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Design of microbial polysaccharide films for food packaging

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Aos meus pais,
Pelo vosso exemplo e amor.

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"Aqueles que passam por nós, não vão sós,
não nos deixam sós. Deixam um pouco de si,
levam um pouco de nós."
- Antoine de Saint-Exupéry

"I am among those who think that science has great beauty. A scientist in his laboratory is not only a technician: he is also a child placed before natural phenomena which impress him like a fairy tale."

- Marie Curie

ABSTRACT

The overuse of synthetic non-biodegradable plastic packaging in the last decades turned into a serious global environmental problem, once recycling and energy recovery are not perfect solutions. To overcome this problem, efforts have been done by researchers around the world to use biodegradable biopolymers from renewable resources as food packaging materials.

In this thesis, FucoPol, a microbial exopolysaccharide has been studied as a sustainable alternative and the following questions were raised:

- Is it possible to produce films of FucoPol and which properties should be improved?
- Which strategies may be applied to improve their behaviour, specially their barrier properties to gases and water vapour?
- Is it possible to use FucoPol films as an alternative to synthetic plastics aiming their use as packaging material?

This study shows that, FucoPol films offer good barrier properties to gases and biodegradability. Their weaker properties, low barrier properties to water, have been improved along the work, using two distinct strategies: formulation of bilayer films of FucoPol and chitosan and use of different coatings techniques in FucoPol films.

The results have shown that, it is possible to improve FucoPol films properties, namely the barrier properties to gases and water vapour, and resistance to liquid water.

FucoPol combined with chitosan as a bilayer film was used for walnuts packaging and it was possible to verify that it can protect food susceptible to lipid oxidation as effectively as synthetic materials.

Keywords: FucoPol, Exopolysaccharide, Biodegradable films, Films characterization, Barrier Properties, Food packaging.

RESUMO

O uso excessivo de embalagens de plástico sintéticas e não biodegradáveis nas últimas décadas tornou-se um sério problema ambiental a nível global, uma vez que as soluções como reciclagem ou incineração para recuperação de energia não são soluções perfeitas. Para ultrapassar este problema, esforços têm sido feitos por investigadores de todo o mundo para usar biopolímeros biodegradáveis de fontes renováveis para produção de materiais para embalagens alimentares.

Nesta tese, FucoPol, um exopolisacárido microbiano foi estudado como uma alternativa sustentável e as seguintes questões foram levantadas:

- É possível produzir filmes de FucoPol e que propriedades podem ser melhoradas?
- Que estratégias podem ser aplicadas para melhorar o seu desempenho, especialmente as suas propriedades barreira a gases e vapor de água?
- É possível usar filmes de FucoPol como alternativa a plásticos sintéticos visando a sua utilização como material para embalagem?

Este estudo demonstra que os filmes de FucoPol têm boas propriedades barreira a gases e são biodegradáveis. A sua propriedade mais fraca é a deficiente barreira à água que foi melhorada ao longo do trabalho, utilizando duas estragégias distintas: formulação de filmes bi-camada de FucoPol e quitosano e uso de diferentes técnicas de revestimento em filmes de FucoPol.

Os resultados mostraram que é possível melhorar as propriedades dos filmes de FucoPol, nomeadamente as propriedades barreira aos gases e vapor de água, e a resistência à agua.

FucoPol combinado com quitosano em forma de filme bi-camada foi utilizado para embalar nozes e foi possível verificar que pode proteger os alimentos susceptíveis de oxidação lipídica tão eficazmente como materiais sintéticos.

Palavras-chave: FucoPol, Exopolissacárido, Filmes biodegradáveis, Caracterização de filmes, Propriedades barreira, Embalagem alimentar.

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Acronyms

ALD atomic layer deposition.

DO dissolved oxygen.

EB elongation at break.

EM elastic modulus.

EPS exopolysaccharide.

EVOH ethylene vinyl alcohol.

GAB Guggenheim–Anderson–de-Boer.

HDPE high density polyethylene.

LDPE low density polyethylene.

LFS liquid flame spray.

Mw molecular weight.

PA polyamide.

PA/PE polyamide/polyethylene.

PBSA polybutylene succinate adipate.

PCL polycaprolactone.

PE polyethylene.

PET polyethylene terephthalate.

PFH perfluorohexane.

PGA polyglycolide.

PHA polyhydroxyalkanoates.

PLA polylactic acid.

PP polypropylene.

PS polystyrene.

PUFAs polyunsaturated fatty acids.

PV peroxide value.

PVC polyvinyl chloride.

RFID radio-frequency identification.

RH relative humidity.

SEM scanning electron microscopy.

TFA trifluoroacetic acid.

TS tensile strength at break.

WCA water contact angle.

WPI whey protein.

WVP water vapour permeability.

Chapter 1

Motivation

Plastic packaging is essential nowadays. However, the huge environmental problem caused by landfill disposal of non-biodegradable polymers in the end of life has to be minimized and preferentially eliminated. The solution may rely on the use of biopolymers, in particular polysaccharides. These macromolecules possess film-forming properties and are able to produce attracting biodegradable materials possible to apply in food packaging. Despite all advantages of using polysaccharides obtained from different sources, some drawbacks, mostly related to their low resistance to water, mechanical performance and price, have hindered their wider use and commercialization. Nevertheless, with increasing attention and research on this field, it has been possible to trace some strategies to overcome the problems and recognize solutions.

1.1 State of the art

Currently food packaging is essential for preservation, protection, storage, among others. Non-biodegradable synthetic plastic packaging has been widely used since they are flexible, light, stable and have low cost. Their extended use and implemented methods to mold and extrude are so established in industry that makes difficult their substitution by another environmentally friendly solution. However, the growth of synthetic plastics use caused by growing of human population has caused a serious environmental problem, the post-consumer plastic waste.

One of the current trends in food industry consists on the substitution of synthetic plastics and non-biodegradable materials by natural compounds, in particular, by biopolymers. Biopolymers obtained from renewble resources are biodegradable and compostable, which makes them suitable to close carbon cycle.

The interest in a particular type of biopolymers - polysaccharides - has been increasing, once they are the most abundant macromolecules in the biosphere. Polysaccharides are high molecular weight carbohydrates, non-toxic, which can be obtained from different sources, such as plants, algae, animals or microorganisms. A high diversity of polysaccharides (chitosan, starch, carrageenan, pullulan, among others) have been used to produce biodegradable films used in packaging and edible coatings in food industry. A detailed description of different polysaccharides and their use in food packaging is presented in Chapter 2.

Polysaccharides obtained by microorganisms (yeast, fungus or bacteria) represent a good alternative to others recovered from different sources, once their production is non dependent on climatic or seasonal effects. Actually, several microbial polysaccharides (xanthan, pullulan, gellan gum, among others) have been used to produce films for food packaging applications.

Polysaccharide films present good barrier properties to gases (in particular oxygen and carbon dioxide) at low and moderate relative humidity. Nevertheless, their main drawback is related with poor barrier to water vapour and their sensitivity to liquid water, which results from their hydrophilic nature.

In this thesis, FucoPol, a fucose-rich exopolysaccharide is used for designing films with potential use in food packaging. FucoPol is a high molecular weight heteropolysaccharide produced by bacterium *Enterobacter* A47 using glycerol by-product from biodiesel industry as carbon source. It is composed by neutral sugars (fucose, galactose and glucose), an acidic sugar (glucuronic acid) and acyl groups (acetate, succinate and pyruvate). Its anionic character, imposed by glucuronic acid, succinate and pyruvate, provide interesting properties such as emulsion and film-forming capacity.

1.2 Questions and goals

Concerning the state-of-the-art and the problems previously raised, the following questions become of significant scientific relevance:

- Is it possible to produce films of FucoPol and which properties should be improved?
- Which strategies may be applied to improve their behaviour, specially their barrier properties to gases and water vapour?
- Is it possible to use FucoPol films as an alternative to synthetic plastics aiming their use as packaging material?

To address these questions, the following research strategy was applied:

1. Development of effective formulations for obtaining films, transparent and malleable enough to manipulate and characterize.

2. Development of systems with two biopolymers (FucoPol and chitosan) to improve FucoPol stand-alone films properties.

3. Coating of FucoPol films with different metal oxide thin layers, to enhance or even introduce new properties.

4. Performance evaluation of the formulated films with food susceptible to lipid oxidation, as case study.

By fulfilling this strategy and consequent goals, it is envisaged a valuable contribution of this thesis for polysaccharide films research, showing different approaches for improving films properties and contributing with relevant discussion in the food packaging field.

1.3 Thesis outline

This thesis presents the work developed during the PhD period and it is organized in seven chapters and one appendix.

Overall, the work described in this thesis encompasses development and characterization of different FucoPol biodegradable films, with potential use in packaging, as alternative to synthetic and non-biodegradable polymers obtained from non-renewable sources.

Once FucoPol is a bacterial exopolysaccharide (EPS), each chapter was developed with polymer obtained from a different batch production. The exceptions were Chapters 4 and 6, where bilayer films were developed with biopolymer from the same batch production.

Each chapter includes a short review of the state of the art, describes the materials and methods used and discusses the results and main conclusions obtained. The methodology used in each individual chapter is detailed in the context of the respective subject.

The work performed during this PhD resulted in three scientific papers, which have been published in international scientific journals, referred in the ISI Web of Science. Furthermore, two more manuscripts, were already prepared to be submitted to international scientific journals, also referred in the ISI Web of Science (Appendix A).

A brief description of the contents of each chapter is described below and Figure 1.1 schematizes its organization.

Chapter 1 - **Introduction** - This chapter introduces the main subject of this thesis, providing some insight on the motivation and outlining the main goals of this work. This chapter also describes the thesis structure.

Chapter 2 - **Background** - Introduces an overview and state of the art of food packaging, focused on polysaccharides. This chapter presents an introduction to food packaging, plastics end of life, alternatives to synthetic polymers, biodegradable polymers and finally polysaccharides. Several polysaccharides are categorized by source and described in detail.

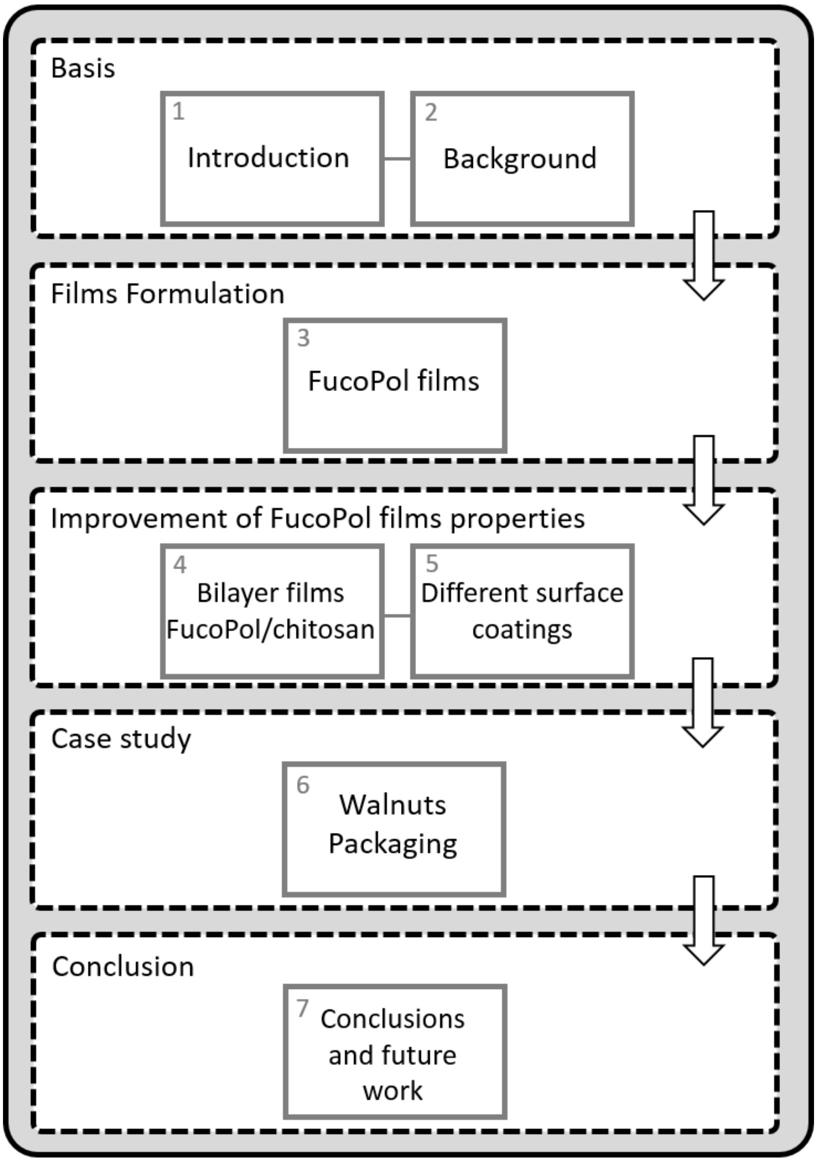


Figure 1.1: Thesis structure

Chapter 3 - **Biodegradable films produced from the bacterial polysaccharide FucoPol** - Focuses on design and characterization of FucoPol films, prepared with citric acid which has plasticizing effect in the polymeric matrix. Characterization in terms of optical, hygroscopic, mechanical and barrier properties was fulfilled to evaluate their performance and possible application as food packaging material.

Chapter 4 - **Development and characterization of bilayer films of FucoPol and chitosan** - Is dedicated to produce bilayer films composed by FucoPol and chitosan, polymers with opposite charges. Characterization of bilayer and stand alone films of FucoPol and chitosan, respectively, was fulfilled in terms of optical, morphologic, hygroscopic, mechanical and barrier properties. From this interaction, bilayer films presented improved properties when compared with stand-alone films. Those referred properties give us a material more suitable to use in food packaging applications, namely in packages for dehydrated food products with high oil content.

Chapter 5 - **Comparison of different coating techniques on the properties of FucoPol films** - This chapter is focused on development of different coatings in FucoPol films surface, performed with different techniques, such as, low-pressure plasma deposition, liquid flame spraying and atomic layer deposition. The characterization of coated films in terms of surface and barrier properties was performed aiming the use of films for food packaging applications.

Chapter 6 - **Evaluation of FucoPol and Chitosan bilayer films performance for packaging of walnuts (*Juglans regia L.*)** - In this chapter, the bilayer films were applied in walnuts packages. Their performance was compared to that of a commercial non-biodegradable polymer, by evaluating the walnuts oil oxidation over time.

Chapter 7 - **Conclusions and future work** - Presents an overview of the overall conclusions of this work and describes suggestions of possible future work aiming the use of FucoPol exopolisaccharide as material to use in food packaging application.

Appendix A - It provides a list of the author's publications, papers in journals accepted and under review and oral and poster communications.

CHAPTER 2

BACKGROUND

Food packaging is essential for products containment, protection, preservation, convenience, to provide information about the product, brand communication, among others. This work is focused on primary packaging that is in direct contact with food, such as bottles, trays or bags [33]. This type of packaging is the most familiar to consumers and is defined as *"a sales unit to the final user or consumer at the point of purchase"* [145].

It should prevent or reduce products damage and food spoilage, reduce or eliminate the risk of adulteration and present food in a hygienic and aesthetically attractive way. Essentially, packaging strategies result from the combination of food science, processing and preservation, once they must extend the shelf life of food products reducing the wastage [33, 202].

Plastic packaging represents almost 40% of the European plastics market and is essential for processing, storing, transporting, protecting and preserving food [156, 158]. In fact, over 50% of all European goods are packaged in plastics, and this commercial success is due to a combination of properties such as flexibility, strength, lightness, stability, impermeability and ease of sterilization. These characteristics make them ideal materials for almost every commercial and industrial consumers [157]. According to the last report of PlasticsEurope - Association of plastics manufacturers (2015), the plastics production has grown globally and is stable in Europe (57 Mtonne per year). This success of plastics comes from the fact that many of them can be molded, extruded, cast and blown in different shapes, films or foams [155, 158].

Polyethylene (PE), polypropylene (PP) and polyethylene terephthalate (PET) are the most used in the packaging sector [10], but polyvinyl chloride (PVC) and polystyrene (PS) are also easily found in food packaging due to their biological resistance and excellent water barrier properties [153].

The real success of plastics in food packaging industry is achieved with combination of all referred characteristics (in particular lightness) and their use to help keeping food fresh and free of contamination [10]. The extended shelf life of food products has been reported for different foods with simple packaging, for example, unwrapped cucumber loses moisture and becomes dull and unsaleable within 3 days, but 1.5 g of plastic wrapping can keep a cucumber fresh for 14 days. Some more complex plastic packaging can extend (three times) the shelf-life of specific products, due to unique properties such as: resealable portioned packs, anti-microbial agents, humidity control systems and modified atmosphere packaging solutions. Furthermore, it is expected that in near future more innovations will be available in large scale and at low price, such as absorbers and emitters of natural occurring gaseous substances to prolong products shelf life, biosensors that detect bacteria or printable radio-frequency identification (RFID) tags to inform about integrity of the products [58, 156].

Plastic films are usually produced by extrusion, co-extrusion, casting, extrusion coating, extrusion lamination and metallization. These processes have advantages and disadvantages depending on the polymer used and the thickness required, usually less than 250 µm [153].

However, the crucial problem of using plastics for packaging is the post-consumer waste, once packaging is by far the largest contributor (63%) of plastic waste [78]. In addition, some materials are difficult to reuse and it is estimated that less than 14% of plastic packaging materials are recyclable [204]. According to the report Plastics – The facts 2014/2015 [158], despite recycling and energy recovery solutions have increased since 2006, the landfill disposal remains the largest solution for plastic in the end-of-life, making 9.5 Mtonne (38% of total plastic waste) in 2012, for EU27+Switzerland and Norway. Because most materials used are non-biodegradable, which remain in environment for long periods of time, they consist on threats to human health as well as to the environment [158].

The other used solution, incineration for energy recovery, has a severe environmental impact (as for most solid wastes or fuels) which can include some airborne particulates and greenhouse gas emissions [78].

According to these facts and knowing that, in the last decades a quick growth in synthetic polymers use has been observed, and it is predicted that value could multiply by four by the year of 2100 as result of growing human population and prosperity, it will be necessary the use of 25% of the world's current oil production just to plastics manufacture [153]. Taking in account this scenario, one valid option to overcome this environmental problem is the use of bio-based polymers from renewable resources.

This chapter provides an overview of the application of biodegradable polymers from renewable resources in packaging materials. A wide range of different polysaccharides, their properties, and their state of the art in research and commercial fields are described and discussed.

2.1 Biodegradable Polymers

For the polymer industry and consumers it is important to distinguish between biopolymers and biodegradable materials. According to American Society for Testing and Materials (ASTM Standard D-5488-84d), a biodegradable material is defined as *"material capable of undergoing decomposition into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of microorganisms, that can be measured by standardized tests, in a specified period of time, reflecting available disposal condition"*. On the other hand, biopolymers are polymeric materials derived totally from renewable resources. While biopolymers are biodegradable, not all biodegradable materials are considered biopolymers. As examples, polycaprolactone (PCL), polyglycolide (PGA) and polybutylene succinate adipate (PBSA) which are biodegradable materials, but not classified has biopolymers because they are produced from non-renewable resources (fossil-sourced chemicals) (Figure 2.1) [153].

Biopolymers are generally classified according to their source:

i Polymers directly extracted or removed from biomass such as polysaccharides (e.g. starch, galactomannans, starch, cellulose) and proteins (e.g. casein, gluten).

ii Polymers produced by chemical synthesis from renewable bio-derived monomers, as polylactic acid (PLA), a thermoplastic aliphatic polyester derived from lactic acid monomers. The monomer itself is produced via fermentation of carbohydrate feedstocks.

iii Polymers produced by microorganisms, like some polysaccharides (e.g. gellan gum, pullulan) and polyhydroxyalkanoates (PHA) [70, 122].

According to the European Bioplastics organization, biopolymers from renewable resources have to be biodegradable and especially compostable, which allows disposal of the package in the soil, being more energy efficient than recycling, so they can act as fertilizers and soil conditioners [70, 185].

Sustainability requires a fine balance between environmental, economic and social concerns. Biopolymers can be considered sustainable in terms of material supply, water and energy use and waste product generation. Moreover, the product viability, human resources and technology development also should be pondered from a point of view of sustainability.

Biodegradable products are usually more expensive than polymers manufactured from petrochemicals, but this circumstance is changing gradually, either by material collection, processing and conversion technologies, as well as economies of scale. With the increasing demands for plastic in the world, the consumer concern about the environment and the use of environmentally friendly products has grown. In addition to that, new regulations have been implemented, namely in EU countries, restricting the use of traditional materials, which led to a great development in biodegradable packaging materials [42].

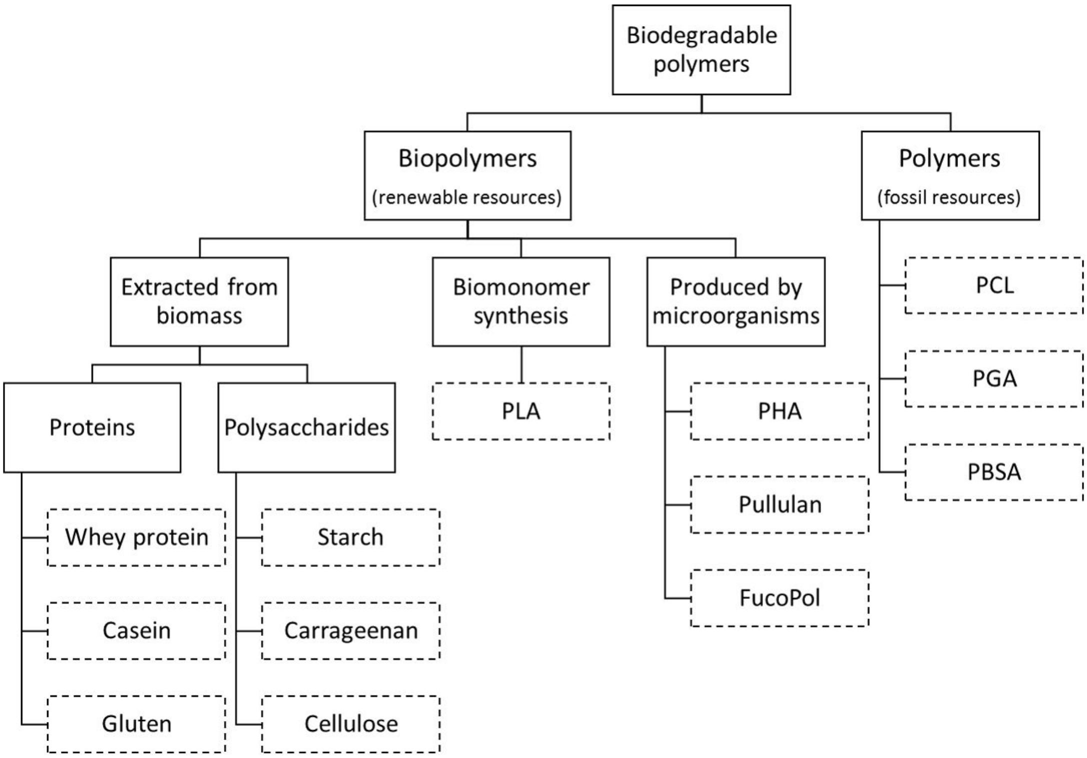


Figure 2.1: Biodegradable Polymers (Adapted from Encyclopedia of Membranes [32])

The idea of using biopolymers (from renewable resources and biodegradable) in packaging, to contribute for a sustainable development is recognized, since it is possible to dispose the plastic waste to be degraded in nature. This solution is particularly interesting for food packaging since this kind of materials are usually contaminated by food residues that constitute a health hazard in sorting and mechanical recycling [42].

2.2 Polysaccharides in food packaging

Polysaccharides are the most abundant macromolecules in the biosphere. These complex carbohydrates constituted by glycosidic bonds are often one of the main structural elements of plants (e.g. cellulose) and animal exoskeletons (e.g. chitin), or have an important role in the plant energy storage (e.g. starch) [195].

A high variety of polysaccharides and their derivatives have been used to produce biodegradable films and thin membranes, and used in several industries, such as food, medical, pharmaceutical and specific industrial processes (e.g. pervaporation) [69]. Polysaccharides have been widely used in food industry, as additives or in packaging films and edible coatings. Polysaccharide films are generally attractive due to their good barrier against oxygen and carbon dioxide (at low or moderate relative humidity) and good mechanical properties. However, their major drawback is related to their low barrier against water vapour due to their hydrophilic nature [70, 169]. The improvement of polysaccharide films have been studied in order to reach satisfactory biopolymer based packaging behaviour, possible to use in industrial applications [169]. In the following sections (2.2.1 - 2.2.4), the attention will be driven to polysaccharides application in food packaging.

2.2.1 Polysaccharides obtained from animals

Chitin and chitosan

Chitin is the second most abundant agro-polymer produced in nature. It appears naturally in the exoskeleton of arthropods and in the cell walls of fungi and yeasts. It is an acetylated polysaccharide composed of N-acetyl-D-glucosamine and is produced commercially by chemical extraction processes from prawns and crabs wastes. Chitin can also be produced by using enzyme hydrolysis or fermentation process, but these processes are not economically feasible yet on industrial scale [13, 195].

Chitosan is obtained from deacetylation of chitin, and different factors (e.g. alkali concentration, incubation time, ratio chitin to alkali, temperature and chitin source) can affected its properties. Chitosan is usually insoluble in water, but may be easily dissolved in acidic solutions. Its distinct characteristics from other polysaccharides rely on its cationic groups along the backbone and its antimicrobial properties against bacteria, yeasts and fungi [13, 19, 170, 195]. The good film-forming properties allow the production of films (thickness >30 µm) and coatings (<30 µm) to act as food preservative. Chitosan films are biodegradable, biocompatible, non-toxic, renewable and commercially available. Furthermore, chitosan films are reported as being semipermeable to gases presenting low oxygen permeability, essential for some food products preservation, and moderate water vapour barrier [19, 52, 57, 151].

Despite those unique properties of chitosan films, many research has been done focused on their improvement. Adding glycerol to chitosan films, and applying thermomechanical treatment (mechanical kneading), it is possible to obtain a kind of thermoplastic material which grants good mechanical properties [53, 195].

The functional properties of chitosan-based films may also be improved by combination with other hydrocolloids. Blends of chitosan and anionic polymers have been reported to have improved mechanical and barrier properties when comparing with stand-alone films. This fact is attributed to the formation of polyelectrolyte complexes through electrostatic interactions between the protonated amino groups of chitosan and the negatively charged side-chain groups in the other biopolymer at the operating pH [52, 114]. Improvements in mechanical properties, better performance in terms of water vapour permeability and lower water solubility have been reported for combinations of chitosan with other polysaccharides, such as, starch, pectin or alginate [96, 114, 215] and proteins, like, gelatin [171] and whey proteins [108], comparing to chitosan stand-alone films.

Lipids are usually added to films to impart hydrophobicity and thereby reduce moisture transfer. A wide range of lipid components is available, such as natural waxes, resins, fatty acids and vegetables oils [71]. A decrease in water susceptibility has been reported for chitosan films with beeswax [89], and decrease in water vapour permeability was described for films with oleic acid [207], neem-oil [177], cinnamon essential oil [139], among others.

The manufacturers and suppliers of chitosan and chitin products are present worldwide. Primex (Iceland) commercializes ChitoClear®, chitosan products that pretend to be based on the purest chitosan possible with potential application in food packaging [165]. Norwegian Chitosan (Norway) trades chitin and chitosan under brand names NorLife and Kitoflok™ respectively, for several applications, including food and beverages [136]. G.T.C. Bio Corporation (China) which is a chitin and chitosan manufacturer, commercializes different grades of both products with a price around 20€/Kg for chitin and between 18-45€ for chitosan (depending on required purity grade) [79].

2.2.2 Polysaccharides obtained from plants

Starch

Starch is the most abundant reserve polysaccharide in plants. As such, it is a renewable resource, biodegradable, produced in abundance at low cost, easy-to-handle and can exhibit thermoplastic behaviour. Starch can be extracted from cereals (e.g. corn, wheat or rice), from tubers (e.g. potato, tapioca or manioc), from grain (e.g. amaranth) or even from nuts (e.g. cashew), but commercially, the main sources of starch are corn, potato and tapioca [13, 195].

Starch granules are insoluble in cold water and are composed of two types of glucose polymers: amylose (the linear polymer which comprises approximately 20% w/w of starch granules) and amylopectin (the branched polymer). Starch properties depend directly on the botanical source, granule size distribution and morphology, genotype, amylose/amylopectin ratio and other factors such as composition, pH, and chemical modifications [13, 42].

This polysaccharide has ability to form films and coatings with very low oxygen permeability, however its applicability as packaging material is dependent on its high hydrophilic character, limited mechanical properties and the retrogradation (increase in crystallinity over time, leading to increased brittleness) [95, 142]. Research has been carried out to overcome these drawbacks, mainly using plasticizers, which increase the chain mobility and improve the flexibility, to create starch plastics with mechanical properties comparable to polyolefin-derived ones. The most used plasticizers are polyols such as glycerol, glycol and sorbitol [2, 91, 129, 187].

Other studied approaches consist on designing blends and composites, as well as starch chemical modification to produce a biodegradable material with appropriate mechanical strength, flexibility and water barrier properties for use as packaging material.

Blending starch with more hydrophobic polymers is widely studied (e.g. PCL or PLA) [12, 82], as well their composites with clay nanoparticles [11, 187].

The producers and traders of plastics based on starch include Novamont (Italy), which commercializes Mater-Bi®, a biodegradable and compostable bioplastic, commercialized in granular form that can be processed using the most common transformation techniques for plastics [137]. In addition, Eco-Go (Thailand) sells finished packaging products (e.g. bowls, food containers and food trays) from cassava and corn starch [50], and Plantic Technologies Limited (Australia) which produces PLANTIC™, a high barrier multilayer sheet for packaging goods, constituted by corn starch and PE and PP [154].

Galactomannans

Galactomannans are neutral polysaccharides obtained from the endosperm of dicotyledonous seeds of several plants, particularly the *Leguminosae*, where they function as carbohydrate reserves [25].

These gums are heterogeneous polysaccharides composed by a β-(1-4)-D-mannan backbone with a single D-galactose branch linked α-(1–6), they differ from each other by the mannose/galactose (M/G) ratio. The three major galactomannans with interest in food and non-food industries are guar gum (*Cyamopsis tetragonolobo* , M/G ratio: 2:1), tara gum (*Caesalpinia spinosa*, M/G ratio: 3:1) and locust bean gum (*Ceratonia siliqua*, M/G ratio: 3.5:1) [25, 148]. But just locust bean gum and guar gum are considered commercially interesting due their availability and price [163].

These natural polysaccharides are commonly used in the food industry, mainly as stabilizers, thickeners and emulsion stabilizers, as well as for the production of edible films and coatings. The galactomannans ability to form very viscous solutions at relatively low concentration and their resistance to pH alterations, ionic strength and heat processing are their main distinct characteristics. The mechanical and barrier properties of galactomannan films and coatings are the basis of their application to improve the shelf-life, safety and quality of food products [25, 70].

Several studies have shown the film-forming properties of different galactomannans, being the mannose/galactose ratio, the degree of substitution and the degree of polymerization, the main parameters affecting edible films properties [125].

Edible films and coatings of galactomannans have been applied for example in fruit and cheese. They have been tested in apples, to decrease the internal oxygen concentration. The sensory analyses revealed that the coated apples maintained consistent quality in firmness, crispness and juiciness [35]. Coatings based on galactomannan, glycerol and corn oil have been applied in cheese, decreasing the transfer rates (water vapour and oxygen), weight loss and colour changes [26].

Cargill (USA) offers various types of locust bean gum and guar gum flour or extracts under the trade name Viscogum™. Also, Chemtotal (Australia) produces and trades galactomannans (guar gum, locust bean gum, tara gum and cassia gum). Other companies producing and commercializing galactomannans include Altrafine Gums (India), with exportation for 90 countries of a wide range of different gums.

Cellulose

Cellulose is the most abundant occurring natural polymer on earth, being the predominant constituent in cell walls of all plants. Cellulose is composed of a unique monomer: glucose under its β-D-glucopyranose form [41]. Due to its regular structure and array of hydroxyl groups, it tends to form strong hydrogen bonded crystalline microfibrils and fibers and is most familiar in the form of paper, paperboard and corrugated paperboard in the packaging context [13, 42].

Its great interest is related with specific properties such as low density, high mechanical strength, low cost, durability, non-toxicity, renewability, biocompatibility, biodegradability, good films-forming performance, chemical stability and ease of making chemical derivatives [41, 48].

The most used raw material source for production of cellulose based products are wood and cotton fibers and in small amounts stalks of sugarcane bagasse. Natural cellulose fibers are low cost, biodegradable and have good mechanical properties, but they are difficult to use for industrial applications due to their hydrophilic nature, insolubility in water and crystallinity [42, 195].

Cellulosic materials are usually used in textiles, fibers and packaging and can be divided into two groups: regenerated and modified cellulose. Chemical reactions are usually performed to improve the thermoplastic behaviour of cellulose, such as etherification and esterification that are conducted on the free hydroxyl groups. Numerous derivatives are commercialized, but the main used for industrial purpose are cellulose acetate, cellulose esters (for molding, extrusion and films) and regenerated cellulose for fibers. To overcome the hard mechanical properties of cellulose, beyond chemical modification, the use of plasticizers and blends with other polymers are also used, being the final mechanical and chemical properties dependent on the blend composition.

To produce cellophane film, for example, cellulose has to be dissolved in aggressive and toxic solutions, and then recast in sulphuric acid. In that way, it is possible to produce a hydrophilic film with good mechanical properties. However, this film is not thermoplastic and cannot be heat-sealed [42].

Nowadays a large number of companies are suppliers of cellulose films. Innovia Films (United Kingdom) presents two different products based on cellulose, Cellophan™ and NatureFlex™, which are biodegradable and compostable, both sold worldwide for food packaging applications (Pre-made bags, tapes, box overwrap, bunch wrap, among others) [90]. Weifang Henglian Films CO. LTD (China) provides food grade cellulose films with different sizes adapted for specific products.

2.2.3 Polysaccharides obtained from algae

Carrageenan

Carrageenan is a naturally occurring hydrophilic, anionic sulfated linear polysaccharide extracted from red seaweeds, specifically from the *Rhodophyceae* family (e.g. *Chondrus crispus*, *Kappaphycus spp.*, *Eucheuma spp.*, and *Gigartina stellata*) [164, 191]. This hydrocolloid is composed of α-D-1,3 and β-D-1,4 galactose residues that are sulfated at up to 40% of the total weight. Carrageenans are classified based on their solubility in potassium chloride, into different types (λ, κ, ι, ε, μ) all composed by 22-35% sulphate groups although these designations do not reflect definitive chemical structures [135]. The κ-carrageenan is the one with less negative charges per disaccharide having excellent properties to form gels and films. When comparing with λ- and ι-carrageenan, the κ-carrageenan films exhibit better mechanical properties [20, 121, 135].

Carrageenan is approved as food-grade additive, and it has been used mainly as emulsifier and stabilizer in flavored milks, dairy products, pet food, dietetic formulas and infant formulas [121, 135].

Carrageenan is also used to produce edible films and coatings, though the reports about its application in coatings are much more common. Carrageenan edible films and coatings and their blends with other polymers were reported to be used in food to preserve fresh cut fruits, by reducing moisture loss and decreasing gas exchange, as well as preventing the discoloration and maintaining texture [17, 159]. Films of carrageenan have also been reported as encapsulating matrices of aroma compounds [55, 83, 191].

FMC (USA) is the largest and the most experienced producer of carrageenan extracts worldwide. Its film-forming carrageenans have brand names of Gelcarin® and Viscarin® [65]. Other important companies in carrageenan market are CP Kelco (USA), Danisco (Denmark), Ceamsa (Spain), and Quest International (The Netherlands). JetNet Corporation (USA) produces carrageenan films, in particular Nutrafilm™ carrageenan film packaging for meat and poultry, and over 300 different styles and sizes of elastic netting [93].

Alginate

Alginate is a linear polysaccharide that is abundant in nature and is synthesized by brown seaweeds (e.g. *Laminaria digitata* and *Ascophyllum nodosum*) and some soil bacteria. It has an anionic character and is water-soluble, consisting of monomeric units of 1-4-linked α-d-mannuronate (M blocks) and β-l-guluronate (G blocks), as well as segments of alternating mannuronic and glucuronic acids (MG blocks). The physical properties of alginates depend on the relative proportion of these three blocks, which are directly related with extraction source [149]. They are appealing film-forming compounds because of their non-toxicity, biodegradability, biocompatibility and low cost. Also, other functional properties have been studied, such as thickening, stabilizing, suspending, gel-producing, among others [13, 191, 209].

Sodium alginate is the most used in industry and was the first by-product from algal purification. Having an efficient brown seaweed extraction, would be interesting to produce an environmentally friendly biopolymer-rich extract for industrial applications, such as, food packaging material, release agents, paper, pharmaceutical and medical uses, among others [191]. Due to the linear structure of alginate, the films are strong, with adequate fibrous structures in solid state, being considered a good filmogenic material [18].

The market of alginate producers is concentrated in few companies, including FMC (USA), Cargill (USA) and DuPont (Danisco) (Denmark). The price of alginate has increased between 2009 and 2013 due to the stronger demand, but become stable in 2014 at 11€/Kg [24].

The properties and applications in food packaging of the polysaccharides obtained from animals, plants and algae are summarized in Table 2.1.

2.2.4 Polysaccharides obtained from microorganisms

Several polysaccharides with film-forming ability can be produced by microorganisms (yeast, fungus or bacteria), such as pullulan, gellan gum, xanthan gum, FucoPol, bacterial cellulose or bacterial alginates. This sub chapter will focus on the most used polymers except bacterial cellulose and alginate referred before.

Pullulan

Pullulan, is a linear, water-soluble and neutral exopolysaccharide (EPS), constituted mainly of maltotriose units connected by α-1,6 glycosidic units and produced by yeast like fungus *Aureobasidium pullulans* using a variety of feedstocks containing simple sugars [13]. The molecular weight of pullulan, ranging from 4.5×10^4 to 6×10^5 Da, is greatly affected by cultivation parameters (temperature, pH, type of carbon source and type of nitrogen source). The commercial production of pullulan began in 1976 by the Hayashibara Company (Japan). Its production was an outgrowth of starch syrup production, noted in 1883. Pullulan films started to be commercialized by Hayashibara in 1982 [30, 161].

Pullulan is biodegradable, non-toxic, tasteless and odourless. It can be used as food additive, as flocculant agent or even as blood plasma substitute, beyond film forming agent. Pullulan films are edible, homogeneous, transparent, printable, heat sealable, flexible and good barriers to oxygen [69, 76, 107]. However, they are water sensitive and mechanically weak [183, 184]. These properties, and the fact of pullulan films inhibit fungal growth, make them a good material for food applications.

Despite all advantages of pullulan, its high cost has limited the use of pullulan and pullulan films in several applications. Research has been carried out on blending pullulan with other biopolymers and additives to produce films with better physicochemical characteristics and mechanical properties. Blends of pullulan with alginate, chitosan,

Table 2.1: Properties and food applications of polysaccharides obtained from animals, plants and algae

Polysaccharide	**Composition**	**Films properties**	**Main films food applications**	**Reference**
Chitin	N-acetylglucosamine	• Biodegradable • Antibacterial and fungistatic properties • Biocompatible and non-toxic • Highly transparent	• Cofee capsules • Food bags • Packaging films	[31, 113, 170, 188]
Chitosan	D-glucosamine N-acetyl-D-glucosamine	• Biodegradable • Biocompatible and non-toxic • Antifungal and antibacterial properties • Good mechanical properties • Barrier to gases • High water vapor permeability • Brittle – need to use plasticizer	• Edible films and coatings (Strawberries, cherries, mango, guava, among others) • Packaging films for vegetables and fruit	[20, 53, 70, 101, 188]
Starch	Glucose	• Biodegradable • Transparent • Odorless and tasteless • Retrogradation • High elongation and tensile strength	• Flexible packaging – Extruded bags – Nets for fresh fruit and vegetables • Rigid packaging • Thermoformed trays and containers for packaging fresh food	[20, 42]
Galactomannans	Mannose Galactose	• Biodegradable • Edible • Semi-permeable barrier to gases	• Edible films and coatings – Fruits – Cheese	[25, 70]
Cellulose	Glucose	• Biodegradable • Good mechanical properties • Transparent • Highly sensitive to water • Resistance to fats and oils • Need to perform modification, use of plasticizer or polymer blend	• Cellophane films	[20, 42]
Carrageenan	Galactose	• Biodegradable • Fragile and ductile behaviour • Usually blended with other polymers	• Coatings – Fruits – Meat • Encapsulation	[20, 191]
Alginate	Mannuronic Glucuronic acid	• Biodegradable • High water vapour permeability • Poor water resistance • Strong and brittle films • Cross-link with calcium	• Coatings • Prevent water loss in fresh cut fruit (apple, papaya, pear and melon) • Inhibition of microbial growth (turkey products) • Microwaveable food (increase warming efficiency)	[20, 70, 191]

cellulose and starch have been reported with improvements in thermal and mechanical properties, low water vapour permeability and low water absorption [99, 103, 198, 203, 212]. Also, composite films of pullulan with lipids and proteins have shown improved properties. Pullulan films with gelatin have demonstrated higher tensile strength and reduced oxygen permeability and cost [216], while the use of rice wax has shown improvements in water vapour barrier properties [183].

Nowadays, apart from the Hayashibara corporation, Shandong Jinmei Biotechnology Co. Ltd. (China) is also a key producer of pullulan (Jinmei Pullulan), which is commercialized in powder or capsules forms, with application in edible films, oral dissolving films, coatings in soft candies, among others [97].

Gellan gum

Gellan gum is an anionic water-soluble exopolysaccharide, produced by *Sphingomonas elodea*, also known as *Auromonas elodea* or *Pseudomonas elodea*. This heteropolysaccharide is a linear high molecular weight (around 5x10^5 Da) compound, with a tetrasaccharide repeating sequence which consists of two residues of β-D-glucose, one of β-D-glucuronic acid and one of β-L-rhamnose [133]. The approximate composition comprises glucose (60%), rhamnose (20%) and glucuronic acid (20%) [162]. Gellan gum was identified as a product with potential commercial value by Kelco (USA) during an extensive screening program of soil and water bacteria. In its original form (high acyl gellan), gellan gum has two acyl substituents (acetate and glycerate). Low acyl gellan gum is obtained with removal of acyl groups [104, 133]. High acyl gellan forms soft, elastic, non-brittle, thermo-reversible gels, and low acyl gellan tends to form firm, non-elastic brittle and thermostable gels [45, 104].

In food industry, gellan gum is usually used as additive (stabilizer, thickening agent and gelling agent), however the applications of gellan gum may be extended to films and coatings for food industry also, such as breading and batters for chicken, fish, cheese, vegetables and potatoes, coatings and adhesion systems. These films and coatings offer advantages, essentially due to their ability to reduce oil absorption by providing an effective barrier. In batters, for example, product crispness is maintained long after frying or baking, which helps to maintain product quality under heating lamps [38].

CP Kelco (USA) is the leading global producer of gellan gum, commercializing Gelrit™ (low acyl) and Kelcogel™ (high acyl). Also, Dancheng Caixin Sugar Industry co. ltd (China) is producer and world-wide seller of high and low acyl gellan.

Xanthan gum

Xanthan gum is an exopolysaccharide produced by *Xanthomonas campestris* using glucose and sucrose as sole carbon source. It was discovered in 1963 at the Northern Regional Research Laboratories (USA) and was the second microbial polysaccharide commercialized. Nowadays is the most extensively studied and widely accepted industrial microbial biopolymer, being the most significant bacterial EPS in global hydrocolloids market [66, 143]. This heteropolysaccharide consists of repeated pentasaccharide units composed by glucose, mannose and glucuronic acid (2 : 2 : 1 ratio) and pyruvate and acetyl substituent groups [72].

Xanthan is water-soluble and non-toxic. It imparts a high viscosity at low concentrations in aqueous media, with a strong shear-thinning behaviour. The rheological properties of xanthan solutions are quite stable in a wide range of pH, ionic strength and temperature values [59, 64].

Xanthan gum has been used in a wide variety of industrial applications, such as food, cosmetic, pharmaceutical, textile, petroleum production or even slurry explosives. In food industry, it is mainly used as additive (suspending and thickening agent) [143, 174]. So far, there is not much information about xanthan films for food packaging, maybe caused by still high cost of xanthan production [143]. Nevertheless, xanthan coatings applied to acerola, showed it is an effective system for reducing the weight loss and the respiration process, keeping the colour and eventually increasing the shelf-life [167].

The major producers include CP Kelco (USA), Danisco (Denmark), Merck (USA), Sanofi-Elf (France) and Jungbunzlauer (Switzerland) that commercialize xanthan with different purity grades and trade names.

The properties and applications in food packaging of the microbial polysaccharides are summarized in Table 2.2.

Table 2.2: Properties and application of microbial polysaccharides in food packaging

Polysaccharide	**Microorganism**	**Composition**	**Films properties**	**Main films food applications**	**Reference**
Pullulan	*Aureobasidium pullulans*	Maltotriose (three glucose)	• Biodegradable • Transparent • Edible • Oil and grease resistant • Heat sealable • High water solubility • Barrier to oxygen	• Coating material • Wrapping material • Blends with other polymers to improvement of mechanical properties • Inner package – Seasoning bag of instant noodles – Instant coffee	[30, 188, 216]
Gellan Gum	*Sphingomonas elodea*	Glucose Rhamnose Glucuronic acid	• Biodegradable • Edible • Lipid barrier • Excellent gas barrier • Good tensile strength	• Edible Coatings – Breadings and batters for chicken, fish, cheese, vegetables and potatoes • Encapsulation of flavor and bioactive ingredients	[69, 133, 213]
Xanthan gum	*Xanthomonas campestris*	Mannose Glucuronic acid Acetate Pyruvate	• Biodegradable • Edible	• Edible coating – Meat (Prevent moisture migration during frying) – Fruit (Extend shelf-life)	[167, 188]

FucoPol

FucoPol is a new high molecular weight exopolysaccharide (2–10x10^6 Da) produced by *Enterobacter* A47 (DSM 23139) using glycerol by-product from biodiesel industry as carbon source. This biodegradable, anionic and water-soluble heteropolysaccharide is composed by fucose (36–38 % mol), galactose (22–24 % mol), glucose (27–33 % mol), glucuronic acid (9–10 % mol) and acyl groups (acetate, succinate and pyruvate), which account for 12–18 wt% of the FucoPol dry weight [7, 199, 200].

FucoPol production at lab-scale has shown productivities and yields comparable to other commercial microbial bacterial polysaccharides, such as xanthan and gellan [64]. Although this polysaccharide is not commercially available yet, the scale up of its production is being developed.

FucoPol has demonstrated flocculating and emulsion stabilizing capacity, comparable to commercial polymers [67]. FucoPol has also shown to have a good thickening capacity in various aqueous formulations (with a wide range of pH and ionic strength) [201]. These functional properties make this polymer a good alternative in several applications in the food, pharmaceutical, cosmetic, textile, paper and petroleum industries.

In this thesis film-forming capacity of FucoPol was tested and their capacity to be used in food industry and packaging material was evaluated and described in the following chapters.

Chapter 3

Biodegradable films produced from the bacterial polysaccharide FucoPol

3.1 Summary

FucoPol, an exopolysaccharide produced by *Enterobacter* A47, grown in bioreactor with glycerol as carbon source, was used with citric acid to obtain biodegradable films by casting. The films were characterized in terms of optical, hygroscopic, mechanical and barrier properties. These films have shown to be transparent, but with a brown tone, imparting small colour changes when applied over coloured surfaces. They were hydrophilic, with high permeability to water vapour ($1.01\text{x}10^{-11}$ $\text{mol}\,\text{m}^{-1}\text{s}^{-1}\text{Pa}^{-1}$), but presented good barrier properties to oxygen and carbon dioxide ($0.7\text{x}10^{-16}$ $\text{mol}\,\text{m}\,\text{m}^{-2}$-$\text{s}^{-1}\text{Pa}^{-1}$ and $42.7\text{x}10^{-16}$ $\text{mol}\,\text{m}\,\text{m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$, respectively). Furthermore, films have shown mechanical properties under tensile tests characteristic of ductile films with high elongation at break, low tension at break and low elastic modulus. Although the obtained results are promising, films properties can be improved, namely by testing alternative plasticizers, crosslinking agents and blends with other biopolymers. Taking into account the observed ductile mechanical properties, good barrier properties to gases when low water content is used and their hydrophilic character, it is foreseen a good potential for FucoPol films to be incorporated as inner layer of a multilayer packaging material.

3.2 Introduction

Petrochemical-based plastics, such as polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP) and polyamide (PA), have been intensively and increasingly used in food packaging because they are manufactured at a low-cost, presenting simultaneously interesting functional characteristics. They are heat sealable, possess good mechanical and thermal properties, as well as, suitable barrier properties to gases, aroma compounds and microorganisms [180, 185]. However, their use must be reduced because they are non-biodegradable and their recyclability is limited, which causes a serious environmental impact [186]. This problem can be overcome by replacing synthetic polymers by natural/bio-based polymers [186]. Most of the bio-based polymers obtained from renewable resources are biodegradable. They may be classified according to the production method or source as: polymers directly extracted from biomass (such as plant or algal polysaccharides and proteins), polymers obtained from renewable bio-based monomers (such as polylactic acid (PLA) or polymers produced by microorganisms (such as polyhydroxyalkanoates (PHA) and bacterial exopolysaccharides (EPS) [44, 122].

Polysaccharides are usually nontoxic and widely available [102]. They have hydrophilic character, usually forming strong films with poor water vapour barrier properties [34, 102, 211]. Nevertheless, polysaccharide films are excellent gas, aroma and lipid barriers and show good mechanical properties. The film forming capacity and film properties of different polysaccharide materials, including, chitosan [49, 52, 57], starch [73, 94, 110, 112], alginate [80, 176] and carrageenan [8] have been intensively studied.

Microbial polysaccharides represent an alternative to others recovered from animal, algal or plant sources, because their production is not dependent on climatic or seasonal impacts. The high molecular structure variability, availability and the properties of these polysaccharides turns them attractive to a wide range of applications, ranging from chemical industry to food, medicine and cosmetics [69]. Some microbial polysaccharides, such as gellan, kefiran and xanthan, have been studied to produce biodegradable films with potential final use on packaging materials [143, 152, 162]. Such microbial polysaccharide films could be applied as primary packaging (as stand-alone films) or coatings. However, their hydrophilic nature limits their use as moisture barrier. Thus, development of biodegradable films based on polymer blends or multilayer films has been explored in order to obtain polymeric matrices with new and improved mechanical, barrier and bioactive properties [69, 182]. For hydrophilic materials (such as polysaccharides) multilayer structures are more advantageous than polymer blends, because the hydrophilic polymer can be sandwiched between hydrophobic materials [56].

In this work, a polysaccharide, FucoPol, was used for the preparation of biodegradable films for food packaging. FucoPol is a recently reported bacterial EPS produced by *Enterobacter A47* (DSM23139) using glycerol as the sole carbonsource [7, 199]. It is a high molecular weight ($4.19\text{x}10^6 - 5.80\text{x}10^6$) heteropolysaccharide composed of sugar residues (fucose, galactose, glucose and glucuronic acid) and acyl groups (pyruvate, succinate and acetate) [67]. It has an anionic character and has interesting functional properties, including emulsion and film-forming capacity [68].

The films were prepared using citric acid, which presents cross-linking and plasticizer properties, already reported for starch [141], gelatin [21, 208] and blends of wheat flour/-PLA films [1]. FucoPol films were characterized in terms of their optical, hygroscopic, mechanical and barrier properties for their potential use either alone, blended or as a layer in a multi-layered film for food packaging.

3.3 Materials and methods

3.3.1 FucoPol production and purification

FucoPol was produced by *Enterobacter A47*, cultivated using glycerol byproduct as carbon source in a 10 L bioreactor (BioStatB-plus, Sartorius), with controlled temperature (30 °C) and pH (6.8). The bioreactor was operated in a batch mode (initial glycerol concentration of $40\,\mathrm{g\,L^{-1}}$) during the first day of cultivation, followed by a fed-batch mode for 3 days (feeding with a $200\,\mathrm{g\,L^{-1}}$ glycerol solution at a constant rate of $20\,\mathrm{g\,L^{-1}h^{-1}}$). The aeration rate (0.125 vvm), volume of air per volume of reactor per minute was kept constant throughout the cultivation, and the dissolved oxygen (DO) concentration was controlled at 10% air saturation by automatic variation of the stirrer speed (400 – 800 rpm) provided by two 6-blade impellers [67]. Overall, $50\,\mathrm{g\,L^{-1}}$ of glycerol were consumed and a final FucoPol concentration of $7.8\,\mathrm{g\,L^{-1}}$ was achieved at the end of the 4 days production run, corresponding to a product yield on substrate of $0.156\,\mathrm{g\,g^{-1}}$.

FucoPol extraction and purification consisted on several steps. Firstly, the culture broth was diluted (1:6) with deionised water for viscosity reduction and centrifuged (1 h, 8875 × g), for cell separation. The cell-free supernatant submitted to thermal treatment (1 h, 70 °C) followed by a second centrifugation (15 min, 8875 × g) to remove precipitated proteins and remaining cells.

The supernatant was then submitted to a diafiltration process,using a hollow fibre membrane module (Model #: UFP-500-E-6A, GE Healthcare), with a 500 kDa cut-off and a surface area of $2800\,\mathrm{cm^2}$, operated at transmembrane pressure below 0.7 bar, to remove low molecular weight contaminants, e.g. salts, glycerol and proteins. After impurities removal, the treated supernatant containing FucoPol was concentrated (5:1) using the same membrane module, switching to an ultrafiltration process mode. The obtained solution was freeze dried (Martin Christ, model Epsilon2–40, Germany) during 48 h and the obtained FucoPol was stored at ambient temperature.

3.3.2 FucoPol films preparation

FucoPol was dissolved in distilled water (1.5% w/w) under stirring, at room temperature, until complete dissolution. Then, citric acid (Panreac Química S.L.U., Barcelona, Spain) was added in a proportion of 1:1 w/w (dry basis) and the solution was let under stirring for at least 12 h for complete homogenization.

After removing the air bubbles under vacuum, 30 mL of solution were transferred to Teflon petri dishes, diameter 100 mm (Bola, Germany) and let to dry at 40 °C, during 15 h, to form a film. The films were stored at a specific relative humidity and temperature,depending on the tests to be performed. Films thickness was measured with a manual micrometre (Brave Instruments, USA).

3.3.3 Colour and transparency

The transparency of films was determined by measuring the transmittance at 600 nm using a spectrophotometer (Heλos α, Thermo Spectronic, UK), and calculated according to Eq. 3.1.

$$Transparency = \frac{-log T_{600}}{x} \tag{3.1}$$

Where T_{600} is the transmittance at 600 nm and x is the film thickness (mm). In addition, the colour alterations on objects caused by application of the prepared films was evaluated by measuring the colour parameters of coloured paper sheets, covered and uncovered by the test films. A Konica Minolta CR-400, USA, colorimeter was used, and the CIELa*b* colour space was applied with the calculation of colour differences (ΔE_{ab}), chroma (C_{ab}) and hue (h_{ab}), with the following equations:

$$\Delta E_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{\frac{1}{2}} \tag{3.2}$$

$$C_{ab} = [a^{*2} + b^{*2}]^{\frac{1}{2}} \tag{3.3}$$

$$h = arctan\left(\frac{b^*}{a^*}\right) \mathrm{x} \frac{180}{\pi}, \text{for } a^* > 0 \; and \; b^* > 0 \tag{3.4}$$

$$h = arctan\left(\frac{b^*}{a^*}\right) \mathrm{x} \frac{180}{\pi} + 180, \text{for } a^* < 0 \tag{3.5}$$

$$\begin{gathered} h = arctan\left(\tfrac{b^*}{a^*}\right) \mathrm{x} \tfrac{180}{\pi} + 360, \\ \text{for } a^* > 0 \; and \;\; b^* < 0 \end{gathered} \tag{3.6}$$

Where L_0^*, a_0^*, b_0^* refer to the colour of the uncovered sheets. Five measurements on different areas of the coloured sheets, with and without films, were carried out.

3.3.4 Water sorption isotherms

Water sorption isotherms were determined by a gravimetric method at 30 °C. Samples with dimensions of 20 mm×20 mm were previously dried at 70 °C during 24 h. The samples were then placed in desiccators with different saturated salt solutions: LiCl, CH_3COOK, $MgCl_2 \cdot 6H_2O$, K_2CO_3, $Mg(NO_3)_2$, $NaNO_2$, NaCl, $(NH_4)_2SO_4$, $BaCl_2$ and K_2SO_4, with a water activity of 0.115, 0.225, 0.324, 0.447, 0.520, 0.649, 0.769, 0.806, 0.920 and 0.977, respectively. Three film replicates for each salt solution were analyzed. The samples were weighed after three weeks, ensuring that the equilibrium has been reached. The Guggenheim–Anderson–de-Boer (GAB) model (Eq. 3.7) was used to fit the experimental sorption data.

$$X = \frac{C k X_0 a_W}{(1 - k a_W)(1 - k a_W + C k a_W)} \tag{3.7}$$

where X is the equilibrium moisture content ($g_{water}/g_{dry\ solids}$) at the water activity (a_W), X_0 is the monolayer moisture content, C is the Guggenheim constant and represents the energy difference between the water molecules attached to primary sorption sites and those absorbed to successive sorption layers, and k is the corrective constant owing to properties of multilayer molecules with respect to the bulk liquid. GAB equation parameters were determined by non-linear fitting using the software package Scientist™, from MicroMath®.

3.3.5 Mechanical properties

Tensile tests were performed using a TA-Xt plus texture analyser (Stable Micro Systems, Surrey, England) performed at 23 °C using film strips (20x70 mm) attached on tensile grips A/TG and stretched at 0.5 mm s^{-1} in tension mode. The tensile stress at break (TS, MPa) was calculated as the ratio of the maximum force to the films initial cross-sectional area. The elongation (strain) at break (EB, %) was determined as the ratio of the extension of the sample upon rupture by the initial gage length. The Elastic Modulus (EM, MPa) was calculated from the slope of the initial linear region of the stress-strain curve. The samples were equilibrated previously (44.3% RH and 22 ± 2 °C). Five film replicas were analyzed.

3.3.6 Water vapour permeability

The water vapour permeability (WVP, mol m^{-1} s^{-1} Pa^{-1}) was measured gravimetrically at 30 °C. The films samples were sealed on the top of a glass cell with a diameter of 44.5 mm and placed in a desiccator containing a saturated salt solution and equipped with a fan to promote air circulation. Room temperature and relative humidity inside the desiccator were monitored over time using a thermohygrometer (Vaisala, Finland). The driving force tested was imposed by using a saturated NaCl solution (RH = 76.9%) inside the cell dish and a saturated CH_3COOK solution outside (RH = 22.5%). The films were previously equilibrated at a relative humidity of 76.9%. The water vapour flux was measured by weighing the cell at regular time intervals during 24 h and the water vapour permeability was calculated by Eq. 3.8,

$$WVP = \frac{N_W\ \delta}{\Delta P_{W.eff}} \tag{3.8}$$

where N_w ($mol\,m^{-2}s^{-1}$) is the water vapour flux, δ (m) is the film thickness and $\Delta P_{w,eff}$ (Pa) is the effective driving force. Three film replicates were analysed.

3.3.7 Gas permeability

The tests were made using a stainless steel cell with two identical chambers separated by the film. The films were equilibrated at 30 °C in a desiccator containing a saturated $MgCl_2 \cdot 6H_2O$ solution, with a water activity of 0.324. The permeability was evaluated by pressurizing one of the chambers (feed) up to 0.4 bar, with pure gas (carbon dioxide (99.998%) or oxygen (99.999%) Praxair, Spain), followed by the measurement of the pressure change in both chambers over time, using two pressure transducers (Druck, PDCR 910 model).

Five independent measurements were made at a constant temperature of 30 °C, using a thermostatic bath (Julabo, Model EH,Germany). The permeability was calculated by Eq. 3.9,

$$\frac{1}{\beta}\left(\frac{\Delta p_0}{\Delta p}\right) = P\frac{t}{\delta} \tag{3.9}$$

where Δp (mbar) is the pressure difference in feed and permeate compartment, P (mol m-$m^{-2}s^{-1}Pa^{-1}$) is the gas permeability, t (s) is the time, δ (m) is the film thickness and β is the geometric parameter of cell, as described by Alves et al. [6].

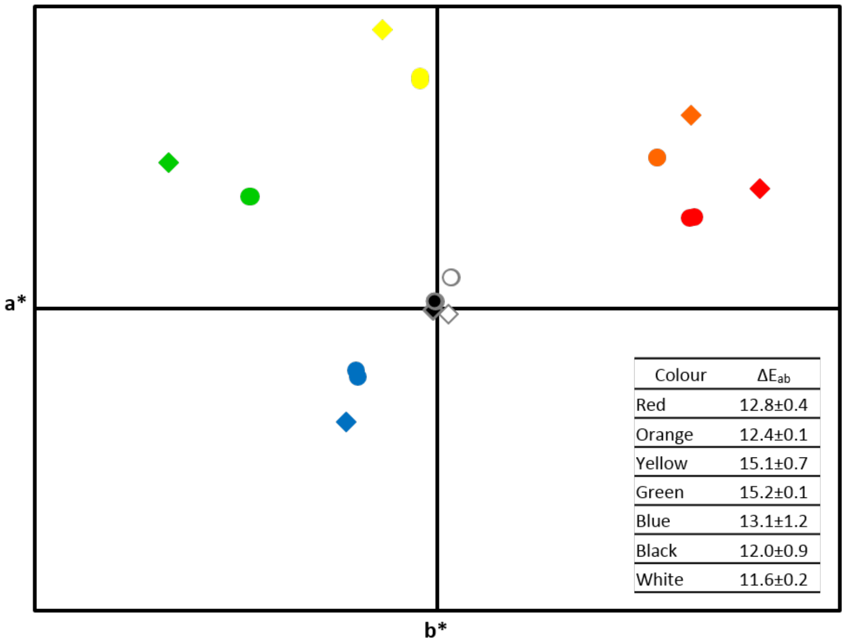
3.4 Results and discussion

FucoPol, produced and purified as described in Section 3.3.1 was characterized and used to prepare the filmogenic solutions to produce the films according to Section 3.3.2. The obtained films were stored in a desicator with a controlled relative humidity (0% RH) and temperature (25 °C) before performing their characterization in terms of their optical, hygroscopic, mechanical and barrier properties.

3.4.1 FucoPol films appearance, colour alteration and transparency

The prepared films are transparent with a slight brownish tone and flexible when handled. They are totally soluble in water indicating the inexistence of cross linking reactions in spite of the use of citric acid. The transparency measured at 600 nm is 3.67 ± 0.57. This value is higher than the obtained for films from other biopolymers, such as chitosan (1.13 ± 0.05) or gelatin (0.67 ± 0.01), although lower than ahipa starch (4.0 ± 0.1), cassava starch (4.7 ± 0.1) or corn starch (4.6 ± 0.1) [112]. The transparency value of FucoPol films is similar to the value obtained for some synthetic films, such as low density polyethylene (LDPE) (3.05) [171].

The colour alteration of objects due to the application of the films was also evaluated by measuring the colour parameters of coloured paper sheets, uncovered and covered by the film sample. Figure 3.1 shows the CIELa*b* colour parameters a* and b*, for all colours tested. It may be perceived that the hue (h_{ab}, angle towards the horizontal axes) does not change significantly with the application of the FucoPol film for the majority of the colours, except for yellow and blue, for which a hue variation was perceived upon film application. In addition, for all cases, the dots move towards the origin,which corresponds to a decrease of colour saturation (chroma, C_{ab}). The colours alteration (ΔE_{ab}) are low (<15.2) (insert in Figure 3.1), but with values representing colour changes that are perceived by the human eye. The colours that present higher colour alterations are yellow, green and blue, because they are the colours furthest from the film colour itself.



Colour	ΔE_{ab}
Red	12.8±0.4
Orange	12.4±0.1
Yellow	15.1±0.7
Green	15.2±0.1
Blue	13.1±1.2
Black	12.0±0.9
White	11.6±0.2

Figure 3.1: Parameters a* and b* of the CIELAB system for coloured paper sheets uncovered (diamonds) and covered (circles) by the test films and calculated colour differences (insert).

3.4.2 Water sorption isotherms

The water sorption isotherms of FucoPol films are presented in Figure 3.2 as well as those of the bacterial polysaccharide GalactoPol, pectin and chitosan films previously reported, for comparison. The obtained experimental data is well described by GAB model. The general isotherm is similar to that of GalactoPol films, although FucoPol films present a higher hydrophilic behaviour. The water vapour sorption capacity is analogous to pectin for water activity values lower than 0.7, but above this value FucoPol has a significantly higher affinity to water vapour. When compared to chitosan films, it can be observed a much higher affinity to water vapour by the FucoPol films, for the entire range of water activity.

3.4.3 Mechanical properties

The film samples presenting a water content of 8 ± 0.4% (on a dry basis), were subjected to tensile tests. The results show that FucoPol films present a typical behaviour of a ductile film: tensile strength at break (TS) values (3.1 ± 0.3 MPa), high elongation at break (EB) (54.9 ± 4%) and low elastic modulus (EM) (2.8 ± 0.2 MPa) (Table 3.1).

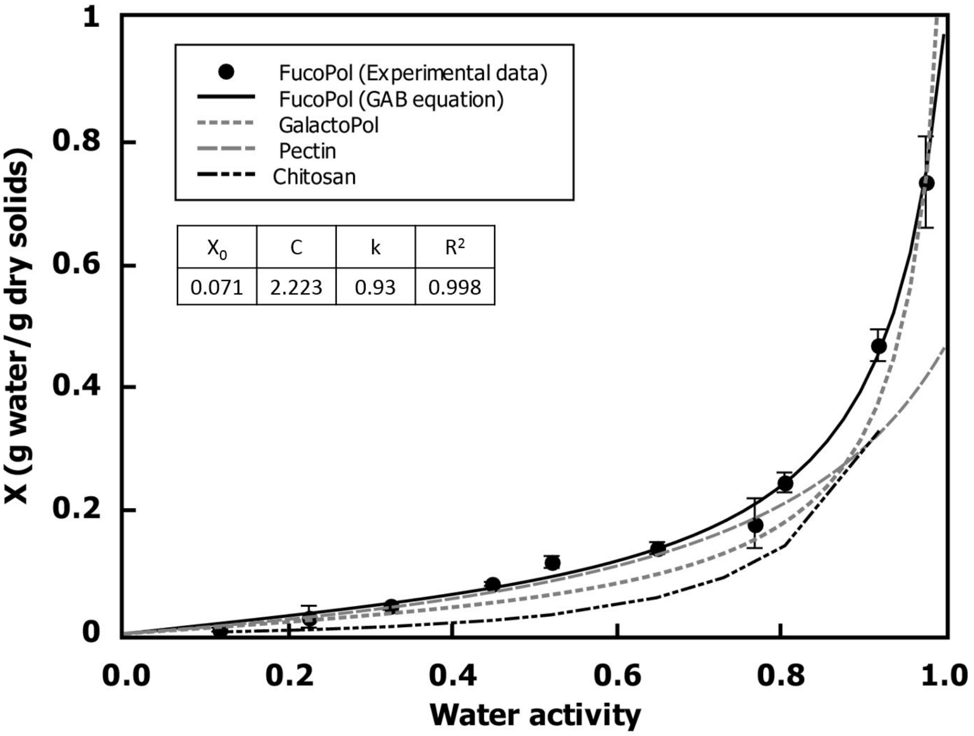


Figure 3.2: Water sorption isotherms of FucoPol films at 30 ºC (symbols) and GAB equation for FucoPol, GalactoPol, pectin and chitosan films (lines).

The mechanical properties of polysaccharide films are strongly dependent not only on the characteristics of the biopolymer used, but also on the addition of plasticizers, cross-linking agents and other additives upon film formation, as well as on the water content of the polymeric matrix during the measurements. This fact turns difficult to compare the mechanical properties of films obtained in different works. Still, it can be noticed that the EB and TS of FucoPol films are close to those reported for films from galactomannans plasticized with glycerol (Table 3.1) [28]. On the other hand, when comparing FucoPol and GalactoPol films, it is found that their properties are completely different. GalactoPol films are rigid, presenting higher TS and EM (51 MPa and 1738 MPa), especially due to the absence of a plasticizer and to evidence of cross-linking [9]. The mechanical properties of FucoPol films reveal a polymeric structure that is not mechanically resistant enough to be used as a stand-alone film. However, there is the potential to be used as a hydrophilic layer incorporated in a multi-layered material.

The mechanical properties of FucoPol films reveal a polymeric structure that is not mechanically resistant enough to be used as a stand-alone film. However, there is the potential to be used as a hydrophilic layer incorporated in a multi-layered material.

Table 3.1: Mechanical properties of EPS films, along with those of other polymers referred in literature

Films	%RH	Tensile strength (MPa)	Elongation at break (%)	Elastic modulus (MPa)	Reference
FucoPol	44.3	3.1 ± 0.3	54.9 ± 4.0	2.8 ± 0.2	Present Study
GalactoPol	44.3	51.0 ± 3.0	9.5 ± 3.9	1738.0 ± 114.0	[9]
Gelatin (unplasticized)	65.0	59.0	3.0	19.6 ± 2.4	[172]
Gelatin (100g glycerol/100g gelatin)	65.0	5.0	190.0	0.035 ± 0.004	[172]
Galactomannan (unplasticized)	54.0	18.6 ± 4.4	3.8 ± 0.1	–	[28]
Galactomannan (2g glycerol/100g galactomannan)	54.0	1.7 ± 0.1	38.7 ± 3.8	–	[28]

3.4.4 Water vapour permeability

The obtained water vapour permeability (WVP) for FucoPol films was (1.01 ± 0.23)x10^{-11} mol m^{-1} s^{-1} Pa^{-1}, for a driving force of 76.9–22.5% RH, which is about two orders of magnitude higher than those reported for synthetic films used in industry (LDPE or PET) (Table 3.2) [77]. However, the WVP is similar to other films obtained from biopolymers (GalactoPol [9], corn starch [73] or gelatin [172]).

Due to their hydrophilic behaviour, which makes them poor barriers to water vapour they can be used as an inner layer sandwiched between hydrophobic materials.

Table 3.2: Water vapour permeability of natural and synthetic films.

Film	ΔRH (%)	WVP (x10^{-11} mol m^{-1} s^{-1} Pa^{-1})	Reference
FucoPol	76.9 – 22.5	1.01 ± 0.23	Present Study
GalactoPol	64.8 – 22.0	1.10 ± 0.20	[9]
Galactomannan	100 – 0	0.47 ± 0.03	[28]
Chitosan	75 – 0	0.25 ± 0.03	[172]
Corn Starch	75 – 0	0.98 ± 0.16	[73]
Gelatin	75 – 0	1.50	[73]
LDPE	90 – 0	0.01	[172]
EVOH	90 – 0	0.09	[109]
PET	90 – 0	0.01	[109]

3.4.5 Gas permeability

The measured permeability of FucoPol films to oxygen (0.7x10^{-16} mol m m^{-2}s^{-1}Pa^{-1}) is two orders of magnitude lower than its carbon dioxide permeability (42.7x10^{-16} mol m m^{-2}s^{-1}Pa^{-1}) (Table 3.3). The lower permeability values to oxygen than to carbon dioxide has been reported by other authors to be characteristic of films from hydrophilic polymers (e.g. wheat gluten, starch, carrageenan/pectin and galactomannans) [8, 27, 74, 77].

Table 3.3: Oxygen and carbon dioxide permeability of natural and synthetic films.

Films	**%RH**	**O_2 Permeability** (x10^{-16} mol m m^{-2}s^{-1}Pa^{-1})	**CO_2 Permeability** (x10^{-16} mol m m^{-2}s^{-1}Pa^{-1})	**Selectivity** (α)	**Reference**
FucoPol	32.4	0.7 ± 0.3	42.7 ± 5.6	62	Present Study
GalactoPol	–	–	2.0 ± 0.3	–	[9]
Starch	100	10.9	264.1	24	[77]
Starch	57	0.0062 ± 0.0013	–	–	[74]
Starch:sorbitol (4:1)	57	0.0014 ± 0.0009	–	–	[74]
Carrageenan/pectin	–	0.4	39	98	[8]
Chitosan	50	2.3 ± 0.1	24.3 ± 2.7	11	[57]
Chitosan	0	0.006	0.018	3	[77]
Cassava starch	75	0.0220 ± 0.0001	–	–	[187]
Cassava starch:glycerol (10:1)	75	0.109 ± 0.0002	–	–	[187]
Galactomannans:glycerol (1:1)	50	0.13 ± 0.03	14.7 ± 0.6	113	[27]
Wheat gluten	91	9.8	245.0	25	[77]
Wheat gluten/beeswax	91	6.9	66.1	10	[77]
LDPE	0	10.03	42.2	4	[77]
PET	0	0.12	0.38	3	[77]
EVOH (10:1)	0	0.0003	–	–	[109]
PP	0	3.01	–	–	[109]
Cellophane	0	0.0134	–	–	[77]

FucoPol films show good barrier properties to oxygen when compared with some synthetic polymers usually used as packaging materials, such as LDPE (10.03x10^{-16} mol m m^{-2}s^{-1}Pa^{-1}) or PET (0.119x10^{-16} mol m m^{-2}s^{-1}Pa^{-1}) [77]. However, their oxygen permeability is higher than that of ethylene vinyl alcohol (EVOH), which is considered for ultra-high barrier applications in dry conditions (0.0003x10^{-16} mol m m^{-2}s^{-1}Pa^{-1}) [6].

When comparing to natural polymers, the oxygen permeability of FucoPol films is similar to that of unplasticized carrageenan/pectin films, and to that of cassava starch and galactomannans films with plasticizers incorporation (Table 3.3).

Regarding FucoPol films carbon dioxide permeability, it is similar to the permeability of LDPE and analogous to other natural polymers (carrageenan/pectin, chitosan, wheat gluten blended with beeswax). However, as reported by Gontard et al. [77] for wheat gluten films, it is essential to bear in mind that the oxygen and carbon dioxide permeability of hydrophilic films is dependent on the water content of the polymeric matrix.

For packaging films, one of the most important parameter is the ratio of CO_2/O_2 permeabilities (selectivity, α), which deter-mines the relative proportion of carbon dioxide and oxygen in the package. The selectivity is also dependent on the relative humidity which determines the water content in the polymeric matrix. For FucoPol films, the obtained selectivity (α = 62) was much higher than the values referred for biopolymers, such as chitosan (α = 11 or α = 3), depending on the water content, wheat gluten (α= 25) or starch (α = 24), and for synthetic polymers, such as LDPE (α = 4) or PET (α = 3), but lower than the obtained for galactomannans (α = 113) (Table 3.3). Comparing FucoPol and LDPE films, it can be observed that the former, show higher barrier properties to oxygen maintaining similar values of carbon dioxide permeability. Thus, FucoPol films can be advantageously used in modified atmosphere packaging without oxygen.

3.5 Conclusions

A bacterial exopolysaccharide, FucoPol, was used to prepare biodegradable films by casting of a filmogenic solution composed of polymer and citric acid.

The films are transparent, but have a brown shade that caused colour changes noticeable to the human eye when placed over a coloured surface. FucoPol films are hydrophilic and soluble in water, which makes them poor barriers to water vapour. By the contrary, FucoPol films present good barrier properties to gases (oxygen and carbon dioxide). The mechanical tests revealed a ductile film, with high elongation at break and a low tension at break and elastic modulus.

Characteristics of FucoPol films indicate its potential application on food packaging, though, the properties can be improved, namely by testing alternative plasticizers, crosslinking agents and blends with other biopolymers. Taking into account the observed ductile mechanical properties, hydrophilic character and good barrier properties to gases for low film's water content, the most promising application of these films may be as an inner layer in a multi-layered film.

Chapter 4

Development and characterization of bilayer films of FucoPol and chitosan

4.1 Summary

Bilayer films of FucoPol and chitosan were prepared and characterized in terms of optical, morphologic, hygroscopic, mechanical and barrier properties, to evaluate their potential application in food packaging. Bilayer films have shown dense and homogeneous layers, and presented enhanced properties when comparing to monolayer FucoPol films. Though, a high swelling degree in contact with liquid water (263.3%) and a high water vapour permeability ($0.75\text{x}10^{-11}\ \text{mol}\,\text{m}^{-1}\text{s}^{-1}\text{Pa}^{-1}$), typical of polysaccharide films, was still observed. However, they presented a low permeability to O_2 and CO_2 ($0.47\text{x}10^{-16}$ $\text{mol}\,\text{m}\,\text{m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$ and $5.8\text{x}10^{-16}\ \text{mol}\,\text{m}\,\text{m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$, respectively). Tensile tests revealed a flexible and resistant film with an elongation at break of 38% and an elastic modulus of 137 MPa. The studied properties, in particular the excellent barrier to gases, impart these bilayer films potential to be used in packaging of low moisture content products, as well as in multilayered hydrophobic/hydrophilic/hydrophobic barriers for food products with a broader range of water content.

4.2 Introduction

Primary packaging, defined as “a sales unit to the final user or consumer at the point of purchase” [145] tends to be the most visible aspect of packaging [15]. The use of synthetic non-biodegradable polymers for primary packaging was tremendous in the last century, mainly because they are low-cost and present good mechanical and thermal properties, and are good barriers to gases, aroma compounds and microorganisms.

Though primary packaging is mandatory for food preservation and protection, the intensive use of synthetic plastics created serious environmental problems because they are non-biodegradable and non-renewable materials.

This severe problem can be minimized using biodegradable natural and bio-based polymers instead of the non-biodegradable synthetic ones [134, 185].

As such, the attention has been driven to the search of alternative materials for use in food packaging. Bio-based films are usually designed from biodegradable, non-toxic and edible polymers (e.g. polysaccharides and proteins) and lipids [70]. The use of blends and multi-layers of those materials are strategies for new composite materials development, with properties that turn them potential synthetic polymers substitutes [19, 46].

Polysaccharides obtained from plant, algae, animal and microbial origin (e.g. starch, alginate, chitosan, gellan gum) have been widely used for edible and/or biodegradable films development [19, 102, 186]. Such films are usually poor barriers to water vapour but good barriers to gases.

FucoPol, one of the microbial polymers referred in the literature, is a fucose-rich exopolysaccharide produced by the bacterium *Enterobacter* A47 [7, 199]. It is a high molecular weight heteropolysaccharide composed of neutral sugars (fucose, galactose, glucose), an acidic sugar (glucuronic acid) and acyl groups (acetate, succinate and pyruvate). Glucuronic acid, together with succinate and pyruvate, are responsible for the anionic character of the biopolymer [67]. FucoPol has film forming capacity and its films have been reported to be transparent, with brown tone, hydrophilic with high permeability to water vapour and good barrier properties to gases (CO_2 and O_2) [60].

Chitosan is derived from chitin, which is the most abundant natural amino polysaccharide and the second most abundant biopolymer in nature. Crustacean shells, a food industry waste, are one of the chitin main sources [168]. Chitosan is a copolymer of β-(1-4)-2-cetamido-D-glucose and β-(1-4)-2-amino-D-glucose units, with the latter usually exceeding 60%. It possesses a cationic character, antimicrobial properties and film forming capacity [52]. Chitosan films have a selective permeability to gases (CO_2 and O_2) and good mechanical properties, but are highly permeable to water vapour that limits their use in food packaging applications.

The improved properties obtained by the combination of different hydrocolloids have been reported for several systems. Blends and bilayer films of chitosan and anionic polymers have been reported to have improved mechanical and barrier transport properties comparing to single component based films. This fact was attributed to the formation of polyelectrolyte complexes through electrostatic interactions between the protonated amino groups of chitosan and the negatively charged side-chain groups in the other biopolymer at the operating pH [52, 114, 138].

Improvement in mechanical properties, better performance in terms of water vapour permeability and lower water solubility have been reported for blends and bilayer films of chitosan with starch, pectin or alginate [96, 114, 215], gelatin [171] or whey [108], comparing to chitosan stand-alone films.

Some authors reported difficulties in total solubilization of one of the polymers in specific conditions and formation of insoluble complexes between polymers in blends preparation [63]. Otherwise, bilayer systems are reported to have better water vapour barrier properties than blend films [108, 171]. In this context, the aim of the present study is to develop bilayer films in combination with chitosan in order to enhance the properties of FucoPol films. The films were characterized in terms of their optical, hygroscopic, surface, mechanical and barrier properties, envisaging their potential use in food-packaging applications.

4.3 Materials and Methods

4.3.1 Materials

FucoPol was produced and purified as described in Chapter 3, section 3.3.1. The freeze dried FucoPol was analysed in terms of chemical composition and average molecular weight. Commercial medium molecular weight chitosan (deacetylation degree 75 – 85 %) was purchased from Sigma (USA). Citric acid monohydrate was purchased from VWR chemicals – BDH Prolabo (UK). Glycerol (analytical grade) was used as plasticizer and purchased from Sigma (USA).

4.3.2 FucoPol chemical composition

FucoPol dried samples (5 mg) were hydrolyzed (2 h at 120 °C) with trifluoroacetic acid (TFA) (0.1 mL TFA 99%), and the hydrolysate was used for the identification and quantification of the constituent sugar monomers by ion chromatography (HPIC), using a CarboPac PA10 column (Dionex), equipped with an amperometric detector. The separation was performed at 30 °C with a gradient of NaOH (0.018 – 0.025 mol L^{-1}) and CH_3COONa (0 – 0.17 mol L^{-1}). Fucose, glucose, galactose and glucuronic acid (Sigma, USA) were used as standards at concentrations between 0.005 and 0.1 g L^{-1}. The acid hydrolysates were also used for the identification and quantification of acyl groups by HPLC with an Aminex HPX-87H column (BioRad) coupled to a UV detector. The analysis was performed using sulphuric acid (0.005 mol L^{-1} H_2SO_4) as eluent, at a flow rate of 0.6 mL min^{-1} and a temperature of 30 °C. Acetate, pyruvate and succinate (Sigma, USA) were used as standards at concentrations between 0.015 and 1.0 g L^{-1}.

4.3.3 FucoPol average molecular weight

The EPS average molecular weight (Mw) were determined by size exclusion chromatography–multi-angle laser light scattering (SEC-MALLS - Wyatt Technology Corporation Dawn Model). The FucoPol solutions (2 g L^{-1}) were prepared in 0.1 M Tris–HCl, NaCl (0.2 mol L^{-1}), pH 8.1 buffer, which was also the SEC mobile phase. The SEC columns (PL aquagel-OH mixed 8 µm, 300×7.5 mm) were equilibrated for 24 h before running the analysis at a flow rate of 0.7 mL min^{-1} at room temperature. Each analysis was conducted in duplicate. Signals from MALLS were recorded in parallel and treated with Astra (V 4.73.04) in order to follow the purity and molecular mass distribution of the polysaccharide. A dn/dc of 0.190 mL g^{-1} was adopted to calculate the Mw.

4.3.4 Films preparation

FucoPol filmogenic solutions were prepared by dissolving freeze dried FucoPol in distilled water (1.5% w/w) under stirring, at room temperature, until complete dissolution. Then, citric acid (50% $w_{\text{citric acid}}/w_{\text{dried polymer}}$) was added and the solution was left under stirring for complete homogenization.

Chitosan films were prepared by dissolving chitosan in an acetic acid (1% w/w) solution, at a concentration of 1.5% (w/w). After stirring overnight at ambient temperature, glycerol (50% $w_{\text{glycerol}}/w_{\text{dried polymer}}$) and citric acid (50% $w_{\text{citric acid}}/w_{\text{dried polymer}}$) were added, followed by stirring for complete homogenization.

Air bubbles were removed under vacuum, and the solution was transferred to Teflon petri dishes and left to dry at 30 °C, during 24 h.

Bilayer films were prepared by a two-step coating technique. Firstly, the FucoPol solution, prepared as described above, was cast onto a Teflon petri dish and then dried at 30 °C until a firm but still adhesive surface was obtained. Then, the chitosan solution, prepared as described above, was cast on the top of FucoPol film and both layers were dried at 30 °C during 24 h.

4.3.5 Morphological characterization

The morphology of the bilayer films was evaluated by scanning electron microscopy (SEM) using a FEG-SEM JEOL JSM7001F (Oxford) equipment, with the acquisition system JEOL software PC-SEM. Samples were coated with chromium sputtering (Quorum Technologies, Q150TES) to enable the observation of surface and cross section. For cross-section observation, the films were cryofractured by immersion into liquid nitrogen.

4.3.6 Colour and transparency

Colour alteration on objects caused by application of the prepared films and their transparency were determined as described in chapter 3, section 3.3.3.

4.3.7 Contact angle measurements

The hydrophilic character of the films was evaluated from the water contact angles of their upper surface. The water contact angles were measured at room temperature (23 °C) using a goniometer (KSV Instruments LTD, CAM 100, Finland) with the software KSV CAM 100. Samples with dimensions of 10 mm x 50 mm were used and the contact angle value was given by the average on both sides of the drop. Three film replicates were analyzed.

4.3.8 Water sorption isotherms

Water sorption isotherms were determined by a gravimetric method at 30 °C. Samples with dimensions of 20x20 mm were previously dried at 70 °C during 24 h. The samples were then placed in desiccators with different saturated salt solutions: LiCl, CH_3COOK, $MgCl_2\,6H_2O$, K_2CO_3, $Mg(NO_3)_2$, NaCl, $BaCl_2$ and K_2SO_4, with a water activity of 0.115, 0.225, 0.324, 0.447, 0.520, 0.769, 0.920 and 0.977, respectively. Three film replicates were equilibrated at each salt solution. The samples were weighed after three weeks, ensuring that the equilibrium has been reached. The Guggenheim–Anderson–de-Boer (GAB) model (Eq. 4.1) was used to fit the experimental sorption data.

$$X = \frac{C\,k\,X_0\,a_W}{(1-k\,a_W)(1-k\,a_W+C\,k\,a_W)} \tag{4.1}$$

where X is the equilibrium moisture content ($g_{\text{water}}/g_{\text{dry solids}}$) at the water activity (a_W), X_0 is the monolayer moisture content, C is the Guggenheim constant and represents the energy difference between the water molecules attached to primary sorption sites and those absorbed to successive sorption layers, and k is the corrective constant owing to properties of multilayer molecules with respect to the bulk liquid. GAB equation parameters were calculated by non-linear fitting using the software OriginLab® Origin 9.

4.3.9 Solubility and swelling degree

For measuring the solubility and swelling degree, samples (20 x 20 mm) were placed 24 h at 70 °C to obtain a dried sample mass (m_1, g). Then the films were placed in 20 mL of deionized water during 24 h under orbital stirring at room temperature. Afterwards, they were weighted (m_2, g), dried again during 24 h at 70 °C and weighted (m_3, g). The solubility (S, %) and swelling degree (Sw, %) were calculated by the following equations:

$$S = \frac{m_3 - m_1}{m_1} \text{ x } 100 \tag{4.2}$$

$$S_W = \frac{m_2 - m_3}{m_1} \text{ x } 100 \tag{4.3}$$

4.3.10 Mechanical properties

Tensile tests were performed in order to determine tensile stress at break (TS, MPa), elongation at break (EB, %) and Elastic Modulus (EM, MPa), as described in chapter 3, section 3.3.5.

The samples were equilibrated previously at 50.3% RH and 23 °C. Five film replicas were analyzed.

4.3.11 Water vapour permeability

The water vapour permeability (WVP, $\mathrm{mol\,m^{-1}s^{-1}Pa^{-1}}$) was measured gravimetrically, and determined as described in chapter 3, section 3.3.6.

4.3.12 Gas permeability

The tests were carried out using a stainless steel cell with two identical chambers separated by the film. The films were equilibrated at room temperature (24 °C) and relative humidity (50%) before being tested. The permeability was evaluated by pressurizing one of the chambers (feed) up to 0.7 bar, with pure gas, either carbon dioxide (99.998%) or oxygen (99.999%) (Praxair, Spain), followed by the measurement of the pressure change in both chambers over time, using two pressure transducers (JUMO, Model 404327, Germany). The temperature was maintained constant at 30 °C, using a thermostatic bath (Julabo, Model EH, Germany). The permeability was calculated by Eq. 4.4, using five independent measurements:

$$\frac{1}{\beta}\left(\frac{\Delta p_0}{\Delta p}\right) = P\frac{t}{\delta} \tag{4.4}$$

Where Δp (mbar) is the pressure difference between feed and permeate compartment, P ($\mathrm{mol\,m\,m^{-2}\,s^{-1}\,Pa^{-1}}$) is the gas permeability, t (s) is the time, δ (m) is the film thickness and β is the geometric parameter of cell, as described by Alves et al. [6].

4.3.13 Statistical analysis

In this work, the obtained data values were statistically analysed by one-way analysis of variance (ANOVA) using OriginLab® Origin 9. Differences between pairs of means were assessed on the basis of confidence intervals using the Tukey test. The least significance difference was P > 0.05.

4.4 Results and discussion

4.4.1 FucoPol characterization

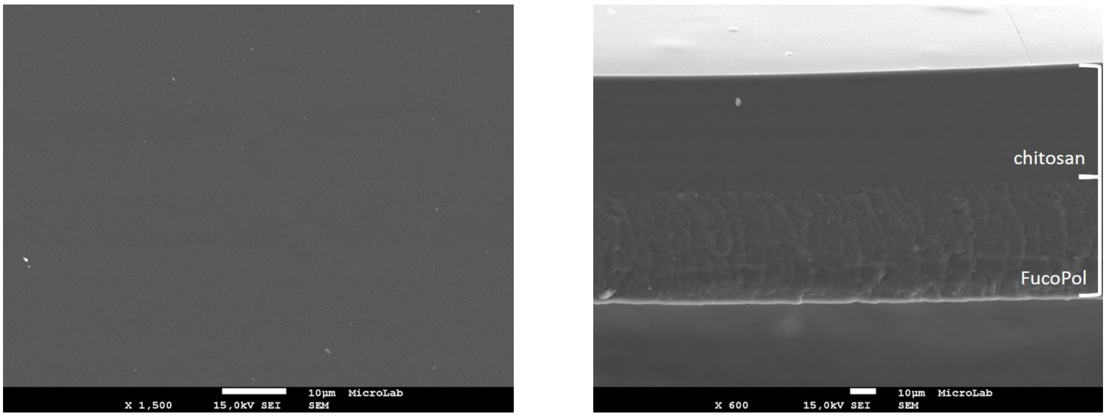
The chemical composition analysis revealed FucoPol was composed of fucose (33 mol%), glucose (25 mol%), galactose (25 mol%) and glucuronic acid (15 mol%) and a total acyl groups content of 18 wt%. The average molecular weight was $4.7\mathrm{x}10^6\ \mathrm{g\,mol^{-1}}$.

4.4.2 Films appearance, colour alteration and transparency

FucoPol, chitosan and bilayer films were uniform and homogeneous by naked eye observation. The bilayer films presented a brownish tone, as well as FucoPol stand-alone films.

The transparency of bilayer films measured at 600 nm was 5.6 ± 0.3, while that of chitosan and FucoPol stand-alone films was 1.9 ± 0.1 and 9.4 ± 0.2, respectively. These results suggest that the transparency value of the bilayer film is the average of the transparency values of the two films and is in the same order of magnitude of that of low density polyethylene (LDPE) which presents a transparency value of 3.05 [171].

Regarding morphology, SEM analysis revealed that FucoPol, chitosan and bilayer films were constituted by a dense structure (Figure 4.1). The chitosan (upper layer) was homogeneous without any pores (Figure 4.1a). The cross-section image (Figure 4.1b) shows two distinct dense layers: the upper chitosan layer was compact and smooth, while the lower FucoPol layer presented a more irregular structure. This characteristic may be attributed to some polymer aggregates of FucoPol that are not completely dissolved, in fact, its filmogenic solution was not fully transparent.



a

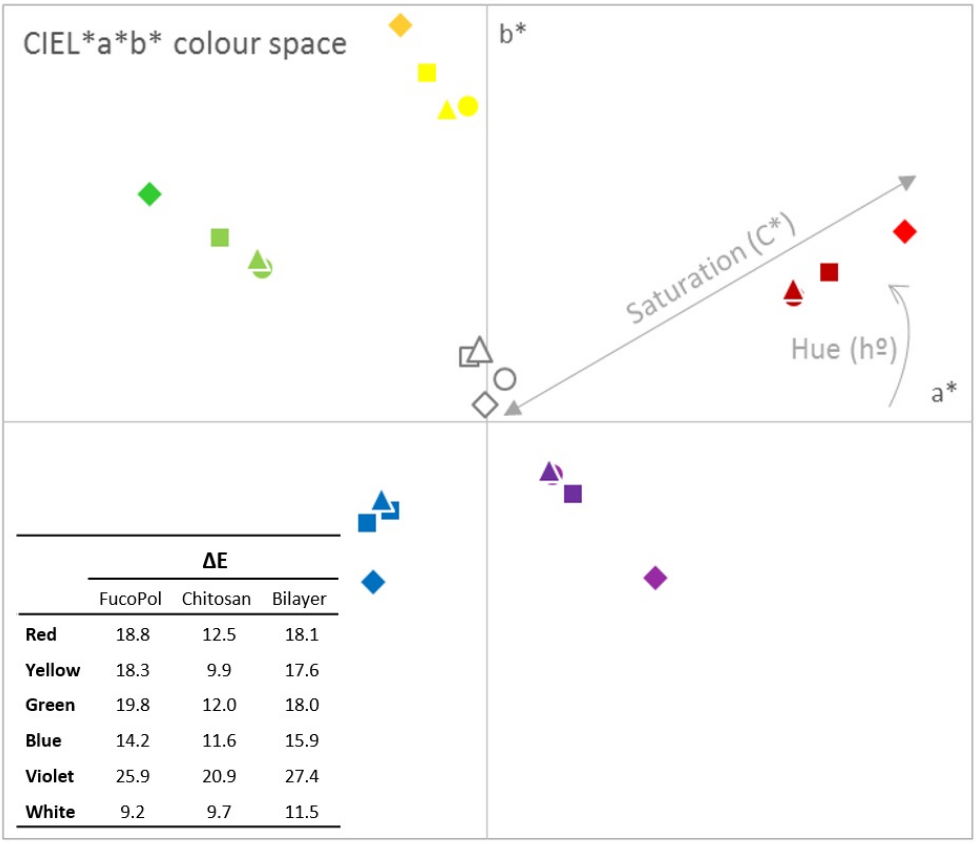
b

Figure 4.1: SEM images of bilayer films (a) Upper surface - chitosan (magnification 1500x) (b) Cross-section (magnification 600x)

Figure 4.2 presents the colour parameters of coloured paper surfaces, uncovered and covered by the films samples. It is possible to observe that the hue (h_{ab}, angle towards the horizontal axes) did not change substantially with films application.

In addition, for all covered coloured surfaces, there was an approximation towards the origin, which corresponds to a decrease in colour saturation (chroma, C_{ab}). The C_{ab} values observed for the bilayer films were similar to that observed for FucoPol films.

From the values of ΔE_{ab} (Insert in Figure 4.2), it may be foreseen that the total colours alteration originated by all films can be perceived by the human eye. The largest ΔE_{ab} was found for the most different colours (violet, green, red) of the original film colour. Overall, the higher differences in colour were induced by FucoPol films, which influenced the behavior of the bilayer films.



	ΔE		
	FucoPol	Chitosan	Bilayer
Red	18.8	12.5	18.1
Yellow	18.3	9.9	17.6
Green	19.8	12.0	18.0
Blue	14.2	11.6	15.9
Violet	25.9	20.9	27.4
White	9.2	9.7	11.5

Figure 4.2: Parameters a* and b* of the CIELAB system for different coloured surfaces uncovered (diamonds - ◊) and covered by FucoPol films (circles - ∘), chitosan films (squares - □) and FucoPol/chitosan bilayer films (triangles - △) and total colour alteration (ΔE) values (insert).

4.4.3 Contact angle measurements

The water contact angle was measured in the upper surface of the films, which was not affected by the flat Teflon petri dish where the films were dried. The obtained values were the average of the first 5s after the water drop deposition. A contact angle of $58.1 \pm 5.0^{\circ}$ was obtained for chitosan stand-alone films revealing its hydrophilic character.

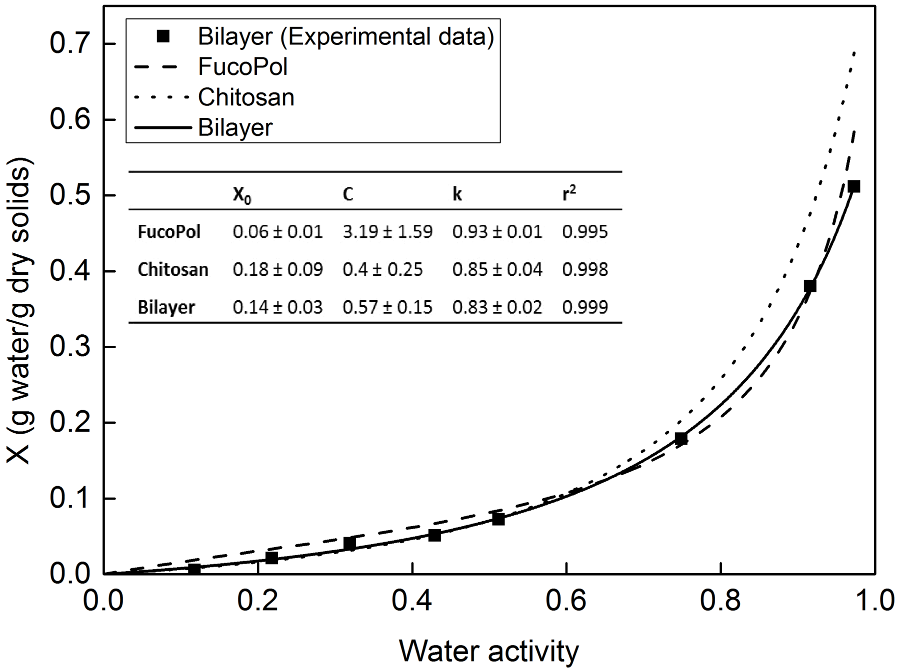
The bilayer films presented a higher contact angle ($70.6 \pm 1.6^{\circ}$), although the upper side layer was composed by chitosan, there was a significant difference in the contact angle values comparing with chitosan stand-alone films.

Similar results were obtained by Kurek et al. for bilayer films of chitosan and whey protein with a contact angle increase from 71º to 94º [108]. This change in the contact angle was attributed to immediate swelling of chitosan layer and surface hygroscopicity. In the present work, a glycerol transference by diffusion from the chitosan filmogenic solution to the lower FucoPol layer upon bilayer formation may also have occurred, resulting on a less hydrophilic chitosan layer surface that increased contact angle.

In addition, the single chitosan film was cast on a completely flat and rigid surface (Teflon surface), while in the bilayer films the chitosan layer was cast on a previously less flat and more flexible FucoPol layer. The contact angles difference could be also a result of small differences on the surface roughness between the two types of films, inherent to the different film formation methods.

4.4.4 Water sorption isotherms

The water sorption isotherms for the bilayer FucoPol/chitosan, FucoPol and chitosan stand-alone films, and respective GAB equation parameters are presented in Figure 4.3.



	X_0	C	k	r^2
FucoPol	0.06 ± 0.01	3.19 ± 1.59	0.93 ± 0.01	0.995
Chitosan	0.18 ± 0.09	0.4 ± 0.25	0.85 ± 0.04	0.998
Bilayer	0.14 ± 0.03	0.57 ± 0.15	0.83 ± 0.02	0.999

Figure 4.3: Water sorption isotherms of FucoPol, chitosan and bilayer films at 30 °C fitted by GAB model (lines), experimental data (symbols) and the obtained GAB parameters (insert).

The obtained moisture content for all films increased slowly for water activity values below 0.75, but above that, a great increase in moisture content with increasing water activity was observed. A similar behavior was referred for other polysaccharide films (e.g. gellan gum, GalactoPol, pectin and starch) [3, 60, 115].

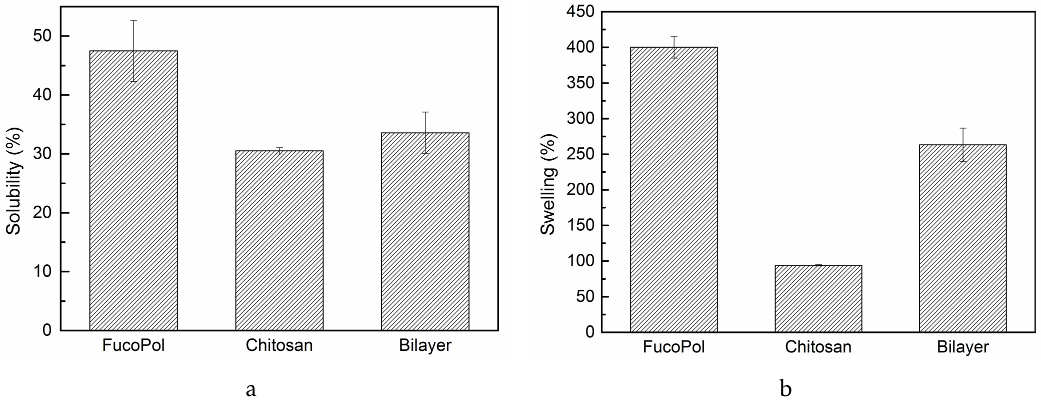
It may be emphasized that chitosan films demonstrated a higher water vapour adsorption capacity than FucoPol and bilayer films, for water activity values above 0.7, which might be related to the concentration of glycerol present. Higher concentration of plasticizer increases the films moisture affinity, due to the hydrophilicity of the plasticizers, presenting hydroxyl groups able to interact with water by hydrogen bonds. This behavior in plasticized films with glycerol has been reported for starch films [115], feather keratin films [173] and alginate films [140] among others.

The experimental data for all films is well described by the GAB model (r^2 = 0.995 – 0.999). The monolayer value (X_0) indicates the maximum amount of water that can be absorbed in a single layer per gram of dry film. X_0 obtained for FucoPol films (0.06 g_{water}/g_{solids}) is in agreement with the values referred in Chapter 3, section 3.4.2 for FucoPol films with citric acid (0.07 g_{water}/g_{solids}), Abdillahi et al. [1] for wheat flour/glycerol/PLA blends with low content in citric acid (0.06 g_{water}/g_{solids}) and Mali et al. [115] for cassava starch films with glycerol (0.05 g_{water}/g_{solids}). Higher X_0 values were obtained for chitosan and the bilayer films (0.18 and 0.14 g_{water}/g_{solids}, respectively), which suggests that the bilayer films X_0 value was controlled by the chitosan layer.

The C parameter is related to the difference of magnitude between the upper water molecules layers and the monolayer. This parameter decreases with increasing plasticizer concentration, which is in agreement with the presented results: the lowest value (0.4) was obtained for chitosan films, which has the highest plasticizer concentration. The *k* parameter that determines the profile at the highest activity range, regulating the upswing after the plateau [197], shows no significant differences among the studied films. The same behavior was reported by Mali et al. [115] and Coupland et al. [37] for cassava starch and whey protein films with different plasticizer content.

4.4.5 Solubility and swelling degree

The results presented in Figure 4.4a reveal that the solubility of FucoPol films may be decreased with the application of a chitosan layer. The bilayer films, with a solubility in water of 33.6 ± 3.6%, were significantly less soluble than FucoPol films (solubility in water of 47.5 ± 5.2%), but not significantly different from chitosan films (solubility in water of 30.5 ± 0.5%). This fact may result from electrostatic interactions between both polymers due to different net charges, beyond hydrogen bonds, as has been reported for bilayers of gelatin and chitosan [150].



a b

Figure 4.4: Solubility (a) and swelling degree (b) of studied films.

Regarding the swelling degree (Figure 4.4b), FucoPol films presented the highest value (400.2 ± 15.1%). High values of swelling (>150%) have been reported for pork gelatin, oxidized potato starch [105] and pectin [146], among others. Moreover, the swelling degree is influenced by the amount of plasticizer used (e.g. glycerol). It is usually higher when high amounts of plasticizer are used, because it increases water uptake. Chitosan films presented the lowest swelling degree (94.0 ± 0.9%), which may be attributed to crosslinking reactions with citric acid. Cui et al. [43] reported the formation of amide bond between citric acid and chitosan amine group, which might be responsible for increased water resistance. As it was observed for solubility in water, the swelling of FucoPol films could be decreased by the combination with the chitosan layer, having in account the bilayer structure, its swelling degree is nearly the average of that of stand-alone films of chitosan and FucoPol, once the bilayers are constituted by half of each polymer. The bilayer films presented a swelling degree of 263.3 ± 23.3%, which is significantly lower than that of FucoPol films which may be attributed to the lower water absorption capacity of chitosan. Still, from the results presented for all films tested, it is foreseen their application in environments with low water content, due to their high affinity to water that may compromise their physical integrity.

4.4.6 Mechanical properties

The film samples were conditioned at 50.3% relative humidity and subjected to tensile tests. The mechanical parameters obtained for FucoPol/Chitosan bilayer films present intermediate values of both polymer layers. It is possible to notice a significant difference of elongation at break (38.4 ± 11.3%) and elastic modulus (137.0 ± 36.8 MPa) when comparing with FucoPol stand-alone films (Table 4.1). The bilayer films were stretched until break and no separation from each layer was noticed, even upon rupture. Bilayer films worked always as a whole system showing good adhesion.

Table 4.1: Mechanical properties of the films tested in this study along with others referred in the literature.

Films	%RH	Tensile strength at break (MPa)	Elongation at break (%)	Elastic modulus (MPa)	Reference
FucoPol	50.3	7.6 ± 2.2	6.6 ± 2.6	237.5 ± 43.7	Present Study
Chitosan	50.3	18.5 ± 7.3	86.7 ± 9.9	20.8 ± 5.2	Present Study
Bilayer	50.3	11.9 ± 6.2	38.4 ± 11.3	137.0 ± 36.8	Present Study
FucoPol (1:1 citric acid)	44.3	3.1 ± 0.3	54.9 ± 4.0	2.8 ± 0.2	[60]
Chitosan	–	22.2 ± 3.9	73.6 ± 8.2	–	[4]
Chitosan	50±2	66	51	19	[63]

FucoPol films with a water content of 11% (on a dry basis) have shown the more stiff characteristics: lower tensile strength at break (TS) (7.6 ± 2.2 MPa), low elongation at break (EB) (6.6 ± 2.6%) and higher elastic modulus (EM) (237.5 ± 43.7 MPa). Comparing to FucoPol films in Chapter 3, it is possible to see the effect of the decrease amount of citric acid in the films composition. The proportion 1:2 in citric acid:FucoPol used is not sufficient to have the plasticized effect described previously for films 1:1 (citric acid:FucoPol). On the other hand, chitosan films, with a water content of 7% (on dry basis), have shown a higher TS (18.5 ± 7.3 MPa), higher EB (86.7 ± 9.9%) and lower EM (20.8 ± 5.2 MPa). The results obtained for chitosan are difficult to compare with the literature, because of the large range of variables involved (different compositions, degree of acetylation, molecular weight, film preparation methods and conditioning conditions). Despite that, similar values of TS and EB were reported by Abugoch et al. [4] and a similar value of EM was reported by Ferreira et al. [63] for films with 1:10 w/w (glycerol/chitosan) (Table 4.1). The plasticizing effect of glycerol is given by the presence of the additive itself, and also by its hygroscopic behavior. The films containing glycerol are able to adsorb more water vapour, increasing the plasticizing degree. In this work, beyond citric acid, which has been described to produce brittle films when used as additive, chitosan films have 50% of glycerol (dry basis) that enabled to obtain flexible chitosan matrices [144].

The mechanical parameters of bilayer films of FucoPol and chitosan present intermediate values of both polymer layers. It is possible to notice significant differences of elongation at break (38.4 ± 11.3%) and elastic modulus (137.0 ± 36.8 MPa) when comparing with FucoPol films.

4.4.7 Water vapour permeability

Water vapour permeability (WVP) is one of the most important properties of bio-based polymer films for food packaging applications since it has direct influence on the shelf life of food products. Polysaccharide films present a high WVP due to their hydrophilic nature.

Table 4.2: Water vapour permeability of natural films.

Film	ΔRH (%)	WVP (x10^{-11} mol m^{-1}s^{-1}Pa^{-1})	Reference
FucoPol	76.9 – 22.5	0.75 ± 0.05	Present Study
Chitosan	76.9 – 22.5	4.13 ± 0.13	Present Study
Bilayer	76.9 – 22.5	1.65 ± 0.40	Present Study
FucoPol (1:1 citric acid)	76.9 – 22.5	1.01 ± 0.23	[60]
Chitosan	65 – 0	1.06 ± 0.05	[150]
Chitosan	75 – 30	1.32 ± 0.21	[108]

FucoPol films exhibited higher water vapour barrier properties, (0.75 ± 0.05)x10^{-11}-mol m^{-1}s^{-1}Pa^{-1}, than chitosan ones (4.13 ± 0.13)x10^{-11} mol m^{-1}s^{-1}Pa^{-1} for a driving force of 76.9–22.5% RH (Table 4.2). A higher WVP value was reported for FucoPol films with higher citric acid content [60]. Regarding the WVP of chitosan, it is difficult to compare the results obtained with the reported by other authors, since the WVP is highly dependent on the driving force used, amount of plasticizer or other additives, molecular weight, among others variables. Kurek et al. [108] and Pereda et al. [150] reported lower WVP values for chitosan films with 30% (w/w) and 28% (w/w) of glycerol contents, respectively (Table 4.2).

The water vapour permeability, for a driving force of 76.9–22.5% RH, obtained for the bilayer films was $(1.65 \pm 0.23) \text{x} 10^{-11}$ $\text{mol}\,\text{m}^{-1}\text{s}^{-1}\text{Pa}^{-1}$, which is an intermediate value significantly different from that of both FucoPol and chitosan films.

It is possible to calculate the permeability of multi-layer films using the permeability and thickness of each individual layer [36]. So, in this work, the permeability of the bilayer films was estimated by:

$$\frac{l_{BL}}{P_{BL}} = \frac{l_F}{P_F} + \frac{l_{Ch}}{P_{Ch}} \tag{4.5}$$

Where l is the thickness and P is the permeability of bilayers (BL), FucoPol (F) and chitosan (Ch) respectively. The permeability obtained using Eq. 4.5, was $1.41 \text{x} 10^{-11}$ mol-$\text{m}^{-1}\text{s}^{-1}\text{Pa}^{-1}$ which is not significantly different from the experimental value. This result shows that is possible to design and predict the permeability characteristics of bilayer films using the permeability values of each layer if the process of building the bilayer system causes no significant interferences at the layers interface.

4.4.8 Gas permeability

Gas barrier properties of polymeric films are essential to study and design materials for food packaging applications. Polysaccharides usually provide good barriers to gases (e.g. oxygen and carbon dioxide) due to their highly packed hydrogen-bonded dense polymer matrix, at low relative humidity (RH), but their permeability to gases increases significantly with increasing ambient moisture, due to the plasticizing effect of water. In this work, the permeability was measured for films previously conditioned at a RH of 55% and the results are presented in Table 4.3.

The oxygen permeability of the FucoPol, chitosan and the bilayer films are one order of magnitude lower than their carbon dioxide permeability values. This behavior is common for hydrophilic polymer films, such as chitosan, pullulan or starch, and has been reported by other authors [57, 77].

Bilayer films presented a significantly higher barrier to oxygen than the control films, with an O_2 permeability of $0.47 \text{x} 10^{-16}$ $\text{mol}\,\text{m}\,\text{m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$. Regarding the barrier behavior to carbon dioxide, bilayer films presented a permeability of $5.8 \text{x} 10^{-16}$ $\text{mol}\,\text{m}\,\text{m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$, which is significantly lower than that of chitosan film. However, the value was not significantly different than that of the FucoPol film.

The permeability to gases of polymeric films is affected by many factors, such as relative humidity, polymer morphology and polymer matrix plasticization. FucoPol has

Table 4.3: Oxygen and carbon dioxide permeability of selected natural and synthetic polymer films.

Films	**%RH**	**O_2 Permeability** ($\times10^{-16}$ **mol m m^{-2}s^{-1}Pa^{-1}**)	**CO_2 Permeability** ($\times10^{-16}$ **mol m m^{-2}s^{-1}Pa^{-1}**)	**Selectivity** (α)	**Reference**
FucoPol	55 ± 5	1.93 ± 0.70	6.53 ± 0.79	2.7	Present Study
Chitosan	55 ± 5	2.35 ± 0.48	15.03 ± 4.25	6.4	Present Study
Bilayer	55 ± 5	0.47 ± 0.19	5.80 ± 0.70	12.6	Present Study
FucoPol (1:1 citric acid)	32.4	0.7 ± 0.3	42.7 ± 5.6	62	[60]
Chitosan	50	2.3 ± 0.1	24.3 ± 2.7	11	[57]
Pullulan	30	0.17	0.72	4.2	[77]
Galactomannan (1:1 glycerol)	50	0.13 ± 0.03	14.7 ± 0.6	113	[27]
HDPE	55 - 75	2.2	35.5	16.4	[206]
LDPE	50 - 75	8.4	26.6	3.2	[206]
EVOH 29	90	0.24	0.165	0.68	[206]

been reported to present significant differences in O_2 and CO_2 permeability, for films prepared with 1:1 citric acid in Chapter 3, when compared to those of this Chapter. This may be attributed to the different plasticization of the polymer matrix and relative humidity at which the films were conditioned (RH=32%). Regarding chitosan films, no significant differences in permeability were noticed with the reported by Fajardo et al. [57] for chitosan films with 50% HR.

Studies with bilayer films with natural and synthetic polymers revealed great improvements in their barrier properties. Ortega-Toro et al. reported higher barrier to oxygen transport of starch and polycaprolactone (PCL) bilayers, when compared to isolated polymer layers [142]. And Schmid et al. reported that whey protein (WPI) coated polyethylene terephthalate (PET) had a significant decrease in O_2 permeability comparing to PET or WPI films [178].

Comparing the FucoPol/chitosan bilayer with synthetic polymers used in packaging (HDPE and LDPE), it is perceived significantly higher barrier properties, both to oxygen and to carbon dioxide. However, the barrier of this bilayer is still lower than that of EVOH films, one of the better hydrophilic gas barriers used in packaging, with a permeability to O_2 of 0.24×10^{-16} mol m m^{-2}s^{-1}Pa^{-1} [132].

For packaging applications, the ratio of CO_2/O_2 permeability values (selectivity - α), is also important since it determines the proportion of those gases inside the package. The selectivity values obtained for the bilayer films (12.6) is similar to those reported for high density polyethylene (HDPE) (Table 4.3).

4.5 Conclusions

Bilayer films of FucoPol and chitosan are homogeneous, flexible and resistant. They are transparent, causing colour alterations on coloured surfaces detectable by human eye. The films presented a high swelling degree in contact with liquid water and a high water vapour permeability, which disables their used in applications that involve the direct contact with high moisture content products. However, they have shown excellent barrier properties to O_2 and CO_2, better than those referred for HDPE. Furthermore, the bilayer films revealed to be significantly less permeable to O_2 than single FucoPol films. Such properties, together with their production from renewable resources, biodegradability and nontoxicity, make these bilayer films good candidates to be used for packaging of low moisture content products. In addition, they also present a good potential as alternatives to some of the synthetic plastic materials from non-renewable resources currently used in multilayered barriers (e.g. ethylene vinyl alcohol).

CHAPTER 5

COMPARISON OF DIFFERENT COATING TECHNIQUES ON THE PROPERTIES OF FUCOPOL FILMS

5.1 Summary

Plasma deposition, liquid flame spray (LFS) and atomic layer deposition (ALD) were used to form inorganic coatings in new exopolysaccharide (FucoPol) biodegradable films. Coated films were characterized in terms of surface, optical and barrier properties in order to evaluate their potential use in food packaging.

FucoPol films presented dense and homogeneous surface with instant water contact angle of 95°. Plasma deposition of perfluorohexane (PFH) on FucoPol surface has not shown significant improvement in the hydrophobic behavior over the time. The FucoPol coating of SiO_2 nanoparticles deposited by LFS and plasma deposition of PFH have shown higher instant water contact angle (135°) caused by coating surface roughness, but this hydrophobic behavior was not stable over time. FucoPol films coated only with TiO_2 deposited by ALD and combination of that with plasma deposition of PFH have shown stable water contact angle during time (90° and 115°, respectively), transparency in the same order of magnitude and significantly lower permeability to water vapour ($3.45\text{x}10^{-11}\,\mathrm{mol\,m^{-1}s^{-1}Pa^{-1}}$ and $3.45\text{x}10^{-11}\,\mathrm{mol\,m^{-1}s^{-1}Pa^{-1}}$ when compared to uncoated films with $5.32\text{x}10^{-11}\,\mathrm{mol\,m^{-1}s^{-1}Pa^{-1}}$). Moreover, films coated with TiO_2-PFH have also shown a permeability to oxygen of $1.70\text{x}10^{-16}\,\mathrm{mol\,m\,m^{-2}s^{-1}Pa^{-1}}$ which is 67 % lower than uncoated films.

5.2 Introduction

Food packaging is essential in the food industry to deliver quality products to the consumer, by reducing food products damage and spoilage, and decreasing or eliminating the risk of adulteration. Synthetic petrochemical-based packaging materials are a common choice due to a combination of properties such as flexibility, strength, lightness, stability, impermeability, heat sealability, low price and ease of sterilization. However, due to their non-degradability, synthetic plastic packaging became the largest contributor for plastic waste [61, 194].

A good potential alternative to overcome this environmental problem relies on the use of bio-based materials from renewable resources, in particular the use of biopolymers, which include polysaccharides.

Polysaccharides are the most abundant macromolecules in the biosphere and can be obtained from plants, algae, animals or produced by microorganisms. Polysaccharides have been used to produce biodegradable films and thin membranes, which have been applied in several areas, including in the food industry [61, 69, 195].

In this work, FucoPol, a high molecular weight exopolysaccharide produced by *Enterobacter* A47, was used to produce biodegradable films. This is an anionic and water-soluble heteropolysaccharide composed by sugars (fucose, galactose, glucose and glucuronic acid) and acyl groups (acetate, succinate and pyruvate). FucoPol films have been characterized in previous chapters, showing to be transparent with brownish tone, ductile behavior, low water vapour barrier properties but high barrier properties to gases, in particular to oxygen [60–62]. However, for use in food packaging, its water repellency and barrier towards gases and water vapour should be improved.

Water repellency (superhydrophobicity and water sorption) and barrier properties (water vapour and oxygen) of polymer films have been enhanced both for synthetic (e.g. polyethylene (PE), polyvinyl chloride (PVC), ethylene vinyl alcohol (EVOH), polyethylene terephthalate (PET) and LDPE) and natural (e.g. cellulose, alginate and pullulan.) materials [40, 194]. Multilayer systems have been employed to improve barrier performance, traditionally by incorporation of an aluminum foil, however this hinders its recyclability [85]. The research for improving barrier properties to water vapour and oxygen is thus important for achieving sustainable solutions. The most common technology to improve such barriers on polymeric substrates consists on the deposition of inorganic thin films of metals or oxides. Deposition of inorganic oxide layers is advantageous over metallic aluminum due to their transparency and microwaveability.

However, the barrier properties of a polymer-coated system depend on numerous factors, such as thickness, composition, density, surface topography and defects in the inorganic layer [54, 194].

In this work three different thin coating techniques were used: plasma deposition, liquid flame spray (LFS) and atomic layer deposition (ALD).

Plasma deposition can be used to deposit functional layers, made of hydrocarbons, hydrocarbons with polar groups, organosilicons, halocarbons and organometallics at low substrate temperature [166]. In this work perfluorohexane (PFH) was used, aiming at achieving a stable hydrophobic behavior of FucoPol films. PFH has been used in several other studies to induce hydrophobic behavior on different surfaces, like wood [130, 205], paper and board [166], silica surfaces [84], among others.

Liquid flame spray (LFS) is a thermal aerosol-based process used to deposit nano metal and metal oxide particles. In this process, a liquid precursor is used and fed together with combustion gases into a specially designed burner in which the precursor is atomized to micron-sized droplets and evaporated in the high temperature flame. The solid nanoparticles that deposit on the substrate are formed through different synthesis reactions of the precursor vapor [196]. The range of materials that can be used as LFS precursor is wide. As examples, iron oxide, manganese oxide, alumina, silica, titanium, silver and palladium nanoparticles, have been successfully produced by LFS [127, 192]. LFS coatings have been used to improve water repellency, i.e. to create superhydrophobic surfaces on different packaging materials, e.g. paper, paperboard and LDPE-coated paper. In particular, extremely water repellent surfaces can potentially be applied in non-wetting and self-cleaning surfaces [128, 193].

In this study, FucoPol films were coated with silicon dioxide (SiO_2) nanoparticles by LFS, which were then coated with a hydrophobic PFH-layer by plasma deposition.

Atomic layer deposition (ALD) is a thin film deposition technique in which the precursor vapours are admitted into a reaction chamber one at a time. During each precursor pulse, a monolayer or sub-monolayer of desirable material is formed. ALD can be operated at room temperature, though somewhat higher temperatures are often used (85-300 °C). It is distinguish by other techniques for its self-limited growth of the deposited materials, which enables the deposition of high quality thin layers over large areas and in roll-to-roll processes. The coating thickness can be controlled by changing the number of ALD cycles used [75, 160, 210].

ALD can be used on thermally sensitive materials such as organic polymers, and can also be used to create organic-inorganic polymer composites as gas diffusion barriers on polymer surfaces [75].

Several studies have been published with ALD thin layers deposited on sensitive packaging materials such as uncoated papers, polymer-coated papers and boards, and synthetic and natural polymer films [86]. From those, several thin coating layers have been studied, namely Al_2O_3, SiO_2 and TiO_2 [86–88, 100, 214] .

In this work, FucoPol film surfaces were coated by a thin layer of titanium dioxide (TiO_2) deposited by ALD.

The aim of the present study is to evaluate different coatings, applied with distinct methods, on FucoPol surfaces targeting improved liquid repellence/hydrophobicity and barriers to gases and vapour, to turn those films available to use as food packaging. It is expected that plasma coatings can improve hydrophobic effects on FucoPol surfaces, LFS treatment can provide water repellence behavior and ALD can induce water repellence and barrier properties.

The characterization in terms of water contact angles, optical, morphological, hygroscopic and barrier properties was performed to compare the properties of the different coatings on the FucoPol films.

5.3 Materials and methods

5.3.1 FucoPol films preparation

FucoPol was dissolved in distilled water (1.5 % w/w) under stirring, at room temperature, until complete dissolution. Then, hydrochloric acid was added until pH reached 3.5 and the solution was stirred until complete homogenization.

After removing the air bubbles under vacuum, the solution was transferred to Teflon petri dishes and let to dry at 30 °C for 24 h, to form a polymer film. The films were stored at a specific relative humidity and temperature, depending on the tests to be performed. Films thickness was measured with a manual micrometer (Brave Instruments, USA).

5.3.2 Plasma deposition of perfluorohexane

Low-pressure plasma deposition was carried out using an in-house constructed reactor consisting of a glass vessel connected to a double-stage rotary vacuum pump (Leybold-Heraeus D 65 B).

Two externally wrapped, capacitively coupled, copper electrode bands were powered by a 13.56 MHz radio-frequency power generator (ENI, Model ACG-3). Perfluorohexane (PFH, Apollo Scientific) monomer was used as a precursor. During plasma deposition the treatment (generator) power was 40 W at a pressure of 18 Pa during 5 min. Based on previous experience, these plasma deposition parameters were chosen to provide an uniform and homogeneous coverage of 30 nm thickness [131]. Deposition was performed on both sides of the tested film.

5.3.3 Liquid Flame Spray coating of SiO_2

The LFS coating was performed using a single nozzle type burner.

Hydrogen and oxygen with flow rates of 50 and 15 L min^{-1}, respectively, were used as combustion gases. Tetraethyl orthosilicate (98 % pure, Aldrich) diluted in 2-propanol (VWR, HPLC Grade) was utilized as the SiO_2 precursor. The concentration of the precursor solution was 50 mg mL^{-1} of silicon metal, the feed rate was 12 mL min^{-1}, the treatment distance was 60 mm and treatment speed was 50 m min^{-1}.

5.3.4 Atomic Layer Deposition of TiO_2

The ALD depositions were performed in a Beneq TFS-500 system using an inner chamber of dimensions ϕ =20 cm and height of 3 cm. The deposition processes consisted of sequential pulsing of $TiCl_4$ and deionized water (type 2). Both reactants were delivered from external sources kept at room temperature. N_2 was used as carrier and purging gas, and was separated from air in a nitrogen generator (Schmidlin UHPN3001 N_2 purifier, >99.999 % N_2+Ar purity). The pulsing times were 1 s $TiCl_4$ - 12 s purge - 0.5 s water - 12 s purge for 1000 consecutive cycles, resulting in a layer thickness of around 42 nm.

Deposition was performed in both sides of the tested films and samples were kept in the dark.

5.3.5 Morphological characterization

The morphology of the films was evaluated by scanning electron microscopy (SEM) using a FEG-SEM JEOL JSM7001F (Oxford) equipment, with the acquisition system JEOL software PC-SEM.

Samples were coated with sputtered chromium (Quorum Technologies, Q150TES) to enable observation of surface and cross section. For cross-sections, the films were cryofractured after immersion into liquid nitrogen.

5.3.6 Contact angle measurements

The static contact angles of water were determined using an optical contact angle instrument (CAM 200, KSV Instruments Oy). The droplet volume used was 5 µL. The treated samples were stored and the measurements were performed in a controlled atmosphere (50 ± 2 % RH, 23 ± 2 °C). Five replicates of each sample were analysed.

5.3.7 Water vapour permeability

Water vapour permeability (WVP, $\mathrm{mol\,m^{-1}s^{-1}Pa^{-1}}$) of coated and uncoated FucoPol films were determined as described in chapter 3, section 3.3.6.

5.3.8 Colour and transparency

Colour and transparency of coated and uncoated FucoPol films were determined as described in chapter 3, section 3.3.3.

5.3.9 Water sorption isotherms

Water sorption isotherms of coated and uncoated FucoPol films were determined by gravimetric method at 30 °C as described in chapter 4, section 4.4.4.

5.3.10 Oxygen permeability

The tests were carried out using a stainless steel cell with two identical chambers separated by the film. The films were equilibrated at room temperature (24 °C) and relative humidity (50 %) before being tested. The permeability was evaluated by pressurizing one of the chambers (feed) up to 0.7 bar, with pure oxygen (99.999%) (Praxair, Spain), followed by the measurement of the pressure change in both chambers over time, using two pressure transducers (JUMO, Model 404327, Germany). The temperature was maintained constant at 30 °C, using a thermostatic bath (Julabo, Model EH, Germany). The permeability was calculated by Eq. 5.1, using five independent measurements:

$$\frac{1}{\beta}\left(\frac{\Delta p_0}{\Delta p}\right) = P\frac{t}{\delta} \tag{5.1}$$

Where Δp (mbar) is the pressure difference between feed and permeate compartment, P ($\mathrm{mol\,m\,m^{-2}\,s^{-1}\,Pa^{-1}}$) is the oxygen permeability, t (s) is the time, δ (m) is the film thickness and β is the geometric parameter of cell, as described by Alves et al. [6].

5.4 Results and discussion

5.4.1 Morphological characterization

SEM analysis revealed that the FucoPol films were dense and homogeneous (Figure 5.1a and 5.1b).

Fucopol films coated with PFH show on their surface, some dispersed agglomerates separated by smooth areas (Figure 5.1d). Still, due to the low coating thickness of PFH, the roughness and appearance shown in SEM image, is similar to uncoated samples. These observations are similar to those previously reported for glass [124] and a thermoplastic elastomer [175] coated with PFH.

FucoPol-LFS (SiO_2)-PFH coated films (Figure 5.1e) show uniformly distributed SiO_2 nanoparticles and agglomerates on the FucoPol surface as characteristic from the LFS process. The cross section image (Figure 5.1f) does not show any alterations, excluding migration of SiO_2 nanoparticles to the inside of the film.

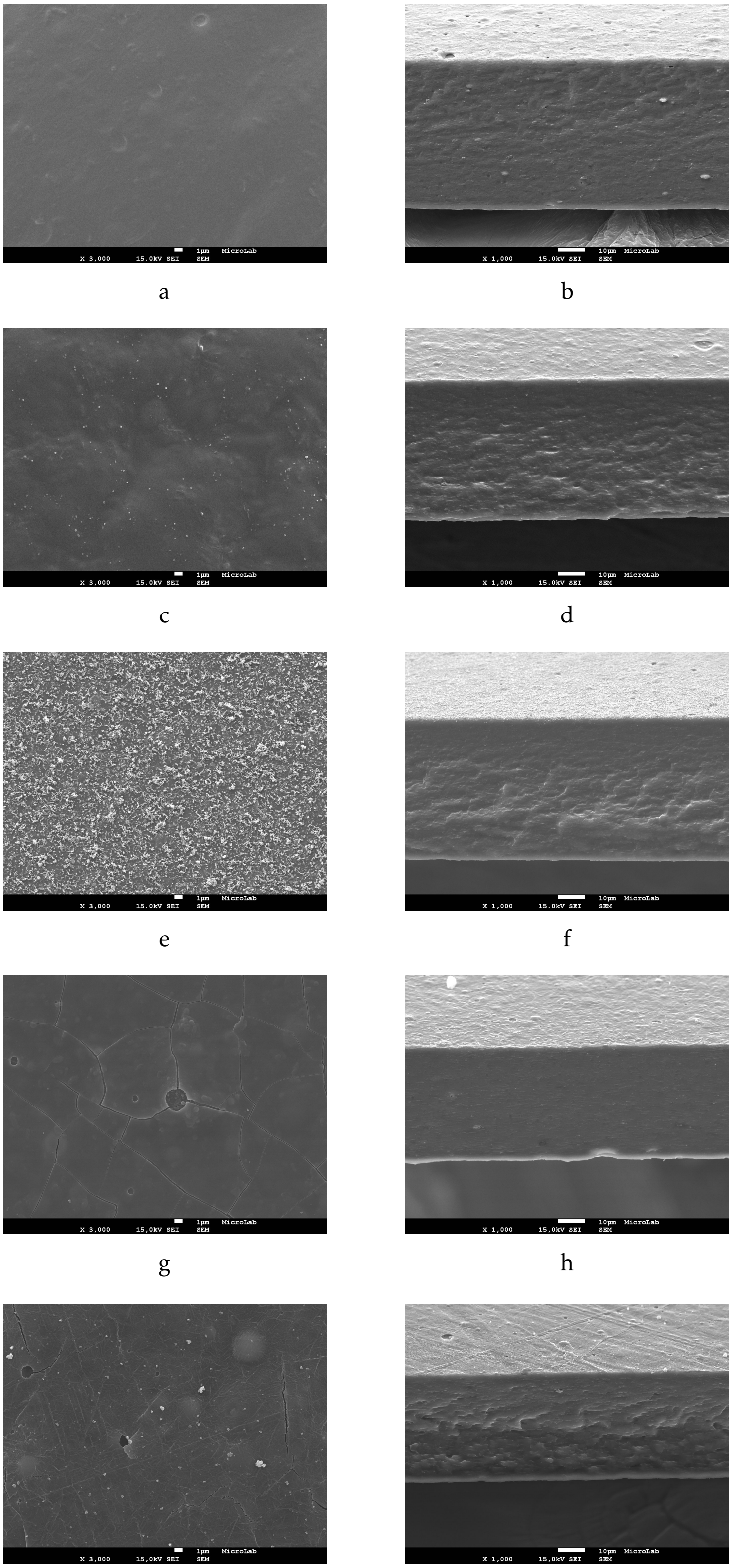
Analysis of the FucoPol-ALD (TiO_2) coated films demonstrate a dense structure in cross section (Figure 5.1h) but also show a cracking effect on the surface (Figure 5.1g) where ALD deposition has occurred. This behavior has been reported before for PET-ALD (Al_2O_3) coated films [106]. Commonly, two polymer properties define their behavior under thermal cycling, the coefficient of thermal expansion and the glass transmission rate. As inorganic materials deposited by ALD have significantly lower coefficient of thermal expansion than coated polymers, this elastic misfit lead to an increase in strain and subsequently cracking under thermal cycling [106, 126].

SEM images of FucoPol-ALD (TiO_2)-PFH coated films (Figure 5.1i and 5.1j) confirm that PFH layer cannot cover completely the cracks caused by ALD deposition, but significant differences can be perceived.

5.4.2 Contact angle measurements

Water contact angle (WCA) measurements were used to characterize the water repellence of FucoPol surfaces (Figure 5.2). WCA was measured on the top surface of FucoPol films, to avoid any effects from the flat Teflon petri dish where the films were dried.

Figure 5.2 shows the static WCA of the plasma (PFH), LFS (SiO_2) and ALD (TiO_2) coated FucoPol film. FucoPol film without any coating has a WCA around 95°. The thin ALD (TiO_2) coating does not change the WCA of Fucopol film. WCA of a smooth and



a b

c d

e f

g h

i j

Figure 5.1: SEM images of surface (a, c, e, g and i) and cross section (b, d, f, h and j) of (a) (b) FucoPol films, (c) (d) FucoPol + PFH coating, (e) (f) FucoPol + LFS (SiO_2) + PFH coating, (g) (h) FucoPol + ALD (TiO_2) coating and (i) (j) FucoPol + ALD (TiO_2) + PFH coating.

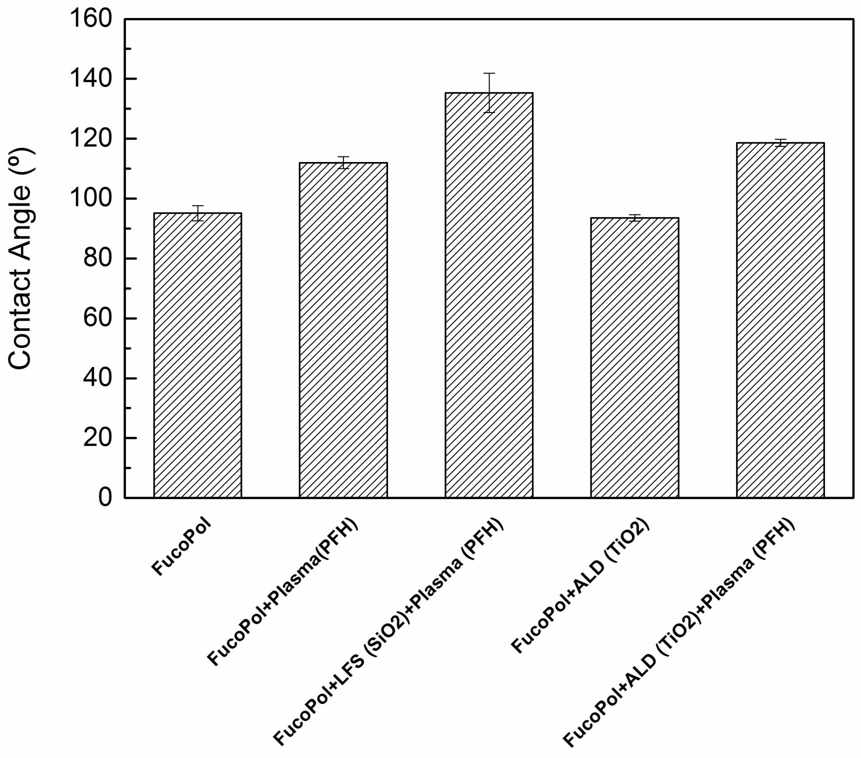


Figure 5.2: Water contact angle of coated and uncoated FucoPol films at time 0 s.

totally clean TiO_2 surface is known to be between 0° and 40°, depending on the surface roughness and UV-exposure. However, surfaces exposed to ambient conditions and aging are hardly completely clean, and the effect of organic molecules that are adsorbed onto the surfaces, decreased the surface energy and smooth TiO_2 surface is usually reported to have CA around 80° [190]. A low pressure plasma deposition of PFH increased the WCA of FucoPol film, as expected. As example, an increase in water contact angle have been reported for poly(ethylene-co-vinyl alcohol) (EVOH) films from 52° to 105° with CF_4 plasma deposition [116]. In this work hydrophobic PFH-layer increased the WCA of FucoPol from 95° to 112°, ALD (TiO_2) coated FucoPol increased the WCA to 119° and LFS (SiO_2) coated FucoPol presented a WCA for 135°. On the contrary to ALD (TiO_2) and Plasma (PFH) coated FucoPol surfaces, the SiO_2 nanoparticle coating increases the roughness of the FucoPol surface, as seen in the SEM images in Figure 5.1e. The surface roughness increased the WCA of hydrophobic surface [23].

However, FucoPol-LFS (SiO_2)-PFH surface do not have micrometer and submicrometer scale structure, which would cause air to be present in the interface between the surface and water, and thus create a superhydrophobic surface, i.e. WCA over 150° [193]. In addition to the static WCA measurement, which represents spontaneous wetting, WCAs as a function of time were followed (Figure 5.3).

FucoPol films with no treatment demonstrate a decrease in water contact angle between 95° and 77° in 120 s. Treatment with low pressure plasma deposition of PFH has

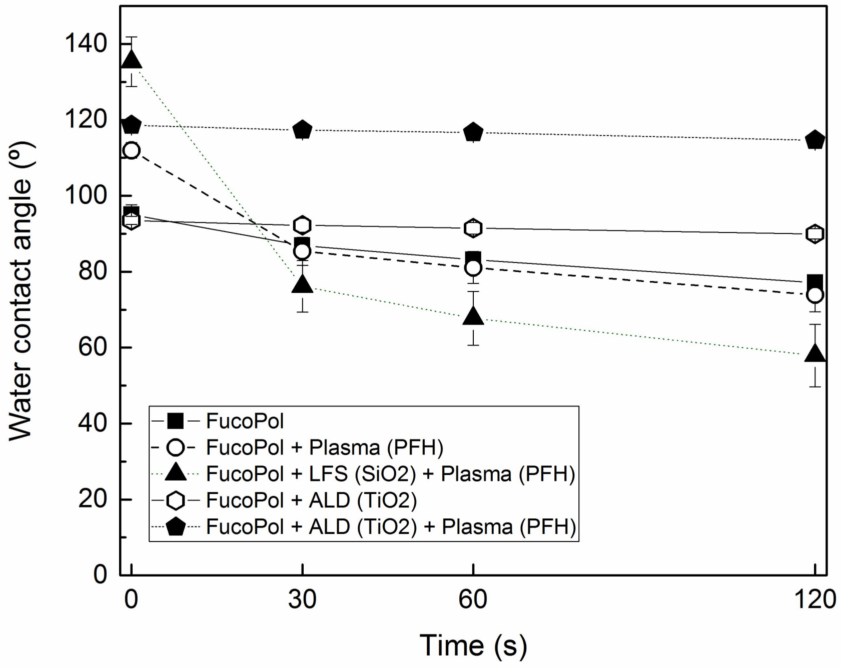


Figure 5.3: The effect of coating treatment in water contact angle of FucoPol films surface.

shown, as expected, an increase in initial value (time 0 s) to 112º, but after that WCA decreased over time and no significant differences to uncoated FucoPol were noticed. Despite a hydrophobic behaviour of the PFH layer, the layer amount and swelling of FucoPol leads to the decrease in WCA to values close to untreated FucoPol films.

The combination of LFS (SiO_2) and plasma treatment with PFH showed a high increase in WCA, but after 120 seconds the WCA decreased to lower values than the WCAs of the other surfaces. WCA on FucoPol films with ALD coatings were stable with time, demonstrated by changes lower than 4º. However, films with ALD (TiO_2)-PFH show the most hydrophobic behaviour, with WCAs between 119º and 115º.

Once the combined LFS (SiO_2)-PFH coating did not demonstrate a stable WCA and resulted in values close to untreated films ($p<0.05$), this coating was removed from the rest of study.

5.4.3 Water vapour permeability

Water vapour permeability (WVP) is an important parameter in packaging material and has a direct influence on the shelf life of food products.

In this study, the same driving force (76.9-22.5 % RH) was used for films with and without coatings to evaluate their performance in the same conditions (Table 5.1). Uncoated FucoPol films present a water vapour permeability of 5.28 $\times 10^{-11}$ mol $m^{-1}s^{-1}Pa^{-1}$.

The plasma deposition of PFH in FucoPol films did not decrease the water vapour permeability. Despite the value was higher than films with no coating, non-significant ($p>0.05$) differences were noticed.

TiO_2 deposition by ALD (with a 42 nm thick layer) demonstrated a significant decrease on water vapour permeability of about 35 %. ALD-grown oxides layers (such as Al_2O_3, SiO_2 and TiO_2) have previously been reported as barrier layers to oxygen and water vapour on polymeric materials (polylactide, pectin, among others) [88, 106].

The addition of PFH to FucoPol-ALD (TiO_2) coated films has shown non-significant differences in the water vapour barrier behavior, in contrast with what was observed with WCA.

From the obtained results above, the deposition of TiO_2 is the most important factor to decrease water vapour permeability and not the hydrophobic layer of PFH.

However, it should be emphasized that barriers to water vapour are not directly proportional to the thickness of the ALD layer. It has been reported that LDPE films with ALD coatings of TiO_2 with 40 nm thickness presented higher water vapour transmission rates than LDPE films with a thickness of 23 nm [106]. It is important to notice that the development of barrier behavior by coatings application in flexible subtracts has to consider other aspects, such as polymer surface modification, polymer-inorganic layer interface modification and inorganic layer structure.

Table 5.1: Water vapour permeability of coated and uncoated FucoPol films.

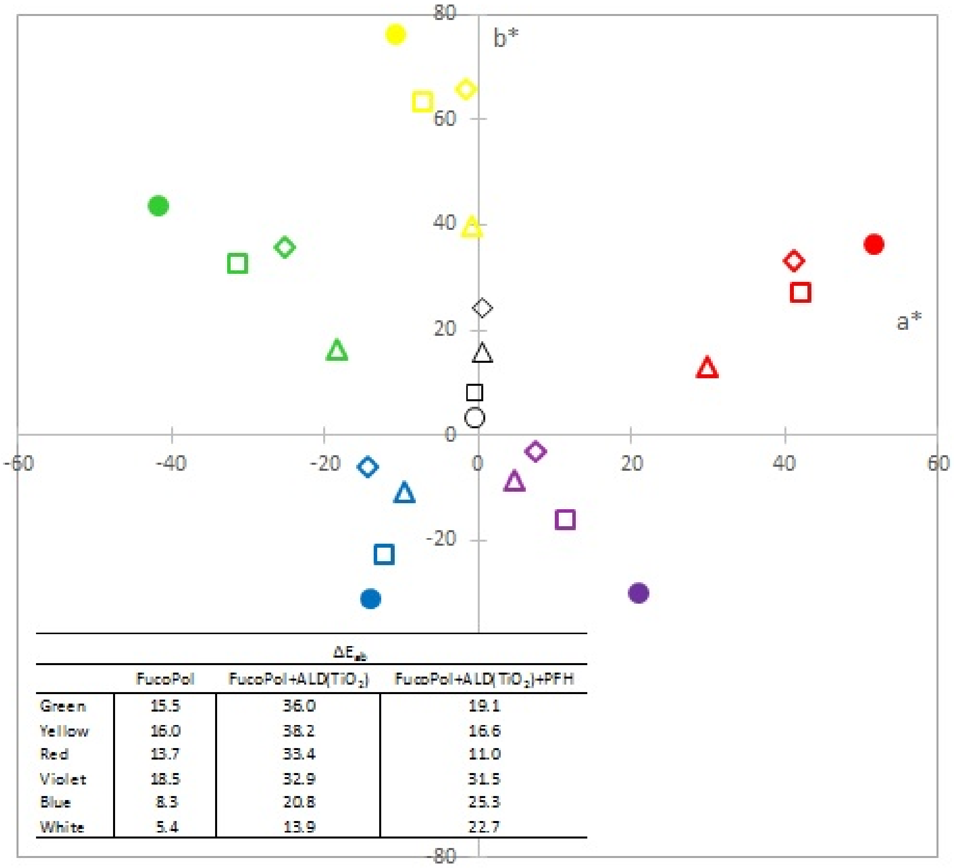
Film	**ΔRH (%)**	**WVP (x10^{-11} mol m^{-1}s^{-1}Pa^{-1})**
FucoPol	76.9 – 22.5	5.32 ± 0.69
FucoPol + Plasma (PFH)	76.9 – 22.5	6.25 ± 0.78
FucoPol + ALD (TiO_2)	76.9 – 22.5	3.45 ± 0.49
FucoPol + ALD (TiO_2) + Plasma (PFH)	76.9 – 22.5	3.40 ± 0.71

Once PFH coating alone did not demonstrate any improvement on WCA over time and in barrier properties to water vapour, FucoPol films with this coating were not considered in the rest of the study.

5.4.4 Colour and transparency

Transparency plays an important role in packaging materials because it is a key factor to good acceptance by end-users when the aim is to show the product inside the package upon purchase. The transparency of FucoPol films measured at 600 nm was 5.9 ± 0.1, while that of FucoPol-ALD (TiO_2) and FucoPol-ALD (TiO_2)-PFH coated films was 9.6 ± 0.3 and 8.4 ± 0.1, respectively, both significantly different from the one of films without coatings . These results suggest that the TiO_2 layer, or formation process of it, affects the transparency of FucoPol films in a negative manner, giving a more yellowish colour. On the other hand, the application of PFH layer, seems to bring a positive effect to transparency of FucoPol-ALD (TiO_2) coating, probably due to a reduction in the scattering of the TiO_2 rough layer.

Colour parameters (a* and b*) of coloured paper surfaces, uncovered and covered by the films samples with several coatings are presented in Figure 5.4. An approximation towards the origin can be noticed for all colours (exception to white). This behavior corresponds to a decrease in colour saturation (chorma, C_{ab}) caused by coatings deposition. Once more, FucoPol-ALD (TiO_2)-PFH films presented a different behavior, and for yellow, green and red colour, these films demonstrate higher colour saturation (near initial values), which are in accordance with the transparency results.



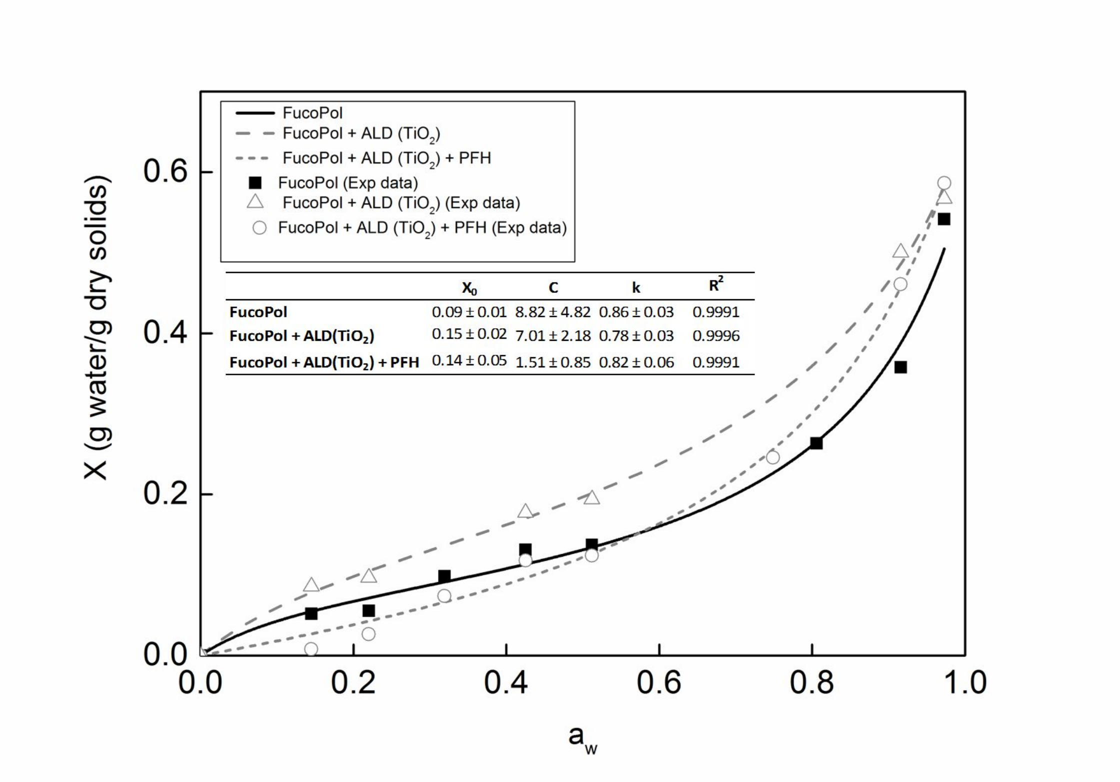
ΔE_{ab}			
	FucoPol	FucoPol+ALD(TiO_2)	FucoPol+ALD(TiO_2)+PFH
Green	15.5	36.0	19.1
Yellow	16.0	38.2	16.6
Red	13.7	33.4	11.0
Violet	18.5	32.9	31.5
Blue	8.3	20.8	25.3
White	5.4	13.9	22.7

Figure 5.4: Parameters a* and b* of the CIELAB system for different coloured surfaces uncovered (circles - ○) and covered by FucoPol films (squares - □), FucoPol films + ALD (TiO_2) coating (triangles - △) and FucoPol films + ALD (TiO_2) + Plasma (PFH) coating (diamonds - ◊) and total colour alteration (ΔE_{ab}) values (insert).

From the values of ΔE_{ab} (Insert in Figure 5.4), it may be foreseen that the total colours alteration originated by system FucoPol films with coating can be perceived by the human eye. In general, higher ΔE_{ab} values were found for films coated only with TiO_2, just for white colour, total colour alteration is significantly higher to films coated with both layer (TiO_2 and PFH).

5.4.5 Water sorption isotherms

The water sorption isotherms for FucoPol films with and without coatings, and respective GAB equation parameters are presented in Figure 5.5. The experimental data for all films are well described by the GAB model (r^2= 0.9991–0.9996). The obtained moisture content for all tested films increased with water activity (a_w), but curves for the three samples films are considerably different.



	X_0	C	k	R^2
FucoPol	0.09 ± 0.01	8.82 ± 4.82	0.86 ± 0.03	0.9991
FucoPol + ALD(TiO$_2$)	0.15 ± 0.02	7.01 ± 2.18	0.78 ± 0.03	0.9996
FucoPol + ALD(TiO$_2$) + PFH	0.14 ± 0.05	1.51 ± 0.85	0.82 ± 0.06	0.9991

Figure 5.5: Water sorption isotherms of FucoPol film coated and uncoated at 30 °C fitted by GAB model and the obtained GAB parameters (insert).

FucoPol films without any coating show the most sigmoidal shape, usually observed for polysaccharides and corresponding to higher C parameter of GAB model, which is responsible for more pronounced form of the "knee" for lower activity range [197]. FucoPol-ALD (TiO$_2$)-PFH sample, as expected, show a less sigmoidal shape, corresponding to lower C parameter and lower water absorption at low water activities, which might be due to the hydrophobic behavior of PFH on top of FucoPol-ALD (TiO$_2$) surface.

The *k* parameter determines the profile at higher activity range, regulating the upswing after the plateau, in this case uncoated FucoPol films have shown the highest value (0.86 ± 0.03) which are in accordance with GAB model fitting curve shape. As reported by Timmermann the upswings determined by constant *k* should not be confused with the upswing due to the third sorption stage ($0.85 < a_w < 0.9$) at highest sorbate activities [197].

The monolayer value (X_0) indicates the maximum amount of water that can be absorbed in a single layer per gram of dry film. In this work higher values of X_0 were obtained for coated films, FucoPol-ALD (TiO_2) and FucoPol-ALD (TiO_2)-PFH coated films have shown 0.15 ± 0.02 and 0.14 ± 0.05 respectively, while uncoated FucoPol films presented a value of 0.09 ± 0.01. This behavior suggests that a layer of TiO_2 allowed adsorption of water, probably due to its rough surface. This behavior could be also related with aging and UV-exposure, once ALD coating can turn more hydrophilic and adsorbing more water with the influence of those two factors. In water sorption isotherms experiments, samples were placed in desiccators during three weeks with light exposure.

5.4.6 Oxygen permeability

The study of gas barrier properties of polymeric films is essential to design food packaging solutions. Usually, polysaccharides are good barriers to gases at low relative humidity (RH), due to their highly packed hydrogen-bonded dense polymer matrix. The increase of ambient moisture however, increases significantly the permeability of polymeric film, due to the plasticization effect of water. In this study, the permeability was measured at RH of 48 % (Table 5.2).

Table 5.2: Oxygen permeability of FucoPol films coated and uncoated, measured at 48 ± 5 % of RH.

Film	**O_2 Permeability** ($\times 10^{-16}$ **mol m m^{-2}s^{-1}Pa^{-1}**)
FucoPol	5.13 ± 0.28
FucoPol + ALD (TiO_2)	3.31 ± 0.41
FucoPol + ALD (TiO_2) + Plasma (PFH)	1.70 ± 0.71

FucoPol-ALD (TiO_2) and FucoPol-ALD (TiO_2)-PFH coated films present a significant ($p<0.05$) lower permeability to oxygen than uncoated FucoPol films (5.13×10^{-16} mol m - m^{-2}s^{-1}Pa^{-1}). The thin TiO_2 layer (42 nm) decreased the FucoPol permeability by 35 % to 3.31×10^{-16} mol m m^{-2}s^{-1}Pa^{-1}. Previous studies have shown that a thin layer applied by ALD can have an enormous effect on the barrier to oxygen. Hirvikorpi et al. reported a decrease of 65 % in oxygen transmission rate to paperboard/PE system coated with 50 nm of Al_2O_3 and 73 % when coated with 50 nm of SiO_2 thin film [87]. Also Kääriäinen et al. reported a decrease of 91 % in oxygen transmission rate to LDPE extrusion coated paper, coated by ALD with 40 nm of TiO_2 thin film [106].

Those decreases are much higher than what was measured in this work, which can be due to the cracks shown in Figure 5.1g. FucoPol has a smooth surface, as such, a 42 nm TiO_2 thin layer may be rather prone to cracking, and therefore a thinner layer could have been better. This behavior was described by Hirvikorpi for paperboard/PLA system coated with Al_2O_3 , where 25 nm of Al_2O_3 presented better barrier properties than 100 nm [86]. However, the improvement detected with FucoPol-ALD (TiO_2) films could be caused by strong bonding between functional OH- groups on FucoPol surface and TiO_2 layer. This behavior is well-known and described in literature for polymers with high content of hydroxyl groups, such as, PVA or cellulose [81].

Plasma PFH coating applied on FucoPol-ALD (TiO_2) coated film can improve the oxygen barrier behavior even more, presenting a permeability of $1.70\text{x}10^{-16}$ mol m m^{-2}-$s^{-1}Pa^{-1}$, which is 67 % lower than for untreated FucoPol films. In this case, PFH can act to reduce the cracking effect of the TiO_2 layer, in a similar manner as reported by Charton et al., which described that the mechanism for permeation through inorganic barrier coatings on polymers is usually dominated by defects in the inorganic barrier layer [29, 194].

5.5 Conclusions

Several coatings were created on FucoPol film surfaces using plasma deposition, liquid flame spraying and atomic layer deposition. Inorganic coatings were used with the aim of improving the surface and barrier properties. FucoPol films coated with ALD (TiO_2) and ALD (TiO_2)-Plasma (PFH) have shown potential use in food packaging, as they presented higher and stable water contact angle, lower water vapour permeability, a transparency in the same order of magnitude and significantly lower oxygen permeability values than the uncoated films. Films coated with SiO_2 nanoparticles presented a rough surface which led to higher instant water contact angle, though not stable over time.

The combination of ALD (TiO_2) and Plasma (PFH) coating has demonstrated to be the best option, presenting a permeability to oxygen ($1.70\text{x}10^{-16}$ mol m $m^{-2}s^{-1}Pa^{-1}$) 67 % lower than that of untreated films and a water vapour permeability of $3.40\text{x}10^{-11}$ mol - $m^{-1}s^{-1}Pa^{-1}$.

CHAPTER 6

EVALUATION OF FUCOPOL AND CHITOSAN BILAYER FILMS PERFORMANCE FOR PACKAGING OF WALNUTS (*Juglans regia L.*)

6.1 Summary

The performance of FucoPol/chitosan bilayer and PA/PE commercial films, was evaluated to storage fresh walnut (*Juglans regia L.*, cultivar Chandler) kernels at accelerated storage conditions (24 h of light, 33 % RH and 38 ºC) and compared with walnuts without any package.

A previous study with extracted walnuts oil was performed to obtain a fast lipid oxidation. The oxygen content inside the package, oil peroxide value and oxidation compounds were monitored. A pronounced increase in peroxide values was noticed along the experiment (14 days), related with increase of oxygen content. Walnut oil does not show significant differences in peroxide values and oxidation compounds when packaged with bilayer or commercial film, but significantly higher values were detected for oil with no package.

As proof of concept, shelled walnut kernels were stored, at the same conditions to evaluate the rancid taste using a trained sensory panel. Minor differences in rancidity were detected for kernels packaged with both barrier materials, however significant differences for walnut with no package were detected and classified as moderate to rancid taste.

Overall, the behavior of the biodegradable bilayer films was found similar to that of the non-biodegradable commercial ones, which makes them a promising sustainable alternative as packaging materials.

6.2 Introduction

Nowadays walnuts are cultivated on southern Europe, northern Africa, eastern Asia, USA and western South Africa, and the high economic interest to food industry is related to the broad variety of products and their uses. Walnuts are commercialized in-shell, shelled, in kernels form or grounded. In addition, they can be consumed as fresh fruit or toasted, as ingredient in bakery or even as flavour [118, 123].

Walnut (*Juglans regia L.*) kernels are highly appreciated not only due to their good organoleptic characteristics, but also because of their health benefits, namely to reduce blood pressure and total cholesterol and to prevent cardiovascular diseases. These properties are associated with their chemical composition, in particular with oil and antioxidants (phytosterols and polyphenols). The oil content may vary from 52% to 74% according to the cultivar and origin [14, 98, 111, 118].

The oil has a high polyunsaturated fatty acids (PUFAs) content, around 57.3 – 76.6 g/100 g, being oleic (13.8 - 33.0 g/100 g), linoleic (49.3 - 62.3 g/100 g) and linolenic (8.0 - 15.4 g/100 g) acids the major fatty acids referred to be present in its composition. The overall and relative contents of PUFAs are important to economic and nutritional value of the nuts, once higher levels of PUFAs are more desirable due to their potential health benefits. However, high PUFAs content limits the nuts shelf life due to their susceptibility to oxidation. This fact leads to the most important quality parameter of walnuts conservation, lipid oxidation, which causes rancid taste and aroma that are unacceptable to the final consumer [14, 217]. The oxidation reactions are directly related to external factors, from the harvest until the conservation period, and are related essentially with oxygen concentration, temperature, relative humidity and light.

Oxygen concentration is considered the most important external factor inducing lipid oxidation. As such, several strategies have been studied to decrease the oxygen effect, such as modified atmosphere packaging with low oxygen levels (composed mainly by nitrogen and carbon dioxide), vacuum packaging, or the use of oxygen absorbers [92, 147]. The availability of oxygen in the package, can also be controlled by using oxygen barrier materials [123] or a coating formulation [98]. The rate of oxidation is independent of oxygen concentration at high oxygen partial pressure, but it is proportional to oxygen concentration at low oxygen partial pressure [120]. Independently of the strategy used, it is essential to minimize oxygen contact with walnut kernels to extend their shelf-life.

Storage temperature has also an important influence on oxidation rate. Several authors reported that higher temperatures (above 21 ºC) induced a faster oxidation than lower ones. According to several authors the optimal storage temperature for walnuts should be always lower than 11 ºC [14, 92, 123]. It was also found that walnuts storage at higher temperatures present higher levels of hexanal, a secondary metabolite of lipid oxidation which is formed by breakdown of linoleic acid hydroperoxides [92].

The relative humidity (RH) can also affect the oxidative rancidity, since moisture influences the rate of oxygen uptake by lipids in foods. Moreover, high values of RH can affect the performance of certain edible films decreasing their barrier properties to oxygen [120].

Light is also an important factor in walnuts storage. Martínez et al. have reported its high effect on the photo-oxidative degradation of walnut oil. In their work, the oil stored in transparent glass bottles exposed to light at room temperature maintains an acceptable quality only for two months of storage, which represents a very short shelf life [117].

As referred before, an efficient oxygen barrier material may be used for walnuts packaging, since the availability of oxygen can be controlled by the oxygen permeability of the packaging material. Several barrier materials (with one or more polymers) have been studied, such as polyamide/polyethylene (PA/PE) [14, 111], low density polyethylene (LDPE), polyethylene terephthalate/polyethylene (PET/PE) [123], polyethylene (PE), ethylene-vinyl alcohol/low-density polyethylene(EVOH/LDPE) [92], among others.

A wide variety of biopolymers has been studied to produce barrier materials, in particular, polysaccharides, due to their good barrier against oxygen at low or moderate relative humidity. These materials are biodegradable, making possible to contribute for reducing plastic waste in the end of service life [61].

In this work a bilayer films composed by two biopolymers (FucoPol and chitosan) and characterized in Chapter 4 was tested as barrier material. FucoPol is a high molecular weight exopolysaccharide produced by bacterium *Enterobacter* A47 (DSM 23139) using glycerol by-product from biodiesel industry as carbon source. This biodegradable anionic heteropolysaccharide is composed by sugars (fucose, galactose, glucose and glucuronic acid) and acyl groups (acetate, succinate and pyruvate).

Bilayer films of FucoPol and chitosan were tested as barrier material to oxygen to be used in walnuts packaging. Storage experiments were carried out with walnut oil and kernels, using the FucoPol/chitosan and commercial PA/PE films for comparison. The performance of the two barrier materials was evaluated in accelerated storage conditions (24 h of light, 33 % RH and 38 °C), monitoring the oxygen content in the package, as well as, the oils peroxide value and oxidation compounds over time.

6.3 Materials and methods

6.3.1 Walnut kernels and oil

Fresh walnut (*Juglans regia L.*, cultivar Chandler) kernels were supplied by H. Reynolds de Souza, Estremoz, Portugal. Oil from walnut kernels was extracted mechanically using a homemade pressing machine composed of screw and a nozzle of 5 mm.

After extrusion, the pressing cake was discharged and the crude oil was centrifuged (15 min, 48384 x g). The clean oil was transferred into amber glass bottles, which headspace was flushed with nitrogen before closing. The bottles were stored at 4 °C before experiments for a maximum period of 24 h.

6.3.2 Bilayer films preparation

Bilayer films of FucoPol and chitosan were prepared by a two-step coating technique as described in chapter 4, section 4.3.4.

FucoPol was produced and purified as described in chapter 3, section 3.3.1. Commercial medium molecular weight chitosan (deacetylation degree of 75 – 85%) was purchased from Sigma (USA). Citric acid monohydrate was purchased from VWR chemicals – BDH Prolabo (UK). Glycerol (analytical grade) was used as plasticizer and purchased from Sigma (USA).

6.3.3 Packaging preparation

Depending on the experiment, walnuts oil (15 mL) or walnut kernels (12 g) were transferred to home-made glass vessels (35 mm diameter, 6 cm height) without cover. The vessels possess an open vial-like (20 mm) where Mininert® Valve (Supelco, USA) was inserted.

In order to have a sealed vessel, after introducing the walnuts kernel or walnuts oil, the top of glass vessel was covered with the test film (bilayer film of FucoPol and chitosan or commercial film) and sealed with a commercial aluminium foil (Avery Dennison, USA). The effective mass transfer area was 2 cm^2. The leak tightness was tested to ensure that the oxygen transfer took place exclusively through the tested films.

The permeability to oxygen of the commercial film (PA/PE 90) is lower than $4.6x10^{-17}$ mol m m^{-2} s^{-1} Pa^{-1} (according to the supplier), and that of the bilayer film was reported in chapter 4 to be $4.7x10^{-17}$ mol m m^{-2} s^{-1} Pa^{-1}.

The flasks with the samples (oil or walnut kernels) were flushed with nitrogen to start with nearly zero oxygen content in the beginning of the experiments.

6.3.4 Storage experiments

Two different experiments were carried out. One of them with walnut extracted oil and the other with shelled walnut kernels. In each case, samples totally exposed to air (labelled as No Package), sealed with bilayer films of FucoPol and chitosan (labelled as Bilayer) and sealed with commercial film (polyamide cast flexible-polietylene (PA/PE 90), purchased from AlemPack (Portugal)) (labelled as Commercial) were used.

For fast lipid oxidation monitoring, unpacked and packed walnut oil was stored in climate chamber (Cassel, Germany) set to 38 ± 2 ºC and 35 % relative humidity (RH) for 14 days with 24 h of light. Oxygen content inside the package and oil analysis (peroxide value, primary decomposition products) were performed on the 1st, 7th and 14th days of storage. Three replicates of each day were analyzed.

In the second experiment, shelled walnuts were packed in the same vessels, with the same sealing method. Unpacked walnuts kernels were maintained at the same light and temperature conditions. Sensory evaluation of packed and unpacked walnuts kernels were performed at the 7th and 11th days (to avoid complete oxidization). Four replicates of each day were analyzed.

6.3.5 Analytical methods

6.3.5.1 Oxygen content

The oxygen content inside the glass vessels was measured along time using a headspace gas analyser Checkmate 3 (Dansensor, Ringsted (Denmark)). Gas analysis was performed by pulling out a headspace gas sample by piercing a syringe needle through Mininert® Valve attached in the glass vessels.

6.3.5.2 Peroxide value of walnut oil

The peroxide value (PV) was determined according to the standard NP EN ISO 3960 (2010) “Animal and vegetable fats and oils - Determination of peroxide value - Iodometric (visual) endpoint determination”.

An oil sample (5 g to estimate peroxide value between 0 - 12 meqO_2 Kg^{-1}) was dissolved in 10 mL of chloroform (analytical grade) under stirring. Glacial acetic acid (15 mL) and potassium iodide (KI) were added to the previous solution and stirred for 1 minute in a capped flask. Flask was left 5 minutes in dark at temperature between 15 - 20 ºC. Then, 75 mL of deionized water was added.

The liberated iodine was titrated with sodium thiosulfate ($Na_2S_2O_3$) solution (0.002 N solution for expected PV < 12 meqO_2 Kg^{-1}, and 0.01 N solution for expected PV > 12 meqO_2 Kg^{-1}) under high stirring, using a starch solution (10 g L^{-1}) as an indicator. Two replicates of each sample were analyzed.

The peroxide value was calculated by the following equation:

$$PV = \frac{VN1000}{m} \tag{6.1}$$

Where, V (mL) is the volume of $Na_2S_2O_3$ solution in the experiment (corrected with blank test), N is the normality of $Na_2S_2O_3$ solution and m (g) is the sample mass.

6.3.5.3 Oxidation compounds of walnut oil

Oil thermal oxidation was indirectly evaluated following the Portuguese standard NP 970:1986-pt “Edible fats and oils. Ultraviolet absorption” [51]. A solution of walnut oil in isooctane (10 g L^{-1}) (Carlo E Erba, France) was analyzed for lipid oxidation. The detection of conjugated diene hydroperoxide products produced in the early stages of lipid oxidation process was evaluated through a direct absorbance measurement at 232 nm [181].

Similarly, conjugated trienes, the secondary products of oxidation, could be detected from UV absorbance in the range of 268-272 nm of the same 10 g L^{-1} oil solution in isooctane. Three replicates of each sample were analyzed.

6.3.6 Sensorial analysis

Sensory evaluation of walnuts kernels was assessed by an expert panel of 5 trained assessors (ISO 8586:2012) [179]. Prior to the sensory evaluation, the walnuts of the same sample, unpackaged and package with bilayer and commercial films at 7^{th} and 11^{th} days, were mixed and each panellist was served a representative sample of walnut. The walnuts were randomly coded with 3-digit (one number and two letters). Sensory descriptive analysis was performed to characterized rancid taste and aroma of the samples. Ratings were based on a six-point numerical unipolar category scale (1 - absent until 6 - very strong). The samples were presented to assessors monadically.

6.3.7 Statistical analysis

In this work, the obtained data values were statistically analysed by one-way analysis of variance (ANOVA) using OriginLab® OriginPro 2016. Differences between pairs of means were assessed on the basis of confidence intervals using the Tukey test. The least significance difference was $p > 0.05$.

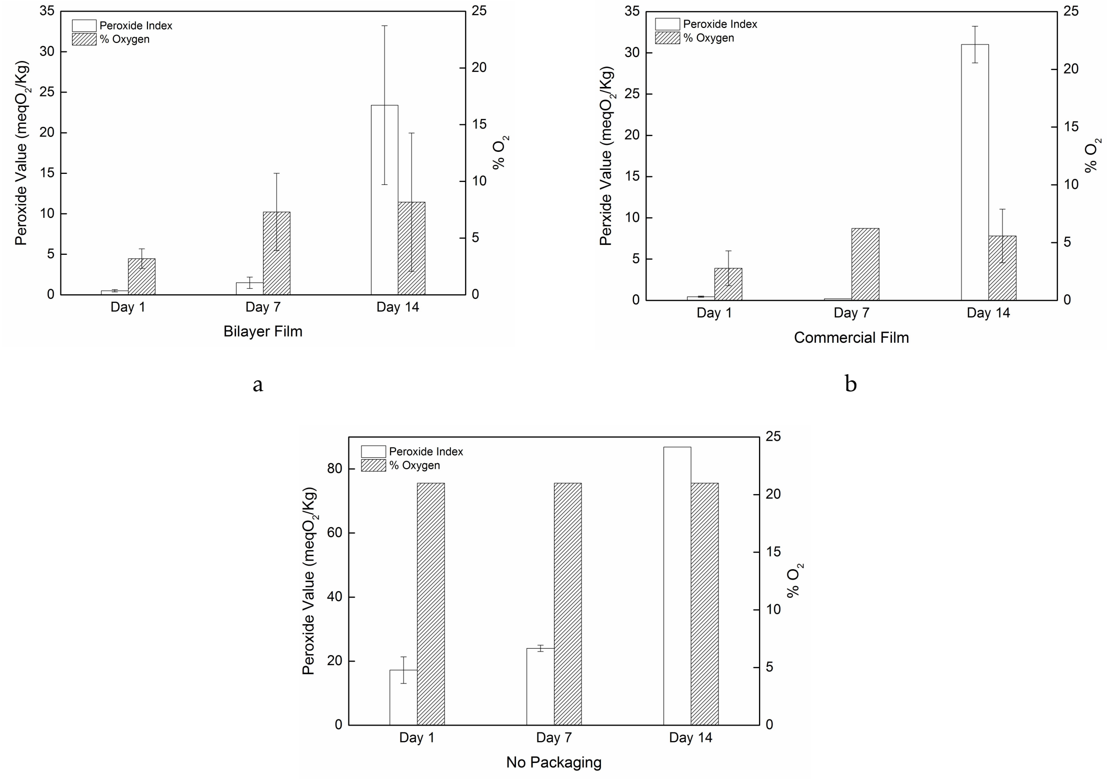
6.4 Results and discussion

6.4.1 Walnut oil storage experiment

Peroxides are the primer products of lipid oxidation and a conventional indicator to quantify oxidative rancidity. The initial peroxide value (PV) of fresh walnut kernels was very low, $\leq 0.1\,\mathrm{meqO_2\,Kg^{-1}}$ walnut oil, which is in accordance with other authors [118, 123, 189]. This fact indicates that the oil extraction method used in this work is effective and does not promote oxidation.

The experiment was performed with 24 h of light, 38 ºC and 35% RH, and the variation of oxygen content inside the packages and PV of walnut oil values over time are shown in Figure 6.1. It may be observed a pronounced increase of PV for both packages (bilayer film and commercial film), with no substantial differences between them. This fact is in line with the oxygen content inside the package, which also increased over time nearly in the same manner. According to Maté et al. nuts at standard atmosphere oxygen concentration are unprotected, while an oxygen content lower than 2.5% represents good conditions for nuts storage [120]. In the present work, the oxygen content increased up to around 5% in the first day of the experiment for both films tested.

Regarding the oil stored in open flasks (no packaging), PV values have also shown a great increase, reaching much higher levels, which is attributed to their contact with 21% of oxygen present in the atmosphere (Figure 6.1c). At day 1, the oil stored without any package, exceeded the peroxide value limits for edible virgin oils ($15\,\mathrm{meqO_2\,Kg^{-1}}$) established by Portuguese law [47]. However, the oil stored in vessels capped with bilayer and commercial films only exceeded that value at day 14. Significant differences ($p<0.05$)



a

b

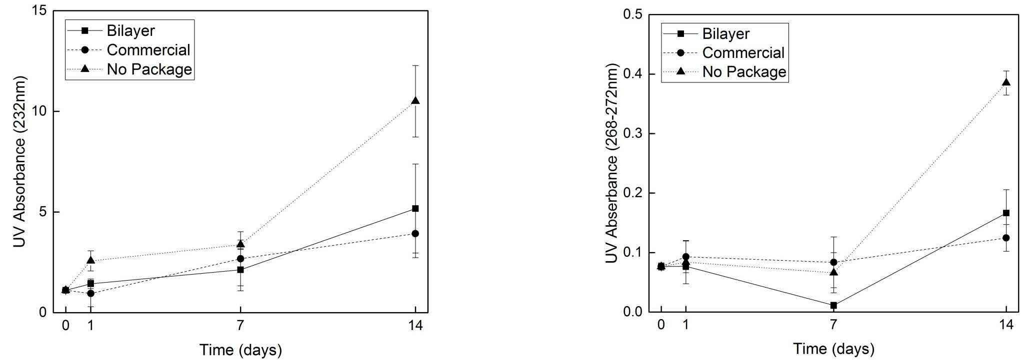
c

Figure 6.1: Oxygen content inside the packages and peroxide value of walnuts oil stored during 14 days in packages with (a) Bilayer film, (b) commercial film and (c) no package.

were observed in PV between packed and no packed walnut oil, though no substantial differences were perceived between both packaging films.

This test was carried out during 14 days, under accelerated oxidation conditions, especially due to deleterious effect of light and increased temperature, as reported for olive oil, sunflower oil, peanut oil, among others [5, 16, 39]. Other storage conditions are referred in the literature, such as the one reported by Mexis et al. that used LDPE and PET/LDPE packages, subjected to light and T=20 ºC, in which after 12 months the PV value observed is similar to that of this work at day 14 (32 meqO$_2$ Kg^{-1}) [123]. In addition, tests performed by Martínez with walnut oil stored in transparent glass bottles in light, at 25 ºC, have shown similar values of PV (22.25 meqO$_2$ Kg^{-1}) after three months of storage [119]. It is worthy to mention that other factors than temperature, light, relative humidity and oxygen content, have to be taken into account on preservation studies, such as the package volume, the mass of product and the internal void volume.

The formation of oxidation compounds of walnut oil was monitored and the results are presented in Figure 6.2. Primary oxidation products (conjugated dienes) were evaluated from the absorbance at 232 nm, Figure 6.2a. The Initial value (1.1 ± 0.1) is in accordance with values referred by Martínez et al., range between 1.0 and 1.2 [118]. Only at day 14 significant differences ($p<0.05$) were noticed between oil with and without package. Still, no significant differences were detected between bilayer and commercial packages. Final values of conjugated dienes for bilayer (5.2 ± 2.2), commercial (3.9 ± 1.2) and no package (10.5 ± 1.8) samples are in accordance with PV values presented in Figure 6.1.



a

b

Figure 6.2: Conjugated dienes (K232) (a) and conjugated trienes (K268-272) (b) from walnut oil during the storage experiment.

The production of secondary oxidation products (conjugated trienes) over time is presented in Figure 6.2b. A similar behaviour can be observed, as only at day 14 significant differences ($p<0.05$) were detected for walnut oil with and without package. Again, no significant differences were noticed between both types of packaging films during all experiment. Those high values of absorbance are correlated with presence of secondary oxidation compounds, which could indicate that light degradation of primary oxidation compounds was simplified and peroxide degradation reaction occurs more rapidly as described by Caponio et al. for extra virgin olive oil [22]. Light is described by same authors as the main cause for the increase in absorbance at 270 nm and loss of oil colour, which also occurred in this experiment, and could be observed at human naked eye.

6.4.2 Walnut kernels experiment

As proof of concept, an experiment with shelled walnut kernels was carried out. Taking into account the results obtained with walnut oil, two samples (at day 7 and day 11) were evaluated in terms of sensory analysis, with a trained panel, aiming at targeting the rancid attribute (aroma and taste). As in the previous experiment, after 7 days, the oxygen content of the samples packed with bilayer film and commercial film was 6.9 ± 0.2 % and 4.3 ± 2.1 %, respectively; it was expected to achieve no significant differences in peroxide values in kernel oil, and a lower value than the limit for peroxide value for edible virgin oils ($15\,\mathrm{meqO_2\,kg^{-1}}$ oil). However, the oil of walnut kernels with no package at the referred day is expected to have a PV above the limit. The second sample was tested at day 11 to obtain data near the limit of the experiment with oil, at which the content of peroxides, and primary and secondary oxidation products, were substantially different when comparing packaged and unpackaged oil.

The results of sensory analysis, presented in Figure 6.3, are in agreement with the results obtained for walnut oil presented previously. Minor differences were found between walnuts packed with bilayer of FucoPol and chitosan film and commercial film, and major differences were found for walnuts with no package, once they were completely exposed to oxygen. In this experiment no samples with very intense rancid taste or aroma were detected by the panellists. Still, the samples stored with no package (for both days) were classified as moderate in terms of those attributes. It was expected the panellists to detect a higher rancid intensity, especially in walnuts kernels with no package, since in the experiment with oil a high amount of secondary oxidation products, responsible for the typical unpleasant sensory characteristics, was detected (Figure 6.2b) [16]. Knowing that initial lipids are radically oxidized into hydroperoxides, which are odourless and tasteless, these compounds are not detected in sensory analysis. In addition, in kernels experiment the oil is more protected from all oxidation reactions as it remains physically entrapped within the fruit. These facts may explain the low rancid taste or aroma detected by the panellists.

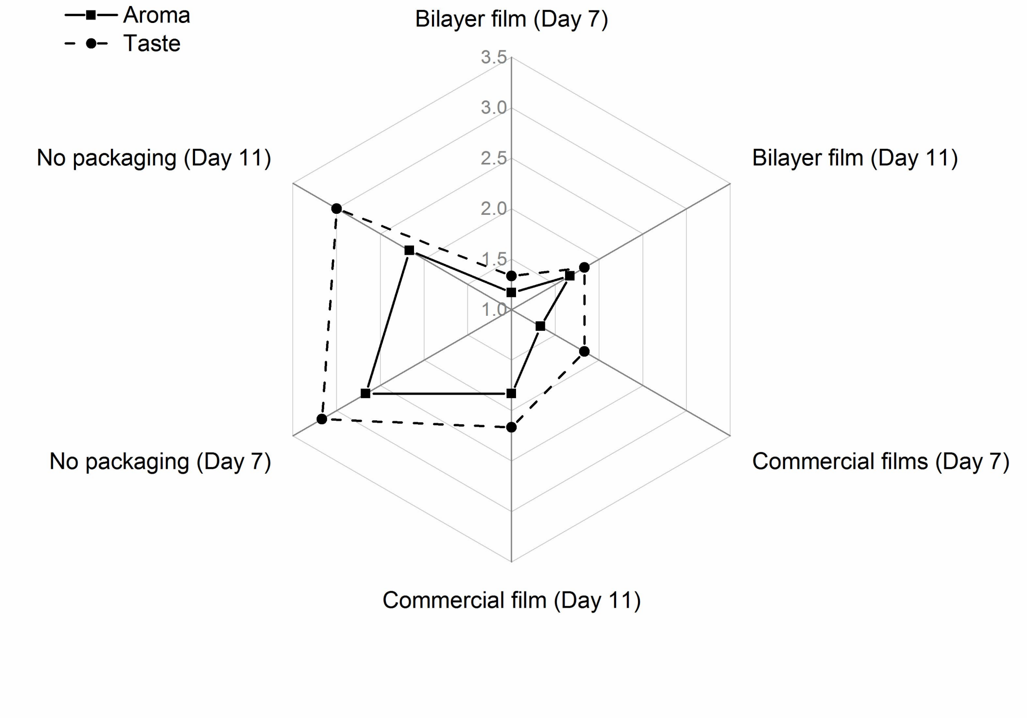


Figure 6.3: Aroma and taste of the walnuts at 7th and 11th days of storage.

6.5 Conclusions

Experiments in accelerated storage conditions (24 h of light, 33 % RH and 38 ºC) for walnuts oil were performed. The oxygen content in the package increased to 5 – 7 %

along the experiment with both films used. The increase of peroxide values is related with the presence of oxygen and follows the same trend as oxygen content. At day 1, oil stored with no package presented a peroxide value above the legal limit ($15\,\mathrm{meqO_2\,kg^{-1}}$), however, films stored with bilayer and commercial films only present values above that limit (23 and $31\,\mathrm{meqO_2\,kg^{-1}}$ respectively) at day 14.

Walnut oil with no package presents conjugated dienes values two times higher than oil packaged with bilayer or commercial films, and this difference is even higher (almost three times) for conjugated trienes.

As proof of concept walnuts kernels were also packaged with FucoPol/chitosan bilayer and PA/PE commercial films, as well as, walnuts with no package aiming at the evaluation of rancid taste in nuts. No significant differences in taste and aroma were detected in walnuts packaged with bilayer films and PA/PE commercial films. However walnuts with no package presented moderate values of rancidity.

From the results obtained, the protection effect of the biodegradable bilayer FucoPol/-chitosan films was found to be similar to that of the non-biodegradable commercial ones (PA/PE), which makes them a promising sustainable alternative as packaging materials.

CHAPTER 7

CONCLUSIONS AND FUTURE WORK

The overuse of synthetic non-biodegradable plastic packaging has caused a serious global environmental problem related with post-consumer waste. The way to overcome this severe problem is currently a strong source of motivation to researchers around the world.

One of the current research paths to apply biodegradable polymers from renewable resources in packaging. This solution is even more interesting to food packaging once, usually, these materials are food contaminated which constitutes a health hazard in sorting and mechanical recycling.

This chapter summarizes the developed work and presents an overall view of what was accomplished. Future goals and work guidelines are also described.

7.1 General conclusions

The work underlying this PhD rises from the need of new biodegradable packaging materials. This study was established on films formulation based on FucoPol, able to use in food industry, in order to face the environmental problem caused by the excessive use of synthetic plastics. This thesis intended to address the following scientific questions:

- Is it possible to produce films of FucoPol and which properties should be improved?
- Which strategies may be applied to improve their behaviour, specially their barrier properties to gases and water vapour?
- Is it possible to use FucoPol films as an alternative to synthetic plastics aiming their use as packaging material?

Firstly, to achieve these goals, a study of biodegradable polymers, in particular microbial polysaccharides used in food industry and their theoretical background and state-of-the-art, was carried out.

FucoPol, an exopolysaccharide produced by *Enterobacter* A47, grown in bioreactor, using glycerol by product from biodiesel industry as carbon source was chosen to develop this PhD work. Once this study had as main goal a positive contribution for the preservation of environment by the use of sustainable reagents, the application of low-cost products, create a distinctive starting point. As FucoPol is a bacterial EPS, each chapter was developed with polymer obtained from the same batch production, and different batch productions were used in the different chapters.

FucoPol films using citric acid as plasticizer were developed and characterized in Chapter 3. FucoPol films were transparent with brownish tone, able to cause colour alteration noticeable at human eye when applied at coloured surfaces. Those films were hydrophilic and soluble in water, which make them poor barriers to water vapour, however, presented good barrier properties to gases (oxygen and carbon dioxide) as reported for several others polysaccharide films. FucoPol films presented ductile mechanical properties.

Based on these results, and answer to the first scientific question, FucoPol films had potential application on food packaging, namely, as an inner layer in a multi-layered film, in order to protect the ductile behaviour and hydrophilic character and use their good barrier properties to gases.

In order to answer the second question and knowing that, FucoPol films show potential to be used in food packaging, the films characteristics can be improved. In that way two main strategies were used:

- Formulation of bilayer films with FucoPol and chitosan
- Use of different coating techniques in FucoPol films.

The obtained bilayer films of FucoPol and chitosan, described in Chapter 4 were homogeneous, flexible, resistant and transparent, and their application in coloured surfaces caused a decrease on the original colour saturation.

Bilyer films presented high swelling degree in contact with liquid water and a high water vapour permeability, which disables their used in applications that involve the direct contact with high moisture content products. Those films have shown excellent barrier properties to gases (O_2 and CO_2), better than some synthetic materials (LDPE and HDPE) and FucoPol stand-alone films. Once more, bilayer films characteristics presented good potential to be used for packaging of low moisture content products, such as nuts or to be used as alternative to some synthetic plastic materials from non-renewable sources currently used in multilayered films (EVOH).

In Chapter 5, a different approach was used, the application of diverse coating materials on FucoPol films surface.

Plasma deposition, LFS and ALD techniques were used to form inorganic coatings aiming the improvement of the surface and barrier properties, in particular, liquid water and moisture resistance.

FucoPol films presented dense and homogeneous surface with instant water contact angle of 95°. Films coated with Plasma deposition (PFH) have not shown significant improvement in the hydrophobic behaviour over the time, but with a coating of LFS (SiO_2)-Plasma (PFH) have shown a higher instant water contact angle (135°) caused by coating surface roughness, but this hydrophobic behavior was not stable over time. In contrast, FucoPol films coated with ALD (TiO_2) and ALD (TiO_2)-Plasma (PFH) exhibited stable water contact angle during time (90° and 115°, respectively).

Moreover, films coated with ALD (TiO_2) and ALD (TiO_2)-Plasma (PFH) have shown a decrease in water vapour permeability of 35 % and 36 % respectively and a decrease in oxygen permeability of 35 % and 67 % respectively, with slightly differences in transparency and colour alterations.

Having in account the studied characteristics, the combination of ALD (TiO_2) and Plasma (PFH) coatings in FucoPol films has demonstrated the best potential use in food packaging.

In order to answer the third question and close this thesis work, a proof of concept was developed in Chapter 6 using walnuts (*Juglans regia L.*).

To establish this work, the commercial film (PA/PE) performance was compared with that of bilayer films of FucoPol and chitosan. Bilayer films were chosen, once from the range of films developed and characterized during this PhD, presented the best characteristics and availability to test a real case study. Walnuts packaged with both films were also compared with walnuts without any package.

Tests with walnuts oil were executed previously to evaluate the conservation under accelerated conditions and obtain a fast lipid oxidation. A pronounced increase in peroxide values was noticed along the experiment, related with increase of oxygen content inside the package. Walnut oil does not present significant differences in peroxide values and oxidation compounds when packaged with bilayer or commercial film, but significantly higher values were detected for oil with no package.

Shelled walnut kernels were stored, at the same conditions to evaluate the rancid taste using a trained sensory panel. Minor differences in rancidity were detected for kernels packaged with both barrier materials, however significant differences for walnut with no package were detected and classified as moderate to rancid taste.

Overall, the protection effect of the biodegradable bilayer films was found similar to that of the non-biodegradable commercial ones, which makes them a promising sustainable alternative as packaging materials.

Figure 7.1 complements the questions raised in Chapter 1, showing their correlation with each study performed and consequent answers.

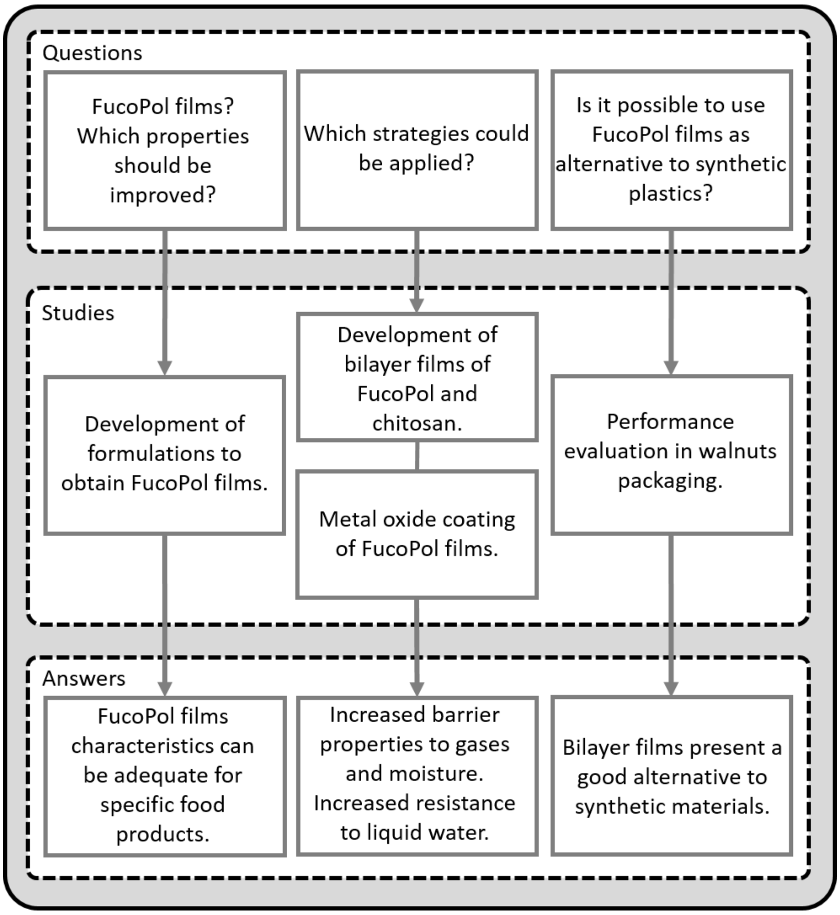


Figure 7.1: Illustration of the final conclusions of this work. The scientific questions, studies implemented and answers for initial questions

7.2 Future work

This PhD research leaves a few aspects unfinished and raises new questions for which additional research effort should be devoted in the future. Concerning that, three work guidelines should be considered for future improvements:

1. Scale up production of FucoPol films.

   This goal includes FucoPol production at large scale, which has already started by BPEG research group at Universidade NOVA de Lisboa. Production of films, it self, should be tested with roller systems. In the case of bilayer films, knife coating should be applied and characterization of films should be carried out to attest their good performance.

2. Further study of coating techniques.

   In this objective, it is intended to optimize the conditions previously tested, such as ALD thickness deposition and PFH quantity. And also to test some possible other metal oxides. The production of these coatings at large scale is also an important work, already being tested by SP Technical Institution of Sweden for other applications.

3. Study of different applications.

   One of the most thrilling aspect of this work is the application in case studies. So, the last path for future work is the application of bilayer films (or other) in food, such as, cheese or fruits.

BIBLIOGRAPHY

[1] H. Abdillahi, E. Chabrat, A. Rouilly, and L. Rigal. “Influence of citric acid on thermoplastic wheat flour/poly(lactic acid) blends. II. Barrier properties and water vapor sorption isotherms”. In: *Industrial Crops and Products* 50 (2013), pp. 104–111.

[2] M. N. Abdorreza, L. H. Cheng, and A. A. Karim. “Effects of plasticizers on thermal properties and heat sealability of sago starch films”. In: *Food Hydrocolloids* 25.1 (2011), pp. 56–60.

[3] H. Abramovič and C. Klofutar. “Water adsorption isotherms of some gellan gum samples”. In: *Journal of Food Engineering* 77.3 (2006), pp. 514–520.

[4] L. E. Abugoch, C. Tapia, M. C. Villamán, M. Yazdani-Pedram, and M. Díaz-Dosque. “Characterization of quinoa protein–chitosan blend edible films”. In: *Food Hydrocolloids* 25.5 (2011), pp. 879–886.

[5] M. Al-Bachir. “Quality characteristics of oil extracted from gamma irradiated peanut (*Arachis hypogea L.*)” In: *Radiation Physics and Chemistry* 106 (2015), pp. 56–60.

[6] V. D. Alves, N. Costa, and I. M. Coelhoso. “Barrier properties of biodegradable composite films based on kappa-carrageenan/pectin blends and mica flakes”. In: *Carbohydrate Polymers* 79.2 (2010), pp. 269–276.

[7] V. D. Alves, F. Freitas, C. A. V. Torres, M. Cruz, R. Marques, C. Grandfils, M. P. Gonçalves, R. Oliveira, and M. A. M. Reis. “Rheological and morphological characterization of the culture broth during exopolysaccharide production by Enterobacter sp”. In: *Carbohydrate Polymers* 81.4 (2010), pp. 758–764.

[8] V. D. Alves, R. Castelló, A. R. Ferreira, N. Costa, I. M. Fonseca, and I. M. Coelhoso. “Barrier properties of carrageenan/pectin biodegradable composite films”. In: *Procedia Food Science* 1 (2011), pp. 240 –245.

[9] V. D. Alves, A. R. Ferreira, N. Costa, F. Freitas, M. A. M. Reis, and I. M. Coelhoso. “Characterization of biodegradable films from the extracellular polysaccharide produced by Pseudomonas oleovorans grown on glycerol byproduct”. In: *Carbohydrate Polymers* 83.4 (2011), pp. 1582–1590.

[10] AmericanChemistryConcil. *Lifecycle of a Plastic Product*. Dec. 2015. URL: `http://plastics.americanchemistry.com/Life-Cycle#uses`.

[11] M. Avella, J. J. De Vlieger, M. E. Errico, S. Fischer, P. Vacca, and M. G. Volpe. “Biodegradable starch/clay nanocomposite films for food packaging applications”. In: *Food Chemistry* 93.3 (2005), pp. 467–474.

[12] L. Averous, L. Moro, P. Dole, and C. Fringant. “Properties of thermoplastic blends: starch–polycaprolactone”. In: *Polymer* 41.11 (2000), pp. 4157–4167.

[13] R. Babu, K. O’Connor, and R. Seeram. “Current progress on bio-based polymers and their future trends”. In: *Progress in Biomaterials* 2.1 (2013), p. 8.

[14] E. Bakkalbaşı, z. M. Yılmaz, I. Javidipour, and N. Artık. “Effects of packaging materials, storage conditions and variety on oxidative stability of shelled walnuts”. In: *LWT - Food Science and Technology* 46.1 (2012), pp. 203–209.

[15] C. Y. Barlow and D. C. Morgan. “Polymer film packaging for food: An environmental assessment”. In: *Resources, Conservation and Recycling* 78 (2013), pp. 74–80.

[16] K. Ben-Hassine, A. Taamalli, S. Ferchichi, A. Mlaouah, C. Benincasa, E. Romano, G. Flamini, A. Lazzez, N. Grati-kamoun, E. Perri, D. Malouche, and M. Hammami. “Physicochemical and sensory characteristics of virgin olive oils in relation to cultivar, extraction system and storage conditions”. In: *Food Research International* 54.2 (2013), pp. 1915–1925.

[17] S. L. S. Bico, M. F. J. Raposo, R. M. S. C. Morais, and A. M. M. B. Morais. “Combined effects of chemical dip and/or carrageenan coating and/or controlled atmosphere on quality of fresh-cut banana”. In: *Food Control* 20.5 (2009), pp. 508–514.

[18] N. Blanco-Pascual, M. P. Montero, and M. C. Gómez-Guillén. “Antioxidant film development from unrefined extracts of brown seaweeds Laminaria digitata and Ascophyllum nodosum”. In: *Food Hydrocolloids* 37 (2014), pp. 100–110.

[19] L. A. M. van den Broek, R. J. I. Knoop, F. H. J. Kappen, and C. G. Boeriu. “Chitosan films and blends for packaging material”. In: *Carbohydrate Polymers* 116 (2015), pp. 237–242.

[20] C. A. Campos, L. N. Gerschenson, and S. K. Flores. “Development of Edible Films and Coatings with Antimicrobial Activity”. In: *Food and Bioprocess Technology* 4.6 (2011), pp. 849–875.

[21] N. Cao, X. Yang, and Y. Fu. “Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films”. In: *Food Hydrocolloids* 23.3 (2009), pp. 729 –735.

[22] F. Caponio, M. T. Bilancia, A. Pasqualone, E. Sikorska, and T. Gomes. "Influence of the exposure to light on extra virgin olive oil quality during storage". In: *European Food Research and Technology* 221.1-2 (2005), pp. 92–98.

[23] A. B. D. Cassie and S. Baxter. "Wettability of porous surfaces". In: *Transactions of the Faraday Society* 40.0 (1944), pp. 546–551.

[24] CBI. *CBI Product Factsheet: Alginates in Europe*. Tech. rep. CBI Market Intelligence, 2015.

[25] M. A. Cerqueira, A. I. Bourbon, A. C. Pinheiro, J. T. Martins, B. W. S. Souza, J. A. Teixeira, and A. A. Vicente. "Galactomannans use in the development of edible films/coatings for food applications". In: *Trends in Food Science & Technology* 22.12 (2011), pp. 662–671.

[26] M. A. Cerqueira, l. M. Lima, B. W. S. Souza, J. A. Teixeira, R. A. Moreira, and A. A. Vicente. "Functional Polysaccharides as Edible Coatings for Cheese". In: *Journal of Agricultural and Food Chemistry* 57.4 (2009), pp. 1456–1462.

[27] M. A. Cerqueira, l. M. Lima, J. A. Teixeira, R. A. Moreira, and A. A. Vicente. "Suitability of novel galactomannans as edible coatings for tropical fruits". In: *Journal of Food Engineering* 94.3–4 (2009), pp. 372–378.

[28] M. A. Cerqueira, B. W. Souza, J. A. Teixeira, and A. A. Vicente. "Effect of glycerol and corn oil on physicochemical properties of polysaccharide films – A comparative study". In: *Food Hydrocolloids* 27.1 (2012), pp. 175 –184.

[29] C. Charton, N. Schiller, M. Fahland, A. Holländer, A. Wedel, and K. Noller. "Development of high barrier films on flexible polymer substrates". In: *Thin Solid Films* 502.1–2 (2006), pp. 99–103.

[30] K. C. Cheng, A. Demirci, and J. M. Catchmark. "Pullulan: biosynthesis, production, and applications". In: *Appl Microbiol Biotechnol* 92.1 (2011), pp. 29–44.

[31] n CHITOPACK. *n-CHITOPACK project - European R&D Project*. 2016. URL: `http://www.n-chitopack.eu/` (visited on 02/22/2016).

[32] I. Coelhoso, F. Freitas, V. D. Alves, and M. A. M. Reis. "Biodegradable Organic Matter". In: *Encyclopedia of Membranes*. Ed. by E. Drioli and L. Giorno. Springer Berlin Heidelberg, 2015. Chap. 52-1, pp. 1–2.

[33] R. Coles, D. McDowell, and M. Kirwan. *Food Packaging Technology*. Taylor & Francis, 2003.

[34] V. Coma. "Polysaccharide-based biomaterials with antimicrobial and antioxidant properties". In: *Polímeros* 23 (2013), pp. 287 –297.

[35] F. D. Conforti and J. A. Totty. "Effect of three lipid/hydrocolloid coatings on shelf life stability of Golden Delicious apples". In: *International Journal of Food Science & Technology* 42.9 (2007), pp. 1101–1106.

[36] K. Cooksey, K. S. Marsh, and L. H. Doar. “Predicting permeability & transmission rate for multilayer materials”. In: *Food Technology* 53.9 (1999), pp. 60–63.

[37] J. N. Coupland, N. B. Shaw, F. J. Monahan, E. Dolores O’Riordan, and M. O’Sullivan. “Modeling the effect of glycerol on the moisture sorption behavior of whey protein edible films”. In: *Journal of Food Engineering* 43.1 (2000), pp. 25–30.

[38] CPKelco. *KELCOGEL® gellan gum Book 5th Edition*. 2007. URL: `http://www.appliedbioscience.com/docs/Gellan_Book_5th_Edition.pdf`.

[39] G. H. Crapiste, M. I. V. Brevedan, and A. A. Carelli. “Oxidation of sunflower oil during storage”. In: *Journal of the American Oil Chemists’ Society* 76.12 (1999), pp. 1437–1443.

[40] M. Creatore, F. Palumbo, R. d’Agostino, and P. Fayet. “RF plasma deposition of SiO2-like films: plasma phase diagnostics and gas barrier film properties optimisation”. In: *Surface and Coatings Technology* 142–144 (2001), pp. 163–168.

[41] J. Credou and T. Berthelot. “Cellulose: from biocompatible to bioactive material”. In: *Journal of Materials Chemistry B* 2.30 (2014), pp. 4767–4788.

[42] M. Cruz-Romero and J. P. Kerry. “Crop-based biodegradable packaging and its environmental implications”. In: *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 3.074 (2008), pp. 1–25.

[43] Z. Cui, E. S. Beach, and P. T. Anastas. “Modification of chitosan films with environmentally benign reagents for increased water resistance”. In: *Green Chemistry Letters and Reviews* 4.1 (2011). Cui, Zheng Beach, Evan S. Anastas, Paul T., pp. 35–40.

[44] C. N. Cutter. “Opportunities for bio-based packaging technologies to improve the quality and safety of fresh and further processed muscle foods”. In: *Meat Science* 74.1 (2006), pp. 131 –142.

[45] G. De Filpo, A. M. Palermo, R. Munno, L. Molinaro, P. Formoso, and F. P. Nicoletta. “Gellan gum/titanium dioxide nanoparticle hybrid hydrogels for the cleaning and disinfection of parchment”. In: *International Biodeterioration & Biodegradation* 103 (2015), pp. 51–58.

[46] F. Debeaufort, J. A. Quezada-Gallo, and A. Voilley. “Edible films and coatings: Tomorrow’s packagings: A review”. In: *Critical Reviews in Food Science and Nutrition* 38.4 (1998), pp. 299–313.

[47] *Decreto-Lei n.o 106/2005 de 29 de Junho do Ministério da Agricultura, do Desenvolvimento Rural e das Pescas*. 2005.

[48] J. Duan, K. O. Reddy, B. Ashok, J. Cai, L. Zhang, and A. V. Rajulu. “Effects of spent tea leaf powder on the properties and functions of cellulose green composite films”. In: *Journal of Environmental Chemical Engineering* 4.1 (2016), pp. 440–448.

[49] P. Dutta, S. Tripathi, G. Mehrotra, and J. Dutta. "Perspectives for chitosan based antimicrobial films in food applications". In: *Food Chemistry* 114.4 (2009), pp. 1173 –1182.

[50] Eco-Go. *Eco Go products*. 2007. URL: `http://www.eco-go.net/products.php`.

[51] *Edible fats and oils. Ultraviolet absorption*. NP 970:1986-pt. Instituto Português da Qualidade. 1986, p. 13.

[52] M. Z. Elsabee and E. S. Abdou. "Chitosan based edible films and coatings: A review". In: *Materials Science and Engineering: C* 33.4 (2013), pp. 1819–1841.

[53] V. Epure, M. Griffon, E. Pollet, and L. Avérous. "Structure and properties of glycerol-plasticized chitosan obtained by mechanical kneading". In: *Carbohydrate Polymers* 83.2 (2011), pp. 947–952.

[54] A. G. Erlat, B. M. Henry, J. J. Ingram, D. B. Mountain, A. McGuigan, R. P. Howson, C. R. M. Grovenor, G. A. D. Briggs, and Y. Tsukahara. "Characterisation of aluminium oxynitride gas barrier films". In: *Thin Solid Films* 388.1–2 (2001), pp. 78–86.

[55] M. J. Fabra, O. Chambin, A. Voilley, J.-P. Gay, and F. Debeaufort. "Influence of temperature and NaCl on the release in aqueous liquid media of aroma compounds encapsulated in edible films". In: *Journal of Food Engineering* 108.1 (2012), pp. 30–36.

[56] M. J. Fabra, M. A. Busolo, A. Lopez-Rubio, and J. M. Lagaron. "Nanostructured biolayers in food packaging". In: *Trends in Food Science & Technology* 31.1 (2013), pp. 79 –87.

[57] P. Fajardo, J. T. Martins, C. Fuciños, L. Pastrana, J. A. Teixeira, and A. A. Vicente. "Evaluation of a chitosan-based edible film as carrier of natamycin to improve the storability of Saloio cheese". In: *Journal of Food Engineering* 101.4 (2010), pp. 349–356.

[58] FAO. *Global food losses and food waste – Extent, causes and prevention*. Tech. rep. 2011.

[59] S. Faria, C. L. de Oliveira Petkowicz, S. A. L. de Morais, M. G. H. Terrones, M. M. de Resende, F. P. de França, and V. L. Cardoso. "Characterization of xanthan gum produced from sugar cane broth". In: *Carbohydrate Polymers* 86.2 (2011), pp. 469–476.

[60] A. R. V. Ferreira, C. A. V. Torres, F. Freitas, M. A. M. Reis, V. D. Alves, and I. M. Coelhoso. "Biodegradable films produced from the bacterial polysaccharide FucoPol". In: *International Journal of Biological Macromolecules* 71 (2014), pp. 111–116.

[61] A. R. V. Ferreira, V. D. Alves, and I. M. Coelhoso. "Polysaccharide-Based Membranes in Food Packaging Applications". In: *Membranes* 6.2 (2016), pp. 1–17.

[62] A. R. Ferreira, C. A. Torres, F. Freitas, C. Sevrin, C. Grandfils, M. A. Reis, V. D. Alves, and I. M. Coelhoso. "Development and characterization of bilayer films of FucoPol and chitosan". In: *Carbohydrate Polymers* 147 (2016), pp. 8–15.

[63] C. O. Ferreira, C. A. Nunes, I. Delgadillo, and J. A. Lopes-da Silva. "Characterization of chitosan–whey protein films at acid pH". In: *Food Research International* 42.7 (2009), pp. 807–813.

[64] I. C. M. A. M. R. Filomena Freitas Vitor D. Alves. "Production and food applications of microbial biopolymers". In: *Engineering Aspects of Food Biotechnology*. Ed. by J. A. Teixeira and A. A. Vicente. CRC Press, 2013. Chap. 3.

[65] FMC. *Section 13 - Carrageenan By Dr. William A. Bubnis.* 2000.

[66] F. Freitas, V. D. Alves, and M. A. M. Reis. "Advances in bacterial exopolysaccharides: from production to biotechnological applications". In: *Trends in Biotechnology* 29.8 (2011), pp. 388–398.

[67] F. Freitas, V. D. Alves, C. A. V. Torres, M. Cruz, I. Sousa, M. J. Melo, A. M. Ramos, and M. A. M. Reis. "Fucose-containing exopolysaccharide produced by the newly isolated Enterobacter strain A47 DSM 23139". In: *Carbohydrate Polymers* 83.1 (2011), pp. 159–165.

[68] F. Freitas, V. D. Alves, A. R. Gouveia, C. Pinheiro, C. A. V. Torres, C. Grandfils, and M. A. M. Reis. "Controlled Production of Exopolysaccharides from Enterobacter A47 as a Function of Carbon Source with Demonstration of Their Film and Emulsifying Abilities". In: *Applied Biochemistry and Biotechnology* 172.2 (2013), pp. 641–657.

[69] F. Freitas, V. D. Alves, M. A. Reis, J. G. Crespo, and I. M. Coelhoso. "Microbial polysaccharide-based membranes: Current and future applications". In: *Journal of Applied Polymer Science* 131.6 (2014), pp. 40047–40058.

[70] F. Galgano. "Biodegradable packaging and edible coating for fresh-cut fruits and vegetables". In: *Italian Journal of Food Science* 27.1 (2015), p. 20.

[71] S. Galus and J. Kadzińska. "Food applications of emulsion-based edible films and coatings". In: *Trends in Food Science & Technology* 45.2 (2015), pp. 273–283.

[72] F. García-Ochoa, V. E. Santos, J. A. Casas, and E. Gómez. "Xanthan gum: production, recovery and properties". In: *Biotechnology Advances* 18.7 (2000), pp. 549–579.

[73] M. A. Garcia, A. Pinotti, and N. E. Zaritzky. "Physicochemical, Water Vapor Barrier and Mechanical Properties of Corn Starch and Chitosan Composite Films". In: *Starch - Stärke* 58.9 (2006), pp. 453–463.

[74] S Gaudin, D Lourdin, P. Forssell, and P Colonna. "Antiplasticisation and oxygen permeability of starch–sorbitol films". In: *Carbohydrate Polymers* 43.1 (2000), pp. 33 –37.

[75] S. M. George. "Atomic Layer Deposition: An Overview". In: *Chemical Reviews* 110.1 (2010), pp. 111–131.

[76] M. Gniewosz and A. Synowiec. "Antibacterial activity of pullulan films containing thymol". In: *Flavour and Fragrance Journal* 26.6 (2011), pp. 389–395.

[77] N. Gontard, R. Thibault, B. Cuq, and S. Guilbert. "Influence of relative humidity and film composition on oxygen and carbon dioxide permeabilities of edible films". In: *Journal of Agricultural and Food Chemistry* 44.4 (1996), pp. 1064–1069.

[78] GreenFacts. *Report Highlights - Trends of plastic waste in the EU*. Dec. 2015. URL: `http://www.greenfacts.org/highlights/2011/02/trends-of-plastic-waste-in-the-eu-environment-options-for-regulatory-initiatives/`.

[79] G.T.C.BioCorporation. *Gtc bio corporation - product show*. 2016. URL: `http://www.bestchitosan.com/e_products/`.

[80] C. H. Gu, J. J. Wang, Y. Yu, H. Sun, N. Shuai, and B. Wei. "Biodegradable multilayer barrier films based on alginate/polyethyleneimine and biaxially oriented poly(lactic acid)". In: *Carbohydrate Polymers* 92.2 (2013), pp. 1579–1585.

[81] H. C. Guo, E. Ye, Z. Li, M.-Y. Han, and X. J. Loh. "Recent progress of atomic layer deposition on polymeric materials". In: *Materials Science and Engineering: C* (2016). Article in press.

[82] A. P. Gupta and V. Kumar. "New emerging trends in synthetic biodegradable polymers – Polylactide: A critique". In: *European Polymer Journal* 43.10 (2007), pp. 4053–4074.

[83] A. Hambleton, M.-J. Fabra, F. Debeaufort, C. Dury-Brun, and A. Voilley. "Interface and aroma barrier properties of iota-carrageenan emulsion–based films used for encapsulation of active food compounds". In: *Journal of Food Engineering* 93.1 (2009), pp. 80–88.

[84] G. Heydari, M. Sedighi Moghaddam, M. Tuominen, M. Fielden, J. Haapanen, J. M. Mäkelä, and P. M. Claesson. "Wetting hysteresis induced by temperature changes: Supercooled water on hydrophobic surfaces". In: *Journal of Colloid and Interface Science* 468 (2016), pp. 21–33.

[85] T. Hirvikorpi. "Thin Al2O3 barrier coatings grown on bio-based packaging materials by atomic layer deposition". PhD thesis. Espoo, Finland, 2011, p. 74.

[86] T. Hirvikorpi, M. Vähä-Nissi, A. Harlin, and M. Karppinen. "Comparison of some coating techniques to fabricate barrier layers on packaging materials". In: *Thin Solid Films* 518.19 (2010), pp. 5463–5466.

[87] T. Hirvikorpi, M. Vähä-Nissi, A. Harlin, J. Marles, V. Miikkulainen, and M. Karppinen. "Effect of corona pre-treatment on the performance of gas barrier layers applied by atomic layer deposition onto polymer-coated paperboard". In: *Applied Surface Science* 257.3 (2010), pp. 736–740.

[88] T. Hirvikorpi, M. Vähä-Nissi, J. Nikkola, A. Harlin, and M. Karppinen. "Thin Al2O3 barrier coatings onto temperature-sensitive packaging materials by atomic layer deposition". In: *Surface and Coatings Technology* 205.21–22 (2011), pp. 5088–5092.

[89] N. M. Hromiš, V. L. Lazić, S. L. Markov, u. G. Vaštag, S. Z. Popović, D. Z. Šuput, N. R. Džinić, A. S. Velićanski, and L. M. Popović. "Optimization of chitosan biofilm properties by addition of caraway essential oil and beeswax". In: *Journal of Food Engineering* 158 (2015), pp. 86–93.

[90] innoviaFILMS. *Cellulose Film Attributes*. 2016. URL: `http://www.innoviafilms.com/Our-Products/Packaging/Cellulose-Film-Attributes.aspx` (visited on 02/12/2016).

[91] F. S. Isotton, G. L. Bernardo, C. Baldasso, L. M. Rosa, and M. Zeni. "The plasticizer effect on preparation and properties of etherified corn starchs films". In: *Industrial Crops and Products* 76 (2015), pp. 717–724.

[92] P. N. Jensen, G. Sørensen, P. Brockhoff, and G. Bertelsen. "Investigation of Packaging Systems for Shelled Walnuts Based on Oxygen Absorbers". In: *Journal of Agricultural and Food Chemistry* 51.17 (2003), pp. 4941–4947.

[93] JetNet. *Nutrafilm™*. 2016. URL: `http://www.jetnetcorp.com/nutrafilm.html` (visited on 02/12/2016).

[94] A. Jiménez, M. J. Fabra, P. Talens, and A. Chiralt. "Edible and Biodegradable Starch Films: A Review". In: *Food and Bioprocess Technology* 5.6 (2012), pp. 2058–2076.

[95] A. Jiménez, M. J. Fabra, P. Talens, and A. Chiralt. "Effect of sodium caseinate on properties and ageing behaviour of corn starch based films". In: *Food Hydrocolloids* 29.2 (2012), pp. 265–271.

[96] M. Jindal, V. Kumar, V. Rana, and A. K. Tiwary. "An insight into the properties of Aegle marmelos pectin–chitosan cross-linked films". In: *International Journal of Biological Macromolecules* 52 (2013), pp. 77–84.

[97] Jinmei. *PRODUCT NAME: PULLULAN*. 2016. URL: `http://www.jinmeibio.com/en/showproduct/?pone=38&id=44` (visited on 02/15/2016).

[98] H.-J. Kang, S.-J. Kim, Y.-S. You, M. Lacroix, and J. Han. "Inhibitory effect of soy protein coating formulations on walnut (*Juglans regia L.*) kernels against lipid oxidation". In: *LWT - Food Science and Technology* 51.1 (2013), pp. 393–396.

[99] P. Kanmani and S. T. Lim. "Development and characterization of novel probiotic - residing pullulan/starch edible films". In: *Food Chemistry* 141.2 (2013), pp. 1041–1049.

[100] M. Kemell, E. Färm, M. Ritala, and M. Leskelä. “Surface modification of thermoplastics by atomic layer deposition of Al2O3 and TiO2 thin films”. In: *European Polymer Journal* 44.11 (2008), pp. 3564–3570.

[101] G. Kerch. “Chitosan films and coatings prevent losses of fresh fruit nutritional quality: A review”. In: *Trends in Food Science & Technology* 46.2, Part A (2015), pp. 159–166.

[102] K. Khwaldia, E. Arab-Tehrany, and S. Desobry. “Biopolymer Coatings on Paper Packaging Materials”. In: *Comprehensive Reviews in Food Science and Food Safety* 9.1 (2010), pp. 82–91.

[103] J.-Y. Kim, Y.-G. Choi, S. R. Byul Kim, and S.-T. Lim. “Humidity stability of tapioca starch - pullulan composite films”. In: *Food Hydrocolloids* 41 (2014), pp. 140–145.

[104] D. M. Kirchmajer, B. Steinhoff, H. Warren, R. Clark, and M. in het Panhuis. “Enhanced gelation properties of purified gellan gum”. In: *Carbohydrate Research* 388 (2014), pp. 125–129.

[105] D. Kowalczyk. “Biopolymer/candelilla wax emulsion films as carriers of ascorbic acid – A comparative study”. In: *Food Hydrocolloids* 52 (2016), pp. 543–553.

[106] T. O. Kääriäinen, P. Maydannik, D. C. Cameron, K. Lahtinen, P. Johansson, and J. Kuusipalo. “Atomic layer deposition on polymer based flexible packaging materials: Growth characteristics and diffusion barrier properties”. In: *Thin Solid Films* 519.10 (2011), pp. 3146–3154.

[107] E. Kristo and C. G. Biliaderis. “Physical properties of starch nanocrystal - reinforced pullulan films”. In: *Carbohydrate Polymers* 68.1 (2007), pp. 146–158.

[108] M. Kurek, S. Galus, and F. Debeaufort. “Surface, mechanical and barrier properties of bio-based composite films based on chitosan and whey protein”. In: *Food Packaging and Shelf Life* 1.1 (2014), pp. 56–67.

[109] J. M. Lagaron, R. Catal´, and R. Gavara. “Structural characteristics defining high barrier properties in polymeric materials.” In: *Materials Science & Technology* 20.1 (2004), pp. 1 –7.

[110] H. Li, X. Gao, Y. Wang, X. Zhang, and Z. Tong. “Comparison of chitosan/starch composite film properties before and after cross-linking”. In: *International Journal of Biological Macromolecules* 52 (2013), pp. 275 –279.

[111] B. Ling, L. Hou, R. Li, and S. Wang. “Thermal treatment and storage condition effects on walnut paste quality associated with enzyme inactivation”. In: *LWT - Food Science and Technology* 59.2, Part 1 (2014), pp. 786–793.

[112] O. V. López and M. A. García. “Starch films from a novel (*Pachyrhizus ahipa*) and conventional sources: Development and characterization”. In: *Materials Science and Engineering: C* 32.7 (2012), pp. 1931 –1940.

[113] Y. Lu, Q. Sun, X. She, Y. Xia, Y. Liu, J. Li, and D. Yang. "Fabrication and characterisation of α - chitin nanofibers and highly transparent chitin films by pulsed ultrasonication". In: *Carbohydrate Polymers* 98.2 (2013), pp. 1497–1504.

[114] Y. Luo and Q. Wang. "Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery". In: *International Journal of Biological Macromolecules* 64 (2014), pp. 353–367.

[115] S. Mali, L. S. Sakanaka, F. Yamashita, and M. V. E. Grossmann. "Water sorption and mechanical properties of cassava starch films and their relation to plasticizing effect". In: *Carbohydrate Polymers* 60.3 (2005), pp. 283–289.

[116] S. Marais, Y. Hirata, C. Cabot, S. Morin-Grognet, M. R. Garda, H. Atmani, and F. Poncin-Epaillard. "Effect of a low-pressure plasma treatment on water vapor diffusivity and permeability of poly(ethylene-co-vinyl alcohol) and polyethylene films". In: *Surface and Coatings Technology* 201.3–4 (2006), pp. 868–879.

[117] M. Martínez, G. Barrionuevo, V. Nepote, N. Grosso, and D. Maestri. "Sensory characterisation and oxidative stability of walnut oil". In: *International Journal of Food Science & Technology* 46.6 (2011), pp. 1276–1281.

[118] M. L. Martínez, D. O. Labuckas, A. L. Lamarque, and D. M. Maestri. "Walnut (*Juglans regia L.*): genetic resources, chemistry, by-products". In: *Journal of the Science of Food and Agriculture* 90.12 (2010), pp. 1959–1967.

[119] M. L. Martínez, M. C. Penci, V. Ixtaina, P. D. Ribotta, and D. Maestri. "Effect of natural and synthetic antioxidants on the oxidative stability of walnut oil under different storage conditions". In: *LWT - Food Science and Technology* 51.1 (2013), pp. 44–50.

[120] J. I. Maté, M. E. Saltveit, and J. M. Krochta. "Peanut and Walnut Rancidity: Effects of Oxygen Concentration and Relative Humidity". In: *Journal of Food Science* 61.2 (1996), pp. 465–469.

[121] D. J. McHugh. "A guide to the seaweed industry - FAO fisheries technical paper 441". In: *FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS* Rome (2003), p. 105.

[122] G. Mensitieri, E. D. Maio, G. G. Buonocore, I. Nedi, M. Oliviero, L. Sansone, and S. Iannace. "Processing and shelf life issues of selected food packaging materials and structures from renewable resources". In: *Trends in Food Science & Technology* 22.2–3 (2011). New challenges in food preservation.Processing – Safety – SustainabilityEFFoST 2009 Annual Meeting, pp. 72 –80. ISSN: 0924-2244.

[123] S. F. Mexis, A. V. Badeka, K. A. Riganakos, K. X. Karakostas, and M. G. Kontominas. "Effect of packaging and storage conditions on quality of shelled walnuts". In: *Food Control* 20.8 (2009), pp. 743–751.

[124] R. Múgica-Vidal, F. Alba-Elías, E. Sainz-García, and M. Pantoja-Ruiz. "Hydrophobicity attainment and wear resistance enhancement on glass substrates by atmospheric plasma-polymerization of mixtures of an aminosilane and a fluorocarbon". In: *Applied Surface Science* 347 (2015), pp. 325–335.

[125] K. S. Mikkonen, H. Rita, H. Helén, R. A. Talja, L. Hyvönen, and M. Tenkanen. "Effect of Polysaccharide Structure on Mechanical and Thermal Properties of Galactomannan-Based Films". In: *Biomacromolecules* 8.10 (2007), pp. 3198–3205.

[126] D. C. Miller, R. R. Foster, Y. Zhang, S.-H. Jen, J. A. Bertrand, Z. Lu, D. Seghete, J. L. O'Patchen, R. Yang, Y.-C. Lee, S. M. George, and M. L. Dunn. "The mechanical robustness of atomic-layer- and molecular-layer-deposited coatings on polymer substrates". In: *Journal of Applied Physics* 105.9 (2009), pp. 093527–12.

[127] J. M. Mäkelä, H. Keskinen, T. Forsblom, and J. Keskinen. "Generation of metal and metal oxide nanoparticles by liquid flame spray process". In: *Journal of Materials Science* 39.8 (2004), pp. 2783–2788.

[128] J. M. Mäkelä, M. Aromaa, H. Teisala, M. Tuominen, M. Stepien, J. J. Saarinen, M. Toivakka, and J. Kuusipalo. "Nanoparticle Deposition from Liquid Flame Spray onto Moving Roll-to-Roll Paperboard Material". In: *Aerosol Science and Technology* 45.7 (2011), pp. 827–837.

[129] C. M. O. Müller, F. Yamashita, and J. B. Laurindo. "Evaluation of the effects of glycerol and sorbitol concentration and water activity on the water barrier properties of cassava starch films through a solubility approach". In: *Carbohydrate Polymers* 72.1 (2008), pp. 82–87.

[130] M. S. Moghaddam. "Wettability of modified wood". PhD thesis. Stockholm, 2015, p. 69.

[131] M. S. Moghaddam, G. Heydari, M. Tuominen, M. Fielden, J. Haapanen, J. M. Mäkelä, M. E. P. Wålinder, P. M. Claesson, and A. Swerin. "Hydrophobisation of wood surfaces by combining liquid flame spray (LFS) and plasma treatment: dynamic wetting properties". In: *Holzforschung: International Journal of the Biology, Chemistry, Physics & Technology of Wood* 70.6 (2016), pp. 527–537.

[132] K. K. Mokwena and J. Tang. "Ethylene Vinyl Alcohol: A Review of Barrier Properties for Packaging Shelf Stable Foods". In: *Critical Reviews in Food Science and Nutrition* 52.7 (2012), pp. 640–650.

[133] E. R. Morris, K. Nishinari, and M. Rinaudo. "Gelation of gellan – A review". In: *Food Hydrocolloids* 28.2 (2012), pp. 373–411.

[134] D. Muscat, B. Adhikari, R. Adhikari, and D. S. Chaudhary. "Comparative study of film forming behaviour of low and high amylose starches using glycerol and xylitol as plasticizers". In: *Journal of Food Engineering* 109.2 (2012), pp. 189–201.

[135] J. Necas and L. Bartosikova. “Carrageenan: a review”. In: *Veterinarni Medicina* 58 (4) (2013), pp. 187–205.

[136] NorwegianChitosan. *Products*. 2016. URL: `http://www.chitosan.no/?page_id=1266`.

[137] Novamont. *Mater-Bi ®*. 2016. URL: `http://www.novamont.com/eng/page.php?id_page=9&id_first=9`.

[138] F. Nowzari, B. Shábanpour, and S. M. Ojagh. “Comparison of chitosan–gelatin composite and bilayer coating and film effect on the quality of refrigerated rainbow trout”. In: *Food Chemistry* 141.3 (2013), pp. 1667–1672.

[139] S. M. Ojagh, M. Rezaei, S. H. Razavi, and S. M. H. Hosseini. “Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water”. In: *Food Chemistry* 122.1 (2010), pp. 161–166.

[140] G. I. Olivas and G. V. Barbosa-Cánovas. “Alginate–calcium films: Water vapor permeability and mechanical properties as affected by plasticizer and relative humidity”. In: *LWT - Food Science and Technology* 41.2 (2008), pp. 359–366.

[141] E. Olsson, C. Menzel, C. Johansson, R. Andersson, K. Koch, and L. Järnström. “The effect of pH on hydrolysis, cross-linking and barrier properties of starch barriers containing citric acid”. In: *Carbohydrate Polymers* 98.2 (2013), pp. 1505 –1513.

[142] R. Ortega-Toro, I. Morey, P. Talens, and A. Chiralt. “Active bilayer films of thermoplastic starch and polycaprolactone obtained by compression molding”. In: *Carbohydrate Polymers* 127 (2015), pp. 282–290.

[143] A. Palaniraj and V. Jayaraman. “Production, recovery and applications of xanthan gum by Xanthomonas campestris”. In: *Journal of Food Engineering* 106.1 (2011), pp. 1 –12.

[144] S. Y. Park, K. S. Marsh, and J. W. Rhim. “Characteristics of Different Molecular Weight Chitosan Films Affected by the Type of Organic Solvents”. In: *Journal of Food Science* 67.1 (2002), pp. 194–197.

[145] E. Parliament and C. Directive. *94/62/EC on Packaging and Packaging Waste, 1994 L0062 - EN - 05.04.2005 - 001.001*. 1994.

[146] S. D. Pasini Cabello, E. A. Takara, J. Marchese, and N. A. Ochoa. “Influence of plasticizers in pectin films: Microstructural changes”. In: *Materials Chemistry and Physics* 162 (2015), pp. 491–497.

[147] S. Pastorelli, L. Torri, A. Rodriguez, S. Valzacchi, S. Limbo, and C. Simoneau. “Solid-phase micro-extraction (SPME-GC) and sensors as rapid methods for monitoring lipid oxidation in nuts”. In: *Food Additives & Contaminants* 24.11 (2007), pp. 1219–1225.

[148] H. A. Pawar and K. G. Lalitha. “Isolation, purification and characterization of galactomannans as an excipient from Senna tora seeds”. In: *International Journal of Biological Macromolecules* 65 (2014), pp. 167–175.

[149] S. N. Pawar and K. J. Edgar. “Alginate derivatization: A review of chemistry, properties and applications”. In: *Biomaterials* 33.11 (2012), pp. 3279–3305.

[150] M. Pereda, A. G. Ponce, N. E. Marcovich, R. A. Ruseckaite, and J. F. Martucci. “Chitosan-gelatin composites and bi-layer films with potential antimicrobial activity”. In: *Food Hydrocolloids* 25.5 (2011), pp. 1372–1381.

[151] M. Pereda, M. I. Aranguren, and N. E. Marcovich. “Water Vapor Absorption and Permeability of Films Based on Chitosan and Sodium Caseinate”. In: *Journal of Applied Polymer Science* 111.6 (2009). Times Cited: 22, pp. 2777–2784.

[152] J. A. Piermaria, A. Pinotti, M. A. Garcia, and A. G. Abraham. “Films based on kefiran, an exopolysaccharide obtained from kefir grain: Development and characterization”. In: *Food Hydrocolloids* 23.3 (2009), pp. 684 –690.

[153] D. Plackett. “Introductory Overview”. In: *Biopolymers: New Materials for Sustainable Films and Coatings*. Ed. by J. W. Ltd and Sons. 2011, pp. 1 –14.

[154] Plantic. *Plantic™*. 2016. URL: `http://www.plantic.com.au/product/plantic-e`.

[155] PlasticPackaging. *Plastic packaging innovation. Safety. Sustainability*. Dec. 2015. URL: `http://www.plasticpackagingfacts.org/plastic-packaging`.

[156] PlasticsEurope. *Plastic Packaging: Born to protect*. Tech. rep. Plastics Europe - Association of plastics manufacturers, 2012.

[157] PlasticsEurope. *Packaging*. Dec. 2015. URL: `http://www.plasticseurope.org/use-of-plastics/packaging.aspx`.

[158] PlasticsEurope. *Plastics – the Facts 2014/2015*. Tech. rep. Plastics Europe - Association of plastics manufacturers, 2015.

[159] A. Plotto, J. A. Narciso, N. Rattanapanone, and E. A. Baldwin. “Surface treatments and coatings to maintain fresh-cut mango quality in storage”. In: *Journal of the Science of Food and Agriculture* 90.13 (2010), pp. 2333–2341.

[160] V. Pore, T. Kivela, M. Ritala, and M. Leskela. “Atomic layer deposition of photocatalytic TiO_2 thin films from TiF_4 and H_2O”. In: *Dalton Transactions* 45 (2008), pp. 6467–6474.

[161] V. D. Prajapati, G. K. Jani, and S. M. Khanda. “Pullulan: an exopolysaccharide and its various applications”. In: *Carbohydr Polym* 95.1 (2013), pp. 540–549.

[162] V. D. Prajapati, G. K. Jani, B. S. Zala, and T. A. Khutliwala. “An insight into the emerging exopolysaccharide gellan gum as a novel polymer”. In: *Carbohydrate Polymers* 93.2 (2013), pp. 670–678.

[163] V. D. Prajapati, G. K. Jani, N. G. Moradiya, N. P. Randeria, B. J. Nagar, N. N. Naikwadi, and B. C. Variya. "Galactomannan: A versatile biodegradable seed polysaccharide". In: *International Journal of Biological Macromolecules* 60 (2013), pp. 83–92.

[164] V. D. Prajapati, P. M. Maheriya, G. K. Jani, and H. K. Solanki. "Carrageenan: A natural seaweed polysaccharide and its applications". In: *Carbohydrate Polymers* 105 (2014), pp. 97–112.

[165] Primex. *Products - ChitoClear®*. 2016. URL: `http://www.primex.is/Products/`.

[166] M. Pykönen, K. Johansson, M. Dubreuil, D. Vangeneugden, G. Ström, P. Fardim, and M. Toivakka. "Evaluation of Plasma-Deposited Hydrophobic Coatings on Pigment-Coated Paper for Reduced Dampening Water Absorption". In: *Journal of Adhesion Science and Technology* 24.3 (2010), pp. 511–537.

[167] L. Quoc, D. Hoa, H. Ngoc, and T. Phi. "Effect of xanthan gum solution on the preservation of acerola". In: *Cercetări Agronomice în Moldova* XLVIII.3 (2015), pp. 89–97.

[168] M. N. V. Ravi Kumar. "A review of chitin and chitosan applications". In: *Reactive and Functional Polymers* 46.1 (2000), pp. 1–27.

[169] J.-W. Rhim, H.-M. Park, and C.-S. Ha. "Bio-nanocomposites for food packaging applications". In: *Progress in Polymer Science* 38.10–11 (2013), pp. 1629–1652.

[170] M. Rinaudo. "Chitin and chitosan: Properties and applications". In: *Progress in Polymer Science* 31.7 (2006), pp. 603–632.

[171] S. Rivero, M. A. García, and A. Pinotti. "Composite and bi-layer films based on gelatin and chitosan". In: *Journal of Food Engineering* 90.4 (2009), pp. 531–539.

[172] S. Rivero, M. García, and A. Pinotti. "Correlations between structural, barrier, thermal and mechanical properties of plasticized gelatin films". In: *Innovative Food Science & Emerging Technologies* 11.2 (2010), pp. 369 –375.

[173] G. Rocha Plácido Moore, S. Maria Martelli, C. Gandolfo, P. José do Amaral Sobral, and J. Borges Laurindo. "Influence of the glycerol concentration on some physical properties of feather keratin films". In: *Food Hydrocolloids* 20.7 (2006), pp. 975–982.

[174] S. Rosalam and R. England. "Review of xanthan gum production from unmodified starches by Xanthomonas comprestris sp." In: *Enzyme and Microbial Technology* 39.2 (2006), pp. 197 –207.

[175] E. Sainz-García, F. Alba-Elías, R. Múgica-Vidal, and M. Pantoja-Ruiz. "Promotion of tribological and hydrophobic properties of a coating on TPE substrates by atmospheric plasma-polymerization". In: *Applied Surface Science* 371 (2016), pp. 50–60.

[176] S. Salmieri, and M. Lacroix*. "Physicochemical Properties of Alginate/Polycaprolactone-Based Films Containing Essential Oils". In: *Journal of Agricultural and Food Chemistry* 54.26 (2006), pp. 10205–10214.

[177] S. Sanuja, A. Agalya, and M. J. Umapathy. "Synthesis and characterization of zinc oxide–neem oil–chitosan bionanocomposite for food packaging application". In: *International Journal of Biological Macromolecules* 74 (2015), pp. 76–84.

[178] M. Schmid, W. Zillinger, K. Müller, and S. Sängerlaub. "Permeation of water vapour, nitrogen, oxygen and carbon dioxide through whey protein isolate based films and coatings—Permselectivity and activation energy". In: *Food Packaging and Shelf Life* 6 (2015), pp. 21–29.

[179] *Sensory analysis d General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors*. ISO 8586:2012. International Organization for Standardization. 2012.

[180] S. Seyedi, A. Koocheki, M. Mohebbi, and Y. Zahedi. "Lepidium perfoliatum seed gum: A new source of carbohydrate to make a biodegradable film". In: *Carbohydrate Polymers* 101 (2014), pp. 349 –358.

[181] F. Shahidi and Y. Zhong. "Lipid Oxidation: Measurement Methods". In: *Bailey's Industrial Oil and Fat Products*. John Wiley & Sons, Inc., 2005.

[182] C. Shi, S. Zhang, M. Li, W. Sun, G. Fan, Y. Jin, J. Yang, and T. Dong. "Barrier and mechanical properties of biodegradable poly(ϵ-caprolactone)/cellophane multilayer film". In: *Journal of Applied Polymer Science* 130.3 (2013), pp. 1805–1811.

[183] F. F. Shih, K. W. Daigle, and E. T. Champagne. "Effect of rice wax on water vapour permeability and sorption properties of edible pullulan films". In: *Food Chemistry* 127.1 (2011), pp. 118–121.

[184] R. S. Singh, N. Kaur, and J. F. Kennedy. "Pullulan and pullulan derivatives as promising biomolecules for drug and gene targeting". In: *Carbohydrate Polymers* 123 (2015), pp. 190–207.

[185] V. Siracusa, P. Rocculi, S. Romani, and M. D. Rosa. "Biodegradable polymers for food packaging: a review". In: *Trends in Food Science & Technology* 19.12 (2008), pp. 634–643.

[186] J. H. Song, R. J. Murphy, R. Narayan, and G. B. H. Davies. "Biodegradable and compostable alternatives to conventional plastics". In: *Philosophical Transactions of the Royal Society B-Biological Sciences* 364.1526 (2009), pp. 2127–2139.

[187] A. C. Souza, R. Benze, E. S. Ferrão, C. Ditchfield, A. C. V. Coelho, and C. C. Tadini. "Cassava starch biodegradable films: Influence of glycerol and clay nanoparticles content on tensile and barrier properties and glass transition temperature". In: *LWT - Food Science and Technology* 46.1 (2012), pp. 110–117.

[188] P. C. Srinivasa and R. N. Tharanathan. "Chitin/Chitosan - Safe, Ecofriendly Packaging Materials with Multiple Potential Uses". In: *Food Reviews International* 23.1 (2007), pp. 53–72.

[189] K. Österberg, G. P. Savage, and D. L. McNeil. "Oxidative stability of walnuts during long term in shell storage". In: *IV International Walnut Symposium*. International Society for Horticultural Science (ISHS), Leuven, Belgium, 2001, pp. 591–597.

[190] S. Takeda, M. Fukawa, Y. Hayashi, and K. Matsumoto. "Surface OH group governing adsorption properties of metal oxide films". In: *Thin Solid Films* 339.1–2 (1999), pp. 220–224.

[191] E. Tavassoli-Kafrani, H. Shekarchizadeh, and M. Masoudpour-Behabadi. "Development of edible films and coatings from alginates and carrageenans". In: *Carbohydrate Polymers* 137 (2016), pp. 360–374.

[192] H. Teisala, M. Tuominen, M. Aromaa, J. M. Mäkelä, M. Stepien, J. J. Saarinen, M. Toivakka, and J. Kuusipalo. "Development of superhydrophobic coating on paperboard surface using the Liquid Flame Spray". In: *Surface and Coatings Technology* 205.2 (2010), pp. 436–445.

[193] H. Teisala, M. Tuominen, M. Aromaa, M. Stepien, J. M. Mäkelä, J. J. Saarinen, M. Toivakka, and J. Kuusipalo. "Nanostructures Increase Water Droplet Adhesion on Hierarchically Rough Superhydrophobic Surfaces". In: *Langmuir* 28.6 (2012), pp. 3138–3145.

[194] V. Teixeira, J. Carneiro, P. Carvalho, E. Silva, S. Azevedo, and C. Batista. "High Barrier Plastics Using Nanoscale Inorganic Films". In: *Multifunctional and Nanoreinforced Polymers for Food Packaging*. Ed. by J. Lagarón. 2011, pp. 285–315.

[195] V. Thakur and M. Thakur. *Handbook of Sustainable Polymers: Processing and Applications*. Pan Stanford Publishing, 2016.

[196] J. Tikkanen, K. A. Gross, C. C. Berndt, V. Pitkänen, J. Keskinen, S. Raghu, M. Rajala, and J. Karthikeyan. "Characteristics of the liquid flame spray process". In: *Surface and Coatings Technology* 90.3 (1997), pp. 210–216.

[197] E. O. Timmermann. "Multilayer sorption parameters: BET or GAB values?" In: *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 220.1–3 (2003), pp. 235–260.

[198] Q. Tong, Q. Xiao, and L.-T. Lim. "Preparation and properties of pullulan - alginate - carboxymethylcellulose blend films". In: *Food Research International* 41.10 (2008), pp. 1007–1014.

[199] C. A. V. Torres, R. Marques, S. Antunes, V. D. Alves, I. Sousa, A. M. Ramos, R. Oliveira, F. Freitas, and M. A. M. Reis. "Kinetics of production and characterization of the fucose-containing exopolysaccharide from Enterobacter A47". In: *Journal of Biotechnology* 156.4 (2011), pp. 261–267.

[200] C. A. V. Torres, R. Marques, A. R. V. Ferreira, S. Antunes, C. Grandfils, F. Freitas, and M. A. M. Reis. "Impact of glycerol and nitrogen concentration on Enterobacter A47 growth and exopolysaccharide production". In: *International Journal of Biological Macromolecules* 71 (2014), pp. 81–86.

[201] C. A. V. Torres, A. R. V. Ferreira, F. Freitas, M. A. M. Reis, I. Coelhoso, I. Sousa, and V. D. Alves. "Rheological studies of the fucose-rich exopolysaccharide FucoPol". In: *International Journal of Biological Macromolecules* 79 (2015), pp. 611–617.

[202] V. Trinetta. "Definition and Function of Food Packaging". In: *Reference Module in Food Science*. Elsevier, 2016.

[203] E. Trovatti, S. C. M. Fernandes, L. Rubatat, D. d. S. Perez, C. S. R. Freire, A. J. D. Silvestre, and C. P. Neto. "Pullulan - nanofibrillated cellulose composite films with improved thermal and mechanical properties". In: *Composites Science and Technology* 72.13 (2012), pp. 1556–1561.

[204] K. S. Tumwesigye, J. C. Oliveira, and M. J. Sousa-Gallagher. "New sustainable approach to reduce cassava borne environmental waste and develop biodegradable materials for food packaging applications". In: *Food Packaging and Shelf Life* 7 (2016), pp. 8–19.

[205] M. Tuominen, H. Teisala, J. Haapanen, J. M. Mäkelä, M. Honkanen, M. Vippola, S. Bardage, M. E. P. Wålinder, and A. Swerin. "Superamphiphobic overhang structured coating on a biobased material". In: *Applied Surface Science* 389 (2016), pp. 135–143.

[206] N. H. Ullsten and M. S. Hedenqvist. "A new test method based on head space analysis to determine permeability to oxygen and carbon dioxide of flexible packaging". In: *Polymer Testing* 22.3 (2003), pp. 291–295.

[207] M. Vargas, n. Perdones, A. Chiralt, M. Cháfer, and C. González-Martínez. "Effect of homogenization conditions on physicochemical properties of chitosan-based film-forming dispersions and films". In: *Food Hydrocolloids* 25.5 (2011), pp. 1158–1164.

[208] M. G. A. Vieira, M. A. da Silva, L. O. dos Santos, and M. M. Beppu. "Natural-based plasticizers and biopolymer films: A review". In: *European Polymer Journal* 47.3 (2011), pp. 254–263.

[209] C. H. T. Vu and K. Won. "Novel water-resistant UV-activated oxygen indicator for intelligent food packaging". In: *Food Chemistry* 140.1–2 (2013), pp. 52–56.

[210] Q. Wang, X. Wang, Z. Wang, J. Huang, and Y. Wang. "PVDF membranes with simultaneously enhanced permeability and selectivity by breaking the tradeoff effect via atomic layer deposition of TiO_2". In: *Journal of Membrane Science* 442 (2013), pp. 57–64.

[211] M. Wihodo and C. I. Moraru. "Physical and chemical methods used to enhance the structure and mechanical properties of protein films: A review". In: *Journal of Food Engineering* 114.3 (2013), pp. 292 –302.

[212] J. Wu, F. Zhong, Y. Li, C. F. Shoemaker, and W. Xia. "Preparation and characterization of pullulan - chitosan and pullulan - carboxymethyl chitosan blended films". In: *Food Hydrocolloids* 30.1 (2013), pp. 82–91.

[213] G. Xiao, Y. Zhu, L. Wang, Q. You, P. Huo, and Y. You. "Production and Storage of Edible Film Using Gellan Gum". In: *Procedia Environmental Sciences* 8 (2011), pp. 756–763.

[214] Y. Xiong, M. Lai, J. Li, H. Yong, H. Qian, C. Xu, K. Zhong, and S. Xiao. "Facile synthesis of ultra-smooth and transparent TiO2 thin films with superhydrophilicity". In: *Surface and Coatings Technology* 265 (2015), pp. 78–82.

[215] Y. X. Xu, K. M. Kim, M. A. Hanna, and D. Nag. "Chitosan–starch composite film: preparation and characterization". In: *Industrial Crops and Products* 21.2 (2005), pp. 185–192.

[216] C. Zhang, D. Gao, Y. Ma, and X. Zhao. "Effect of gelatin addition on properties of pullulan films". In: *J Food Sci* 78.6 (2013), pp. C805–10.

[217] L. Zwarts, G. P. Savage, and D. L. McNeil. "Fatty acid content of New Zealand-grown walnuts (Juglans regia L.)" In: *Int J Food Sci Nutr* 50.3 (1999), pp. 189–94.

APPENDIX A

LIST OF PUBLICATIONS

A.1 Articles in scientific journals with review and transfer of copyright

Ana R. V. Ferreira, Vítor D. Alves, Isabel M. Coelhoso; Polysaccharide-Based Membranes in Food Packaging Applications (Review); Membranes; 6 (2) (2016) 22.

Ana R. V. Ferreira, Cristiana A. V. Torres, Filomena Freitas, Christian Grandfils, Maria A. M. Reis, Vítor D. Alves, Isabel M. Coelhoso; Development and characterization of bilayer films of FucoPol and chitosan; Carbohydrate Polymers; 147 (2016) 8–15.

Ana R. V. Ferreira, Cristiana A. V. Torres, Filomena Freitas, Maria A. M. Reis, Vítor D. Alves, Isabel M. Coelhoso; Biodegradable films produced from the bacterial polysaccharide FucoPol; International Journal of Biological Macromolecules; 71 (2014) 111-116.

A.2 Manuscripts in submission in scientific journals with review and transfer of copyright

Ana R. V. Ferreira, Janne Haapanen, Jyrki M. Mäkelä, Jon E. Bratvold, Ola Nilsen, Mikko Tuominen, Vítor D. Alves, Isabel M. Coelhoso; Comparison of different coating techniques on the properties of FucoPol films; to be submitted to Surface and Coatings Technology.

Ana R. V. Ferreira, Margarida Moldão-Martins, Isabel M. Coelhoso, Vítor D. Alves; Evaluation of FucoPol and Chitosan bilayer films performance for packaging of walnuts (*Juglans regia L.*); to be submitted to LWT - Food Science and Technology.

A.3 Related articles in scientific journals with review and transfer of copyright

Cristiana A. V. Torres, **Ana R. V. Ferreira**, Filomena Freitas, Maria A. M. Reis, Isabel Coelhoso, Isabel Sousa, Vítor D. Alves; Rheological studies of the fucose-rich exopolysaccharide FucoPol; International Journal of Biological Macromolecules; 79 (2015) 611-617.

Isabel M. Coelhoso, **Ana Rita V. Ferreira**, Vitor D. Alves; Biodegradable Barrier Membranes Based on Nanoclays and Carrageenan/Pectin Blends; International Journal of Membrane Science and Technology, 1 (2014) 23-30.

Cristiana A. V. Torres, Rodolfo Marques, **Ana R. V. Ferreira**, Sílvia Antunes, Christian Grandfils, Filomena Freitas, Maria A. M. Reis; Impact of glycerol and nitrogen concentration on *Enterobacter* A47 growth and exopolysaccharide production; International Journal of Biological Macromolecules; 71 (2014) 81-86.

A.4 Oral communications

1. Ana R. V. Ferreira, Cristiana A. V. Torres, Filomena Freitas, Maria Reis, Vítor D. Alves, Isabel M. Coelhoso; Design of FucoPol/Chitosan films for food packaging applications; 2nd EuCheMS Congress on Green and Sustainable Chemistry (EuGSC); Lisboa – Portugal; October 4-7, 2015.

2. Ana R. V. Ferreira; Vitor D. Alves; Isabel M. Coelhoso; Bi-layered films of FucoPol and chitosan for food packaging applications; 3rd International Symposium International Meeting on Packaging Material/Bioproduct Interactions (MATBIM 2015); Zaragoza – Spain; June 17-19, 2015.

3. Ana R. V. Ferreira, Cristiana A. V. Torres, Filomena Freitas, Maria Reis, Vítor D. Alves, Isabel M. Coelhoso; Design of films for food applications based on the microbial polysaccharide FucoPol; XII ENCONTRO DE QUÍMICA DOS ALIMENTOS; Lisboa - Portugal; September 10-12, 2014.

4. Ana R. V. Ferreira, Cristiana A. V. Torres, Filomena Freitas, Maria Reis, Vítor D. Alves, Isabel M. Coelhoso; Preparation and characterization of microbial polysaccharide FucoPol films; 2nd international conference on Bio-based Polymers and Composites (BiPoCo2014); Visegrád - Hungary; August 24-28, 2014.

5. Ana R. V. Ferreira, Cristiana A. V. Torres, Filomena Freitas, Maria Reis, Vítor D. Alves, Isabel M. Coelhoso; Characterization of FucoPol films for food packaging; International Conference "Eco-sustainable Food Packaging Based on Polymer Nanomaterials"COST ACTION FA0904; Rome – Italy; February 26-28, 2014.

6. Ana R. V. Ferreira, Cristiana A. V. Torres, Filomena Freitas, Maria Reis, Vítor D. Alves and Isabel M. Coelhoso; Design of biodegradable films produced from a novel

microbial polysaccharide; Workshop EcoBioCap inserido no European Symposium on Biopolymers (ESBP2013); Lisboa – Portugal; October 9, 2013.

7. Cristiana A. V. Torres, Ana R. Ferreira, Elsa Bastos, Isabel Coelhoso, Maria A. M. Reis, Filomena Freitas, Isabel Sousa, Vítor D. Alves; FucoPol: a new polysaccharide for viscosity control and stabilization of water in oil emulsions; European Symposium on Biopolymers (ESBP2013); Lisboa – Portugal; October 7-9, 2013.

8. Ana R. V. Ferreira, Cristiana A. V. Torres, Filomena Freitas, Maria Reis, Vítor D. Alves, Isabel M. Coelhoso; Biodegradable films based on a new microbial polysaccharide for food applications; Engineering With Membranes (EWM 2013); Saint-Pierre d'Oléron – France; September 3-7, 2013.

9. Cristiana A. V. Torres, Ana R. Ferreira, Elsa Bastos, Isabel Coelhoso, Maria A. M. Reis, Filomena Freitas, Isabel Sousa, Vítor D. Alves; FucoPol as thickening agent and stabilizer of water in oil emulsions; Iberian Meeting on Rheology (IBEREO); Malaga – Spain; September 5-6, 2013.

10. Keynote: A. R. Ferreira, V. Alves, I. Coelhoso; Design of biodegradable films for food packaging; Food and Agriculture (FA) COST Action Fa 0904 – Eco-sustainable food packaging based on polymer nanomaterials, Marinha Grande – Portugal; July 4-5, 2013.

11. A. Rita Ferreira, Vítor D. Alves and Isabel M. Coelhoso; Design of edible coatings based on a new microbial polysaccharide for the improvement of food products quality; 5th Young Scientist Conference and 9th ECNP Short course on functional polymers; Prague – Check Republic; April 22-24, 2012.

12. Ana Rita Ferreira, Vitor D. Alves, Isabel M. Coelhoso; Design of biodegradable films using biomaterials from renewable resources; Network of Young Membrains 13 (NYM13) – Enschede – Holland; July 21-23, 2011.

A.5 Poster communications

1. Ana R. V. Ferreira, Cristiana A. V. Torres, Filomena Freitas, Maria Reis, Vítor D. Alves, Isabel M. Coelhoso; Biodegradable fucopol films for food packaging applications; 8th European Symposium on Biopolymers (ESBP 2015); Rome – Italy; September 16-18, 2015.

2. Ana Rita V. Ferreira; Janne Haapanen, Jyrki M. Mäkelä, Ola Nilsen, Vitor D. Alves; Isabel M. Coelhoso, Mikko Tuominen; New coating techniques used in FucoPol films; 3rd International Symposium International Meeting on Packaging Material / Bioproduct Interactions (MATBIM 2015); Zaragoza – Spain; June 17-19, 2015.

3. Ana R. V. Ferreira, Cristiana A. V. Torres, Filomena Freitas, Maria Reis, Vítor D. Alves and Isabel M. Coelhoso; Design of biodegradable films produced from a novel microbial polysaccharide; European Symposium on Biopolymers (ESBP2013); Lisboa – Portugal; October 7-9, 2013.

4. A.R. Ferreira, Cristiana A. V. Torres, Filomena Freitas, Maria Reis, Vítor D. Alves and Isabel M. Coelhoso; Biodegradable Film Blends Using a Novel Microbial Polysaccharide and Chitosan; 11th International Conference of the European Chitin Society (EUCHIS 2013); Porto – Portugal; May 5-8, 2013.

5. A. R. Ferreira, V. D. Alves, I. M. Coelhoso, Design of dense edible films and coatings using a new microbial polysaccharide, XXIX EMS Summer School on Membranes, Nancy – France, July 10-13, 2012.

6. A. Rita Ferreira, Filomena Freitas, M. Ascensão Reis, Vitor D. Alves, Isabel M. Coelhoso; Biodegradable films for packaging using a novel microbial polysaccharide obtained from renewable resources; 2nd International meeting on Material/Bioproduct Interaction (MATBIM 2012); Dijon – France; April 22 – 25 2012.

7. Ana Rita Ferreira, Vítor D. Alves, Isabel M. Coelhoso; Biodegradable films for food packaging from renewable resources; 11th International Chemical and Biological Engineering Conference (CHEMPOR 2011), Lisboa – Portugal, September 5-7, 2011.