DEVELOPMENT OF A NOVEL ACTIVE PACKAGING SYSTEM BASED ON LAYER-BY-LAYER DEPOSITION OF NATURAL ANTIOXIDANTS AND ANTIMICROBIALS TO EXTEND FOOD PRODUCTS' SHELF LIFE

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ABSTRACT

DEVELOPMENT OF A NOVEL ACTIVE PACKAGING SYSTEM BASED ON LAYER-BY-LAYER DEPOSITION OF NATURAL ANTIOXIDANTS AND ANTIMICROBIALS TO EXTEND FOOD PRODUCTS' SHELF LIFE

In recent years, Layer-by-Layer (LbL) assembly of thin films has been extensively investigated for many purposes, particularly for its functionality and simplicity of fabrication. Through LbL assembly; antioxidant agents have been incorporated into active packaging systems in different forms, mainly including sachets, physical adsorption on packaging material surface, multilayer films which contact with food packaging surface. In previous studies, active packaging system associated with LbL depositions were investigated, but no detailed investigation has been found about the application of controlled releasing-active packaging system on fresh-cut fruits. In this study, 20 bilayers of chitosan and alginate; respectively; were coated on amorphous polyethylene terephthalate (A-PET) sheets by dipping method. The LbL treated PET films were used for active packaging of fresh-cut peaches. Total soluble solids content and titratable acidity demonstrated that LbL active coating did not interfere with the natural postharvest behaviour. A lower weight loss was observed for LbL coated peach samples, suggesting that the presence of layer structure on PET strips could act as a barrier against fruit water loss. After 7 days of storage, the LbL treated samples have shown the best preservation of carotenoids, the lower PPO activity and higher phenolic index. Microbiological analysis has shown that all the microorganism types which were investigated were affected by the active packaging system. Sensory results were really encouraging, as well. As a conclusion, LbL coating has been proved for being a useful technique to produce controlled-release active packaging systems on fresh-cut fruits.

ÖZET

GIDA ÜRÜNLERİNİN RAF ÖMRÜNÜ UZATMAK İÇİN DOĞAL ANTİOKSİDANLARIN VE ANTİMİKROBİYELLERİN KATMANLI DEPOZİSYONUNA DAYALI YENİ AKTİF AMBALAJLAMA SİSTEMİNİN GELİŞTİRİLMESİ

Son yıllarda aktif bileşenlerin katmanlı depozisyonuyla ambalaj materyallerine kaplanması uygulama kolaylığı, fonksiyonelliği ve daha birçok sebep dolayısıyla araştırılmalara konu olmaktadır ve bu teknik katman katman kaplama (LbL) olarak anılmaktadır. Katmanlı depozisyon ile oluşturulan aktif ambalajlama sistemi sayesinde; antioksidan, antimikrobiyal, antifungal vb. doğal aktif bileşenler raf ömrü suresince gıdalarda meydana gelebilecek bozulmaları önlemektedir. Güncel çalışmalarda katmanlı depozisyon ile oluşturulan aktif ambalajlama sistemi çokça araştırılmış fakat aktif bileşenlerin kontrollü salınımı ile oluşturulan aktif ambalaj sistemlerinin taze kesilmiş meyvelere uygulanması üzerinde durulmamıştır. Bu çalışmada, aljinat (20 katman) ve kitosan (20 katman) sıra ile A-PET ambalaj materyaline daldırma yöntemiyle toplamda 40 katman olacak şekilde kaplanmıştır. Bu çok katmanlı kaplama sistemi aktif bileşenlerin gıda maddesine raf ömrü boyunca kademeli salınımı için uygulanmıştır. Taze dilimli şeftali örnekleri bu aktif ambalaj sistemi ile kaplanmıştır. Ağırlık kaybı analizi, titre edilebilir asitlik gibi analizler bu sistemin meyvenin su kaybını önlemek için koruyucu bir bariyer oluşturduğunu kanıtlamıştır. Raf ömrü sonunda bu sistemin mikrobiyolojik analizde araştırılan tüm mikroorganizma grupları üzerinde son derece etkili olduğu belirlenmiştir. Kimyasal, fiziksel ve mikrobiyolojik analizler duyusal analiz ile de doğrulanmış ve aktif ambalajlı şeftaliler panelistler tarafından görünüm ve renk bakımından daha yüksek skorlar almıştır. Sonuç olarak; katmanlı depozisyon sisteminin çok kullanışlı bir aktif ambalajlama sistemi olduğu ve şeftalilerin raf ömrünü uzatmaya katkı sağladığı kanıtlanmıştır.

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CHAPTER 1

INTRODUCTION

The interest for slightly processed, simply prepared and ready-to-eat 'fresh' food products is increasing rapidly. Consumers want to eat natural foods which do not include any synthetic ingredients, and are produced without using any technologies that have potential risks to human health and the environment (Gol et al., 2013). This situation generates a greater demand for products that retain stability and quality while having a longer shelf life. These facts result in the creation of major challenges for food safety and quality. Recently, innovative ways to inhibit microbial growth in the foods while maintaining quality, freshness, and safety have been investigated. One option is to use innovative packaging systems to provide safety, quality and longer shelf life (De Roever 1998, Devlieghere, et al. 2004). The expression food packaging refer to both the objects and activities related to the operation of food products packaging. The history of packaging, especially of food packaging started a long time ago, indeed we can suppose that humans were hiding food before they learned how to cook and transform it (Piergiovanni et al., 2009). In this way, food packages were basically used to provide barrier and protective functions to protect the food stuff against the physical and environmental damages (Han, 2000). During the last decades, the food industries have seen several changes in packaging technology and applications, due to the new consumer demands and market trends. These requests can be summarized in high quality, freshness and extended shelf life of food products, coupled to the use of handling and resistant packaging made with lighter, cheaper and recyclable materials (Galdi, 2006). Packaging is also used as a way to communicate and interact with consumers: the shape of a pack, its colour and appearance can contribute to commercial success of that product and it is possible to report some useful information on the packaging (nutritional information, advice for use, recipes). Another important function of food packaging is related to the convenience that it can offer: as an example; the attitude to be applied in a microwave, easy opening devices and many more. Lastly, packaging solutions are also important for logistic consideration, in fact through the optimisation of packaging, according to the logistic needs, it's possible to save money

and shorten the time of delivering. All of that primary functions are summed in a lot of different types of packaging, that involved different traditional and innovative materials and technologies (such as high barrier materials, aseptic packaging, vacuum packaging, etc.) Recently new packaging functionalities emerged from consumers and food producer demands, connected to lifestyle change, retailing practice and an increased attention to food quality, safety and environmental impact (Majid et al., 2016). Therefore, food packaging started to have not only an inertial and passive role but also an active function on the food it encloses or on the surrounding environment to offer new or better functionalities. In addition to these aspects, there have been a number of specific packages that have both created new food categories and changed the way that we can deliver a product to the consumer (Limbo, 2016)

CHAPTER 2

LITERATURE REVIEW

2.1. General Aspect of Fresh-Cut Fruit Storage

"Fresh-cut produce" is defined as any fresh fruit or vegetable or their combination that has been physically altered from its original form, but remains in a fresh state. Regardless of commodity, it has been trimmed, peeled, washed and cut into 100% usable product that is subsequently bagged or pre-packed to offer consumers high nutrition, convenience and value while still maintaining freshness (IFPA, 2003)

The market sales of ready-to-use fresh vegetable have grown rapidly in the past decades as a result of changing in consumer habits. These types of products are satisfying consumer demands for fresh and healthy food while offering also convenience in quick preparation meal (Soliva - Fortuny et al., 2003).

The problem for this kind of products is their vulnerability. When fruits are cut, several alterations can appear: enzymatic browning, microbial spoilage, loss of weight and firmness, degradation of nutraceutical and organoleptic properties.

After cutting, immediate physical effects occur, such as mechanical shock of tissue, removal of protective epidermal layer or cell fluids on cut surface. Enzymatic reactions can happen due to the deterioration of the cellular structure (Saltveit, 2003).

Phenolic compounds, present into the vacuole, in contact with polyphenol oxidase (PPO), inside the plastids, react and produce coloured polymers (Figure 2.1); this is the onset of enzymatic browning. Enzymatic browning process consist of two parts, i.e. the hydroxylation of phenols and following oxidation that causes the formation of o-quinones, brown pigments, by PPO in the presence of oxygen (Toivonen & Brummel, 2008). Sensitivity of the fruit also makes it exposed to microbial and/or chemical contaminants. After that, several physiological effects take place and there is an increase of the respiration rate, in comparison with the integer fruit (Watada, 1996).

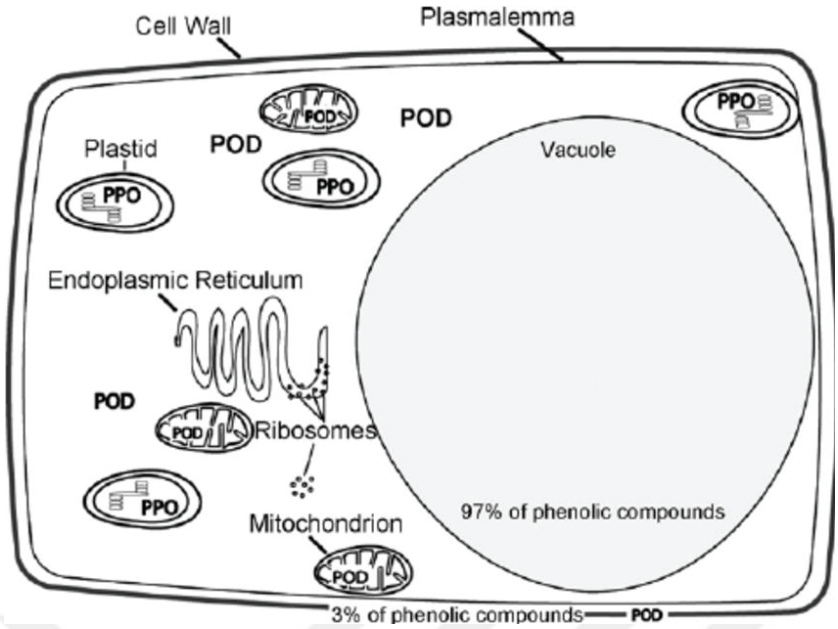


Figure 2. 1. The localization of phenolic compounds and phenolic oxidizing enzymes (PPO: polyphenol oxidase; POD: phenol peroxidase). (Toivonen & Brummel, 2008).

Also, the ethylene production rate is altered and there is an increase of other biochemical reactions (discoloration and colour, texture, aroma and flavour, nutritional quality, etc.) (Cantwell, 1999). Preservation of chlorophyll in vegetables, red to purple anthocyanins, yellow, orange and red carotenoids in both fruit and vegetables is of vital importance to maintain quality (Garcia & Barrett, 2002).

Consumers take product appearance into consideration as a primary criterion, and colour is probably the main factor considered (Kays, 1999), for this reason in the most industrialized areas of the world (Europe, North America, part of Asia) a huge amount of fresh product waste is present (FAO, 2011) For this reason, several solutions are put in place to avoid or limits fresh-cut decay: acidulants, chelating, complexing and reducing agents, edible coatings, antimicrobial compounds, high pressure, pulsed light, reducing temperature, heat treatments, etc. (Bhat, Alias, & Paliyath, 2012).

2.2. Packaging Solutions

2.2.1. Traditional Food Packaging

Traditional food packaging is meant for mechanical supporting of otherwise non-solid food, and protecting food from external influences, like microorganisms, oxygen, off-odours, light etc. and, by doing so, guaranteeing convenience in food handling and preserving the food quality for an extended time period. Packaging functions could be classified into 5 categories: containment, protection, communication, convenience, and logistic. Containment function of packaging is sometimes underestimated, but it is still very important especially for free-flowing products (liquid or pulverous), that don't have their own shape and they need to be contained in every phase of their production, storage, and distribution. The key safety objective for these traditional materials in contact with foods is to be as inert as possible, i.e., there should be a minimum of interaction between food and packaging (Dainelli et al., 2008).

2.2.2. Package Developments

In addition to broad developments in materials, there have been a number of specific packages that have both created new food categories and changed the way that we can deliver a product to the consumer. Metal cans, now typically made of tin-plated steel, have been in use since the early 1800s. It was not until the 1950s that aluminium cans were first manufactured and used. Today, aluminium cans are very widely used, particularly for carbonated beverages. The first aluminium cans were opened with a can opener, similar to the way other metal cans are opened. The first ring pull was introduced in 1963. This facilitated opening a can and being able to drink directly from it. The first ring pulls were not attached to the can and caused a concern that someone could choke on them. It was not until 1975 that what is called the stay tab was introduced, which is a ring tab that stays attached to the can. Another package widely used by the carbonated beverage industry is the 2 L plastic beverage bottle made of polyethylene terephthalate (PET). The concept for the bottle was introduced by Pepsi in 1970, with a patent on the bottle issued in 1973. It is interesting to note that this is one of the few packages in the United States that uses a metric size as its standard. The challenge in using PET is that it must provide a barrier to both carbon dioxide and flavours while not contaminating the product with components of the PET that can migrate from the package to the product. Acetaldehyde is one residual component that can be present in PET and can create undesirable flavours in the product if it is not

closely controlled. The challenge for smaller bottles was that the carbonation would be lost via permeation through the PET as a smaller bottle has a larger surface to volume ratio. Smaller bottles are in use today but most of these are either multilayer or have a coating to add the barrier needed (Risch, 2009).

2.2.3. Defining Active Packaging and Intelligent Packaging

To understand what active and intelligent packaging have to offer the world of packaging, it is important to clarify what each phrase means. Active packaging is accurately defined as "packaging in which subsidiary constituents have been deliberately included in or on either the packaging material or the package headspace to enhance the performance of the package system" (Robertson, 2006). This phrase emphasizes the importance of deliberately including a substance with the intention of enhancing the food product. Active packaging is an extension of the protection function of a package and is commonly used to protect against oxygen and moisture (Huff, 2008).

Intelligent packaging can be defined as "packaging that contains an external or internal indicator to provide information about aspects of the history of the package and/or the quality of the food" (Robertson, 2006). Intelligent packaging is an extension of the communication function of traditional packaging, and communicates information to the consumer based on its ability to sense, detect, or record external or internal changes in the product's environment (Huff, 2008).

Figure 2. 2. Traditional and innovative functionalities of food packaging (Maksimović et al., 2015).

2.2.4. Active Packaging

Active packaging is defined as a package system that deliberately incorporates components that release or absorb substances into or from the packaged food or the environment surrounding, to extend the shelf life or to maintain or improve the condition of food (Regulation (CE) No. 450/2009 (29/05/2009)). Many different active agents can be incorporated into packaging includes organic acids, enzymes, bacteriocin, fungicides, natural extracts, ions and ethanol as well as different materials can be used to include them, e.g. papers, plastics, metals or mixtures of these materials (Dainelli et al., 2008). Therefore, up to now a lot of different types of active packaging solutions have been developed despite of a small commercial application related to the lack of a specific regulation until 2009 (when Regulation CE No. 450/2009 was enacted) and also to the difficulty of finding cheap and easy solutions. Anyway, various research studies in this area are in progress because the global market of active packaging is increasing and it represents an innovative and effective way to improve food shelf life and reduce food waste, a big and controversial problem of our society. The most important types of current active packaging solutions can be classified into two categories: the one; removing undesirable substances and the other one; releasing desirable substances (Labuza & Breene, 1989).

2.2.5. Removal of Undesirable Substances

This functionality can be exercised through different methods: by making the packaging materials able to absorb undesirable substances itself or by introducing an accessory component responsible for the absorption activity within the package (usually a small sachet, or an active label). To achieve this goal physical or physicochemical absorption can be used, as well as chemical reactions that transform undesirable substances into harmless ones. The substances that need to be removed are often gaseous like water vapour, ethylene, oxygen, carbon dioxide, and volatile substances (Ahvenainen & Hurme, 1997).

The texture of food products can be affected by humidity; to remove water vapour it's possible to apply some common substances like silica gel or calcium chloride that are usually collocated into little permeable sachet inside the pack (Galdi,

2006). In addition to this solution for humidity control in packaged dried foods, several companies manufacture moisture absorbent pads for liquid water control in high a_w foods such as meats, fish, poultry, fruit and vegetables. This kind of pads are usually made of two layers of water permeable plastic films (such as PVOH) between which there is a superabsorbent substance, able to absorb more water than its own weight (Ahvenainen & Hurme, 1997). Typical superabsorbent polymers include polyacrylate salts, carboxymethyl cellulose (CMC) and starch copolymers which have a very strong affinity for water. Another possibility to remove exudates from high a_w food is the application of expanded materials that can collect liquids inside their structure (Galdi, 2006).

Ethylene-removing active packaging system are developed to slow down the over- ripening process of fruit and vegetables. Indeed, ethylene is a plant hormone that promotes the respiration rate and senescence of fruits, vegetables, and flowers (Saltveit, 1999) Different substances, such as active carbons and silica gels are good absorbers of ethylene and are usually incorporated into plastic film although this leads to their partially deactivation. Ethylene scavenger substances like potassium permanganate can also be used: it removes ethylene in storage spaces through an oxidation reaction that breakdown ethylene's double bond (Ozdemir & Floros, 2004).

Carbon dioxide scavengers and absorbers are used in active packaging to prevent the instauration of anaerobic metabolism in packed vegetables or the undesirable swelling of toasted product packs (like for example coffee pack). For these purposes calcium or sodium hydroxide can be used as it reacts with carbon dioxides to produce carbonate and water (Galdi, 2006).

Other active packaging systems are designed to remove undesirable volatile substances that come from food degradation, like hexanal and others bad-smelling lipid oxidation products. Different solutions have been developed to solve this problem. Examples are the synthesis of plastic polymers with functional groups able to react with undesirable aldehydes and the incorporation of aluminium oxides into plastic film to reduce the sensorial impact of volatile amines (Lopez- Rubio et al., 2004). Anyway the use of this active packaging solutions is controversial as it can hide some sensorial warnings often used by consumer to differentiate between fresh and safe products from altered ones (Day, 2008).

Oxygen scavengers are by far the most commercially used type of active packaging and the market has been steadily growing over the last several years. Indeed,

oxygen is involved in many different processes, like chemical and enzymatic oxidation reactions, degradation of pigments and aromas, aerobic respiration and also microorganism proliferation, whose kinetics is appositively affected by oxygen concentration; therefore, every solution able to reduce oxygen amount inside the pack leads to an improved food shelf life. Ferrous oxide is the most commonly used oxygen scavenger (Galdi, 2006).

Alternatively, non-metallic oxygen scavengers have also been developed to avoid the potential contamination of food with metals. Non-metallic scavengers include organic reducing agents such as ascorbic acid, ascorbate salts or catechol and enzymes like glucose oxidase or ethanol oxidase which can be incorporated into sachets, adhesive labels or immobilised onto packaging film surfaces (Robertson, 2006).

Oxygen scavengers can be applied alone or in combination with MAP technology (modified atmosphere packaging that is based on the modification of food surrounding gas composition in order to enhance perishable food preservation). If adequately designed, they can be used alone, eliminating the need for MAP machinery, thus increasing packaging operation speeds. However, it is more common commercially to remove most of the atmospheric oxygen by MAP and then use a relatively small and inexpensive scavenger to eliminate the residual oxygen within the food package (Day, 2003; Robertson, 2006).

2.2.6. Release of Desirable Substances

Some useful flavouring agents and other important substances for food preservation such as antioxidants and antimicrobials can be incorporated into packaging systems to be released into food products during their storage instead being used in food formulations as ingredients or additives (Piergiovanni et al., 2012). This technique allows the release of active substances in the correct amount, gradually during storage time and in the place where they are more useful.

Antimicrobial active packaging can be achieved in different way: by including pads or sachets containing volatile antimicrobial agents into packages, by incorporating antimicrobial agents directly into packaging polymers and by using polymers that are antimicrobial by themselves (Kapetanakou, & Skandamis, 2016). A lot of antimicrobial substances with different stability, chemical and antimicrobial properties are used in

these types of active packaging solutions. The most commonly applied antimicrobials are: volatile substances such as natural essential oils, ethanol, sulphur dioxide, chlorine dioxide and non-volatile substances such as enzymes, bacteriocin, silver ions, and organic acid. Nowadays antimicrobial active packaging research is focused on the development and optimization of controlled release systems. Indeed, several studies have proved that a gradual and continues release of antimicrobial substances into food is more effective than one-time addition because that avoid the microbial adaptation phenomena (Zhang et al., 2004).

Non-volatile substances are gradually released through a migration process controlled by diffusion coefficients of the antimicrobials into the packaging and by its solubility into the packed food (Bhunia et al., 2013) For volatile substances, the migration process is also influenced by temperature and sometimes by vapour pressure (in this case a direct contact with food is not necessary). Non-volatile antimicrobial substances can be incorporated into packaging materials during the extrusion phase, adding them just as they are or as master batch (polymer granules with high concentrations of the active substance to improve its dosage and distribution) (Kapetanakou, & Skandamis, 2016).

An alternative technique which is useful especially for thermal-sensitive active substances is to deposit them as a coating on the packaging surface. Recently, Gherardi (2016) have also proposed the incorporations of active substances into the adhesive layers that join different laminating materials, avoiding substances thermal degradation and high production cost.

In opposition volatile substances are absorbed on a solid support and then collocated into a very permeable, or with micro-holes' sachet, inside the package. Moreover, a microencapsulation technique was also tested: active compounds are trapped into microcapsule made of cyclodextrins or synthetic resins and then added to packaging materials during production phase or deposited on them as a coating (Xu, 2015).

Microbial growth and oxidation phenomena are the main causes of food spoilage so antioxidants are also often used alone or in combination with antimicrobials in active food packaging. First-line efforts to retard lipid oxidation usually involve addition of antioxidants, most commonly as ingredients in food formulations (hereafter referred to as instant addition). In this approach, antioxidants are consumed as lipid oxidation progresses, and inhibition ends when all the antioxidants react. However, it is not

always possible to add sufficient antioxidants to stabilize food products over long periods due to legal limits on antioxidant concentrations as well as to conversion of antioxidants to pro-oxidants at high concentrations (Zhu et al, 2012). Therefore, antioxidants are incorporated or coated on a packaging material with the purpose to be delivered at the food surface during commercialization at an appropriate rate. Both primary (free radical scavenging) and secondary (chelators, UV absorbers, oxygen scavengers, and singlet oxygen quenchers) antioxidants can be used in these applications (Galdi, 2006). An example of an efficient antioxidant substance is BHT (Butylated hydroxyl toluene), a free radical scavenger, which is incorporated into polyolefin to prevent their alteration as well as to avoid food oxidation through BHT gradual release. Natural substances are also currently applied, such as α-tocopherol that can be easily incorporated into plastic film during the extrusion, thanks to its high thermal stability (Kaplan & Singh, 2003).

Packaging material surfaces may also be coated with antioxidant substances and, in this case a support material is usually required as a vehicle to carry active agents onto packaging surfaces (Tian et al., 2013). (Figure 2.3)

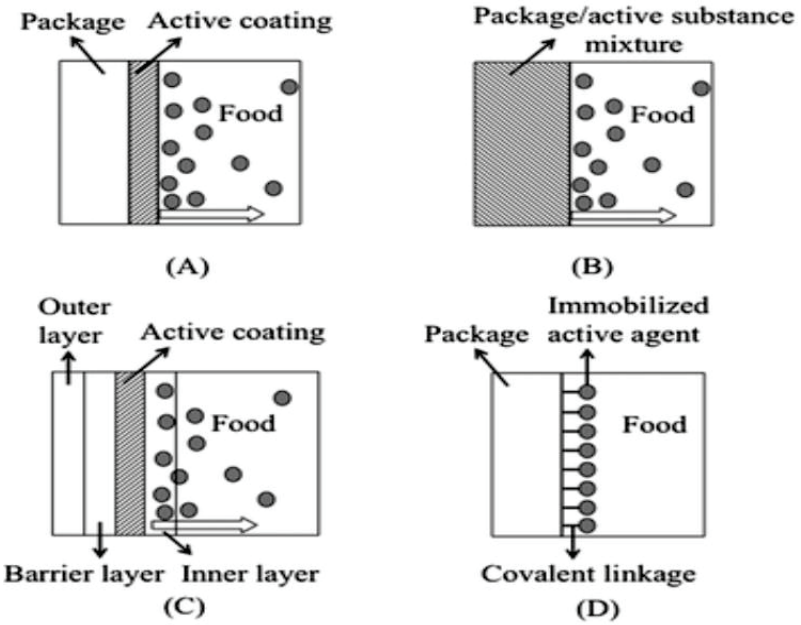


Figure 2. 3. Different application forms of antioxidant packaging systems and the migration of antioxidant agent in each system: (A) coating an active layer to the package surface; (B) incorporating active agent into the package polymeric matrix; (C) multilayer active film; and (D) covalently immobilized active package. (Tian et al, 2013).

2.3. Depositions of Coating on Plastic Materials

The term coating is generally used to refer to a procedure that leads to the deposition of a thin layer of a fluid or melted material on a support surface which is often represented by a plastic film, but it may also be a paper foil, a metallic or a glass surface. Coating deposition is commonly used to improve performances of food packaging materials like barrier, mechanical and surface properties and also to add active substances on packaging surfaces in order to obtain active materials. Usually the thickness of the coating materials are between 0.5 and 15 μm (Silvestre et al., 2011) Traditional approaches to coat plastic materials are the deposition of substances dissolved in water or in an organic solvent followed by the evaporation of the solvent or the deposition of a thicker layer of a melted polymer with a special technique which is known as extrusion coating (Diaio et al., 2014). In the first case, support surface energy, as well as continuity and resistance of the deposited materials after solvent evaporation, are crucial. In the second case, the melted material has to be fluid and able to stick to the support. An innovative approach for coating deposition is the Layer-by-Layer (LbL) technique. This method was developed starting from Idler's studies (1966) on colloidal particles and it became interesting since 1990, when Decher et al. (1997), demonstrated polyelectrolyte can be used in this technique.

2.3.1. Layer-by-Layer (LbL) Coating Technique

A spontaneous absorption of a polyelectrolyte on an opposite-charged surface is the basis of layer-by-layer coating technique and it offers an easy way for multilayer formation, allowing a variety of materials to be incorporated within the film structures (Ariga et al., 2007). LbL coating procedure, shown in Figure 2.4, requires a surface-charged support and involves several deposition steps. For example; a negative charged plastic film is dipped into a polycationic solution in order to obtain an absorption of polycations on the plastic surface, through a charge overcompensation process that leads to a positively charged surface. Plastic support is then washed to remove unlinked polycations and subsequently dipped into a polyanionic solution, producing another absorption and the reversal charge of surface. After another washing treatment, a bilayer

coated support is obtained. Consequential repetition of that procedure leads to a higher layer numbers (Ariga et al., 2007).

One of the most important advantages of the LbL technique is its simplicity since beakers and tweezer are the only apparatus required. Also the variability of the applicable materials, and number of layers are prominent advantages (Ariga & Hill, 2007).

Despite of the simple procedure required, LbL assembly mechanisms are not yet fully understood. Electrostatic interactions between opposite charges were first identified as the main driving force of LbL assembly, but subsequent studies have demonstrated that other different physicochemical interactions, such as hydrogen bonds and hydrophobic interactions, may contribute to layer formations. Thermodynamics also play a big role on LbL assembly (Klitzing, 2006).

Different multilayer structures and thicknesses can be achieved by changing the type of polyelectrolytes and solution conditions. For weakly charged polyelectrolytes, a sharp maximum in film thickness at intermediate charge density was observed (Yoo et al., 1998). This is probably because higher density charge, related to extreme pH values of the polyelectrolyte solutions (very low pH for polycations and very high for polyanions), produce higher repulsion between polymer chains which leads to more flat structures and lower thickness of the deposited layers. Extreme pH values of polyelectrolyte solutions can also produce a neutralisation of the coated surface when it is dipped into the next solution.

The presence of coexisting electrolyte species can also affect the layer thickness, in fact, chaotropic anions are able to produce a larger thickness and a stronger roughness of the absorbed layer, due to the partial screening of charges along the polyelectrolyte chains, allowing polymer to assume a coil conformation.

Another important effect is determined by the type of polyelectrolytes: it seems that a certain degree of hydrophobicity produce a stronger increase in multilayer thickness (Klitzing, 2006).

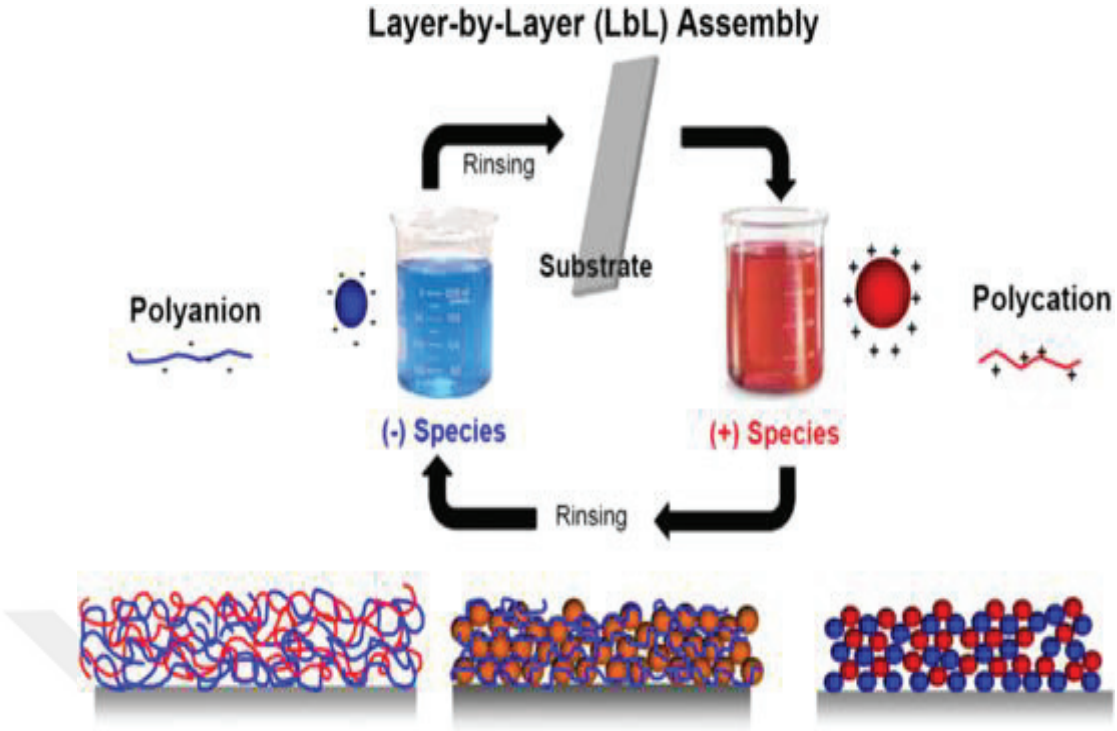


Figure 2. 4. Outline of LbL deposition through electrostatic interaction (Krogman et al., 2013)

Furthermore, polyelectrolyte properties affect LbL growing mechanism: a linear growth is common for strong polyelectrolytes, while an exponential growth has been observed and described in literature for some biologically relevant polyelectrolytes like polypeptides and polysaccharides (Picart et al. 2002, Elbert et al., 1999). That exponential growing seems caused by vertical mobility of charged chains inside the coating already deposited, leading to an increased interaction between polyelectrolytes, proportionally to the increased thickness of the coating (Figure 2.5).

Therefore, it is possible to conclude that LbL coating structures and qualities strongly depend on experimental conditions and specific optimization studies are necessary for every different system (Lavalle et al., 2004).

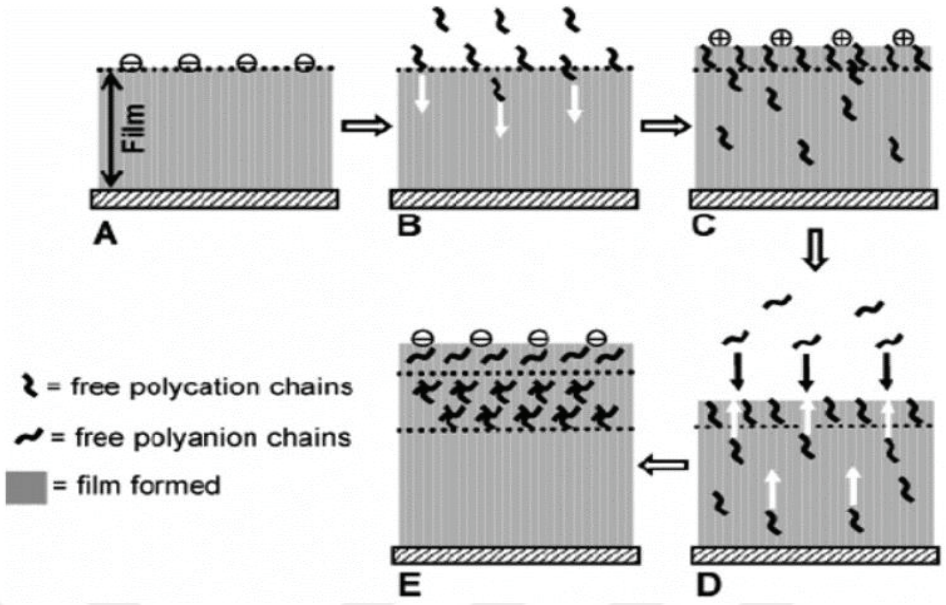


Figure 2. 5. Molecular image of exponential multilayer growth (Klitzing, 2006).

2.3.2. Potential Applications of LbL Technique

This technique has been widely used in diverse areas of chemical and biological fields with different purposes. For instance, Feng (2014) has employed LbL coating technique to produce a pH-responsive nanocarries for an effective and biocompatible drug delivery system. Shiratori and co-workers (2001) have fabricated LbL films from PAH, on a glass filter in order to create an effective filtration system to remove environmentally problematic gases such as ammonia gases and aldehyde species.

In the field of food packaging, LbL has already been applied in experimental research to obtain high barrier materials employing sustainable and environmentally friendly coating made of chitosan and cellulose nanocrystal (Fei, 2011). Another reported application of LbL technique is the production of edible bio-based coating on mangoes to improve their shelf life (Medeiros et al., 2012). There are also some studies that applied LbL coating technique on plastic supports in order to obtain active surfaces with antimicrobial properties (Del Hoyo-Gallego et al., 2016), but no application of those materials on food has been reported in literature.

2.3.3. Research Fields Using LbL Assembly Technique

LbL assembly has been widely used in an extremely large amount of aspects from the energy or physical fields to aspects of medical delivery. Specifically, solar-energy conversion, anti-reflection coatings, biosensors, solid-state ion-conducting materials, controlled drug-releasing coatings, and separation membranes (Xu, 2015). The LbL assembly approach offers the possibility to fabricate ultrathin films as well as controlling the film thickness. It offers functional coatings on solid substrates supported by alternate exposure to positive or negative kinds with spontaneous deposition of the oppositely charged polyelectrolytes, biological species, even the metallic or inorganic nanoparticles (Srivastava & Kotov, 2008) Due to the controlled thickness and the functional groups achieved in films fabricated from the LbL assembly method, more research areas are utilizing this method for the aim of advancing their current products.

2.3.4. Materials Used for LbL Assembly

Polyelectrolyte and proteins are the two most prominent materials for LbL assembly. Materials processed by LbL assembly should possess interactions in and between the bilayers. The material processed by LbL assembly, to some extent, will help bring in some functional groups in order to help improve the quality of the structures. Now, numerous researchers reported the successful LbL assembly fabrication on synthetic linear polymers, copolymers, organic components, polymeric microgels, and polyelectrolytes, stabilized micelles, as well as complexes of these species (Xu, 2015).

The sequential build-up of polymers via the LbL technique provides an efficient and versatile means for depositing functional polymer coatings on surfaces (Pinheiro et al. 2012, Weiss et al. 2006). Thus, a variety of functional thin films can be produced using the LbL assembly technique. Thin films, typically <1 μm thick, are created by alternately exposing a substrate to positively and negatively charged molecules or particles. Each individual layer may be 1–100 nm thick depending on the linear charge density and molecular weight of the adsorbing polymers, extent of film hydration and ionic strength, temperature, deposition time, counter ion and pH of the species being deposited (Zhong, Li, & Haynie, 2006). Some advantages when these coatings are at the

nano-scale are high stability on the substrate surface, facility of preparation (Peng et al., 2001) and lower concentration of materials required (Hinrichsen et al., 2003).

2.3.5. Polyethylene Terephthalate (PET) Film Properties

The support and the matrix materials are often chosen among the common plastics used for packaging, as polyolefin, polyesters, polyamide, polystyrenes, etc. (Arvanitoyannis & Oikonomou, 2012). Polyethylene terephthalate (PET) films are among the most common packaging materials for food products. The development of efficient technologies for its depolymerization for monomers reuse is highly encouraged, since current recycling rates are still very low. Polyethylene terephthalate (PET) is a synthetic aromatic polyester composed of ethylene glycol (EG) and terephthalic acid (TPA) units, which is extremely versatile and used in a variety of applications, such as clothing and technical textiles and packages (e.g., water and soft drink bottles, salad domes and biscuit trays), with an annual worldwide production over 50 million tons (de Castro, et al., 2017). Stretched PET provides a good barrier against carbon dioxide, making it an ideal container for carbonated soft drinks. The repeat unit of PET is shown in Figure 2.6.

The synthesis of PET is a well-documented two step polymerization. PET is typically made in a continuous melt-phase polymerization, followed by a solid-stating process. The first step is the combination of ethylene glycol and either terephthalic acid (TPA) or dimethyl terephthalate (DMT). Synthesis of PET using DMT requires a catalyst; typical catalysts are acetates of lithium, calcium, magnesium, zinc, or lead, or oxides of lead or tin (Matthews, 2007).

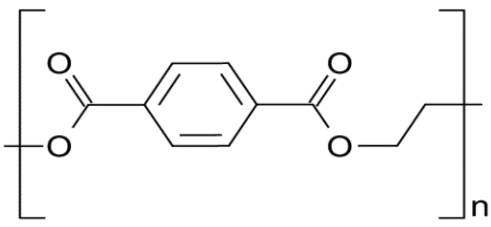


Figure 2. 6. PET repeat unit

2.4. Trends in the Usage of Natural Substances in Food Packaging

Over the last few decades the use of synthetic polymers, as food packaging materials, has increased enormously due to their advantages over other traditional materials such as glass or tin plate. Indeed, the large variety of materials and compositions available, allowed the adoption of the most suitable design for the specific needs of each product (Decker et al., 2010). Some recent problems such as the increasing oil's cost, the wide amount of packaging waste and the impact of their disposal have increased the desire for alternative solutions, which could be more eco-friendly (Hopewell et al., 2009). In this context, the development of biodegradable films for food packaging applications is an interesting and challenging prospective. In principle, biodegradable polymers can also be manufactured from petrochemical raw materials, but the most promising solutions in this fields are the bio-based polymers. Bio-based polymers are defined as polymers that are fully or partially produced from renewable raw materials. Bio-based polymers are generally classified into 3 categories: (1) natural polymers extracted from animal and plant sources: such as starch, cellulose, gluten and caseins; (2) polymers produced by chemical synthesis employing renewable bio-based monomers: such as polylactate (PLA); and (3) polymers produced by genetically modified bacteria or by microorganism through a fermentation process of natural substrates: such as polyhydroxy alkanoates (PHA) (Siracusa et al., 2008).

In the field of food packaging, there is an increasing trend in the use of this bio-based materials, although with some limitations, mostly related to their sensibility to water (both liquid and vapour) which often prevent their applications when high barrier properties are required (Koller, 2014). Natural substances are not only applied to produce bio-based food packaging materials. Indeed, another increasing tendency is the replacement of synthetic food additives with natural ones. This trend, driven by customer demands, has influenced the fields of food formulation as well as the active packaging sector. Various natural substances, coming from animal, plant or microbiological sources are currently applied for this purpose; Table 2.1 shows the most commonly applied natural antioxidants while Table 2.2 shows natural substances which are mainly applied for their antimicrobial properties. Most of these substances show both antioxidant and antimicrobial activity.

Table 2. 1. Most commonly applied natural antioxidants in active packaging production (Sanches-Silva et al., 2014).

ANTIOXIDANT	SOURCE AND EFFICACY
Tocopherols	Apolar antioxidant compounds, abundant in olive, peanuts, eggs, α-tocopherols is the most effective compounds, acting as a radical scavenger.
Quercetin	Flavonol compound obtainable from diverse vegetal sources, it acts as a singlet oxygen quencher and reactivating primary antioxidants.
Ferulic Acid	Fenolic acids, obtainable from grains and fruits. It acts as a radical scavenger.
Caffeic acid	Fenolic acids, was first founded in coffee extract, but widely presents in other fruit and vegetables.
Catechins	Flavonol compounds, abundant in green tea and cocoa, with diverse antioxidants activity depending from molecular structure.

The use of natural active compounds is often limited by their sensorial impact on food. A possible strategy to overcome this problem is to search for good sensorial-matching between active compounds and foods, such as rosemary extract and chicken meat products (Appendini, & Hotchkiss, 2002).

Table 2. 2. Most commonly applied natural antimicrobials in active packaging production (Appendini, & Hotchkiss, 2002).

ANTIMICROBIAL	SOURCE AND EFFICACY
Citral	Aldehydic compounds coming from citrus essential oils, water-soluble, and effective against *Salmonella* and *Listeria*.
Carvacrol	5-isopropil-2methylphenol, extracted from oregano, volatile, effective against *E. coli*, *S. aureus*.
Menthol	Cristallin substance obtained from mint essential oils, effective against *E. coli*, *S. aureus*, *Salmonella*.
Acetic acid	Produced by *Acetobacter aceti* and *Gluconobacter suboxydans*, effective against *E. coli, L. monocytogenes, Salmonella*.
Lysozyme	Globular protein from egg albumen, effective against Gram + and Gram -, *E. coli, P. fluorescens, S. aureus*.
Nisin A	Bacteriocins produced by *Lactococcus lactis*, effective against Gram + and endospores
Sakacin	Bacteriocins produced by *Lactobacillus sakei*, effective against *Enterobacteriaceae, L. monocytogenes, S. aureus*.

2.4.1. Chitin and Chitosan:

Chitin, poly (*β*-(1→4)-*N*-acetyl-D-glucosamine), is a natural polysaccharide first identified in 1884. This biopolymer is synthesized by an enormous number of living organisms and it is the most abundant bio-polymer after cellulose. The main commercial sources of chitin are crab and shrimp shells, but this polymer is also produced by fungi and yeast as well as by other living organisms belonging to lower plants and animal kingdoms (Younes, & Rinaudo, 2015). Chitosan is a straight-chain copolymer composed of D-glucosamine and N-acetyl- D -glucosamine being obtained by the partial deacetylation of chitin (Figure. 2.8).

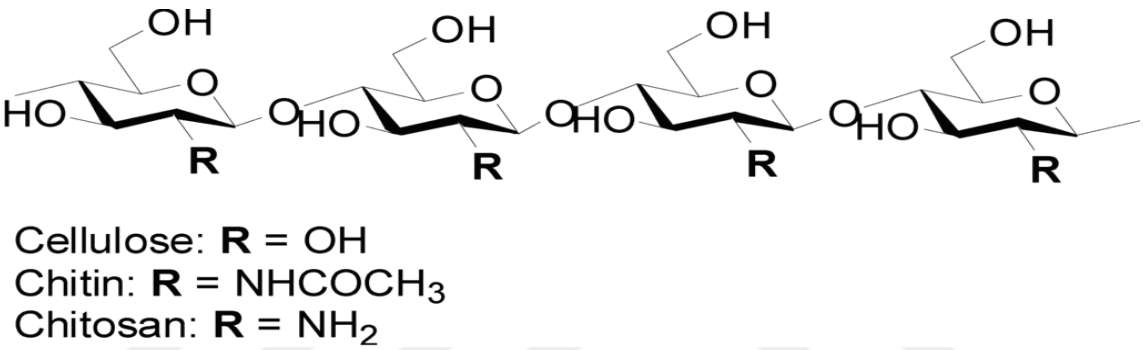


Figure 2. 7. Chemical structures of chitin, chitosan and cellulose (Azuma et al., 2015)

The partial deacetylation of chitin is commonly realized in the solid state under alkaline conditions (concentrated NaOH) or by enzymatic hydrolysis in the presence of a chitin deacetylase. These reactions lead to a heterogeneous distribution of acetyl groups along the chains, because of chitin semi-crystalline morphology. This distribution is very important for the control of solution properties; as heterogeneous products are soluble only under acidic conditions. Higher water soluble products could be obtained through the reacetylactions of highly deacetyleted chitin in the presence of acetic anhydride, leading to a random distributions of the acetyl groups. Chitosan solubility properties, as well as its biodegradability and reactivity are also influenced by the proportions of acetylated and non-acetylated D-glucosamine units. The amino groups (pH from 6.2 to 7.0) are completely protonated in acids with pH smaller than 6.2 making chitosan soluble. Chitosan is one of the few pseudonatural cationic polymers

and thus, it finds many applications due to this interesting character (Azuma et al., 2015).

In the field of active food packaging, chitosan is widely applied because of its high antimicrobial activity against various microorganisms (bacteria and fungi), which makes it useful to improve food shelf life. The exact mechanism of chitosan antimicrobial action is still not completely known, but different mechanisms have been proposed. One explanation for chitosan antimicrobial character resides in its positively charged amino group which interact with negatively charged microbial cell membranes, leading to the leakage of proteins and other intracellular constituents of the microorganisms. Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of toxins and microbial growth (Cuero et al. 1991). It also activates several defence processes in the host tissue (El Ghaouth et al. 1992), acts as a water binding agent, and inhibits various enzymes. These activities mainly depend on three characteristics of chitosan: molecular weight (Mw), acetylation degree (DA), and pH, showing a higher antimicrobial effect with decreasing Mw, DA, and pH (Lago et al., 2014).

Chitosan can be applied to produce casting film, to be coated or incorporated inside packaging materials (plastic or cellulose based) and to obtain edible coating. It is also suitable for the incorporation of others molecules, which is useful to enhance its active properties (Aider, 2010). Abdollahi and co-workers (2012) developed a chitosan based film, with the incorporation of rosemary essential oils to gain an antimicrobial-antioxidants material for food applications.

Chitosan is also considered one of the most promising polymer for biomedical and pharmaceutical applications due to its biodegradability, biocompatibility, antimicrobial, non-toxicity and also anti-tumour properties. In these fields chitosan is commonly applied to produce nanoparticles, microspheres, hydrogels, films and fibres for various specific applications (such as drugs delivery) (Aider, 2010).

2.4.2. Alginate

Alginate is a naturally occurring biopolymer that is used in the biotechnology industry. Alginate has been used successfully for many years in the food and beverage industries as a thickening agent, a gelling agent and a colloidal stabilizer ((Kohli, 2006).

Alginate also has several unique properties that have enabled it to be used as a matrix for the entrapment and/or delivery of a variety of proteins and cells. These properties include: (1) a relatively inert aqueous environment within the matrix; (2) a mild room temperature encapsulation process free of organic solvents; (3) a high gel porosity which allows for high diffusion rates of macromolecules; (4) the ability to control this porosity with simple coating procedures, and (5) dissolution and biodegradation of the system under normal physiological conditions (Gombotz, & Wee, 2012).

2.4.2.1. Alginate Chemistry

2.4.2.1.1 Sources of Alginate

Commercial alginates are extracted primarily from three species of brown algae (kelp). These include *Laminaria hyperborea, Ascophyllum nodosum*, and *Macrocystis pyrifera*. Other sources include *Laminaria japonica, Eclonia maxima, Lesonia negrescens* and *Sargassum* species. In all of these algae, alginate is the primary polysaccharide present and it may comprise up to 40% of the dry weight. Alginate is found in the intracellular matrix where it exists as a mixed salt of various cations found in sea water such as Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Na^{+}. The native alginate is mainly present as an insoluble Ca^{2+} crosslinked gel. Bacterial alginates have also been isolated form *Azotobacter vinelandii* and several *Pseudomonas* species (Rehm & Valla, 1197).

2.4.2.1.2. Chemical Structure

Alginates are a family of linear unbranched polysaccharides which contain varying amounts of 1, 4′-linked β-D-mannuronic acid and α-L-guluronic acid residues (Figure. 2.9). The residues may vary widely in composition and sequence and are arranged in a pattern of blocks along the chain. These homopolymeric regions of β-D-mannuronic acid blocks and α-L-guluronic acid blocks are interdispersed with regions of alternating structure (β-D-mannuronic acid–α-L-guluronic acid blocks). The composition and extent of the sequences, and the molecular weight determine the physical properties of the alginates. The molecular variability is dependent on the organism and tissue from

which the alginates are isolated. For example, alginates prepared from the stipes of old *L. hyperborea* kelp contain the highest content of α-L-guluronic acid residues while alginates from *A. nodosum* and *L. japonica* have a low content of α-L-guluronic acid blocks (Gombotz & Wee, 2012). Alginates do not have a regular repeating unit and the distribution of monomers along the polymer chain cannot be described by Bernoullian statistics (Painter, 1983).

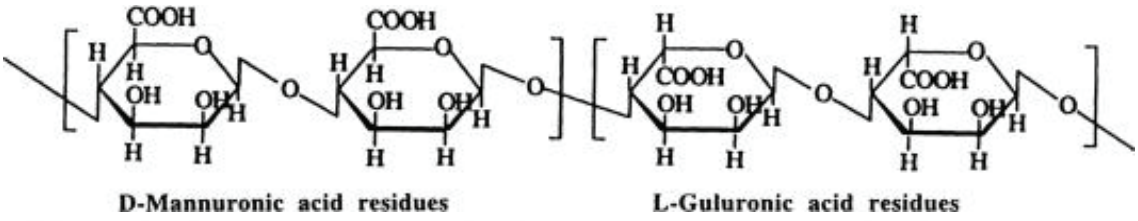


Figure 2. 8. Structure of alginate showing both β-D-mannuronic acid and α-L-guluronic acid residues. (Wayne et al., 2012).

Analytical characterization of alginates is more difficult than for other polysaccharides since acid hydrolysis can lead to destruction of the uronic acids. Circular dichroism spectroscopy has been used to match the linear spectra of the alginate to model samples of well characterized homopolymeric blocks (Morris et al., 1980). NMR spectroscopy has contributed significantly to our understanding of alginate structure. This technique can determine the monomer composition as well as the frequencies of the four possible diad (nearest neighbour) structures F_{GG}, F_{MG}, F_{MM} and F_{GM} (G = α-L-guluronic acid; M = β-D-mannuronic acid). NMR can also provide an estimate of the eight possible triad frequencies and the average block length (Grasdalen et al., 1981).

Gel formation by calcium-alginate system depends on many factors; in general gel strength increases by increasing number of G residues and calcium ion concentration, and when the gel preparation process is conducted at a low pH and temperature. Also alginate molecular weight (between 32.000 and 400.000 g/mol) can affect the hydrogels mechanical properties and solutions viscosity. Higher molecular weight leads to higher solution viscosity and higher post-gelling elastic module (Fang et al., 2007).

Because of its biocompatibility and interesting physical properties, alginate is widely applied in the field of food formulations as a thickener and stabilizer agent.

Sodium alginate, potassium alginate, ammonium alginate and calcium alginate are currently approved as food additives and encoded as E-401, E-402, E-403, E-404, respectively. These additives are commonly applied for preparing sauces, frozen desserts and beverages (Imeson, 2011). Moreover, new interesting prospective for alginate food applications are emerging. For example, the use of alginate films with suitable barrier properties for aroma encapsulations have been reported recently, demonstrating their applicability as an edible coating to extended fresh cut fruit shelf life (Hambleton et al., 2009).

Alginate has also demonstrated great utility and potential as a biomaterial for many biomedical applications, particularly in the areas of wound healing, drug delivery, in vitro cell culture, and tissue engineering (Lee et al., 2012).

2.4.3. Green Tea Extract

Green tea is a tea variety exclusively obtained from *Camellia sinensis* (or *Thea Chinensis*) leaves. *Camellia sinensis* is an evergreen plant that grows primarily in tropical and temperate regions of Asia which mainly include China, India, Sri Lanka, and Japan. It is also cultivated in several African and South-American countries. *Camellia sinensis* derivate beverages are widely consumed all over the world and generally categorized into 3 categories: not fermented (green tea), semi-fermented (Oolong tea) and fermented (black tea). Recently, tea extracts became interesting for the market because of their antioxidant power; green tea extracts shown the best favourable properties, demonstrating antioxidants, and antimicrobial, antitumor, and antiviral activity (Chan et al., 2011).

The chemical composition of tea leaves has been well documented. The main constituents of tea leaves are polyphenols, mainly catechins, which usually represent 25–35% of the dry weight of green tea leaves. The tea catechins belong to the family of flavonoids (Yilmaz, 2006) and possess two benzene rings referred to as the A- and B-rings. In addition, catechin molecules contain a dihydropyran heterocycle (the C-ring) that has a hydroxyl group on carbon 3 (Senanayake, 2013). Epigallocatechin gallate (EGCG) is the most abundant and characteristic catechin in green tea leaves and extracts (EGCG in green tea is 10 % higher than in black tea and 2.5 % higher than in oolong tea). Other characteristic compounds present in green tea leaves are: (+)-

catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epicatechin gallate, (−)-epigallocatechin (Figure. 2.10). Green tea extract also commonly contains phenolic acids (gallic acid included) flavonols (mainly quercetin, kaempferol, myricetin, and their glycosides) theobromine, caffeine, vitamins (mainly A, B, C), pigments (clorofille) and minerals (Erickson, 2011).

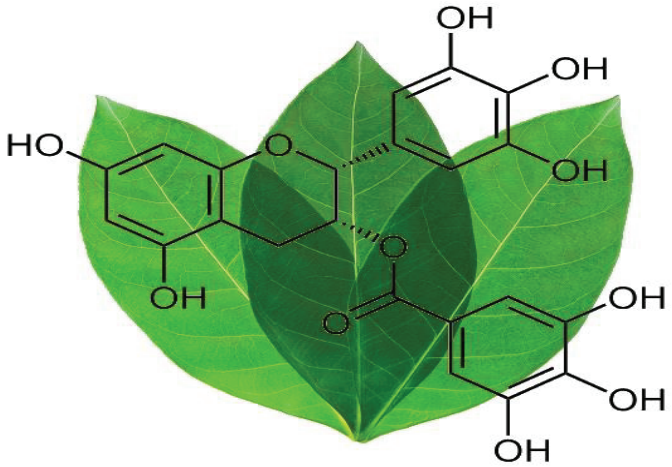


Figure 2. 9. Chemical structure of catechin found in green tea extract (Zaveri, 2006).

Green tea antioxidant properties are related to its high polyphenols content. Several studies have demonstrated that green tea polyphenols are exceptional electron donors and effective scavengers of physiologically relevant reactive oxygen species (Guo et al., 1999, Michalak, 2006). Green tea catechins are also able to chelate redox active transition-metal ions (Michalak, 2006). Because of these properties, there is an increasing interest for green tea extracts incorporations into oxidation-sensitive foods and several studies have reported promising results for bread, biscuits, dehydrated apples and various meat products. Green tea extracts can also be applied to produce active packaging materials; Siripatrawan (2010) developed an active chitosan based film with the incorporation of green tea extracts, Carrizo and co-workers (2016) demonstrated the extended shelf life of two fatty foods by a new antioxidant multilayer packaging containing green tea extract.

2.5. Fresh-Cut Fruit and Vegetables

The market sales of ready-to-use (RTU) fresh fruits have grown rapidly in recent decades as a result of changes in consumer attitudes. Indeed, these products allowed to satisfy consumer demands for fresh and healthy food (encouraged by EFSA recommendations) while offering also convenience (no preparation time required). Fresh-cut produce graduated to retail during the 1990s, especially for lettuce, cabbage, carrots, and other analogous vegetables (Soliva-Fortuny et al. 2003). The high microbial loads of these products were traditionally reduced through a cleaning in flowing chlorinated water and a distribution under ensured controlled refrigeration (Ahvenainen, 1996). Therefore, a lot of different ready-to-eat greens were launched on the markets, contributing to increase consumption. Anyway, the association of chlorine with the potential formation of carcinogenic chlorinated compounds in water has called into question the use of chlorine in food processing. Moreover, chlorine only delay microbiological spoilage, while not exhibit any benefits on biochemical and physiological disorders of fresh-cut products. These disorders, manifested as cut consequences, rapidly lead to colour, texture, and flavour degradation, limiting products shelf life. Therefore, various alternative solutions to chlorine washing treatments started to be used and research studies are still continuing in this fields to achieve better results (Rico et al., 2008).

Calcium lactate has been applied as an alternative to chlorine for delicate fruits with a high senescence index, such as grapefruit, peaches, fresh-cut cantaloupes and apples. Calcium lactate acts as a firming agents and it shows an effectiveness against microbiological spoilage similar to chlorine. The treatment with organic acids, such as ascorbic acid or citric acid, has also shown positive results, reducing the microbiological load and slowing down surface oxidations (Rico et al., 2007).

Modified atmosphere packaging (MAP) is another alternative preservation technique already in use in the fresh-cut industry. Indeed, low levels of O_2 and high levels of CO_2 reduce the respiration rate, with the benefit of delaying senescence, and act also slowing down microbiological spoilage, extending the storage life of fresh products (Toivonen et al., 2009). As shown in Tables 2.3 and 2.4, the recommended atmosphere gas concentrations for preservation depend on the product (Oliveira et al., 2015).

A major concern about product safety is still associated with the use of MAP. Indeed, the desired suppression of spoilage microorganisms extend product shelf life but may create opportunities for the growth of slower growing pathogenic bacteria. (Rosnes et al., 2003) Moreover, reported studies on the effects of MAP on fresh cut products properties such as colour, texture and flavour are often contradictory, manifesting the needs for further investigations.

Recently an increasing interest has been addressed to the use of active coating for enhancing fresh-cut products' shelf life. Active coating can be applied on the package or

directly on the food, allowing the control of moisture transfer, gas exchange, oxidations, and microbiological spoilage. These active coatings are frequently obtained employing natural edible biopolymer, which are incorporated with other active substances (antimicrobials or antioxidants) to be released inside food during storage. For example; in a study; Kapetanakou and co-workers (2016) showed the worth noting suppression of *Aspergillus carbonarius* growth on the skin or the flesh of apples and pears coated with Na-alginate containing 0.3% or 0.9% (v/v) of cinnamon essential oil compared to controls.

Edible coating and surface treatments may be applied in combination with MAP technology, as a feasible way to enhance fresh cut products shelf life. Indeed, the use of hurdle technology recently started to become effective in various applications. The hurdle technology, or combined methods technology (CMT), is based on combining low levels of two or more preservation factors in order to gain a synergic effect which make the product stable. This approach typically leads to minimal sensory and nutritional changes,

Table 2. 3. Modified atmosphere storage recommendations for selected fresh-cut fruits. (Oliveira et al., 2015).

Product:	**Temp. (°C)**	**O_2**	**CO_2**
Sliced apple	0-5	≤ 1	-
Cubed cantaloupe	0-5	3-5%	6-15%
Cubed honeydew	0-5	2%	10%
Sliced kiwifruit	0-5	2-4%	5-10%
Sliced orange	0-5	14-21%	7-10%
Sliced peach	0	1-2%	5-12%
Sliced pear	0-5	0.50%	≤ 10%
Sliced persimmon	0-5	2%	12%
Arils (seed coating) pomegranate	0-5	-	15-20%
Sliced strawberry	0-5	1-2%	5-10%

Table 2. 4. Modified atmosphere storage recommendations for selected fresh-cut vegetables. (Oliveira et al., 2015)

Product:	**Temp. (°C)**	**O_2**	**CO_2**
Chopped green leaf lettuce	0-5	0.5-3%	5-10%
Chopped or shredded iceberg lettuce	0-5	0.5-3%	10-15%
Chopped red leaf lettuce	0-5	0.5-3%	10-15%
Chopped romaine lettuce	0-5	0.5-3%	5-10%
Sliced mushrooms	0-5	3%	10%
Sliced or diced onion	0-5	2-5%	10-15%
Diced peppers	0-5	3%	5-10%
Sliced or whole-peeled potato	0-5	1-3%	6-9%
Sliced rutabaga	0-5	5%	5%
Cleaned spinach	0-5	0.8-3%	10%
Sliced tomato	0-5	3%	3%
Broccoli	0-5	2-3%	6-7%
Shredded cabbage	0-5	5-7.5%	15%
Shredded sticks or sliced carrots	0-5	2-5%	15-2%
Sliced leek	0-5	1-3%	5-10%

which makes the products more acceptable than those obtained by conventional methods (one single factor applied at a high level). The most important hurdles commonly used in food preservation are based on controlling temperature, water activity, acidity, redox potential and the use of preservatives, modified atmosphere and competitive microorganisms (e.g., lactic acid bacteria) (Leistner, 1999).

Several studies are reported in literature for various fresh-cut products, for example, Ban and co-workers (2015) have demonstrated the synergic effects of sodium-chlorite, N, O-carboxhilmethyl chitosan coating and active MAP, on quality maintenance of minimally processed *Citrus Grandis*. Gupa and co-workers (2012) have investigated the effect of citric acid treatment in combination with gamma radiation and MAP for extending minimally processed French beans shelf life. Besides, further studies are necessary to improve these approaches. Indeed; the selection of hurdles need to be tailored carefully to the quality attributes of every single product.

CHAPTER 3

OBJECTIVE

3.1. Aim of the Thesis:

The objective of this study was to develop and investigate a novel active packaging system for extending food products' shelf life. This promising system which is called "Layer-by-Layer (LbL) deposition" is an active coating system based on deposition of the subsequent alternative natural polymers and biopolymers on PET film surfaces, by means of mechanism of attraction between opposite charges. Active properties of LbL deposition were ensured by dipping into chitosan solution (antimicrobial natural polymer), and alginate solution incorporating green tea. The structure of layers' deposition was applied to release of those active substances gradually into the food products.

Firstly; the active coating system was developed and then, the properties of PET films which were coated by LbL technique were investigated. After the development of this active coating system, *in vivo* studies have been carried out for peaches in order to estimate positive effects of the LbL deposition on shelf life of fresh cut peaches.

CHAPTER 4

MATERIAL AND METHODS

4.1. Materials and Chemicals

In this study, shellfish chitosan (degree of deacetylation of 85 % and a molecular weight from 50.000 to 60.000) (Giusto Faravelli Spa, Milan, Italy), alginic acid sodium salt from brown algae (viscosity of 2 % solution at 25 °C) (Sigma Aldrich), and green tea extract (tannins ≥ 69 %, humidity ≤ 8 %) (Dal Cin Gildo S.p.a, Concorrezzo, Italy) were used for LbL assembly. Amorphous polyethylene terephthalate (A-PET) (200 nm thickness) was used as the plastic support for LbL coating. Samples for AFM analysis were obtained by using glass slides.

Fluorescein 5(6)-isothiocyanate (mixtures of 2 components, > 90 %) and DPPH 2,2-diphenyl-1-picrylhydrazyl) were purchased by Sigma Aldrich. Tryptic soy broth (TSB), malt extract broth (MEB), violet red bile agar (VRBA) and *Pseudomonas* agar base, which were employed for the microbiological analyses were purchased by Merck, Germany.

Peaches (Prunus persica L. Batch cv 'Alexandra") for shelf life trials were purchased from the wholesale market in Milan, Italy at commercial maturity.

4.2. Preliminary Test

4.2.1. Characterisation of Antimicrobial Properties of Chitosan

Antimicrobial properties of chitosan were primarily tested in order to obtain detailed information about its efficiency against diverse Gram (+) and Gram (–) bacteria as well as against moulds and yeasts. Strains belongs to official collection of *Escherichia coli, Pseudomonas putida, Listeria innocua, Staphylococcus aureus, Saccharomyces cerevisae, Aspergillus niger* and *Penicillium chrysogenum* were used. In order to determine the minimum inhibitory concentration (MIC) of chitosan against the

microorganisms listed above, 5 ml of TSB or MEB were poured in test tubes, singularly inoculated with microorganisms and added with chitosan solutions at different concentrations (between 0.25 to 5 g/L). After 24 h (bacteria) or 5 days (yeast and moulds) incubation, absorbance of the inoculated tubes was read at 600 nm. When growth of moulds exceeded the possibility of evaluating spectrophotometrically culture turbidity, the MIC was determined through visual inspection, taking into account either the mycelial or the conidial development (Piva, 2016).

4.2.2. Characterisation of Antioxidant Properties of Green Tea Extract

Antioxidant capacity of green tea extract was first tested through the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. DPPH is an alcohol-soluble substance that produces purple coloured solution with a maximum absorbance at 517 nm. It reacts with oxygen scavenger substances to produce its reduced form DPPH-H, with a colour change: from a purple solution to a yellow one. The reaction can be easily monitored with a visible spectrophotometric analysis. In this experiment, DPPH was diluted in methanol to obtain 1.00 ± 0.03 absorbance units at 517 nm. The DPPH solution (2.94 mL) was placed in a cuvette where 60 µL of green tea solutions at different concentrations were added (between 1 and 5 ppm). Another cuvette was prepared adding 60 µL of methanol. The absorbance measure was carried out after incubation for 30 min at 30 ± 1 °C and used to draw a curve, DPPH decay % vs green tea concentration, and to find out the EC50parameter (antioxidant concentration that lead to DPPH 50 % decay).

$$\text{DPPH decay \%} = (A_{517}\ \text{DPPH} - A_{517}\ \text{sample})/A_{517}\text{DPPH} * 100 \qquad (4.1)$$

The same procedure was applied for Trolox, a synthetic equivalent of Vitamin E, which was used as a reference standard (Piva, 2016).

4.3. Preparation of LbL Coating

Chitosan and alginate water dispersions, 0.2 % (w/v), were separately prepared by dissolving the powder in 2 % (v/v) of acetic acid for chitosan, only water for alginate, at 25 °C for 3 h under stirring. The pH value was adjusted to 4 and 6.5 for

chitosan and alginate solution, respectively, adding 0.01 M NaOH and HCl. Then green tea extract (0.35% (w/v)) was added into alginate solution. The employed concentrations of alginate and chitosan were chosen according to literature (Medeiros et al. 2012 Carneiro-da-Cunha et al., 2009, del Hoyo-Gallego, 2016). The amount of green tea extract (0.35 % (w/v)) was chosen due to its maximum solubility in water. The pH of solutions were chosen in order to achieve an intermediate density charge (positive for chitosan and negative for alginate), that may lead to a thicker deposition, as reported in literature. Chitosan and alginate solutions were used for LbL assembly on A-PET and glass supports, as reported below.

A-PET sheets were rinsed with distilled water, methanol, and distilled water once more for removing lipids and contaminants. After drying at room temperature, they were submitted to corona treatment (BD-20 high frequency generator, Electro-Technic Products, Inc., Chicago, IL, USA) to increase surface energy and generate a negative-charge surface. For preparing the coated glass samples intended to AFM analysis, glass slides were charged treating them with Piranha Solution (96 % H_2SO_4: 30 % H_2O_2 = 3:1 v\v) (Cras et al., 1999).

A-PET sheet was subsequently cut by 15 x 9 cm strips, which were dipped firstly into the chitosan solution (that is positively charged) for 1 min; after that, the strips were rinsed in distilled water for 15 s in order to remove the excess of chitosan and then dried by filtered compressed air. Following, strips were dipped into the alginate-green tea extract solution for 1 min; rinsing and drying steps were subsequently applied as the same as chitosan. After that, a bilayer chitosan-alginate was deposited on the support. This procedure was repeated in order to obtain up to 40 layers (20 bilayers) coated PET samples. The same treatments were applied for coating the glass slides. In order to study the LbL assembly and its posterior degradation, other PET samples were prepared employing fluorescein-isothiocyanate labeled chitosan (FITC-CS).

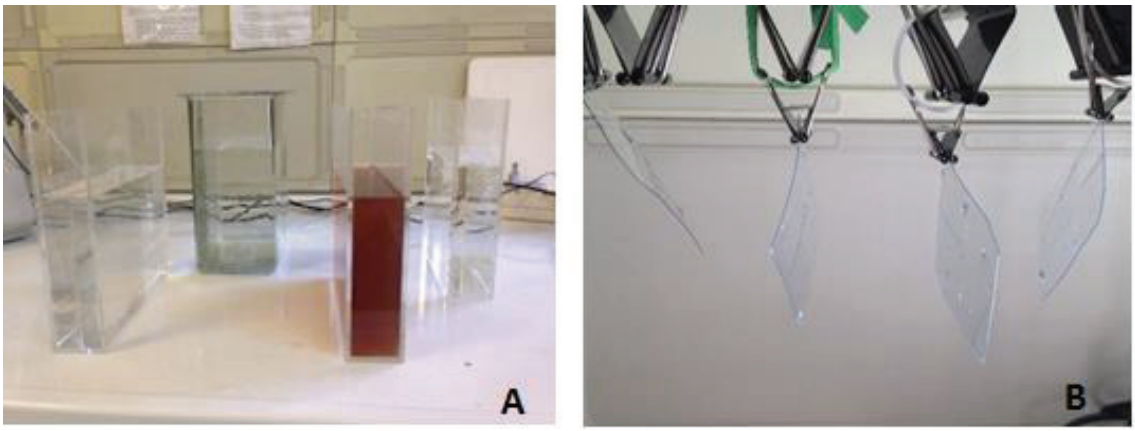


Figure 4. 1. Employed solutions for LbL assembly: chitosan 0.2% w/v, water, alginate 0.2 % w/v incorporated with 0.35 % w/v green tea extract, water (from left to right) (A); PET strips after washing with distilled water (B).

FITC-CS was synthesized using the following procedure reported by del Hoyo-Gallego and co-workers (2016): 2 mg/mL of fluorescein-isothiocyanate in methanol (150 mL) was added into 1 % (w/v) chitosan in 0.1 M acetic acid solution; after 3 h of reaction in the dark at ambient temperature, FITC-CS was precipitated in 0.2 M NaOH and washed several times with distilled water until a clear supernatant was obtained. The FITC-CS was then dissolved in water to obtain a 0.2 % solution which was employed for the LbL assembly as previously reported (except for the green tea extract that was not added inside alginate for avoiding possible interference between compounds for absorbance measurement).

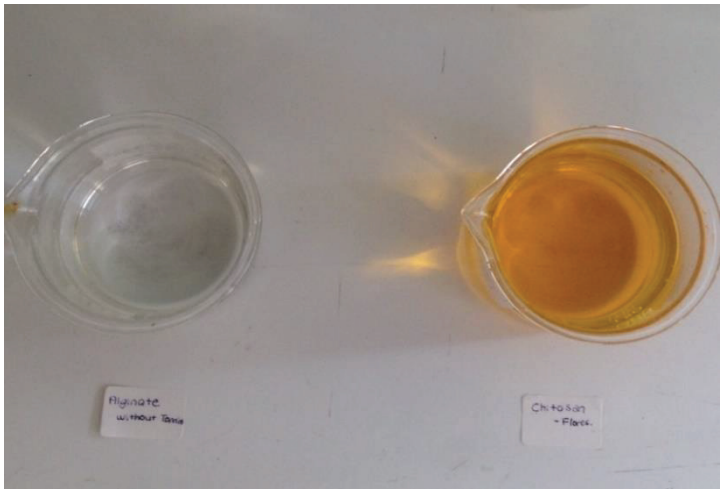


Figure 4. 2 Employed solutions for FITC-CS assay: alginate solution 0.2% (w/v) without green tea extract and chitosan 0.2% (w/v).

4.4. Characterization of LbL Assembly

4.4.1. Contact Angle Analysis

Contact angle measurement is a commonly used method to quantify the wettability of a solid surface. The wettability degree of a surface and the respective measure of contact angle depend on surface tension of the liquid and interfacial energies (Ghosh, 2009).

In this study, contact angle measurements were performed on uncoated A-PET surface and on each successive alginate and chitosan layers, in order to confirm the occurred deposition and to follow the multilayer film assembly. The sessile drop method was applied, employing an optical contact angle apparatus, (OCA 15 Plus – Data Physics Instruments GmbH, Filderstadt, Germany) equipped with a video measuring system with a high-resolution CCD camera and a high performance digitizing adapter (Newman and Kwok, 1999). The SCA20 software (Data Physics Instruments GmbH, Filderstadt, Germany) was used for data acquisition.

Using a syring (Hamilton, Switzerland), 4 ± 0.5 µL droplets of Milli-Q water were serially deposited on the surface of samples at the starting time and 20.5 ± 0.3 °C. A total of 15 replicates of contact angle measurements were carried out.

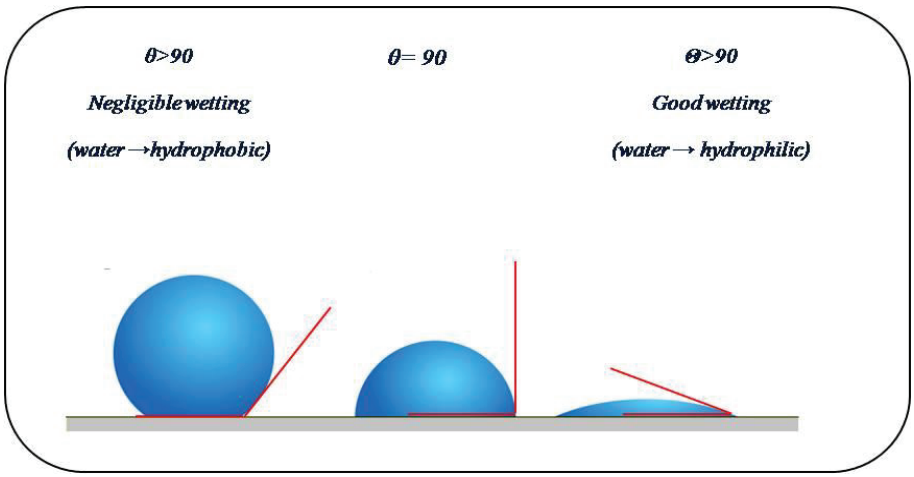


Figure 4. 3. Liquid drop deposited on a solid surface (https://fmps.fbk.eu/contact-angle-platform)

4.4.2. UV-Visible Spectrophotometry

The chitosan-alginate LbL coating mechanism of assembly was investigated using the fluorescein-isothiocyanate labelled chitosan. Indeed, fluorescein-isothiocyanate is easily detectable through fluorescence or absorbance spectroscopy (Khalfan et al., 1986). An UV-vis spectrophotometer (model L650 with a 150 mm integrating sphere, Perkin-Elmer, Milan, Italy) was used to monitor the absorbance at 490 nm (as reported by del Hoyo-Gallego et al., 2016) of the coated PET samples after the deposition of 5, 10, 15, and 20 layers of FITC-CS (9, 19, 29, 39 total layers). The obtained absorbance measurements were then used to produce a curve, absorbance vs number of layers, which was used to understand the coating mechanism of assembly (Piva, 2016).

4.4.3. Atomic Force Microscopy

An atomic force microscopy (AFM, AlphaSNOM, WITec GmbH, Germany) was used to study the thickness of the deposited multilayers and also to analyse their morphologies.

This analysis involved 4 different LbL-coated glass samples: 20 layers coated (last one alginate), 21 layers coated (last one chitosan), 40 layers coated (last one alginate), and 41 layers coated (last one chitosan). For thickness measurements, the LbL coating was gently scratched in order to expose part of the glass support and measure the thickness of the coating. Topography images were acquired with soft tapping mode at low oscillation amplitudes, stabilized by an amplitude-modulation feedback system based on the optical level deflection method (Putman, et al. 1992). Standard AFM probes have been used.

4.6. UV-Visible Spectrophotometry

The coating kinetics of release was investigated using the FITC-CS and alginate coated PET samples, which were submitted to a longer extraction treatment and employed to monitor the increasing of the simulant solution absorbance during time.

Indeed, this increasing trends can be related to a migration of FITC-CS from the LbL coating structure to the simulant solution, allowing to follow the speed of release. Absorbance monitoring was performed until a constant value was observed, as that meant a complete extraction occurred (Hoyo-Gallego et al., 2016). An UV-vis spectrophotometer (model L25, Perkin-Elmer, Milan, Italy) was used to measure the absorbance at 490 nm.

4.7. *In Vitro* Antimicrobial Evaluation

Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Listeria monocytogenes and yeast and moulds were selected as representative microorganisms. The culture media were chosen as MEA for yeast and mould and TSA for the the others. A total of 5 mL of culture were transferred into 6 sterile test tubes and a drop of each representative microorganism was put into every culture tube. To dilute the microorganism tubes; 800 µL pure culture was mixed with 200 µL culture with microorganism in a cuvette. The same step was repeated for each bacteria. After all preliminary steps; 200 µL diluted microorganism was put into each representative Petri dishes and 20 mL of culture media (MEA or TSA; according to the bacteria type) was added into each Petri dish. One Petri dish has been prepared with MEA culture media without microorganism inoculation as control (CTR). Then, PET films coated with LbL deposition technique were cut in squares of 2 cm × 2 cm. When the culture media dries; 2 pieces of PET squares were put over the culture media and one Petri dish with inoculated bacteria has been prepared with uncoated PET squares for comparison. Finally, all dishes were incubated at optimum conditions for each microorganism (Mascheroni et al., 2014).

4.8. In Vivo Application

4.8.1. Peaches

An *in vivo* application of the novel active coating system was carried out on peaches (*Prunus persica*) (5 kg) that were purchased from the open market in Milan,

Italy at commercial maturity. In order to investigate the active coating effects on the fresh cut peaches' shelf-life, two different treatments were compared: samples stored with LbL active coated PET strips (LbL samples), samples stored with uncoated PET strips (CTR samples). The employed procedure for preparing the samples is reported below:

Fruits were pre-washed with distilled water, sanitized for 2 min in chlorinated water (1.5 g/L sodium hypochlorite), rinsed with distilled water and gently dried by hand. Peaches with skin were cut into slices of about 1.5 cm thickness (25 ± 3 g each slice), using a sterile stainless-steel knife. Three slices (75 ± 9 g) were placed on a Styrofoam tray, LbL coated PET samples (40 layers strips, 8 x 3 cm and 8 x 8 cm respectively) were

inserted among each peach slices and on the bottom of the tray; for control (CTR) peaches slices were placed with untreated A-PET strips as control samples; the trays with slices were put into OPP bags (Oriented polypropylene bag, 20 μm thickness, 38 x 20 x 6 cm) and stored at 4 ± 1 °C up to 7 days. Each treatment was carried out in duplicate. Samples were then collected after 2, 4 and 7 days. For color evaluation, specific samples of LbL and CTR were prepared and monitored during 7 days of storage.

Figure 4. 4. '*Prunus persica'* peaches

Figure 4. 5. Peach slices assembled with LbL coated PET strips (on left). Peach slices assembled with LbL coated PET strips after packaging with OPP bag (on right).

4.9. Physicochemical Analysis of Peach Samples

The in vivo applications of the LbL active coating on peaches has involved a monitoring of the ripening process through the evaluation of the total soluble solid contents and the titratable acidity. In order to identify an antioxidant effect due to LbL coating, colour evaluation, carotenoids content determination, total phenolic compounds assay and polyphenol oxidase activity measurement were performed; the employed procedure are reported below:

Peaches' colour evaluation was carried out using the Minolta CR-300 chromameter (Konica Minolta Sensing, Inc., Japan). Three measurements were performed on each side of peach slices. The instrument was calibrated using a standard white plate. Results of the colour measurements are reported using the CIE L*a*b* System, where L* is lightness, a* is index of green-red (+a* is the red direction, while –a* is the green direction) and b* index of yellow-blue (+b* is the yellow direction, -b* is the blue direction). In addition to quantifying the overall colour difference between a sample and standard colour, delta E* is intended to be a single number metric for Pass/Fail tolerance decisions (Schanda, 2007). Total colour difference between samples was calculated according to the equation (4.2.).

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \qquad (4.2.)$$

Where ΔL is the lightness difference, Δa is the difference in red and green and Δb is the difference in yellow and blue.

Peaches weight loss (WL) during storage was calculated by the differences between the initial and final weight (after 7 d) of samples. The value was expressed as a relative percentage and calculated as follows (4.3.):

$$WL\ \% = \frac{(\mathrm{Wi}-\mathrm{Wf})}{Wi} * 100 \qquad (4.3.)$$

Where W_i is the initial weight and W_f is the weight measured during storage.

Fruit firmness was measured with digital penetrometer (53205, TR Turoni, Forlì, Italy) and expressed as kg/cm^2; two measurements were carried out for each slice.

Chemical analyses were carried out on peach juice obtained from slices (50 g for each sample) by an electronic juicer (Moulinex, France).

Fruits total soluble solid content (TSS %) was determined by a digital refractometer (Atago Co., Ltd, Tokyo, Japan model PR-32) and expressed as °Brix.

Titratable acidity was measured by titrating 1:10 diluted juice (obtained from 50 g of samples) using 0.1 M NaOH through an automatic titrator (Compact 44-00, Crison Instruments, SA, Barcelona, Spain) and expressed as % malic acid.

To determine the carotenoid content, de Ritter method was used (de Ritter et al., 2010); 5 g of sample were mixed for 20 min with 50 mL of extracting solvent (hexane/acetone/ethanol, 50:25:25, v/v). The organic phase was recovered and then used for analysis after suitable dilution with hexane. Total carotenoid determination was carried out on an aliquot of the hexane extract by measuring absorbance at 450 nm in a spectrophotometer Lambda 25 (Perkin Elmer, MA, Milan, Italy). Total carotenoids were calculated as follows (4.4.):

$$\beta - carotene\ content(\mu g\ 100\ g) = \frac{Abs*V*D*100*100}{W*Y} \qquad (4.4.)$$

Where V is total volume extract, D the dilution factor, W the sample weight and Y the percentage of dry matter content of the sample. The analyses were performed in triplicate for each sample. For the determination of polyphenol oxidase (PPO) activity, a buffer solution (1:1) at pH 6.5 was prepared using 1 M NaCl and 5% polyvinylpolypyrrolidone; peach samples (5 g) were mixed with the buffer solution and homogenized using an Ultra-Turrax DI25 (IKA Works, Germany). The homogenate

mix was centrifuged at 12,000 rpm for 30 min at 4 ºC (Centrifuge Rotofix 32A, Hettich, Germany.). The supernatant was collected and filtered, to obtain the enzymatic extract, required for enzyme activity determination. According to the method of Soliva-Fortuny et al. (2001) and Kołodziejczyk and co-workers (2010) PPO activity was determined spectrophotometrically, adding 3 mL of 0.05 M catechol and 75 μL of extract into a quartz cuvette. The changes in absorbance at 400 nm were recorded every minute up to 3 min. One unit of PPO activity was defined as a change an increase of 0.001 unit of absorbency/min per g of peach at 420 nm (Lee et al., 1990). All determinations were performed in triplicate.

4.10. Antimicrobial Activity of LbL Deposition on Peach Slices

To survey the microbial growth on peaches, the total aerobic count was monitored as well as the psychrophilic bacteria and yeast and mould counts; the employed procedures were reported below:

After 0, 2, 4 and 7 d, a portion of sample (10 g) were transferred aseptically into a Stomacher bag (400 mL PE, Barloworld, France) containing 90 mL of sterile peptoned water (10 g/L bacteriological peptone, Costantino, Italy) and blended in a Stomacher (Star Blender LB 400, Biosystem, Belgium) at high speed for 4 min. Ten-fold dilution series of the obtained suspension were made in the same solution for plating. The following culture media were used: TSA (Tryptic Soy Agar) for mesophiles, and MEA (Malt Extract Agar) for yeast and moulds. Colonies were counted after incubation at 30 °C for 24 h for mesophiles, 10°C for 10 d for psychrophiles, 25 °C for 5 d for yeast and moulds (Rollini, 2016). Counts were performed in duplicate and reported as logarithms of the number of colony forming units (log cfu/g peach), and means and standard deviations (SD) were calculated.

4.11. Gas Chromatography Analysis

Fruits of selected cultivars of peaches which are coated by LbL treated PET strips (LbL) or uncoated PET strips (CTR) were seal packaged (in OPP bags) and stored at 4 °C. Every sampling day of *in vivo* analysis, gas measurements were taken. Gases were measured by extracting 4 mL of the internal atmosphere of the bags with a syringe

and injecting into a gas chromatograph with a thermal conductivity detector (TCD) (Lurie et al., 1993). Analyses were carried out using a gas chromatograph equipped with a thermal conductivity detector (Hewlett Packard 5890 Series II Gas Chromatograph, Milan, Italy) at 150 °C. A 0.4 mL aliquot of headspace of the pouches equilibrated at 25 °C was withdrawn through an adhesive septum stuck to the cover film, using a manual sampling syringe (dynatech, Batonrouge, Milan, Italy) and then injected into the gas chromatograph. A Porapaqs 80/100 mesh column (2 m x 2 mm) at 70 °C and 200 kPa was used. Nitrogen at 27 mL/min was used as a carrier gas. Peak area integration was performed using Chrom-Card (Chrom-Card Data System, v. 1.18, Thermo Scientific™).

4.12. Sensory Evaluation of Peaches

After taking the sample for microbiology analysis, rest of the samples were used for sensory analysis. A sensory panel was performed with 15 semi-trained panelists to evaluate the quality of peaches at 0, 2, 4, 7 days of storage. Panelists were staffs and students at Food Science and Technology Department of University of Milan and they were asked to rate given samples for appearance, colour, and odour by using difference test. Minimum score was chosen as 0 (dislike extremely in comparison to other sample) and maximum value was chosen as 4 (like extremely in comparison to other sample). Ballot used in sensory panel is shown in Figure 4.6.

4.13. Statistical Analysis

All data were analyzed by using one-way analysis of variance (ANOVA). Significant difference was considered at $p \leq 0.05$ and Tukey's test was used to determine statistical significant difference between treatments with Minitab Software 16 (Minitab Inc., State College, Pa., USA). Each data point was obtained as an average of 3 determinations and the error bars represented the standard deviation in the graphs. Capital and minor letters show significant difference ($p \leq 0.05$) for each treatment and among treatments for each storage time, respectively.

VISUAL AND OLFACTORY ATTRIBUTES OF PEACHES

For each descriptor, please consider the differences between 2 samples and choose the better sample and give the highest score for your better sample.

Peaches Colour Appearance

Please, after observing carefully the two samples (**A and B**), give a score following the structured scale.

Minimum value (0): dislike extremely comparison to other sample
Maximum value (4): like extremely comparison to other sample

Sample A

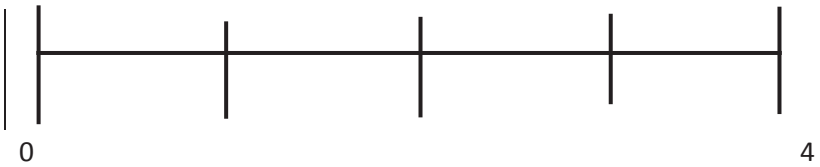
Sample B

Peaches Odour

Please, after observing carefully the two samples (**A and B**), give a score following the structured scale.

Minimum value (0): unpleasant odour
Maximum value (4): pleasant odour

Sample A



Sample B

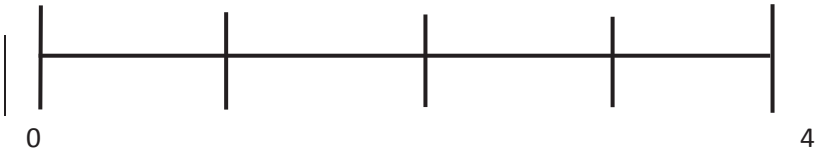


Figure 4. 6. Ballot used in sensory panel

CHAPTER 5

RESULTS and DISCUSSION

5.1. Chitosan and Green Tea Extract Properties

Microbiological assays for chitosan MIC (minimal inhibitory concentration) determination have shown that chitosan displays the highest efficacy against *P. chrisogenum* (MIC 0.5 g/L) while lowest efficacy was observed for *S. aureus* and *P. putida* (MIC 4.5 g/L) (Table 5.1).

Table 5. 1. Observed minimal inhibitory concentrations (MIC) of chitosan.

Species	**MIC (g/L)**
S. aureus	4.5
E. coli	3.0
P. putida	4.5
L. innocua	4.0
A. niger	3.0
P. chrisogenum	0.5

Green tea antioxidant capacity was calculated interpolation with the EC50 parameter from the curve followed reported (Figure 5.1), which was obtained plotting DPPH % decay vs green tea concentration. The same curve and parameter were carried out for Trolox, which was used as a reference standard. The obtained EC50 values were

2.2 ± 0.14 for Trolox and 4.1 ± 0.11 mg/L for the green tea extract, which indicates a high antioxidant value compared to the once reported in literature for other natural extracts (Table 5.2.) since the lower the EC50 the higher the antioxidant activity of a compound is (Paixão et al., 2007).

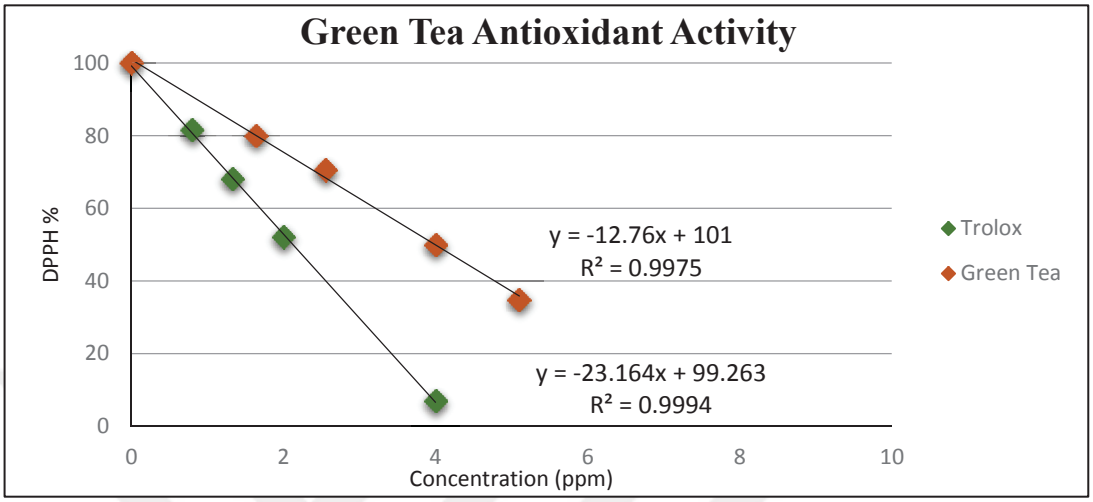


Figure 5. 1. Observed DPPH % decay for increasing concentrations of green tea and Trolox.

Table 5. 2. Reported values of EC50 for natural substances (Filippazzo, 2015, Ruela et al. 2011).

Natural substances	EC50 value (mg/L)
Grape peel	6.14
Blueberries	9.39
Guaranà	45.08
Posidonia Oceanica	72.42
Verbena litoralis	31.08
Vitex poligama	21.94

5.2. LbL Assembly

LbL coating assembly was first confirmed through contact angle measurements. Optical contact angle (OCA) measurements have proved that a different surface has been obtained after each step of new layer deposition. As shown in Figure 5.2, regular

alternating values of OCA (higher for chitosan, lower for alginate) were obtained during LbL assembly on PET.

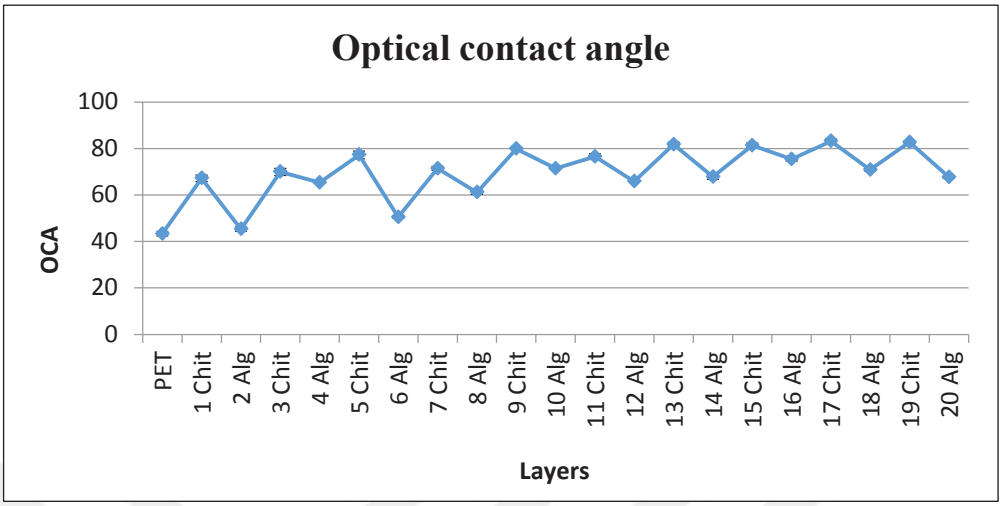


Figure 5. 2. Contact angle values of uncoated A-PET and A-PET coated with 20 alternated layers of chitosan and alginate.

As summarized in Table 5.3 after the deposition of the 9th layer, chitosan surface OCA measurements started to be fixed at 81.8 ± 1.3 while for alginate at 69.8 ± 3.5 ($p<0.05$). The higher variability observed for alginate layer surfaces could be attributed to an interference due to green tea tannins incorporation.

Table 5. 3. Contact angle mean values for alginate and chitosan layers demonstrating statistically significant differences between surface properties.

# Layer	CHITOSAN	# Layer	ALGINATE
9	79.9 ± 0.2	10	71.4 ±0.03
11	81.7 ± 1.1	12	65.8 ± 0.03
13	81.3 ± 0.9	14	67.8 ± 1.3
15	81.3 ± 0.25	16	75.4 ± 0.7
17	83.2 ± 0.9	18	70.9 ± 0.5
19	82.7 ± 0.7	20	67.7 ± 0.49
Mean:	81.8 ± 1.3	**Mean:**	68.6 ± 3.4

The LbL coating assembly was also confirmed by the increasing values of absorbance observed on PET samples during the deposition of following layers, employing the FITC-CS/ALG.

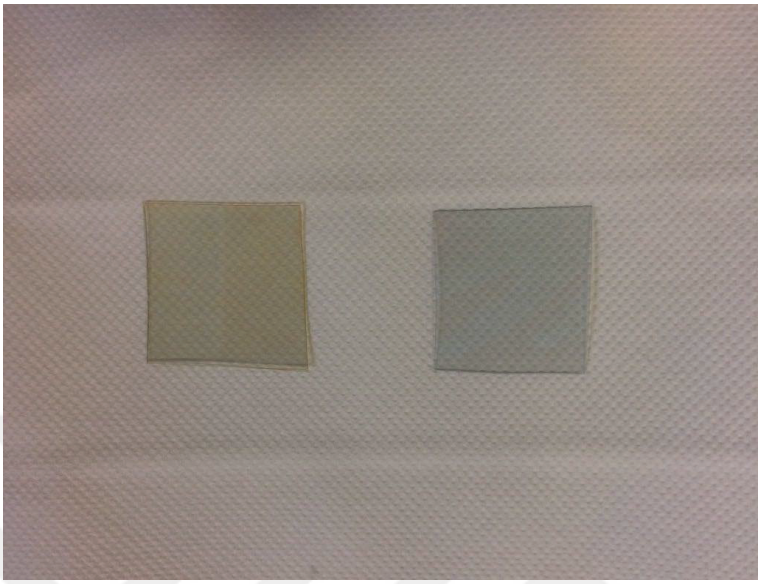


Figure 5. 3. FITC-CS/ALG coated PET samples (40 layers) and uncoated PET.

Figure 5.3 shows the FITC-CS/ALG (40 layers) coated PET samples, in which the colour change was noticeable by naked eyes.

As shown in Figure 5.4, a linear growth of PET absorbance was obtained, suggesting that thick constant layers were deposited.

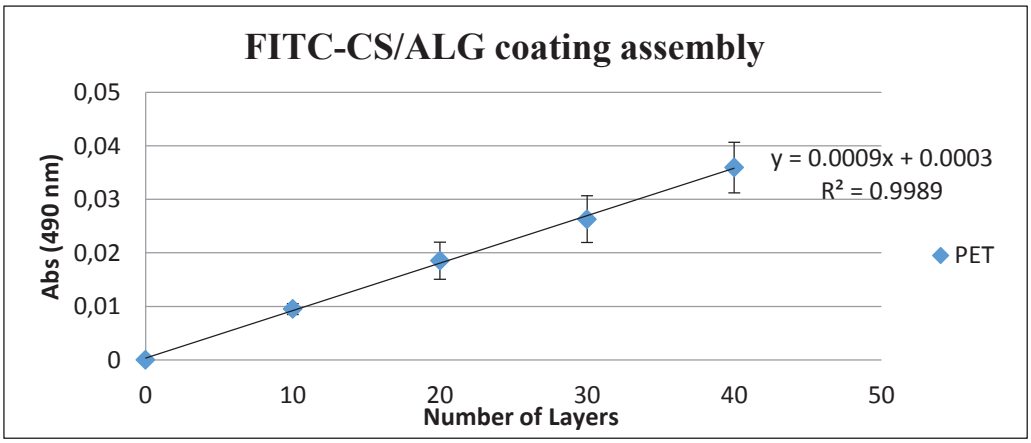


Figure 5. 4. Increasing absorbance of PET sample during FITC-CS/ALG coating assembly.

LbL coating kinetics of releasing was further investigated by submitting the FITC-CS/ALG coated PET samples (40 layers) to a longer extraction process and monitoring the absorbance of the simulant solution over time. As shown in Figure 5.5 a gradual release of the coating was observed during 196 h (8 d). For the first 144 h (6 d) the coating release has occurred at a constant speed (as shown in Figure 5.6), after that moment a deceleration start, leading to a constant value at 244 h (10 d).

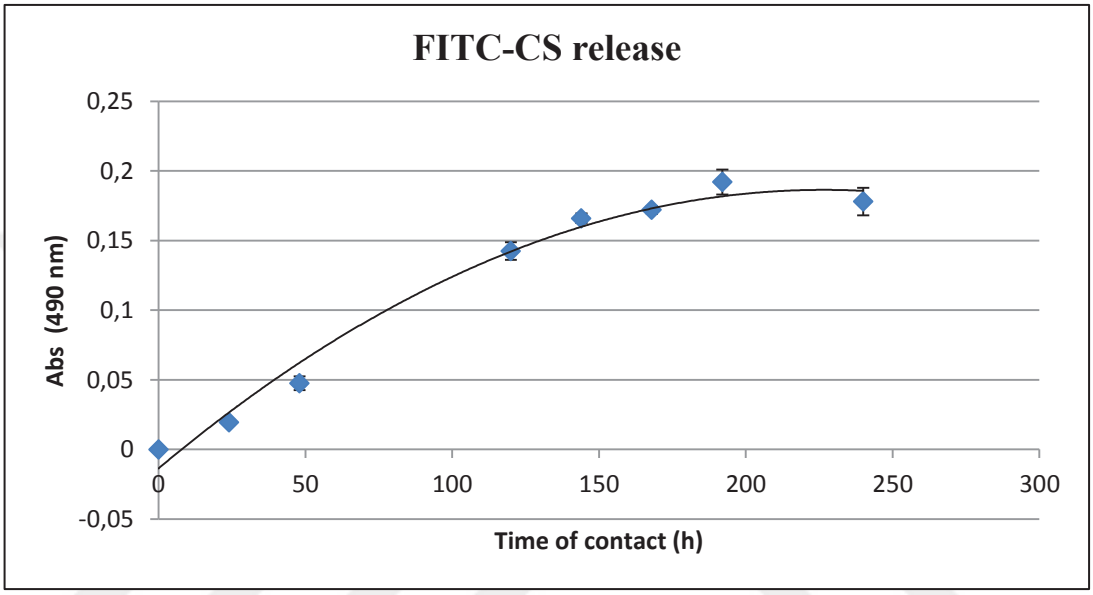


Figure 5. 5. Kinetics of FITC-CS release over 244 h of extraction (10 d).

Through a comparison between this observed FITC-CS/ALG coating kinetic of release and the one observed for the incorporated antioxidants species, it's possible to observe a faster release of the incorporated compounds, which could be related to their migrations through the coating. Indeed, for the antioxidant compounds a decreasing

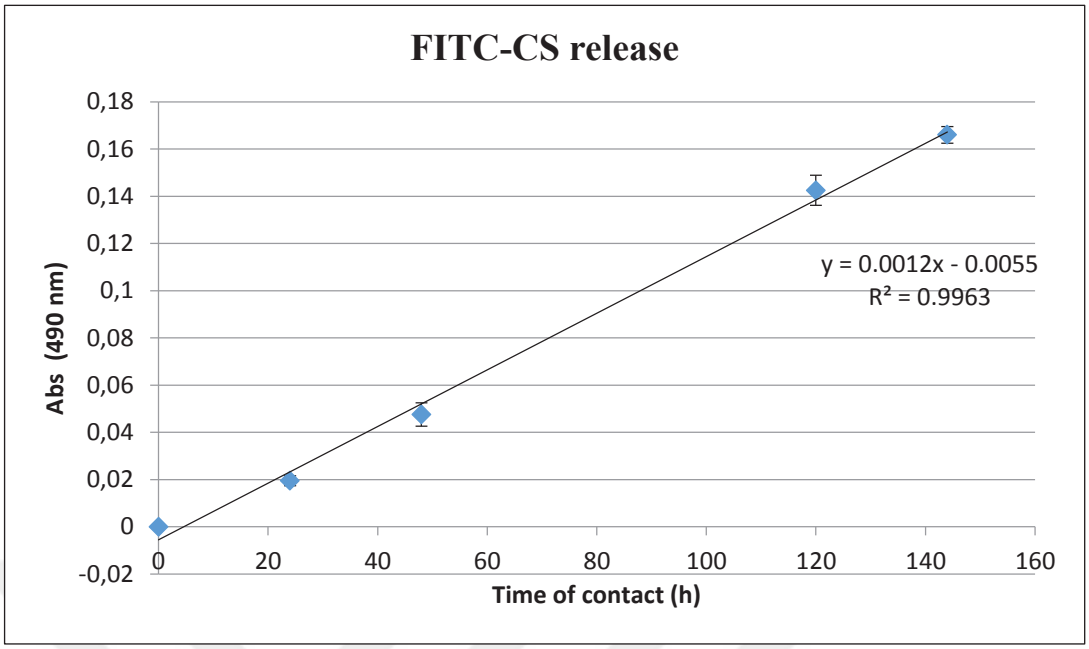


Figure 5. 6. Kinetics of FITC-CS release over the first 144 hours of extraction (6 days).

speed of release was observed before 48 h of extractions, which will probably lead to a complete extraction in few more hours, while the FIT-CS/ALG coating release did not display any decrease until 144 h (6 d).

At last, AFM topography images on the LbL coated glass slides have shown a difference in surface roughness between the alginate and the chitosan deposited layers as shown in Figure 5.7.

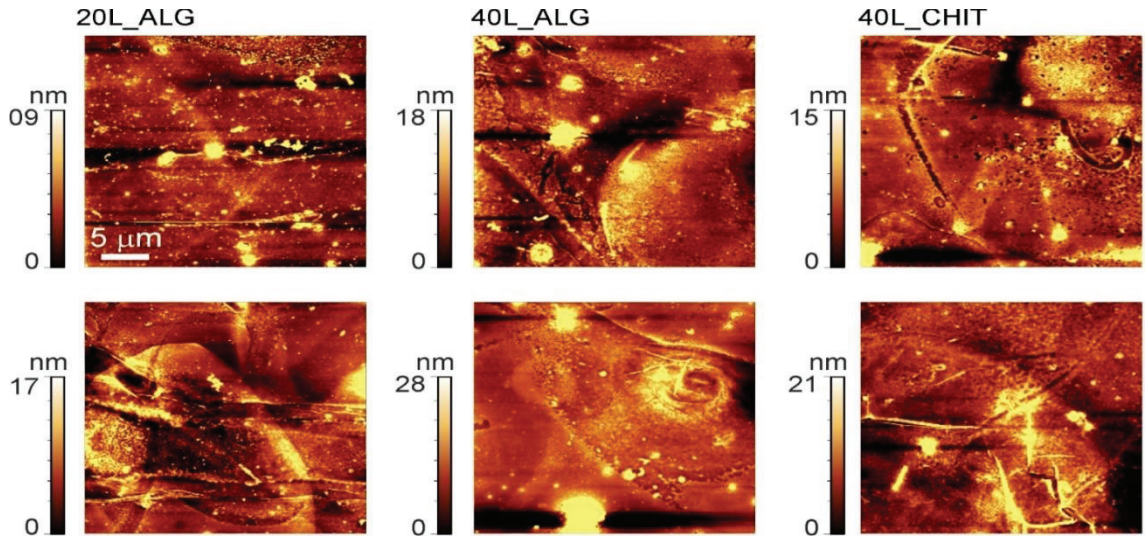


Figure 5. 7. AFM topography images of chitosan and alginate layer surfaces.

The LbL coating thickness determination through the AFM measurements are shown in Figure 5.8.

According to AFM measurements; the more layer built, the more thickness increased. While the thickness of LbL treated PET sheet with 20 layers was found 30 nm, the PET which was coated with 40 layers was found 65 nm. A linear correlation between thickness and number of layers was observed.

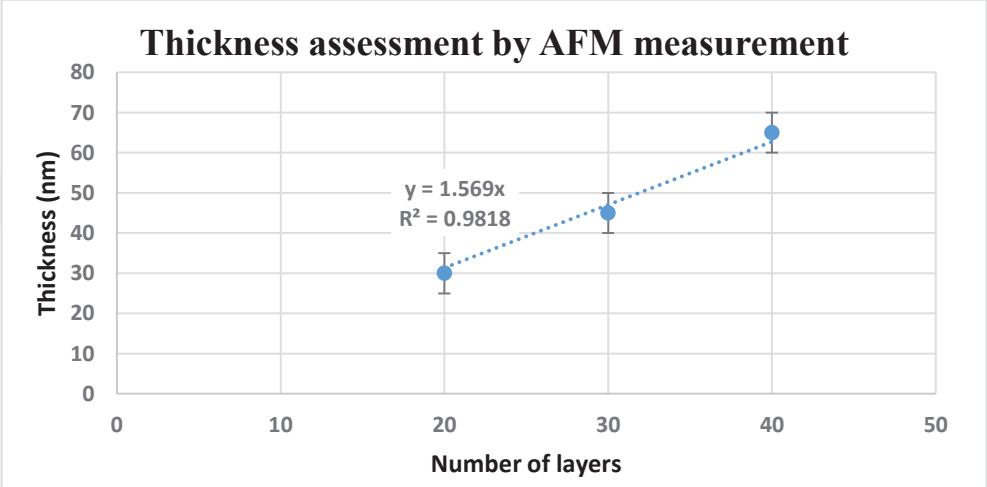


Figure 5. 8. Plot of thickness assessment by AFM measurements.

5.4. In Vitro Antimicrobial Activity

The influence of the antimicrobial compounds at which the coated PET films have been investigated on some specific target microorganisms. It was found in Figure 5.9 that all the LbL films coated with 40 layers had bacterial inhibition activity for *S. aureus* species.

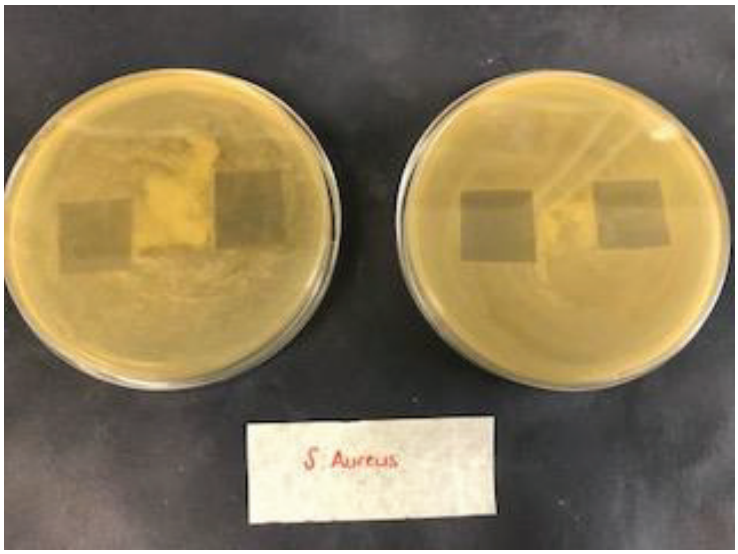


Figure 5. 9. Bacterial inhibition effect of LbL coated PET films on *S. aureus*.

The similar result was found for the samples treated with *Pseudomonas* (Figure 5.10) while the same result was not found in *Listeria spp*.

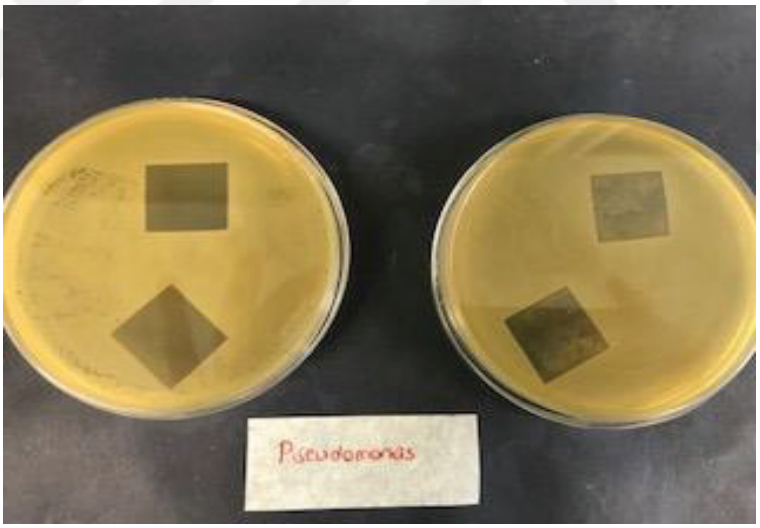


Figure 5. 10. Bacterial inhibition effect of LbL coated PET films on *Pseudomonas.*

5.5. LbL Coating in Vivo Application:

5.5.1. Shelf Life Studies of Peaches

In vivo applications of the LbL active coating on peaches have involved a monitoring of the ripening process through the evaluation of total soluble solids content and titratable acidity during storage. The observed trends for these parameters (shown in Figures 5.11 and 5.12) followed the normal postharvest behaviour and no significant differences were observed between the treatments ($p>0.05$), demonstrating that LbL active coating did not interfere with the pomological behaviour.

Weight loss was significantly different between the treatments and was summarized in Table 5.4. After 4 and 7 d of storage, the peaches packed with LbL PET displayed a lower weight loss than the control peaches (packed with uncoated PET between slides). Therefore, the presence of coated PET strips between peach slides positively affected their shelf life through a reduction of water lost during storage. Results did not show significant differences between the treatments ($p > 0.05$).

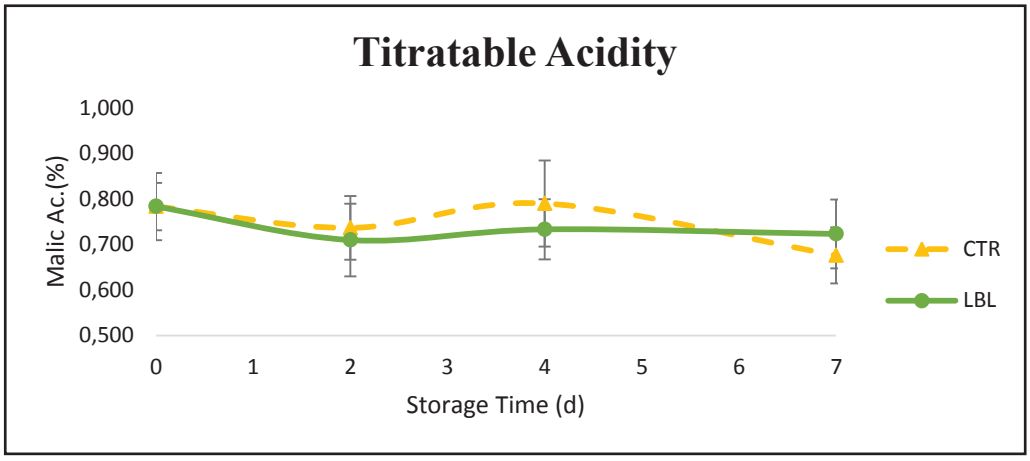


Figure 5. 11. Evolution of titratable acidity over 7 d of storage. No significant differences were observed between the treatments ($p > 0.05$).

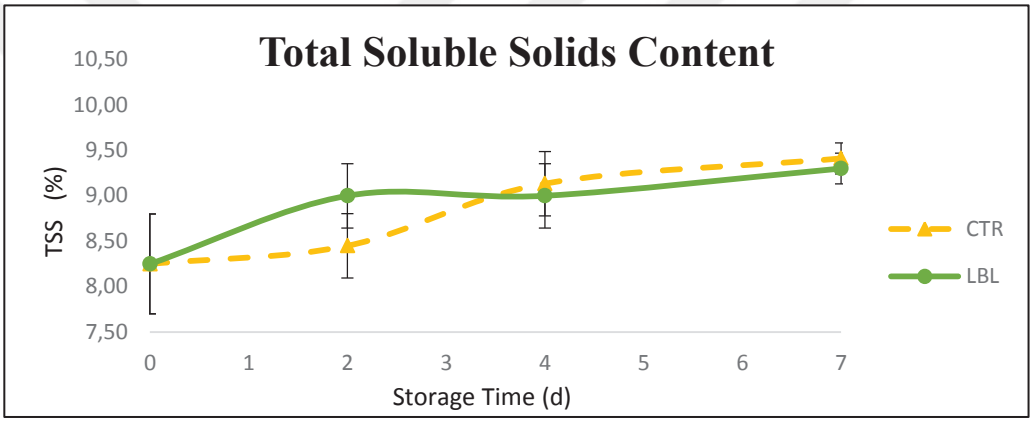


Figure 5. 12. Evolution of total soluble solids content over 7 d of storage. No significant differences observed between the treatments ($p > 0.05$).

Table 5. 4. Evolution of weight loss over 7 d of storage.

WEIGHT LOSS %			
	T2	**T4**	**T7**
CTR	0.17 ± 0.10 bA	1.50 ± 0.28 aB	2.48 ± 0.68 aB
LBL	0.24 ± 0.19 bA	1.25 ± 0.50 aB	2.26 ± 0.70 aB

Data are means ±SD (n=3)

[a-b]: Different letters indicate a significant difference between treatments

[A-B]: Different letters indicate a significant difference between storage time.

No significant differences in samples' firmness were observed ($p>0.05$). This behaviour could be related to the cold damage, known as *leatheriness*, which occurs frequently for peaches and lead to harder texture and dry fruits (Lurie et al. 2005).

Table 5. 5. Evolution of firmness over 7 d of storage.

FIRMNESS kg cm^{-1}				
	T0	**T2**	**T4**	**T7**
CTR	3.97 ± 0.58	3.42 ± 0.94	4.29 ± 0.35	4.46 ± 0.89
LBL	3.97 ± 0.58	3.63 ± 0.64	3.82 ± 0.50	5.36 ± 0.45

Data are means ± SD (n=3)

The changes in colour of peaches had similar trends for L*, a*, b* parameters for both treatments. The L* behaviour during storage was shown in Figure 5.13.

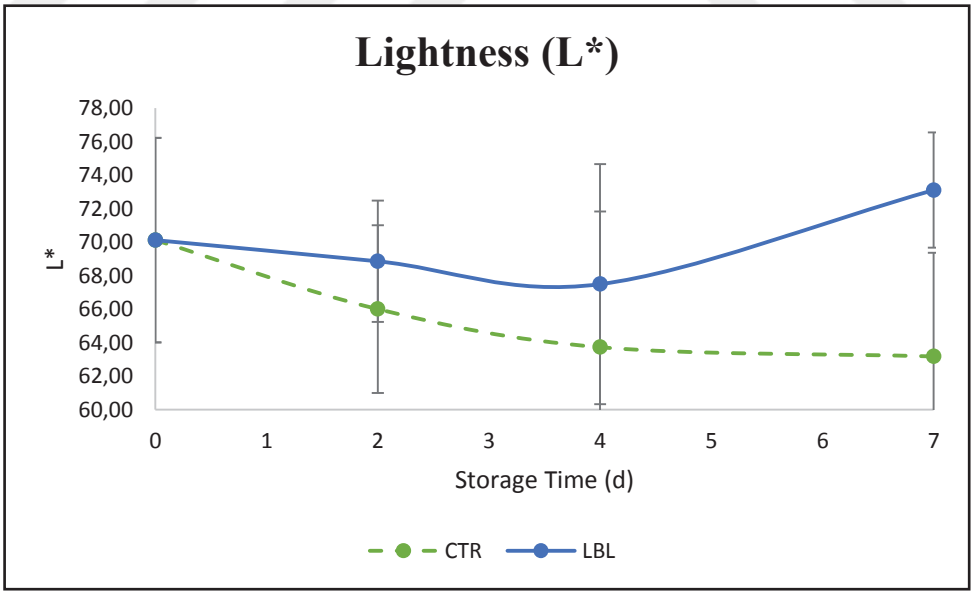


Figure 5. 13. Evolution of lightness (L*) over 7 d of storage.

The reduction in lightness is generally associated with browning in minimally processed peaches. However, in our samples there was an increase in the L* values which could be related to an increase in the brightness of the slice surface due to reversible surface dehydration. Other reported studies described this behaviour for mature-green soft-flash peaches and carrots (Gonzales-Buesa et al. 2011).

Lower increase of L* was observed for CTR (uncoated) suggesting a browning tendency, while LbL samples had higher increase. The difference between the treatments was not significant ($p > 0.05$).

The a* behaviour was shown in Figure 5.14., for all treatments an increasing trend was observed.

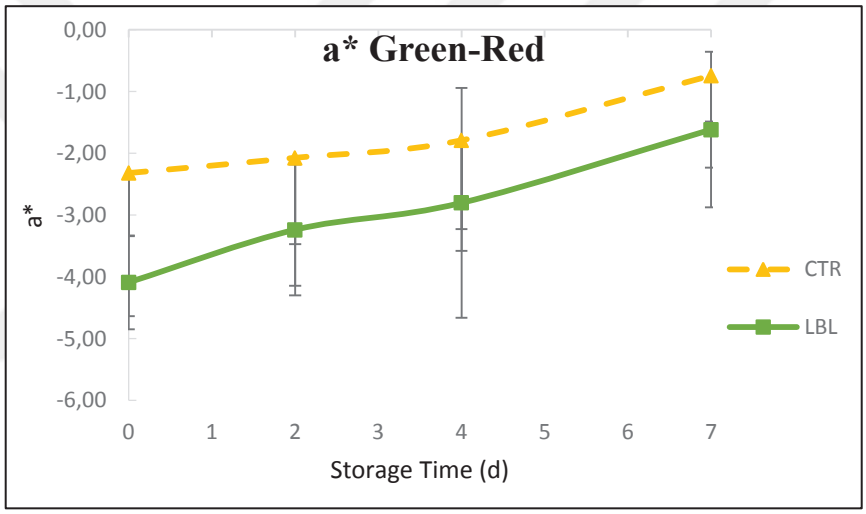


Figure 5. 14. Evolution of a* over 7 d of storage.

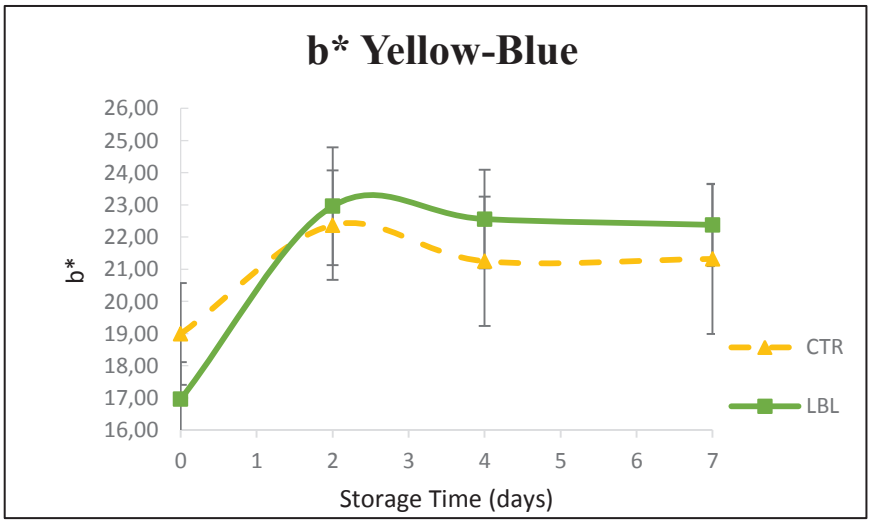


Figure 5. 15. Evolution of b* over 7 d of storage.

This observed behaviour could be related to chlorophyll degradations process, indeed an increase of a* suggests a color change from green (-a*) to red (+a*). For b* values, an increase was observed at 2 d of storage, a slightly decrease at 4 d and it stayed constant until day 7 (Figure5.15).

The development of enzymatic browning is generally reflected by a decrease in both L^* value (which can be associated to the darkening of the fruit), and h° angle (which was linked to the browning at the surface) (Rocha & Morais, 2003). The L*a*b* color measuraments were employed in order to calculate ΔE (Table 5.6 and 5.7) values after 7 d of storage. The values given below were used to determine if the total colour difference was visually obvious (Baixauli et al.; 2008);

$\Delta E^* < 1$ colour differences are not obvious for the human eye

$1 < \Delta E^* < 3$ colour differences are not appreciative by the human eye

$\Delta E^* > 3$ colour differences are obvious for the human eye

Table 5. 6. ΔE* Values in comparison with first day of storage.

ΔE*	**2**	**4**	**7**
CTR	5.10	6.51	7.29
LBL	5.60	4.31	3.70

When the treatments were compared with the first day results; it was clear that both of the treatments have an alteration which can be recognizable by eyes since the ΔE* value was higher than 3 until the last day of storage, but after 4 d of storage, ΔE* value of CTR sample decreased and ΔE* value of LbL sample was still higher than 3. When the reference was taken as CTR samples for each day, as shown in Table 5.7, for all days, ΔE* values were higher than 3 and this means that LbL treated peach samples had better colour and appearance than CTR samples for each sampling day.

Control slices showed a great decrease in the colour parameters, chroma and hue angle which decreased during storage and reached their minimum value 7.08 at the last day of storage (Table 5.8). In contrast, significantly higher hue values were observed for the LbL coated peach slices throughout storage. At the end of storage, decline in hue

angle of LbL was almost 6 times less than decline in CTR values. Chroma values of CTR first increased and then decreased until the end of storage while LbL samples had higher chroma (C*) values throughout storage (Table 5.8).

Table 5. 7. ΔE* values of LbL treated peaches in comparison with CTR for each sampling day.

ΔE	**2**	**4**	**7**
LBL	4.07	3.97	9.96

Table 5. 8. Changes in other color parameters during storage of fresh cut peach slices packed with LbL and ucoated PET (CTR).

Treatment	**Day**	**C***	**Hue °**
	0	39.33	87.42
CTR	2	42.13	44.02
	4	40.11	31.64
	7	40.98	7.08
LBL	2	44.77	86.73
	4	42.74	87.10
	7	41.43	43.51

As shown in Figure 5.16, from a visual evaluation, the LbL treated peaches had the most desirable colour and appearance.

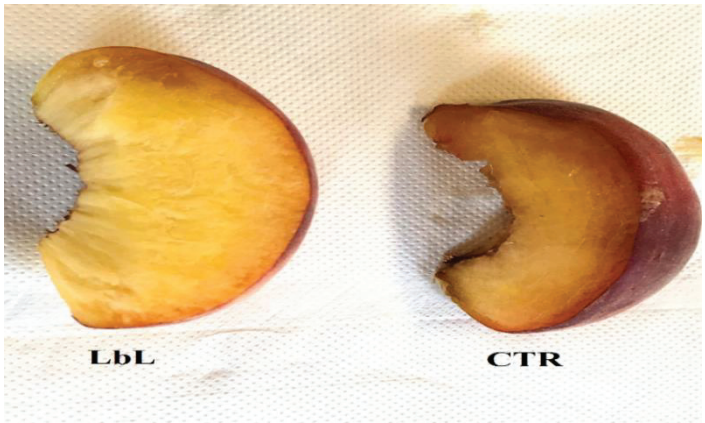


Figure 5. 16. Visual appearance of LbL treated peach slices (LbL), uncoated PET peach slices (CTR) after 7 d of storage.

Carotenoid content determination has shown a high variability between samples (Table 5.9). However, after 7 d of storage the LbL treated peaches have shown better preservation of carotenoid contents (Figure 5.17).

Table 5. 9. Evolution of carotenoid content.

CAROTENOID CONTENT (µg/100 g)				
	T0	**T2**	**T4**	**T7**
CTR	90.3 ± 18.9 aB	38.3 ± 19.11 aA	32.6 ± 11.0 aA	49.3 ± 6.3 aA
LBL	90.3 ± 18.9 aB	52.7 ± 24.7 aA	42 ± 24.6 aA	80.2 ± 2.7 bA

Data are means ±SD (n=3)

[a-b]: Different letters indicate a significant difference between treatments

[A-C]: Different letters indicate a significant difference between storage times.

PPO activity measurements have shown an increasing tendency for CTR samples, resulting in significant higher values for uncoated peaches after 7 d of storage. For the LBL treated peaches no significant increase of PPO activity was observed after 7 d of storage. The PPO activity was significantly lower in LbL samples than uncoated samples (Figure 5.18). This behaviour could have been explained by the binding of the enzyme to chitosan, which is a cationic polysaccharide recognized as an excellent protein binder. The same result was observed in a study which determined the effect of chitosan coating on shelf life of fresh-cut mushroom. (Eissa, 2006).

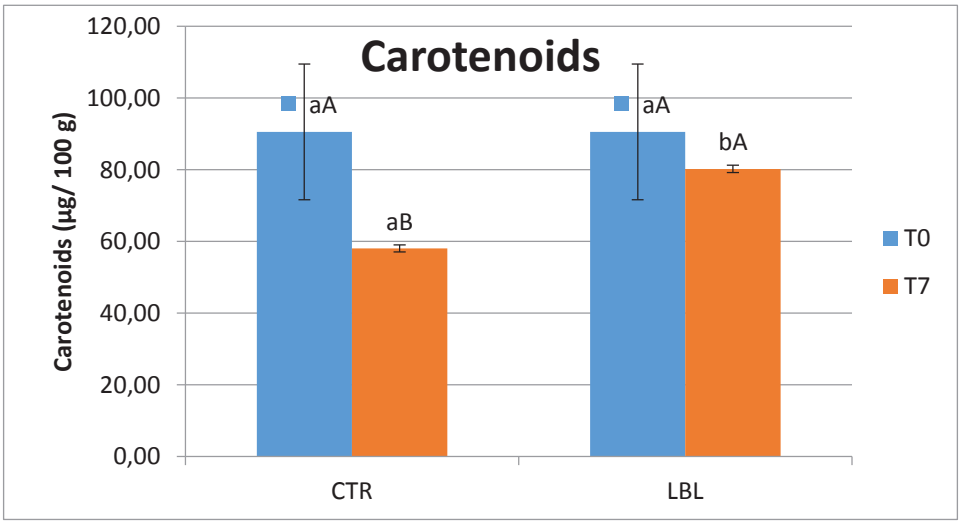


Figure 5. 17. Evolution of total carotenoids after 7 d of storage.

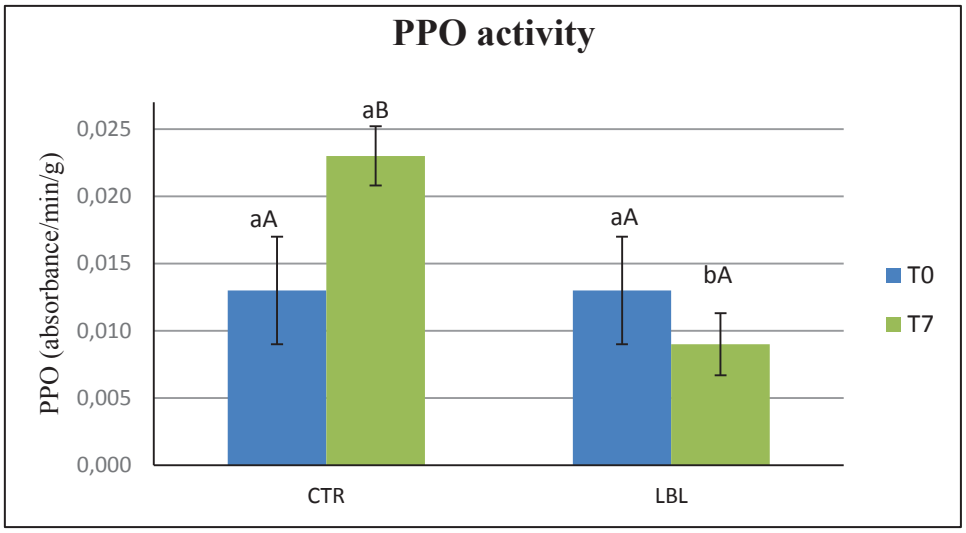


Figure 5. 18. Evolution of PPO activity after 7 d of storage for the LbL treated peaches (LBL), the uncoated peaches (CTR).

[a-b]: Different letters indicate a significant difference between treatments

[A-B]: Different letters indicate a significant difference between storage times.

The total phenolic contents were determined by the Folin–Ciocalteu colorimetric method (Singleton and Rossi 1965) and results were expressed as gallic acid equivalents (mg/L). As seen in Figure 5.19, total phenolic content (mg/L) was higher in LbL peaches (173.68 mg/L) than CTR peaches (130.20 mg/L) at the end of the storage. The absorbance measurements of phenolic compound were showed in Figure 5.20. LbL treated PET coated peach samples had higher phenolic content than control peaches; however, phenolic content of both treatment was similar until the 7th day of storage but after 1 week of storage LbL samples have shown higher phenolic contents than control samples. This could be assumed that decline in total phenols concentration was prevented by LbL deposition due to antioxidant activity of green tea extract. As a result, addition of the green tea extract into the alginate solution was significantly effective ($p > 0.05$) in terms prevention of phenolic compounds' loss.

The DPPH is a stable radical with a maximum absorption at 517 nm that can easily undergo scavenging by antioxidant (Lu and Yeap Foo, 2001). As presented in Table 5.10., LbL treatment on PET sheets have a potential antioxidant activity on fresh-cut peaches, achieved by scavenging abilities observed against DPPH.

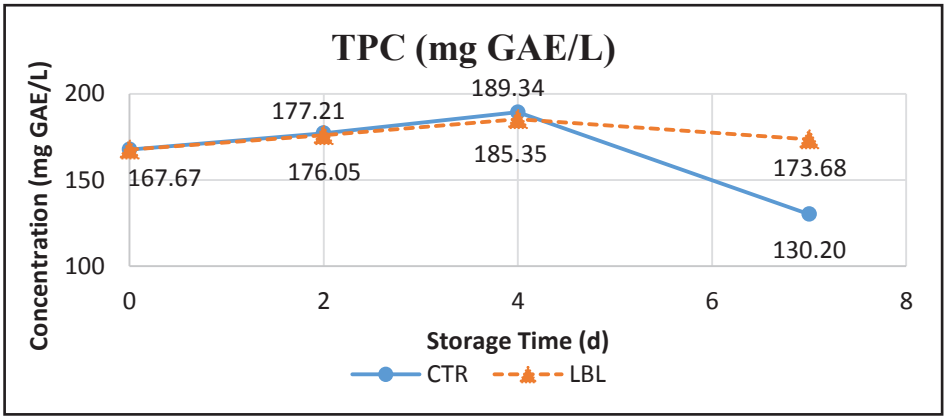


Figure 5. 19. The total phenolic content (mg gallic acid equivalent/L).

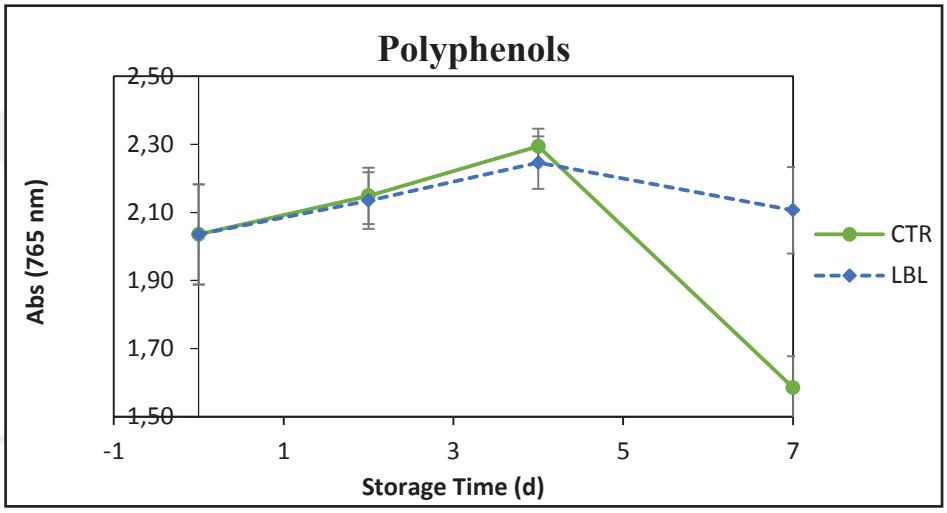


Figure 5. 20. Evaluation of polyphenols over 7 d of storage for the LbL treated peaches (LBL), the uncoated peaches (CTR).

Table 5. 10. DPPH % over 7 d of storage for the LbL treated peaches (LBL) and the uncoated peaches (CTR).

DPPH %	**0**	**2**	**4**	**7**
CTR	24.26 ± 0	36.79 ± 1.8	15.478 ± 1.4	6.138 ± 0.5
LBL	24.26 ± 0	33.58 ± 4.5	9.14 ± 0.1	3.130 ± 2.0

5.5.2. Microbiological Assay Results

Due to the results of microbiological analysis this novel active packaging system was effective for all types of microorganisms which were investigated in this study on fresh-cut peaches. While psychrophile microorganism counts increased during storage for control peaches, LbL results were much less than CTR counts as shown in Figure 5.21.

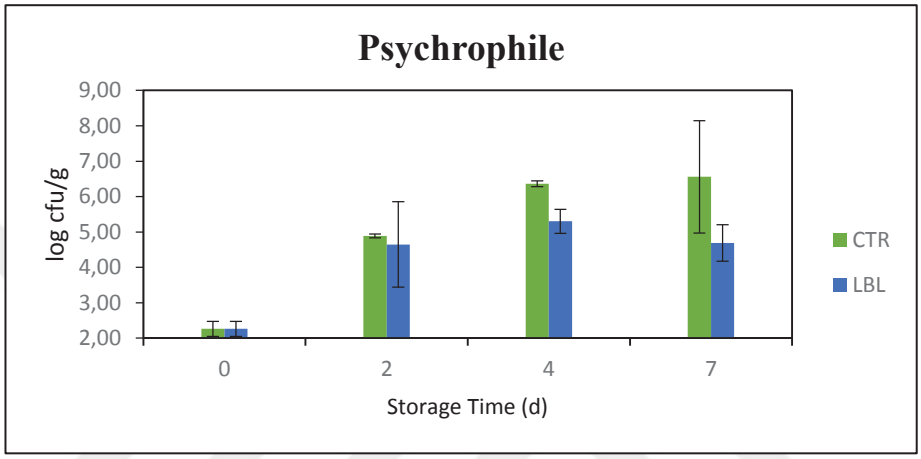


Figure 5. 21. Evolution of psychrophile counts over 7 d of storage for the LbL treated peaches (LBL) and control peaches (CTR).

Microbial analysis showed that after 2 d of storage there was an increase in total aerobic count up to 6.5 log cfu/g in control samples, while it was increasing to 5 log cfu/g in peaches stored with LBL active package until the 4th day. After 4 d of storage, a decline has been observed in microbial population of LbL samples up to 4 log cfu/g, while microbial population of CTR samples continued to increase (Figure 5.22). The fact which provides to obtain this decline should be associated with the antimicrobial effect of LbL deposition.

Time course of yeasts and moulds populations resembles those reported by Siroli et al. (2014) for minimally processed apples dipped in different antimicrobials comparatively: shelf life of fresh cut fruit is affected by microbial growth, independently from the addition of natural antimicrobials, the end of shelf life is mainly determined by changes in colour and not by the increase of microorganisms.

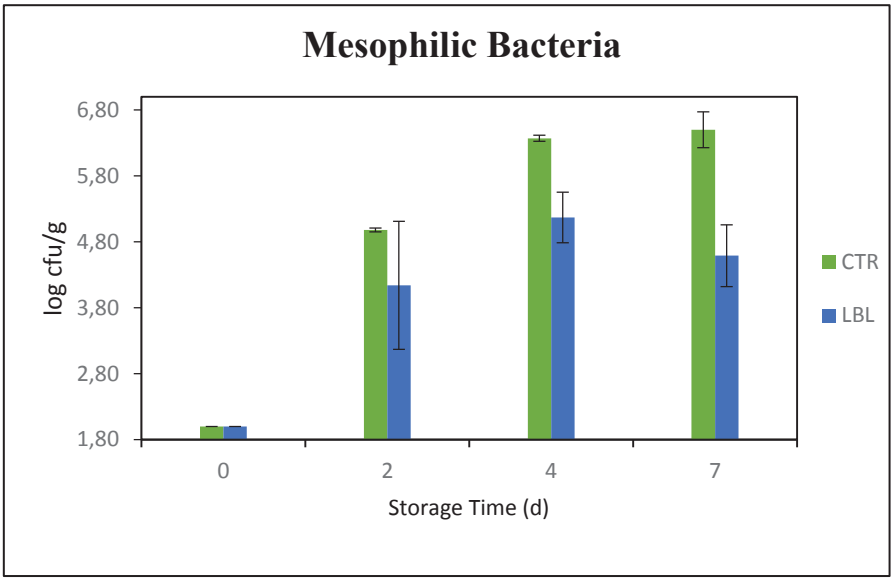


Figure 5. 22. Evolution of total aerobic count over 7 d of storage.

As shown in Figure 5.23, both microbial population of CTR and LbL treatments showed an increase during 1 week of storage. But the increase in CTR treatment has been found much higher than LbL treatment on every sampling day. It could be concluded that this active packaging system is effective on inhibition of the yeast and mould growth.

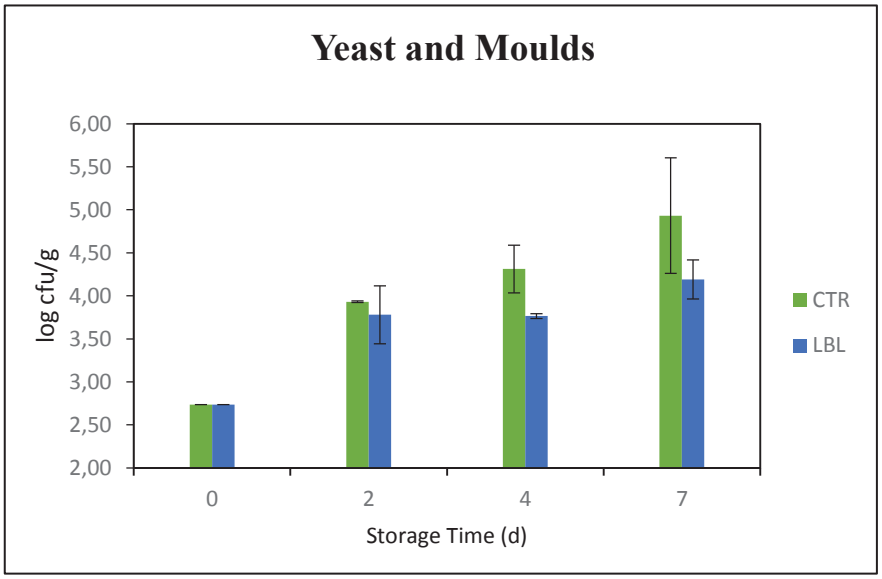


Figure 5. 23. Evolution of yeast and mould counts over 7 d of storage.

5.5.3. Gas Chromatography Analysis

In this study; both the LbL and CTR samples were packed with ambient air (0.033 % CO_2, 20.986 % O_2, and 78.084 % N_2). Every sampling day, gas composition of packages was measured. Since the final gas level in a packaged food system depends on film permeability and product respiration rate (Bai et al., 2001), both treatments showed different results during storage. As shown in Figure 5.24, tendency of both CO_2 results were found the same, both treatments showed an increase until 4th day of storage and showed a decrease until the end of storage. But increase in CO_2 percentage of LbL package was almost 2 times higher than CTR samples.

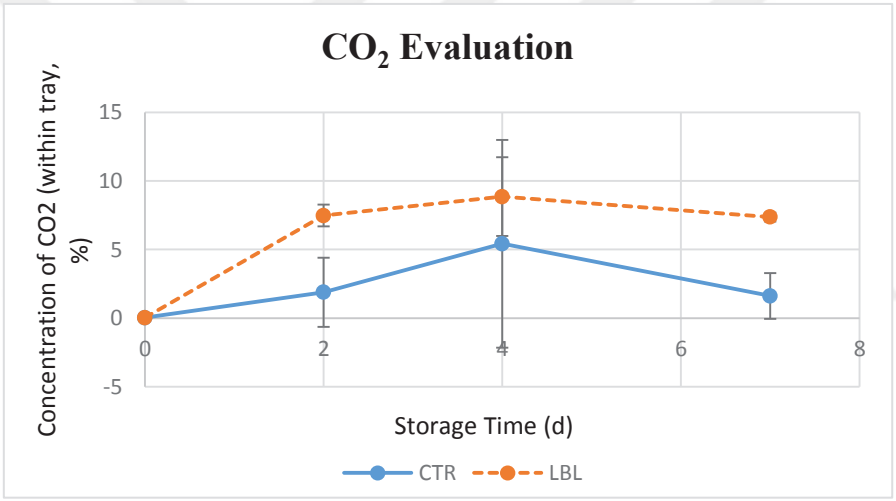


Figure 5. 24. CO_2 exchange dynamics in OPP packed peaches over 7 d of storage at 4°C.

The results of O_2 evaluation for both CTR and LbL treatments have showed a decrease until the 4th d of storage, but decrease in LbL samples were found significantly different ($p > 0.05$) (Figure 5.25). After 4th day of storage, while O_2 level of CTR samples was increasing sharply, LbL samples showed a slight increase in O_2 level. Those results may rise from the antimicrobial effect of chitosan since O_2 level in LbL samples was lower than CTR samples during storage. At the end of storage, both the O_2 and CO_2 levels within the tray of CTR and LbL were found significantly different than each other ($p > 0.05$).

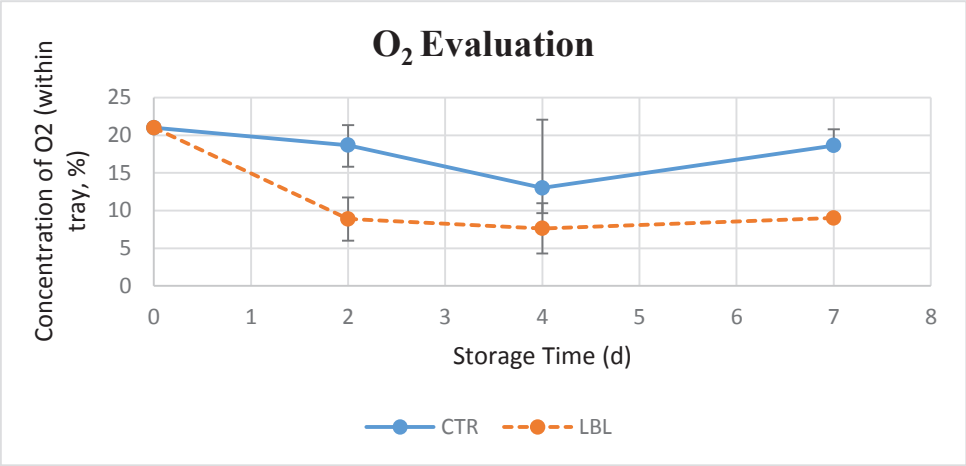


Figure 5. 25. O 2 exchange dynamics in OPP packed peaches over 7 d of storage at 4°C.

Fresh cut fruit and vegetables, which are extremely perishable and more susceptible to spoilage than the whole commodities, could show different exchange dynamics within treatments. In the present study, the dynamics of exchange in O_2 and CO_2 percentages within the bags showed totally different attitudes for CTR and LbL samples. To sum up; these results may arise from the fact that LbL deposition increase the thickness of the PET sheets and provide better barrier properties for the package. It is fact that the peach trays which are coated by LbL PET sheets have power to change the environment of packages.

5.5.4. Sensory Evaluation of the Peaches

Effect of LbL technique on PET films which were used as packaging material for peaches have been evaluated by sensory panel. The results for sensory attributes of LBL treated peaches are summarized in Table 5.11. All sensory attributes were similar for LBL treated peaches while control groups had a decreasing attitude. All the attributes had higher scores for LBL treated peaches until the end of storage. Appearance-color attributes of peaches were ranked as the highest in comparison with control samples. Odour of peaches were found better in LbL treatment during 4 d of storage, but odour was found more pleasant in control samples at the end of storage. Since the explanation of the unpleasant odour by panelists was similar to acidic odour definition, the source of the odour of LbL samples in general may have been connected with the usage of acetic acid for preparation of the chitosan solution. As a conclusion,

when LbL assembled PET strips have been used for fresh-cut peaches, desire should have been increased by consumers. In terms of demonstrating the preference of the LbL samples in terms of desire, the results were obvious by naked eyes, as well. As a suggestion for further studies, acetic acid could be removed from chitosan solution after dissolving chitosan in acidic environment.

Table 5. 11. Sensory attributes of control peaches group with respect to LBL treated peaches during 7 d of storage.

		Days of storage			
Sensory attributes	**Sample**	**0**	**2**	**4**	**7**
Colour	CTR	4.00 ± 0.0	2.20 ± 0.9	2.23 ±1.1	1.73 ± 0.7
	LBL	4.00 ± 0.0	3.13 ± 0.8	3.33 ± 0.9	3.20 ± 0.6
Odour	CTR	4.00 ± 0.0	2.57 ± 0.8	2.77 ± 1.1	2.83 ± 1.0
	LBL	4.00 ± 0.0	2.86 ± 1.2	2.86 ± 1.0	2.20 ± 1.0

CHAPTER 6

CONCLUSION AND FUTURE PERSPECTIVES

In the first step of this thesis, the properties of the PET films coated with layer-by-layer technique was investigated. Chitosan and alginate solution linked with green tea extract was applied on PET films by layer-by-layer deposition. Then, film properties were evaluated by in vitro assays. Multilayers construction was demonstrated by optical contact angle surface measurements, atomic force microscopy and UV-Visible spectroscopy.

In vitro assay allowed us to prove the incorporation of green tea antioxidant compounds inside the alginate layers and their gradual release from the coated PET during time of extraction. Chitosan gradual release was confirmed by the observed increase of simulant solution absorbance over 10 days of contact with the FITC-CS/ALG coated PET samples. It was found that the construction of layer coating showed a linear tendency, as demonstrated by the increase of PET absorbance during the LbL assembly employing fluorescein-isothiocyanate labeled chitosan (FITC-CS).

Preliminary tests have proved the convenience of chitosan and green tea extract as natural active substances to prevent microbiological spoilage and food oxidation on fresh cut fruits; respectively. Based on the results, the in vitro approach with simulant solutions, typically overestimate the extraction capacity of food, therefore the observed kinetics could be different during in vivo applications.

Since some researchers were investigated only properties of the coated PETs, the crucial point of this study was to investigate the effect of the LbL deposition on the shelf life of fresh-cut peaches. And the *in vivo* assays of this study have showed interesting and promising results. The monitoring of peach TSS contents and titratable acidity has demonstrated that the LbL active coating does not interfere with the natural postharvest behaviour. A lower weight loss was observed for LbL treated peach samples, suggesting that the presence of coated PET strips could act as a barrier against fruit water loss and we can conclude that more water vapour change has been found in

CTR samples than LbL samples. Promising results were obtained from carotenoid content evaluation, PPO activity measurements and total phenolic contents. Mainly, after 7 days of storage the LbL treated samples have shown the best preservation of carotenoids, the lowest PPO activity and more phenolic content has been observed in LbL samples. The observed carotenoids preservation and higher phenolic content should be linked to the release of green tea antioxidants from the coating, while the lowest PPO activity could be related to chelating properties of chitosan and alginate.

Microbiological monitoring of peaches has shown that all types of the microorganisms which were investigated during this study affected by this novel active packaging system. While psychrophile microorganism counts increased during storage for control peaches, LbL counts were much less than CTR counts. For both mesophilic and psychrophilic microorganism counts showed that the colonies showed an increasing trend for both treatments until the 4^{th} d of storage. However; the increase in LbL treatment was always lower than CTR counts. After 4^{th} d of storage, LbL treatment shows a decline in counts while the CTR counts continued to increase. Due to those promising microbiological results, it could be concluded that gradual release of active substances was effective in the food product. Overall these *in vivo* results allowed us to suppose that the release of active substances from the LbL coating occurs also during the contact with food, and that release could be effective to produce positive effects on its shelf life. According to best of our knowledge no detailed information were obtained about the kinetic of release of coating on food and about its effectiveness compared to a traditional addition. With this study, a novel promising active packaging study of fresh-cut peaches was added to the literature. Moreover; based on sensory results, appearance and colour of the LbL treated packed peaches were promising and those samples may have been desired by consumers. In comparison with the control peaches, it was obvious to separate the LbL deposition treated peaches even by naked eyes.

As a conclusion, the shelf life of fresh-cut peaches was extended up to 7 days. With this study; the LbL coating technique has been proven as a useful technique to produce controlled-release active packaging system for extending the shelf life of fresh-cut peaches according to all results that were obtained in this study. Since it is an easy and cheap procedure, it is possible to use this system also for industrial applications. However, more detailed studies are needed to optimize the rate of active substances release and their effectiveness on fresh fruit and vegetables which are different than

peaches. To achieve this goal, the effect of diverse factors should be investigated; as examples, different solution pH's can be applied for the LbL assembly to find the most effective construction, various active substances should have been applied to achieve the best effectiveness. Moreover, further studies are required to demonstrate that the described system could be more effective than conventional food preservation.

REFERENCES

Abdollahi, M., Rezaei, M., & Farzi, G. (2012). Improvement of active chitosan film properties with rosemary essential oil for food packaging. International Journal of Food Science & Technology, 47(4), 847-853.

Ahvenainen, R. (1996). New approaches in improving the shelf life of minimally processed fruit and vegetables. Trends in Food Science & Technology, 7(6), 179-187.

Ahvenainen, R., & Hurme, E. (1997). Active and smart packaging for meeting consumer demands for quality and safety. Food Additives & Contaminants, 14(6-7), 753-763

Aider, M. (2010). Chitosan application for active bio-based films production and potential in the food industry. LWT-Food Science and Technology, 43(6), 837-842.

Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. Innovative Food Science & Emerging Technologies, 3(2), 113-126.

Ariga, K., Hill, J. P., & Ji, Q. (2007). Layer-by-layer assembly as a versatile bottom-up nanofabrication technique for exploratory research and realistic application. Physical Chemistry Chemical Physics, 9(19), 2319-2340.

Arvanitoyannis, I. S., & Oikonomou, G. (2012). Active and intelligent packaging. In Modified Atmosphere and Active Packaging Technologies (pp. 627-662). CRC Press.

Azuma, K., Izumi, R., Osaki, T., Ifuku, S., Morimoto, M., Saimoto, H., & Okamoto, Y. (2015). Chitin, chitosan, and its derivatives for wound healing: old and new materials. Journal of functional biomaterials, 6(1), 104-142.

Bai, J. H., Saftner, R. A., Watada, A. E., & Lee, Y. S. (2001). Modified atmosphere maintains quality of fresh- cut cantaloupe (Cucumis melo L.). Journal of Food Science, 66(8), 1207-1211.

Baixauli, R., Salvador, A., & Fiszman, S. M. (2008). Textural and colour changes during storage and sensory shelf life of muffins containing resistant starch. European Food Research and Technology, 226(3), 523-530.

Ban, Z., Feng, J., Wei, W., Yang, X., Li, J., Guan, J., & Li, J. (2015). Synergistic effect of sodium chlorite and edible coating on quality maintenance of minimally processed citrus grandis under passive and active MAP. Journal of Food Science, 80(8), C1705-C1712.

Bhat, R., Alias, A. K., & Paliyath, G. (Eds.). (2012). Progress in food preservation. John Wiley & Sons.

Bhunia, K., Sablani, S. S., Tang, J., & Rasco, B. (2013). Migration of chemical compounds from packaging polymers during microwave, conventional heat treatment, and storage. Comprehensive Reviews in Food Science and Food Safety, 12(5), 523-545.

Cantwell, M., & Suslow, T. (1999). Fresh-cut fruits and vegetables: aspects of physiology, preparation and handling that affect quality. In Annual Workshop Fresh-Cut Products: Maintaining Quality and Safety (Vol. 5, pp. 1-22).

Carneiro-da-Cunha, M. G., Cerqueira, M. A., Souza, B. W., Souza, M. P., Teixeira, J. A., & Vicente, A. A. (2009). Physical properties of edible coatings and films made with a polysaccharide from Anacardium occidentale L. Journal of Food Engineering, 95(3), 379-385.

Carrizo, D., Taborda, G., Nerín, C., & Bosetti, O. (2016). Extension of shelf life of two fatty foods using a new antioxidant multilayer packaging containing green tea extract. Innovative Food Science & Emerging Technologies, 33, 534-541.

Chan, E. W., Soh, E. Y., Tie, P. P., & Law, Y. P. (2011). Antioxidant and antibacterial properties of green, black, and herbal teas of Camellia sinensis. Pharmacognosy research, 3(4), 266.

Chi-Zhang, Y., Yam, K. L., & Chikindas, M. L. (2004). Effective control of Listeria monocytogenes by combination of nisin formulated and slowly released into a broth system. International Journal of Food Microbiology, 90(1), 15-22.

Coles, R., McDowell, D., & Kirwan, M. J. (Eds.). (2003). Food packaging Technology (Vol. 5). CRC Press.

Cras, J. J., Rowe-Taitt, C. A., Nivens, D. A., & Ligler, F. S. (1999). Comparison of chemical cleaning methods of glass in preparation for silanization. Biosensors and bioelectronics, 14(8), 683-688.

Cuero, R. G., Duffus, E., Osuji, G., & Pettit, R. (1991). Aflatoxin control in preharvest maize: effects of chitosan and two microbial agents. The Journal of Agricultural Science, 117(02), 165-169.

Dainelli, D., Gontard, N., Spyropoulos, D., Zondervan-van den Beuken, E., & Tobback, P. (2008). Active and intelligent food packaging: legal aspects and safety concerns. Trends in Food Science & Technology, 19, S103-S112.

Day, B. P. (2008). Active packaging of food. Smart packaging technologies for fast moving consumer goods, 1-18.

de Castro, A. M., Carniel, A., Junior, J. N., da Conceição Gomes, A., & Valoni, É. (2017). Screening of commercial enzymes for poly (ethylene terephthalate) (PET) hydrolysis and synergy studies on different substrate sources. Journal of Industrial Microbiology & Biotechnology, 1-10.

de Ritter E., Purcell A.E. (1981). “Carotenoids Analytical Methods.” Carotenoids as colorants and vitamin A precursors. Ed: Bauernfeind. JC. Academic Inc London Ltd. 815-820.

de Roever, C. (1998). Microbiological safety evaluations and recommendations on fresh produce. Food Control, 9(6), 321-347.

Decher, G. (1997). Fuzzy nanoassemblies: toward layered polymeric multicomposites. Science, 277(5330), 1232-1237.

Decker, E. A., Elias, R. J., & McClements, D. J. (Eds.). (2010). Oxidation in foods and beverages and antioxidant applications: management in different industry sectors. Elsevier.

del Hoyo-Gallego, S., Pérez-Álvarez, L., Gómez-Galván, F., Lizundia, E., Kuritka, I., Sedlarik, V., & Vila-Vilela, J. L. (2016). Construction of antibacterial poly (ethylene terephthalate) films via layer by layer assembly of chitosan and hyaluronic acid. Carbohydrate Polymers, 143, 35-43.

Diao, Y., Shaw, L., Bao, Z., & Mannsfeld, S. C. (2014). Morphology control strategies for solution-processed organic semiconductor thin films. Energy & Environmental Science, 7(7), 2145-2159.

Duran, M., Aday, M. S., Zorba, N. N. D., Temizkan, R., Büyükcan, M. B., & Caner, C. (2016). Potential of antimicrobial active packaging containing natamycin, nisin, pomegranate and grape seed extract in chitosan coating to extend shelf life of fresh strawberry. Food and Bioproducts Processing, 98, 354-363.

Eissa, H. A. (2007). Effect of chitosan coating on shelf life and quality of fresh- cut mushroom. Journal of Food Quality, 30(5), 623-645.

El Ghaouth, A., Arul, J., Asselin, A., & Benhamou, N. (1992). Antifungal activity of chitosan on post-harvest pathogens: induction of morphological and cytological alterations in Rhizopus stolonifer. Mycological research, 96(9), 769-779.

Elbert, D. L., Herbert, C. B., & Hubbell, J. A. (1999). Thin polymer layers formed by polyelectrolyte multilayer techniques on biological surfaces. Langmuir, 15(16), 5355-5362.

Erickson, J. (2011). Determination of the concentration of caffeine, theobromine and gallic acid in commercial teasamples. Concord. Coll. J. of Anal. Chem, 2, 31-35.

Fabra, M. J., Flores-López, M. L., Cerqueira, M. A., de Rodriguez, D. J., Lagaron, J. M., & Vicente, A. A. (2016). Layer-by-layer technique to developing functional nanolaminate films with antifungal activity. Food and Bioprocess Technology, 9(3), 471-480.

Fakhouri, F. M., Martelli, S. M., Caon, T., Velasco, J. I., & Mei, L. H. I. (2015). Edible films and coatings based on starch/gelatin: Film properties and effect of coatings on quality of refrigerated Red Crimson grapes. Postharvest Biology and Technology, 109, 57-64.

Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., Funami, T., Williams, P. A., & Li, L. (2007). Multiple steps and critical behaviors of the binding of calcium to alginate. The Journal of Physical Chemistry B, 111(10), 2456-2462.

FAO (Food and Agriculture Organization)

http://www.fao.org/save-food/resources/keyfindings/en/ (2011).

Farris, S., Schaich, K. M., Liu, L., Piergiovanni, L., & Yam, K. L. (2009). Development of polyion-complex hydrogels as an alternative approach for the production of bio-based polymers for food packaging applications: a review. Trends in Food Science & Technology, 20(8), 316-332.

Feng, W., Zhang, Q., Hu, G., & Huang, J. X. (2014). Mining network data for intrusion detection through combining SVMs with ant colony networks. Future Generation Computer Systems, 37, 127-140.

Filippazzo, J. C., Rice, E. L., Faherty, J., Cruz, K. L., Van Gordon, M. M., & Looper, D. L. (2015). Fundamental parameters and spectral energy distributions of young and field age objects with masses spanning the stellar to planetary regime. The Astrophysical Journal, 810(2), 158.

Functional materials & photonics structures

https://fmps.fbk.eu/contact-angle-platform

(accessed date August 2017)

Galdi, M. R. (2006). Design and production of active films for food packaging application. Department of Chemical and Food Engineering. University of Milan, Italy Ph. D. Course in Chemical Engineering (VII Cycle-New Series).

Garcia, E., & Barrett, D. M. (2002). Preservative treatments for fresh-cut fruits and vegetables. Fresh-cut fruits and vegetables, 267-304.

Gherardi R, Becerril R, Nerin C, & Bosetti O. (2016). Development of a multi-layer antimicrobial packaging material for tomato puree using an innovative technology. LWT - Food Sci Technol. 72:361-367.

Ghosh, P. (2009). Colloid and interface science. PHI Learning Pvt. Ltd.

Gol, N. B., Patel, P. R., & Rao, T. R. (2013). Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan. Postharvest Biology and Technology, 85, 185-195.

Gombotz, W. R., & Wee, S. F. (2012). Protein release from alginate matrices. Advanced Drug Delivery Reviews, 64, 194-205.

González- Buesa, J., Arias, E., Salvador, M. L., Oria, R., & Ferrer- Mairal, A. (2011). Suitability for minimal processing of non- melting clingstone peaches. International Journal of Food Science & Technology, 46(4), 819-826.

Grasdalen, H., Larsen, B., & Smisrod, O. (1981). 13C-NMR studies of monomeric composition and sequence in alginate. Carbohydrate Research, 89(2), 179-191.

Gupta, S., Chatterjee, S., Vaishnav, J., Kumar, V., Variyar, P. S., & Sharma, A. (2012). Hurdle technology for shelf stable minimally processed French beans (*Phaseolus vulgaris*): A response surface methodology approach. LWT-Food Science and Technology, 48(2), 182-189.

Hambleton, A., Debeaufort, F., Bonnotte, A., & Voilley, A. (2009). Influence of alginate emulsion-based films structure on its barrier properties and on the protection of microencapsulated aroma compound. Food Hydrocolloids, 23(8), 2116-2124.

Han, J. H. (2000). Antimicrobial food packaging. Novel food packaging techniques, 8, 50-70.

Hinrichsen, L., Harborth, J., Andrees, L., Weber, K., & Ungewickell, E. J. (2003). Effect of clathrin heavy chain-and α-adaptin-specific small inhibitory RNAs on endocytic accessory proteins and receptor trafficking in HeLa cells. Journal of Biological Chemistry, 278(46), 45160-45170.

Hopewell, J., Dvorak, R., & Kosior, E. (2009). Plastics recycling: challenges and opportunities. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 364(1526), 2115-2126.

Huff, K. (2008). Active and intelligent packaging: innovations for the future. Department of Food Science & Technology. Virginia Polytechnic Institute and State University, Blacksburg, VA, 1-13.

IFPA (2003) Flexible packaging material basics. In: Gorny JR (ed) Packaging design for fresh-cut produce. International Fresh-cut Produce Association, Alexandria, pp 1–3

Imeson, A. (Ed.). (2011). Food stabilisers, thickeners and gelling agents. John Wiley & Sons.

Kapetanakou, A. E., & Skandamis, P. N. (2016). Applications of active packaging for increasing microbial stability in foods: natural volatile antimicrobial compounds. Current Opinion in Food Science, 12, 1-12.

Kaplan, D., & Singh, A. (2003). U.S. Patent Application No. 10/536,810.

Kays, S. J. (1999). Preharvest factors affecting appearance. Postharvest Biology and Technology, 15(3), 233-247.

Khalfan, H., Abuknesha, R., Rand-Weaver, M., Price, R. G., & Robinson, D. (1986). Aminomethyl coumarin acetic acid: a new fluorescent labelling agent for proteins. The Histochemical Journal, 18(9), 497-499.

Klitzing R.V. (2006). "Internal structure of polyelectrolyte multilayer assembly." Physical Chemistry Chemical Physics 8, 5012–5033 | 5021.

Kohli, S. A. (2006). Alginate and its applications. SK Patel College of Pharmaceutical Education & Research.

Koller, M. (2014). Poly (hydroxyalkanoates) for food packaging: Application and attempts towards implementation. Applied Food Biotechnology, 1(1), 3-15.

Kolodziejczyk, K., Milala, J., Sójka, M., Kosmala, M., & Markowski, J. (2010). Polyphenol oxidase activity in selected apple cultivars. Journal of Fruit and Ornamental Plant Research, 2(18), 51-61.

Krogman, K. C., Cohen, R. E., Hammond, P. T., Rubner, M. F., & Wang, B. N. (2013). Industrial-scale spray layer-by-layer assembly for production of biomimetic photonic systems. Bioinspiration & Biomimetics, 8(4), 045005.

Labuza, T. P., & Breene, W. M. (1989). Applications of "active packaging" for improvement of shelf- life and nutritional quality of fresh and extended shelf-life foods. Journal of Food Processing and Preservation, 13(1), 1-69.

Lago M.A, Sendón R, Rodríguez-Bernaldo de Quirós A., Sanches-Silva A, Costa H.S, Sánchez-Machado D.I, Soto Valdez H, Angulo I., Aurrekoetxea G.P, Torrieri E., López-Cervantes J. (2014). Preparation and characterization of antimicrobial films based on chitosan for active food packaging applications. Food and Bioprocess Technology, 7, 2932–2941.

Lavalle, P., Picart, C., Mutterer, J., Gergely, C., Reiss, H., Voegel, J. C. & Schaaf, P. (2004). Modeling the buildup of polyelectrolyte multilayer films having exponential growth The Journal of Physical Chemistry B, 108(2), 635-648.

Lee, C. Y., Kagan, V., Jaworski, A. W., & Brown, S. K. (1990). Enzymatic browning in relation to phenolic compounds and polyphenoloxidase activity among various peach cultivars. Journal of Agricultural and Food Chemistry, 38(1), 99-101.

Lee, K. Y., & Mooney, D. J. (2012). Alginate: properties and biomedical applications. Progress in Polymer Science, 37(1), 106-126.

Leistner, L. (1999). Combined methods for food preservation. Food science and technology-New York-Marcel Dekker-, 457-486.

Li, F., Biagioni, P., Finazzi, M., Tavazzi, S., & Piergiovanni, L. (2013). Tunable green oxygen barrier through layer-by-layer self-assembly of chitosan and cellulose nanocrystals. Carbohydrate Polymers, 92(2), 2128-2134.

Limbo, S., & Khaneghah, A. M. (2014). Active packaging of foods and its combination with electron beam processing. Electron Beam Pasteurization and Complementary Food Processing Technologies, 195.

Lopez-Rubio, A., Almenar, E., Hernandez-Muñoz, P., Lagarón, J. M., Catalá, R., & Gavara, R. (2004). Overview of active polymer-based packaging technologies for food applications. Food Reviews International, 20(4), 357-387.

Lu, Y., & Foo, L. Y. (2001). Antioxidant activities of polyphenols from sage (*Salvia officinalis*). Food Chemistry, 75(2), 197-202.

Lurie, S. (1993). Modified atmosphere storage of peaches and nectarines to reduce storage disorders. Journal of Food Quality, 16(1), 57-65.

Lurie, S., & Crisosto, C. H. (2005). Chilling injury in peach and nectarine. Postharvest Biology and Technology, 37(3), 195-208.

Majid, I., Nayik, G. A., Dar, S. M., & Nanda, V. (2016). Novel food packaging technologies: Innovations and future prospective. Journal of the Saudi Society of Agricultural Sciences.

Maksimović, M., Vujović, V., & Omanović-Miklić anin, E. (2015). Application of internet of things in food packaging and transportation. International Journal of Sustainable Agricultural Management and Informatics, 1(4), 333-350.

Manzocco, L., Plazzotta, S., Maifreni, M., Calligaris, S., Anese, M., & Nicoli, M. C. (2016). Impact of UV-C light on storage quality of fresh-cut pineapple in two different packages. LWT-Food Science and Technology, 65, 1138-1143.

Mascheroni, E., Figoli, A., Musatti, A., Limbo, S., Drioli, E., Suevo, R., & Rollini, M. (2014). An alternative encapsulation approach for production of active chitosan–propolis beads. International journal of food science & technology, 49(5), 1401-1407.

Matthews, T., (2007). Surface properties of Surface properties of poly (ethylene terephthalate). (Doctoral dissertation, The University of Toledo).

Medeiros, B. G. D. S., Pinheiro, A. C., Carneiro-da-Cunha, M. G., & Vicente, A. A. (2012). Development and characterization of a nanomultilayer coating of pectin and chitosan–Evaluation of its gas barrier properties and application on 'Tommy Atkins' mangoes. Journal of Food Engineering, 110(3), 457-464.

Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies, 15(4), 523.

Morris, E. R., Rees, D. A., & Thom, D. (1980). Characterisation of alginate composition and block-structure by circular dichroism. Carbohydrate Research, 81(2), 305-314.

Oliveira, M., Abadias, M., Usall, J., Torres, R., Teixidó, N., & Viñas, I. (2015). Application of modified atmosphere packaging as a safety approach to fresh-cut fruits and vegetables–A review. Trends in Food Science & Technology, 46(1), 13-26.

Ozdemir, M., & Floros, J. D. (2004). Active food packaging technologies. Critical reviews in food science and nutrition, 44(3), 185-193.

Paine, F. A. (Ed.). (2012). Modern processing, packaging and distribution systems for food. Springer Science & Business Media.

Painter TJ (1983) Algal polysaccharides. In: Aspinall GO (ed) The polysaccharides. Academic, New York, pp 196–286

Paixão, N., Perestrelo, R., Marques, J. C., & Câmara, J. S. (2007). Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines. Food Chemistry, 105(1), 204-214.

Peng, C., Chow, A. H., & Chan, C. K. (2001). Hygroscopic study of glucose, citric acid, and sorbitol using an electrodynamic balance: Comparison with UNIFAC predictions. Aerosol Science & Technology, 35(3), 753-758.

Picart, C., Mutterer, J., Richert, L., Luo, Y., Prestwich, G. D., Schaaf, P., & Lavalle, P. (2002). Molecular basis for the explanation of the exponential growth of polyelectrolyte multilayers. Proceedings of the National Academy of Sciences, 99(20), 12531-12535.

Piergiovanni, L., & Limbo, S. (2010). Food packaging: Materiali, tecnologie e soluzioni. Springer Science & Business Media.

Pinheiro, V. B., Taylor, A. I., Cozens, C., Abramov, M., Renders, M., Zhang, S., & Herdewijn, P. (2012). Synthetic genetic polymers capable of heredity and evolution. Science, 336(6079), 341-344.

Piva, G., (2016). “Characterization and development of different methods to extend shelf life of fresh cut fruit”. (Doctoral dissertation, University of Palermo).

Putman, C. A., De Grooth, B. G., Van Hulst, N. F., & Greve, J. (1992). A detailed analysis of the optical beam deflection technique for use in atomic force microscopy. Journal of Applied Physics, 72(1), 6-12

Rehm, B. H. A., & Valla, S. (1997). Bacterial alginates: biosynthesis and applications. Applied microbiology and biotechnology, 48(3), 281-288.

Rico, D., Martin-Diana, A. B., Barat, J. M., & Barry-Ryan, C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends in Food Science & Technology, 18(7), 373-386.

Rico, D., Martín-Diana, A. B., Barry-Ryan, C., Frías, J. M., Henehan, G. T., & Barat, J. M. (2008). Use of neutral electrolysed water (EW) for quality maintenance and shelf-life extension of minimally processed lettuce. Innovative food science & emerging technologies, 9(1), 37-48.

Risch, S. J. (2009). Food packaging history and innovations. Journal of Agricultural and Food Chemistry, 57(18), 8089-8092.

Robertson, G. L. (2006). Modified atmosphere packaging. Food Packaging: Principles and Practice, 313-328.

Rocha, A. M. C. N., & Morais, A. M. M. B. (2003). Shelf life of minimally processed apple (cv. Jonagored) determined by colour changes. Food Control, 14(1), 13-20.

Rollini, M., Nielsen, T., Musatti, A., Limbo, S., Piergiovanni, L., Hernandez Munoz, P., & Gavara, R. (2016). Antimicrobial performance of two different packaging materials on the microbiological quality of fresh salmon. Coatings, 6(1), 6.

Rosnes, J. T., Sivertsvik, M., & Skara, T. (2003). Combining MAP with other preservation techniques. Novel Food Packaging Techniques, 288-290.

Ruela, H. S., Leal, I. C., de Almeida, M. R., Santos, K., Wessjohann, L. A., & Kuster, R. M. (2011). Antibacterial and antioxidant activities and acute toxicity of Bumelia sartorum Mart., Sapotaceae, a Brazilian medicinal plant. Revista Brasileira de Farmacognosia, 21(1), 86-91.

Saltveit, M. E. (1999). Effect of ethylene on quality of fresh fruits and vegetables. Postharvest biology and technology, 15(3), 279-292

Saltveit, M. E. (2003). Fresh-cut vegetables. Food Science and Technology-New York-Marcel Dekker-, 691-712

Sanches-Silva, A., Costa, D., Albuquerque, T. G., Buonocore, G. G., Ramos, F., Castilho, M. C., & Costa, H. S. (2014). Trends in the use of natural antioxidants in active food packaging: a review. Food Additives & Contaminants: Part A, 31(3), 374-395.

Schanda, J. (Ed.). (2007). Colorimetry: understanding the CIE system. John Wiley & Sons.

Senanayake, S. N. (2013). Green tea extract: Chemistry, antioxidant properties and food applications–A review. Journal of Functional Foods, 5(4), 1529-1541.

Shiratori, S., Inami, Y., & Kikuchi, M. (2001). Removal of toxic gas by hybrid chemical filter fabricated by the sequential adsorption of polymers. Thin Solid Films, 393(1), 243-248.

Silvestre, C., Duraccio, D., & Cimmino, S. (2011). Food packaging based on polymer nanomaterials. Progress in Polymer Science, 36(12), 1766-1782.

Siracusa, V., Rocculi, P., Romani, S., & Dalla Rosa, M. (2008). Biodegradable polymers for food packaging: a review. Trends in Food Science & Technology, 19(12), 634-643.

Siripatrawan, U., & Harte, B. R. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. Food Hydrocolloids, 24(8), 770-775.

Siroli, L., Patrignani, F., Serrazanetti, D. I., Tabanelli, G., Montanari, C., Gardini, F., & Lanciotti, R. (2015). Lactic acid bacteria and natural antimicrobials to improve the safety and shelf-life of minimally processed sliced apples and lamb's lettuce. Food Microbiology, 47, 74-84.

Soliva-Fortuny, R. C., & Martín-Belloso, O. (2003). New advances in extending the shelf-life of fresh-cut fruits: A review. Trends in Food Science & Technology, 14(9), 341-353.

Srivastava, S., & Kotov, N. A. (2008). Composite layer-by-layer (LBL) assembly with inorganic nanoparticles and nanowires. Accounts of chemical research, 41(12), 1831-1841.

Tian, F., Decker, E. A., & Goddard, J. M. (2013). Controlling lipid oxidation of food by active packaging technologies. Food & Function, 4(5), 669-680.

Toivonen, P. M., & Brummell, D. A. (2008). Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. Postharvest Biology and Technology, 48(1), 1-14.

Toivonen, P. M., Brandenburg, J. S., & Luo, Y. (2009). Modified atmosphere packaging for fresh-cut produce. Modified and Controlled Atmospheres for the Storage, Transportation, and Packaging of Horticultural Commodities, 464-486.

Uz, M. (2009). Preparation of controlled release antimicrobial food packaging materials (Master's thesis, İzmir Institute of Technology).

Watada, A. E., Ko, N. P., & Minott, D. A. (1996). Factors affecting quality of fresh-cut horticultural products. Postharvest Biology and Technology, 9(2), 115-125.

Weiss, J., Takhistov, P., & McClements, D. J. (2006). Functional materials in food nanotechnology. Journal of Food Science, 71(9), R107-R116.

Xu, L. (2015). Layer-by-Layer Directly-Assembly of Polyelectrolyte Multilayers with Foaming Structures (Doctoral dissertation, University of Akron).

Yam, K. L., Takhistov, P. T., & Miltz, J. (2005). Intelligent packaging: concepts and applications. Journal of Food Science, 70(1).

Yilmaz, Y. (2006). Novel uses of catechins in foods. Trends in Food Science & Technology, 17(2), 64-71.

Yoo, D., Shiratori, S. S., & Rubner, M. F. (1998). Controlling bilayer composition and surface wettability of sequentially adsorbed multilayers of weak polyelectrolytes. Macromolecules, 31(13), 4309-4318.

Younes, I., & Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. Marine drugs, 13(3), 1133-1174.

Zaveri, N. T. (2006). Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. Life sciences, 78(18), 2073-2080.

Zhong, Y., Li, B., & Haynie, D. T. (2006). Fine tuning of physical properties of designed polypeptide multilayer films by control of pH. Biotechnology Progress, 22(1), 126-132.

Zhu, X., Schaich, K. M., Chen, X., Chung, D., & Yam, K. L. (2012). Target release rate of antioxidants to extend induction period of lipid oxidation. Food Research International, 47(1), 1-5.

APPENDIX A

CALIBRATION CURVES

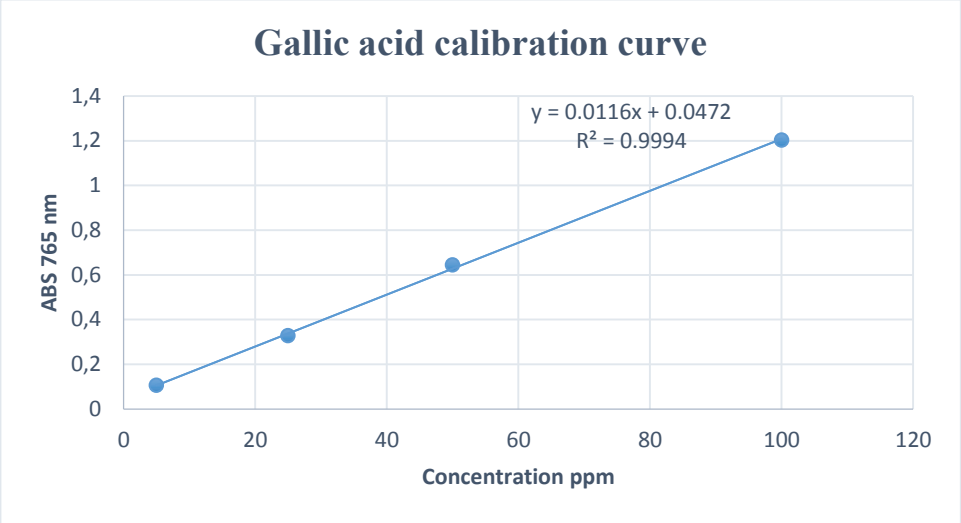


Figure A. 1. Calibration curve of Gallic acid

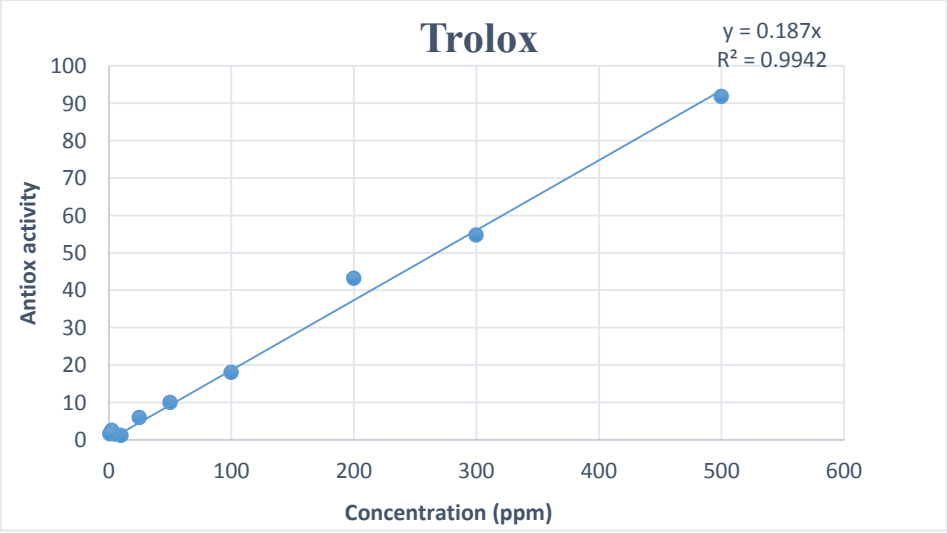


Figure A. 2. Calibration curve of Trolox (6-Hydroxy-2,5,7,8 - tetra-methylchromane-2-carboxylic acid)