum muticum</i> 	<b>Market Value</b> Colourful Flavourful  <b>Plasticizers</b> Cross-linking agent	 → Sachet Packaging

Fig. 3. Seaweed as an active component and its potential applications.

### 5.1. Mechanical properties

The quality of polymer films intended for food packaging is highly dependent on the mechanical properties such as tensile strength, elastic modulus and elongation at break. These measurements indicate the ability of the film to withstand various stresses that occur during the processing, handling and storage of packaged food without losing its integrity. The elastic modulus describes the force per unit area which is necessary to lengthen a film sample to a specific extent. The tensile strength is a measurement used to determine the amount of force per unit area which is applied when the film is broken. And the elongation at break provides the percentage of change in the length of the film when the film is broken (Bastarrachea et al., 2011). Several studies were carried out to investigate the effects of seaweed polysaccharide incorporation on the mechanical properties (Table 3). In over half of the reviewed studies the incorporation of seaweed polysaccharides into the respective polymer led to an increase in tensile strength. Seaweed polysaccharides combinations which showed these results consisted of agar and sugar palm starch (SPS), alginate and PLA, alginate and PHB, alginate and polyvinyl acetate (PVA) as well as FUC and PVA. A similar pattern was also observed regarding the tensile modulus and the elongation at break, which increased and decreased respectively (Eghbalifam et al., 2015; Jumaidin et al., 2016; Ribeiro Lopes et al., 2017; Kostic et al., 2019; Yao et al., 2020). However, the PHB/KC and PHBV/FUC blend showed a decrease in the tensile modulus (Goonoo et al., 2017).

In a study by Eghbalifam et al. (2015) a PVA and sodium alginate (SA) blend was developed. To determine the tensile properties of the samples a universal tensile machine was used which measured at a crosshead speed of 50 mm per min. The samples were initially 40 mm long, 10 mm wide and 100–150  $\mu\text{m}$  thick. The results showed that the tensile modulus of pure PVA was 500 MPa which increased to 710 MPa after the addition of SA leading to a total increase by 210 MPa. The tensile strength also showed a slight increase from 42 MPa to 45 MPa after the incorporation of the polysaccharide while the elongation at break was at 152% and decreased to 56% after adding SA. Despite the reduction in flexibility due to the incorporation of the rigid polysaccharide, the study found that the polymer blend would still be suitable for most applications.

In a study by Jumaidin et al. (2016) regarding the development of a

biodegradable SPS/agar film with different agar content. The tensile strength of the film was determined at a temperature of  $23 \pm 1$  °C, a relative humidity of  $50 \pm 5\%$  and a crosshead speed of 5 mm/min. The results showed an improvement in tensile strength with increasing agar content from  $\sim 10.000$  MPa with 0 wt% agar to  $\sim 14$  MPa and  $\sim 13$  MPa with 30 wt% and 40 wt% respectively. The tensile modulus also steadily increased with the addition of agar and led to an increase by  $\sim 500$  MPa from  $\sim 1500$  to  $\sim 2000$  MPa at 0 wt% and 40 wt% respectively. The addition of agar in the SPS also had an effect on the elongation at break which decreased from  $\sim 3\%$  at 0 wt% agar to  $\sim 0.75\%$  at 40 wt% agar. The increase in tensile strength might be ascribed to the good miscibility of the SPS and the agar due to the similar chemical structure as well as the phase compatibility of the two components. Overall agar has better mechanical properties than SPS which can be attributed to the more entangled network structure.

Goonoo et al. (2017) created a polymer blend with varying polysaccharide contents containing FUC and PHBV and the tensile properties of the films were determined at 27 °C, 60% relative humidity and a crosshead speed of 10 mm/min. For the FUC/PHBV blend a decrease in tensile modulus and therefore increased flexibility was noted. The lowest tensile modulus was observed in the 70/30 and 50/50 PHBV/FUC blends with 37.4 MPa and 35.8 MPa, respectively which suggested better miscibility, thus reducing the brittleness of the polymer. The elongation at break varied widely with the highest value of 8.48 mm/mm being measured in 80/20 PHBV/FUC blend, while the lowest result of 1.72 was noted in a 95/5 PHBV/FUC blend (Goonoo et al., 2017).

### 5.2. Physical properties

Changes in the morphology after the incorporation of components such as seaweed polysaccharides are hard to generalize since they are highly dependent on the polymer-seaweed interaction. They are however important to investigate, since the interaction between the two substances could lead to cavities and other changes which could have an impact on further engineering properties (Bastarrachea et al., 2011). PVA/SA film developed by Eghbalifam et al. (2015) and used scanning electron microscopy (SEM) to study the surface morphology. The analysis of the SEM image showed a homogenous texture of the blend film,

**Table 3**  
Application and impact of seaweed incorporation into polymer film.

Film material	Application	Changes in properties	References
Chitosan (Cs)/ $\kappa$ -carrageenan ( $\kappa$ -CG) film	Food packaging/improve the film properties	<ul style="list-style-type: none"> <li>• Higher flexibility, smooth and uniform surface</li> <li>• Decreased water solubility, water vapour permeability</li> <li>• Improved surface hydrophobicity</li> <li>• Increase in tensile strength, decrease in elongation at break</li> </ul>	Shahbazi et al. (2016)
CG/Cs/allyl isothiocyanate coatings	Improve shelf life of vacuum packaged chicken	<ul style="list-style-type: none"> <li>• Good coating properties</li> <li>• Improved action as a gas barrier</li> <li>• Enable delayed release of bioactive compounds</li> </ul>	Olaimat et al. (2014)
Cs/ $\kappa$ -CG complex	Increases functionality of meat products	<ul style="list-style-type: none"> <li>• Higher antimicrobial activity against <i>B. cereus</i> and <i>B. subtilis</i></li> <li>• Slower release rate</li> <li>• Transparency throughout pH range</li> </ul>	Dima et al. (2014)
$\kappa$ -CG/- starch hydrogel	Designing pharmaceutical and food formulations	<ul style="list-style-type: none"> <li>• Improves bio-adhesive characteristics</li> <li>• Improved drug release</li> </ul>	Lefnaoui and Moulai-Mostefa (2014)
PLA/agarCG/clay bio-nano composite film	Food packaging	<ul style="list-style-type: none"> <li>• Higher tensile strength, water vapour permeability, water uptake ratio and water solubility</li> <li>• Improvement in water resistance</li> <li>• Increased thermal stability</li> </ul>	Rhim (2013)
$\kappa$ -CG/polydextrose high-solid matrix	Food and pharmaceutical industries	<ul style="list-style-type: none"> <li>• Amorphous nature</li> <li>• Exhibits diffusional mobility of <math>\alpha</math>-linolenic acid</li> <li>• Determine stability and quality control</li> </ul>	Paramita et al. (2015)
Polylysine/ $\kappa$ -CG/pectin complexes	Antimicrobial delivery systems for foods and beverages	<ul style="list-style-type: none"> <li>• Stronger complexes through electrostatic attraction</li> </ul>	Lopez-Pena and McClements (2014)
Alginate/Nanocrystalline cellulose	Polymeric packaging films for food	<ul style="list-style-type: none"> <li>• Increase in tensile strength and modulus</li> <li>• Decrease in elongation at break</li> <li>• Lower water vapour permeability</li> <li>• Decrease in swelling properties</li> <li>• Improvement in mechanical, barrier and thermal properties as well as chemical interactions</li> </ul>	Huq et al. (2012)
Agar/nanoclay	Biodegradable food packaging	<ul style="list-style-type: none"> <li>• Increase in tensile strength</li> <li>• Decrease in water vapour permeability, contact angle of water, water vapour absorption and water solubility</li> <li>• Increase in swelling ratio</li> </ul>	Rhim (2011)
Alginate/Lemongrass essential oil	Quality retention of fresh cut pineapples	<ul style="list-style-type: none"> <li>• Decrease in respiration rate</li> <li>• Lower weight loss, increased firmness, decreased change in colour</li> <li>• Decrease in microbiological activity after the addition of lemongrass</li> <li>• Less changes in sensory attributes and morphology of fresh cut fruit</li> </ul>	Azarakhsh et al. (2014)
Salty Alginate	Susceptor during microwave heating	<ul style="list-style-type: none"> <li>• Better heat distribution</li> <li>• Acting as susceptor</li> </ul>	Albert et al. (2012)
Carrageenan/Locust Bean Gum/organically modified nano-clay	Shelf life extension of food	<ul style="list-style-type: none"> <li>• High aggregation degree</li> <li>• Higher tensile strength and elongation at break</li> <li>• Bactericidal effect on <i>L. monocytogenes</i></li> <li>• Delayed thermal degradation</li> </ul>	Martins et al. (2013)
Carrageenan/grapefruit seed extract	Active food packaging	<ul style="list-style-type: none"> <li>• Increase in UV barrier properties</li> <li>• Higher moisture content</li> <li>• Reduced water contact angle</li> <li>• Decrease in tensile strength and elastic modulus</li> <li>• Increase in elongation at break</li> <li>• Antimicrobial activity especially against gram-positive bacteria</li> </ul>	Kanmani and Rhim (2014)
$\kappa$ -carrageenan/Montmorillonite/Zataria multiflora Boiss essential oil	Antimicrobial packaging for food	<ul style="list-style-type: none"> <li>• Lower moisture content</li> <li>• Decrease in water solubility, water vapour permeability, tensile strength</li> <li>• Improved Elongation at break</li> <li>• Inhibitory effect on <i>S. aureus</i>, <i>B. cereus</i>, <i>E. coli</i>, <i>S. typhimurium</i>, <i>P. aeruginosa</i></li> </ul>	Shojaee-Aliabadi, Mohammadifar et al. (2014)
Agar/Nanocrystalline cellulose/savory essential oil	Active packaging for improving the safety and shelf life of foodstuff	<ul style="list-style-type: none"> <li>• Decrease in water solubility, water vapour permeability</li> <li>• Increase in water contact angle, swelling ratio and viscosity</li> <li>• Decrease in tensile strength and elongation modulus</li> <li>• Increase in elongation at break</li> </ul>	Atef et al. (2015)
Acacia lignin-alginate film	Active, antimicrobial, antioxidant film for food packaging	<ul style="list-style-type: none"> <li>• Homogeneous, stretchy, easily handled and non-sticky</li> <li>• Lower moisture content, lower water solubility</li> <li>• Increased swelling properties</li> <li>• Decreased tensile strength and light absorbance</li> <li>• Lower degradation temperature</li> <li>• Moderate cytotoxic effect</li> <li>• Antimicrobial activity against gram-positive bacteria e.g. <i>S. aureus</i></li> <li>• Consistent drug release behaviour</li> </ul>	Aadil et al. (2016)



**Table 4**  
Changes in film properties after the incorporation of seaweed polysaccharides.

Film material	Change in properties	References
FUC/Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)	<ul style="list-style-type: none"> <li>• Homogenous distribution</li> <li>• Higher flexibility</li> <li>• High interaction between biopolymers</li> <li>• Increased hydrophilicity and surface free energy</li> <li>• High miscibility</li> <li>• Slight decrease in crystallinity</li> </ul>	Goonoo et al. (2017)
FUC/Poly vinyl alcohol (PVA)	<ul style="list-style-type: none"> <li>• Higher cross-linking density</li> <li>• Increase in tensile strength and modulus</li> </ul>	Yao et al. (2020)
FUC/Collagen	<ul style="list-style-type: none"> <li>• Increased thermo stability</li> <li>• Smaller pore size and fibril-like structure</li> <li>• Good miscibility, Increased hydrophilicity</li> </ul>	Perumal et al. (2018)
K-carrageenan/PHB	<ul style="list-style-type: none"> <li>• High disruption of surface properties</li> <li>• Decrease in elasticity modulus</li> <li>• Increase in hydrophilicity and surface free energy</li> <li>• Partially miscible</li> </ul>	Goonoo et al. (2017)
K-carrageenan/PHBV	<ul style="list-style-type: none"> <li>• Lower crystallinity</li> <li>• High disruption of surface properties</li> <li>• Higher tensile modulus</li> <li>• Increase in hydrophilicity and surface free energy</li> <li>• Partially miscible</li> <li>• Lower crystallinity</li> </ul>	Goonoo et al. (2017)
Alginate/PVA	<ul style="list-style-type: none"> <li>• Lower crystallinity and melting temperature</li> <li>• Homogenous texture and uniform distribution</li> <li>• Perfect miscibility without phase separation</li> <li>• Increased tensile strength and tensile modulus</li> <li>• Decrease in elongation at break</li> <li>• No antimicrobial activity</li> </ul>	(Eghbalifam et al., 2015; Fan et al., 2019)
Alginate/PHB	<ul style="list-style-type: none"> <li>• Increase in crystallinity</li> <li>• No change in glass transition or melting temperature, decrease in thermal stability</li> <li>• More pronounced rough and open texture</li> <li>• Higher hydrophilicity, lower water contact angles</li> <li>• No change in water vapour permeability</li> <li>• Increase in tensile strength and modulus</li> </ul>	Ribeiro Lopes et al. (2017)
Alginate/PLA	<ul style="list-style-type: none"> <li>• No considerable change in elongation at break</li> <li>• No change in thermal properties</li> <li>• Higher tensile modulus</li> <li>• Decrease in elongation at break</li> </ul>	Kostic et al. (2019)
Agar/Sugar palm starch	<ul style="list-style-type: none"> <li>• Improved tensile strength and modulus</li> <li>• Decrease in elongation</li> <li>• Smooth surface, no cluster, good miscibility</li> <li>• Increase in glass transition and melting temperature</li> <li>• Increased moisture absorption and swelling</li> </ul>	Jumaidin et al. (2016)

which indicates perfect miscibility and no phase separation. A SEM with 10 kV acceleration voltage was used in the study conducted by Jumaidin et al. (2016) to investigate the morphology of an SPS and agar film with varying agar content. The SEM image showed the homogeneous and smooth surface without clusters and no phases of SPS after the addition of agar into the blend, indicates a good miscibility and interaction between the two film components. However, with a higher agar content the surface started to show cleavage structures which might be due to polymer-polymer bonding and a higher filler content in the film matrix.

Ribeiro Lopes et al. (2017) used a SEM analysis on PHB film samples to determine the morphological changes caused by esterified alginate which was added to PHB blend. The changes in the morphological structure of the PHB blend showed a dense, rough and open texture on the surface after the incorporation of the polysaccharide. The analysis of the developed  $\kappa$ -CG/PHB blend by Goonoo et al. (2017) showed the highest disruption of the surface morphology of the film after the addition of  $\kappa$ -CG. The addition of 10 wt%  $\kappa$ -CG led to the formation of a porous structure, while a 50/50 blend of PHB and  $\kappa$ -CG showed a spherical protrusion and phase separation was visible in the SEM images of a 70/50 PHBV/ $\kappa$ -CG blend. The images from the PHBV/ $\kappa$ -CG blend showed less drastic disruption indicating an overall higher miscibility. However, no drastic variation was visible in the FUC/PHBV blend, round structures were noticeable indicating a homogenous distribution of the polysaccharide within the PHBV film (Goonoo et al., 2017).

### 5.3. Chemical properties

The chemical properties such as hydrophobicity or hydrophilicity, composition of film with functional groups in a packaging play an important role to evaluate the safety and efficiency of food packaging. The incorporation could differ widely due to the different components and their interaction with each other. Goonoo et al. (2017) used energy dispersive X-ray (EDX) spectroscopy, in combination with an SEM as well as a field emission (FE)-SEM and the sessile drop method with an OCA 15plus instrument to determine the chemical composition of the films. The sessile-drop method was used to determine the contact angles of the three blend films which are a) containing  $\kappa$ -CG and PHB, b) a combination of  $\kappa$ -CG and PHBV and c) a blend of FUC and PHBV. The contact angles showed that the addition of the seaweed polysaccharides led to increased hydrophilicity, with the PHB/ $\kappa$ -CG film being slightly less hydrophilic than the PHBV/ $\kappa$ -CG and the PHBV/FUC blends which measured similar contact angles. The surface free energy was also investigated by measuring the static contact angles using diiodomethane (99%) and glycerol (99.6%). Both  $\kappa$ -CG blends showed an increased surface free energy after the addition of 10 wt% of the polysaccharides. This indicates a change in the surface component, meaning that the hydrophilic hydroxyl and sulphate groups of the  $\kappa$ -CG take part in polar interactions thereby increasing the surface energy. The EDX analysis further confirmed the presence of the polysaccharides on the blend surface and showed the existence of sulphur in all three samples and

potassium in samples containing  $\kappa$ -CG (Goonoo et al., 2017).

Fourier transform infrared (FT-IR) spectroscopy was utilized by Jumaidin et al. (2016) to detect changes in functional groups in the film after they incorporated the agar into the SPS. The spectra showed no significant changes in the measured peak, however small peak shifts to lower wave numbers were noticed. These indicate increased intermolecular hydrogen bonding which points towards the compatibility and interaction between the two components. The moisture absorption of the film increased after the incorporation of the agar which might be attributed to the fact that it is a sulphated polysaccharide with a higher hydrophilicity.

Overall, these studies show that an increase in hydrophilicity was the most common change in chemical properties after the addition of the seaweed. This is due to the anionic nature of the seaweeds and results in increased moisture absorption, swelling and lower water contact angles. However, the results show that changes in crystallinity depend not only on the seaweed, but on the interaction between the polymer and the polysaccharide.

#### 5.4. Thermal properties

Thermal properties such as melting temperature ( $T_m$ ) and glass transition temperature ( $T_g$ ) are important properties, suggesting the level of association between the different polymer chains. They are also needed to ascertain if the polymers are suitable in food packaging and provide information which has to be considered during the process (Bastarrachea et al., 2011). Thermal analysis by Goonoo et al. (2017) had showed that both blends containing  $\kappa$ -carrageenan (KC) are partially miscible in the amorphous regions and immiscible in the crystalline regions. This leads to an increase in the crystallisation temperature and a decrease in the enthalpy of crystallisation which was higher in the KC/PHBV blend. The FUC/PHBV film showed a partial miscibility in the amorphous regions at a low FUC concentration, and miscible at high concentrations, which might be due to the higher amount of sulphate groups in FUC. Two melting points were measured for the PHBV-films, however the melting transition for FUC blends with a higher concentration decreased, while they did not change in the KC/PHBV blend. Moreover, on the incorporation of fucoidan into collagen detected improved thermal properties after the addition of the seaweed (Perumal et al., 2018). On the addition of sodium alginate to PVA film X-ray diffraction and differential scanning calorimetry analysis indicated a slightly lower melting temperature (Eghbalifam et al., 2015). Also, it was observed on the incorporation of alginate to PLA led to no changes in thermal properties (Kostic et al., 2019). However, a decrease in thermal stability after the addition of alginate to PHB (Ribeiro Lopes et al., 2017). A blend containing agar and sugar palm starch resulted in an increased glass transition temperature as well as a higher melting temperature (Jumaidin et al., 2016).

The impact of the seaweed incorporation onto the thermal properties is highly dependent on the substances used in the blend. Due to different grades of miscibility, crystallinity and overall interaction the effects on the melting and crystallisation temperature can differ widely. While the addition of FUC into collagen led to improved thermal properties the inclusion of alginate into PHB led to a decrease, whereas, the results of an alginate/PLA blend showed no changes.

#### 6. Effect of polysaccharide incorporation on antimicrobial property

Antimicrobial packaging is a key factor when it comes to active packaging, since food safety is one of the biggest concerns in the food industry. To ensure sustainable and safe packaging the incorporation of natural antimicrobial agents such as chitosan etc. is a key factor to consider (Padgett et al., 1998). Several studies on crude seaweed extracts containing a mix of polysaccharide were proven by agar diffusion test to exhibit antimicrobial activity. In a study conducted by

Alboofetileh et al. (2019) polysaccharides of the brown seaweed *Nizamuddiniana zanardinii* were extracted through conventional and non-conventional methods to evaluate the impact of the different extraction methods on biochemical characteristics and the polysaccharide composition. This study found that mixed fucoidans extracted through microwave and subcritical water-extraction inhibited the growth of *E. coli*. While, fucoidans extracted through enzyme-ultrasound, ultrasound microwave and subcritical water-extractions showed antimicrobial effects against *P. aeruginosa* with a minimal inhibitory concentration (MIC) of 2 mg/mL. The results also showed an antiviral effect on HSV-2 infections from all fucoidan independent from the extraction method. Chotigeat et al. (2004) found that crude fucoidan which was extracted from brown seaweed *Sargassum polycystum* exhibited inhibitory effects against *V. harveyi*, *S. aureus* and *E. coli* at a MIC of 12.0, 12.0 and 6.0 mg/mL respectively. The growth of *E. coli* was found to be inhibited by crude sulphated polysaccharide from the red algae *G. ornata*. The extracts which showed the antimicrobial activity were extracted in the third extraction step at 80 °C (Amorim et al., 2012). These studies, however, only utilized crude seaweed extracts not the polysaccharides.

Pure saccharides such as carrageenan, alginate etc. being incorporated into a polymer film which showed low to no inhibitory effect against the bacteria such as *E. coli*, *L. monocytogenes* etc. Velasco and Fundador (2020), had studied the use of durian starch, carrageenan and carvacrol films. The durian starch/carrageenan control films showed no inhibitory effect on *S. aureus* in the disk diffusion assay. A complete inhibition of the gram-positive bacteria was noted after 24 and 48 h pre-diffusion of the film containing 10 wt% carvacrol (Velasco & Fundador, 2020). Similarly, the pure carrageenan based composite film did not exhibit antibacterial effects against *E. coli* or *L. monocytogenes*, after the addition of nano-clay or nano silver (Dou et al., 2018). Another study also showed no antimicrobial properties against gram-positive and gram-negative food-borne pathogenic bacteria of an agar blend. Only after the addition of a blend containing six different copper nanoparticles exhibiting strong antimicrobial activity (Shankar et al., 2014).

A study using aqueous seaweed extract from *Kappaphycus alvarezii* showed antimicrobial activity of the crude extract against the gram-positive bacteria *S. aureus* and *B. cereus* with a higher inhibition of *S. aureus*. The disk diffusion test exhibited no antibacterial activity against gram-negative bacteria such as *E. coli* and *P. fluorescens*. Moreover, Kanatt et al. (2020) had incorporated the seaweed extract into PVA film, where the results showed a good zone of inhibition against both of the gram-positive bacteria, thus proving that the antimicrobial activity of the extract could be retained (Kanatt et al., 2020). Overall, the studies regarding crude seaweed extract had showed a big potential on the antimicrobial activity of the seaweed polysaccharides, however further research is required to define the bioactive abilities of the pure seaweed polysaccharides. While the studies regarding the polysaccharide film did not show any inhibition of food borne pathogens *E. coli*, *L. monocytogenes* or *S. aureus*, they still show that components such as nano-clay, copper nanoparticles, carvacrol etc. can be easily incorporated into the polymer, thereby making it possible to be used as active antimicrobial packaging.

#### 7. Effect of polysaccharide incorporation on antioxidant property

Lipid oxidation has a big impact on the quality of food and food products. Agents, which are created in the oxidation process, such as free radicals have mutagenic, carcinogenic and cytotoxic properties and cause severe health problems. Lipid oxidation can also lead to a decrease in shelf life and nutritional value of food (Ahmed et al., 2016). Antioxidant packaging's are used to decrease the amount of lipid oxidation and protein degradation within the packaging. Active packaging with antioxidant properties is a promising alternative to traditional packaging where the antioxidants are incorporated or coated on the film. Natural

antioxidants such as plant extracts and essential oils are receiving more interest due to the tendency of reducing synthetic additives in packaging since natural additives are safer and offer multiple health benefits (López-de-Dicastillo et al., 2012).

The polysaccharides of the green seaweed (*Ulva lactuca*), the red seaweeds (*Gracilaria lemaneiformis* and *Sarcodia ceylonensis*), and the brown algae (*Durvillaea antarctica*) were extracted through microwave-assisted extraction. The antioxidant activity was then measured through an ABTS radical scavenging activity assay (DPPH), the scavenging activities of hydroxyl radical, nitrite scavenging activity and the determination of the reducing power. The results of the DPPH showed that the polysaccharide from the green seaweed showed the highest activity while the polysaccharide extracted from the brown seaweed and from *Sarcodia ceylonensis* also showed a noticeable effect on the DPPH. The *Gracilaria lemaneiformis* showed only weak antioxidant activities as it is a non-sulphated polysaccharide. The *Sarcodia ceylonensis* and green seaweed polysaccharide showed high hydroxyl radical scavenging activity, while the brown seaweed polysaccharide had a more pronounced reducing power (He et al., 2016). Kanatt et al. (2020) determined the antioxidant activity of aqueous seaweed extracts from *Kappaphycus alvarezii* with a DPPH assay, the iron chelation activity, a  $\beta$ -carotene bleaching assay and a reducing power assay (Kanatt et al., 2020). The overall results of these assays showed that the aqueous seaweed extract had a good antioxidant activity, especially when compared to methanolic or ethanolic seaweed extract. The aqueous seaweed extract was incorporated into a PVA film and also tested on the antioxidant activity by using a DPPH assay. The results showed that the addition of aqueous seaweed extract enhanced the radical scavenging activity significantly compared to pure PVA as well as with seaweed extract extracted through ethanol, methanol or water.

Further studies have shown that the Lambda-carrageenan has a high antioxidant activity and have proven, that the oxidative deterioration of food can be averted through the inclusion of polysaccharides rich in antioxidant (Rocha de S., Micheline C. et al., 2007). Several studies investigated the antioxidant properties of a carrageenan/mulberry extract film through a DPPH assay (Liu et al., 2019, 2020; Shojaee-Aliabadi, Hosseini, et al., 2014). The results showed a general quantity dependent antioxidant activity of carrageenan which was enhanced through the addition of mulberry extract. Similar results were achieved in a study investigating the antioxidant activity of a  $\kappa$ -carrageenan film containing plant essential oils. The results of the DPPH assay indicated a slight antioxidant activity of the pure  $\kappa$ -carrageenan film due to its naturally occurring polyphenols, however, these results were enhanced through the addition of the plant essential oils (Shojaee-Aliabadi, Hosseini, et al., 2014).

Overall, the results showed that most polysaccharides exhibit antioxidant activity to varying degrees, these properties can be used in antioxidant packaging. However, to enhance the antioxidant properties components like plant essential oils, mulberry extract etc. can be added to guarantee the reduction of lipid oxidation and increase the safety of the food. Additionally, the antioxidant activity of different seaweed polysaccharides/extracts should be further investigated to reach the full potential of seaweed as an antioxidant agent in active food packaging.

## 8. Migration of active compounds from package to food

Antioxidant and antimicrobial packaging often rely on active components incorporated into the film to unfold the desired effect onto the product. To determine whether the production of such a film is feasible, the investigation of the release rate and the diffusivity of the active agent from the film into the food is required. For this purpose, it is necessary that the release of the component is neither too rapid, causing migration to the internal part of the food, nor too slow, meaning the inhibitory concentration cannot be reached.

To determine the use of an alginate/pectin blend with added natamycin as an antimicrobial film Bierhalz et al. (2012) carried out a

natamycin migration test. The tests samples were transferred from one beaker containing distilled water to another containing the same multiple times until no natamycin migration could be detected. The concentration in the beaker was measured by a UV/VIS spectrophotometer, the maximum amount released, the accumulated mass released as well as the diffusion coefficient were determined. The results showed, that the release from a pure pectin film took place over a period of 30 h, while the natamycin release from the composite film was depleted in 70 h and this period further increased to over 800 h for the pure alginate (Bierhalz et al., 2012). A significantly reduced diffusion rate of natamycin after the addition of alginate, show a higher compatibility of the natamycin with the alginate rather than the pectin. In a study conducted on the migration of sorbic acid from a PLA and fucus based film with 0.5% sorbic acid was placed in 95 wt% ethanol or 10 wt% ethanol. Regular samples were taken from the liquid over a period of time and the migration kinetics were calculated. The results showed a slower percentage of release of sorbic acid from the PLA and fucus film at 40 °C and 95 wt% than the pure PLA film, however, the percentages of release from the pure PLA at 10 wt% ethanol were significantly lower at both temperatures. Overall, the diffusion coefficients were slightly higher in the PLA/fucus blend, which could be explained by the weak immobilisation of the antimicrobial agent in this film.

Aristizabal-Gil et al. (2019) studied the release kinetics of calcium from alginate films with different food simulants (ethanol, acetic acid and oleic acid). The release profile for Ca was observed to be similar in all the simulants, with an increase in cumulative release up to 24 h and a stationary phase afterwards. The maximal amount, which was released, however, varied from 15% in ethanol to 6% in acetic acid and 38% in oleic acid. The nanoparticle concentration also had a major impact on the release rate, since a smaller concentration of nanoparticles is fully dispersed within the alginate matrix, thereby forming stronger interactions and releasing the agents at a slower rate. A larger number of nanoparticles tend to aggregate into bigger particles, thus reducing the interaction between the alginate and the compounds which leads to a faster release. Further, the low pH in the acetic acid simulant led to lower interaction between the nanocomposite and the film thus increasing the (total) release rate in comparison to ethanol. Higher (total) release rates were also noted in the oleic acid simulant which can be explained by the high solubility of the nanoparticles in the simulant, while the alginate film was not soluble in the oleic acid.

## 9. Legal aspects of the use of seaweed in food

Seaweed can, despite its many positive properties, also accumulate undesirable compounds and toxic metals such as arsenic, lead, mercury etc. This concerns an increasing importance due to the industrialisation of coastal areas which can lead to a higher accumulation of toxic compounds in seaweed (Circunção et al., 2018).

However, so far France is the only European country that has set clear regulations on 24 seaweeds and the maximum amount of toxic metals when they are used for human consumption (Holdt & Kraan, 2011). Legislations in place in the European Union are European Commission Regulation (EC No 629/2008) which regards the use of seaweed as a food supplement and sets a maximum amount of 3 mg/kg dry weight for cadmium. Commission Regulation (EC) No. 629/2008 of 2 July 2008 amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in (2008) and the (EC No. 1275/2013) which sets a maximum level of arsenic in complementary feed/feed meals to 40 and 10 mg/kg (moisture content of 12%), Commission Regulation (EU) No 1275/2013 of 6 December 2013 Amending Annex I to Directive 2002/32/EC of the European Parliament (2013) respectively. However, there is no legislation in place regarding other toxic metals. The same lack of legislation can be observed in Asian countries as well as in the USA and South America (Circunção et al., 2018).

## 10. Conclusion and future perspective

With the increasing awareness of the consumer for sustainable products, the incorporation of seaweed into natural polymers shows a big potential use in the future of food packaging. It can decrease the amount of synthetic packaging used and thereby lower the quantity of plastic pollution in our environment. The antioxidant/antimicrobial properties of the seaweed could have a beneficial effect on the shelf life of food and reduce the amount of food waste caused by spoilage. However, there needs to be more research done on the effect of the seaweed on the shelf life of specific food products. For broader use of seaweed in foodstuffs and packaging there should be an overall improvement regarding the legislation of toxic metals and further, possibly harmful compounds which can accumulate, to make the usage of seaweed safe and risk free. The mechanical properties of the film need to be adapted to the specific field of use, this can be done by using a blend of polymers or natural plasticiser. Overall, it shows to be a promising possibility for more sustainable and active films with room for improvement. The use of seaweed as biodegradable packaging which are more feasible and considerable alternative to current methods, leading to further research towards the optimisation of the process to make it a possible competitor on the market. Furthermore, more detailed research should go into the specific fields in which seaweed would have the biggest advantages over common packaging material due to its mechanical, antibacterial, antioxidant and release properties. The improvement of existing polysaccharide membranes by using additives such as lipids, different polymer blends and a higher variety in, possibly modified, polysaccharides are also crucial to create a more sustainable approach for food packaging.

## Declaration of competing interest

The authors declare no conflict of interest.

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