# DEVELOPMENT OF BIO-BASED COATINGS AS FLEXIBLE FOOD PACKAGING APPLICATIONS

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# ABSTRACT


## DEVELOPMENT OF BIO-BASED COATINGS AS FLEXIBLE FOOD PACKAGING APPLICATIONS

The sustainable packaging solutions require the development of materials based on renewable resources and efficient production methods, which are important nowadays. In this study, a multilayer coated flexible polyethylene terephthalate (PET) sheets were produced by Layer-by-Layer (LbL) assembly using chitosan and pectin bio-based materials incorporated with galls extract (GE) as an active agent. These fabricated films were firstly characterized and results of increasing the depositing of chitosan and pectin from 0 to 60 layers indicated that the deposition of biopolymers successfully improve the performance of flexible films. The optimization of the deposition different number of layer on PET (20 – 40 and 60 layers) was carried out by the study of the surface characterization and the shelf-life tests in order to find out surface modification. The coated LbL films were applied on fresh-cut '*Abate Fetel*' pears and color, microbial, sensory assays were carried out during 7 days of storage at 4 ºC. At the end of storage, in terms of all tests, the LbL treated samples were presented better preserved compared to the control samples (treated base film). In addition, the release of active agents from multilayer films was evaluated using LbL films in contact with food and gradual reduction was observed.

This research demonstrates the feasibility of LbL coating, which acts as a carrier of active substances to enhance the quality of fresh-cut products as an active packaging system.

# ÖZET

## ESNEK GIDA AMBALAJ UYGULAMALARI İÇİN BİYO-BAZLI KAPLAMALARIN GELİŞTİRİLMESİ

Sürdürülebilir ambalaj alanındaki çözümler, günümüzde önemi giderek artan yenilenebilir kaynaklara bırakmakta ve verimli üretim yöntemlerine dayalı materyallerin geliştirilmesini gerektirmektedir. Bu çalışmada, aktif madde olarak galla ekstraktı (GE) ilave edilerek, kitosan ve pektin biyo-esaslı malzemeler ile katman katman kaplama (LbL) tekniği kullanılarak çok katmanlı esnek polietilen tereftalat (PET) materyali üretilmiştir. Bu yöntemle elde edilen filmler ilk olarak karakterize edilmiştir. Kitosan ve pektinden oluşan katman sayısının giderek artması (0'dan 60'a kadar), yüzeyde biriken biyopolimerlerin esnek filmlerin performansını başarılı bir şekilde geliştirdiğini göstermiştir. Yüzeyde oluşan modifikasyonun anlaşılması amacıyla yüzey karakterizasyonu ve farklı katman sayılarının (20 – 40 ve 60 katman) optimizasyonunu gerçekleştirmek amacıyla raf ömrü deneyleri yapılmıştır. LbL filmler, taze kesilmiş '*Abate Fetel*' armutları üzerine uygulanmış ve 4 ºC'de 7 gün depolama süresince meyvenin renk, mikrobiyal ve duyusal kalitesindeki değişimi belirlenmiştir. Depolama sonunda, tüm testler açısından, LbL ile muamele edilmiş numunelerin, kontrol numunelerine (orijinal PET ile muamele edilmiş) kıyasla daha iyi korunduğu gözlenmiştir. Ek olarak, çok tabakalı filmlerden aktif maddelerin salınması, raf ömrü çalışmasında gıda ile temas halinde olan LbL filmleri kullanılarak değerlendirilmiş ve aktif maddenin depolama sırasında kademeli olarak azaldığı gözlemlenmiştir.

Bu araştırma, bir ambalaj sistemi ve aktif maddelerin taşıyıcısı olarak LbL kaplamanın taze kesilmiş ürünlerin kalitesini arttırmak için uygulanabilirliğini göstermektedir.

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# CHAPTER 1

# INTRODUCTION

Fresh-cut fruits and vegetables are mostly called minimally processed products when taken into consideration the degree of processing (Yousuf et al. 2018). The opportunities in the sale of fresh-cut fruit and vegetables are continuously increasing not only in developed countries but also in underdeveloped and developing countries by the increasing request for such products. This is mostly due to the advanced in product development and packaging technologies as well as the demand for fresh foods that keep their fresh-like characteristics, which are easier and faster to consume with the lifestyle change of consumers.

In spite of the rapid growth in demand for fresh-cut fruits, the limitations in the production of these products are still hurdle to the industry. Since fresh-cut produces show physiological response to minimal processing operations, which damage and wound the fruit's living tissues. As a consequence, the wounding of plant tissues induces ethylene production and increases the respiration rate and susceptibility to microbial attack, which decrease the product's quality. The post-cutting life and quality of fresh-cut commodities are generally determined by their susceptibility to tissue softening, color changes, reduction in nutritional quality, off-odor, and microbiological deterioration. Moreover, these qualities are assessed subjectively by consumers, and the first priority of consumer acceptance is already known. Therefore, in addition to offering a convenient and fresh-like product to consumers, the main aim of the further studies is to research on new ways to extend the fresh-cut product shelf-life and to maintain sufficient quality. Pear (Pyrus spp.) is one of the most consumed fruits in the world for its characteristic flavor, crispness, and sweetness. Pear is a rich source of vitamin C, minerals and phytochemicals, especially phenolics. Mostly freshly consumed pears are one of the groups of produce that are frequently associated with deterioration due to changes in color, texture, odor, and biochemical parameters.

One alternative is the use of active packaging to extend the shelf-life of fresh-cut fruits, as well as to improve the gas and moisture barrier properties, quality attributes and microbial protection during storage. Active food packaging is currently one of the most

important parts of food production and distribution all over the world due to keeping food quality and safety better than traditional packaging by interacting with product and/or environment.

Currently, research studies have been focused on bio-based materials as raw material for food packaging due to their biodegradability and safety from petroleum-based polymeric materials that is a major global concern of environmental problems (Cazon et al. 2017). However, it is known that application of a stand-alone bio-based film and coatings as food packaging represents difficulties, because of some drawbacks such as poor mechanical properties and the high sensitivity to humidity. In an attempt to overcome this problem, a promising approach can be used of bio-based materials on conventional flexible plastic films (e.g. polyethylene terephthalate and polypropylene) to fabricate a multilayer coated film to enhance specific properties of the substrate, such as barrier, mechanical, optical and thermal properties. Sequential layer by layer assembly (LbL) of biopolymers on solid supports is a basic technique to produce of multi-nanolayer structured films. The LbL technique is on the basis of the successive deposition of oppositely charged polyelectrolyte solutions on various charged substrate due to their electrostatic attraction (Fabra et al. 2013). Coating of plastic materials with biopolymers produced from natural sources are considered as an advantage since several active agents (antimicrobial and/or antioxidant compounds) can be incorporated into the polymer matrix in order to reduce the growth of microorganisms or browning and oxidation, respectively. In addition, incorporation of many active ingredients in the coating material, which is expected to be released to the food for their specific function, can help their controlled release on the food surfaces to be effective.

In this study, the surface of the polyethylene terephthalate film was coated with a bio-based chitosan and pectin polymers by incorporation of antioxidant agent for flexible food packaging applications. These combination of bio-based material with conventional flexible packaging were prepared as an alternative way to accelerate the development of sustainable solutions for biodegradable polymers. In order to determine the effectiveness of these films as an active food packaging application in the protection of the fruit, mechanical, microbiological, and sensory properties were investigated.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1. Fresh Produce

### 2.1.1. Pear

Pear (Pyrus spp.) belongs to the family of Rosaceae, tribe Pomaceae. It is a native fruit of temperate region of Europe, North America, North Africa. It is the fifth most important fruit in world production and is growing in more than 50 countries around the world (Kolniak-Ostek 2016), mostly in China, Europe, and the United States. China production of pear is largest with reaching 19.000.000 t in 2018, followed by European Union with 2.336.000 t, while Turkey has a total production of 420.000 t per annum, the 5th highest in the world (USDA 2018).

Although there are more than 2000 pear varieties, only a few are important for production. It is widely consumed through the whole world as fresh fruit when fully mature, and also in processed products, canned, puree, jams, juice, dried fruit, so on (Brahem et al. 2017).

Many studies have done on pear chemical composition due to its desirable taste, which makes it more popular among consumers, and high digestibility (Salta et al. 2010, Chen et al. 2007, Colaric et al. 2006). In some studies, it is shown that pear fruit being rich in phytochemicals, especially phenolics may provide a good source of antioxidants and antiinflammatory properties for health benefits (Kolniak-Ostek 2016). These phenolic compounds also make contribution to the sensory characteristics of fruit, such as color and appearance, firmness, flavor (Brahem et al. 2017).

Pear polyphenolic compounds belong to wide classes of flavonoids, (flavonols and anthocyanins), hydroxyphenolic acids and simple phenolics (Brahem et al. 2017, Öztürk et al. 2015). Pear peel possesses high concentrations of flavonols and anthocyanins but are found at low levels in the core and flesh.

In addition, pear is a nutrient-dense fruit with excellent source of dietary fiber, sugars, vitamins and minerals (Brahem et al. 2017, Yim and Nam 2016) and is low in calorie.

Table 2.1. Nutritional composition of pear (USDA 2018)

| Nutrients | Units | Value per 100 g pear |
|---|---|---|
| Water | g | 83.96 |
| Protein | g | 0.36 |
| Total lipid (fat) | g | 0.14 |
| Fiber, total dietary | g | 3.1 |
| Carbohydrate | g | 15.23 |
| Energy | kcal | 57 |
| Sugars, total | g | 9.75 |
| Calcium, Ca | mg | 9 |
| Potassium, K | mg | 116 |
| Sodium, Na | mg | 1 |
| Vitamin C, total ascorbic acid | mg | 4.3 |

### 2.1.2. Fresh-cut fruits

Globally, fruits and vegetables processing are the major food processing industries. Sufficient consumption of fruits and vegetables is not only related to reducing the risk of cancer and chronic diseases, but also prevents the development of diseases by increasing the intake of vitamins, minerals, dietary fiber, carotenoids and flavonoids (Grassmann et al., 2002, Gaziano et al. 1993). Since 1995, production of fresh-cut fruit and vegetables that are ready-to-eat has increased of 10% per year (Barth 2000) due to changes in modern consumers' lifestyles (Yousuf et al. 2018, Brody et al. 2008). Although, it is not common most of underdeveloped and developing countries, a significant increase in the value and volume of this sector is expected in the coming years with a growing trend for such products (Yousuf et al. 2018). Because it is interesting alternative from the industry as a ready-to-use product for today's busy consumers who look for healthy, 'quick' and minimally processed food with quality (Garret 2002, Oms-Oliu 2010).

The term ‘fresh-cut products’ defines as ‘any fruit or vegetable that has been only washed, peeled, cut and bagged or packaged to offer consumers while remaining freshness’ (IFPA 2018).

However, the main difficulty with fresh-cut fruit is to manipulate quality attributes (colour, texture, flavor and nutritional value) of their shelf life that is generally not longer than two weeks, but is long enough to ensure efficient marketing (Putnik et al. 2017, Edelenbos et al. 2017 ). With time, the overall quality and shelf-life of fresh-cut fruits are reduced by various factors, such as tissue softening, browning, off-flavors and microbial decay. It is well known that, in the case of fresh-cut fruits, physiological and microbiological disorders, and metabolic changes of the products accelerate by minimally processing, washing, peeling, cutting etc. (Rojas-Grau et al. 2009, Corbo et al. 2010). Prior to being packaged for consumption, each step can potentially have an impact on quality and safety of the product. Especially, cutting promotes faster deterioration of cut-fresh fruits than their whole counterparts, since injured tissue increases respiration rate by consuming more carbon substrates and producing of reactive oxygen species. In addition, this increase in respiration rate leads to delocalization of enzymes and biochemical deteriorations such as enzymatic browning, undesirable volatile production and softening. Preparation steps may increase also microbial spoilage on their wounded surfaces because of possible contamination by bacteria, yeast and mold from fruit peel to fruit flesh where microorganisms can grow easily when exposed to nutrient rich fruit juices (Sipahi et al. 2013, Leite et al. 2017).

All these numerous factors can bring about negative effects on the production and distribution of fresh-cut fruits, thus, limiting their marketing (Pristijono et al. 2006).

### 2.1.3. Sensory characteristics

Non-microbial and microbial deterioration are two general categories that describe deterioration in fruits (Putnik et al. 2017). In essence, sensory quality of fresh-cut fruits, including appearance, color, texture is generally evaluated by consumers at the time of purchase. These characteristics are influenced by many factors and fall below the acceptable level, especially those associated with wounds and cuts, which are very remarkable in white-flesh fruits, like apples and pears (Toivonen and Brummell 2008).

Browning in fresh-cut fruits is a major non-microbial spoilage that occurs very early during storage on the cutting-surface (Putnik et al. 2017). It decreases the nutrient content in fruits and accelerates the end of the post-cutting life (Kader 2002). Furthermore, this non-microbial spoilage is a critical quality property to the customer's purchase decision, although it is not harmful to human health (Putnik et al. 2017). Because consumers usually evaluate the quality of products according to their appearance and color first, which determines whether a product is accepted or rejected (Barrett et al. 2010).

The main obstacles to limiting shelf life of fresh-cut pears slices are in general textural breakdown and surface browning (Çandır 2017). Anjou, Red Anjou, and Rocha pear slices were shown to browning after processing as fresh-cut products (Gorny et al. 2000, Abreu et al. 2011).

Table 2.2. Main reasons of quality loss in freshly cut fruits and vegetables (Ma et al. 2017, Çandır 2017).

| **Fresh-cut fruits** | **Main causes of degradation** |
|---|---|
| Apple sliced | Browning |
| Pear sliced | Browning |
| Orange sliced | Juice leakage, off flavors |
| Watermelon cubed | Juice leakage, softening |
| Pineapple cubed | Browning, leakage |
| Strawberry sliced | Loss of texture, juice, color |
| Peach sliced | Browning |

Browning may arise from enzymatic or non-enzymatic (chilling injury, mineral elements, ethylene production ect.) reactions (Corzo-Martinez et al. 2012). The studies reported that enzymatic reactions are the main cause of browning during fruit processing and storage (Li et al. 2017) and results from oxidation of phenols (Kou et al. 2015). This browning is mostly related to the total amount of phenolic compounds and the levels of polyphenol oxidase (PPO) enzyme, which is the key enzyme.

After tissue integrity is damaged, phenolic compounds contact with PPO (Oms-Oliu et al. 2010). This copper containing enzyme in the presence of oxygen catalyzes monophenols to o-diphenols and o-diphenols to quinone, then this quinone products are formed of colored melanins as a result of reaction with amino acid groups (Temiz and

Ayhan 2017), so that phenols are transformed into brown polymeric pigments (from quinones to melanins). The other responsible enzyme is peroxidase (POD), which carries out single-electron oxidation in various compounds in the presence of water (Cefola et al. 2012). Massola et al. 2011, have observed that loss of cellular compartmentalization gives accelerate to browning as a result of oxidation by releasing phenolics stored in vacuoles and increasing POD levels. The effect of PPO on the phenolic compounds released during the cutting process was observed to cause discoloration on sliced pear surfaces (Çandır 2017).

Texture is one of the product quality attributes that related to mechanical properties, closely depend on tissue deterioration (Cantwell and Suslow 2002). The consumer expects not to be altered firmness, crispness and crunchy texture of such fruits by processing or storage. It is well known that consumers and/or panelists perceive textural parameters with the sense of touch (Ma et al. 2017).

Flavor is another factor to sustain in fruit products. It is derived from various volatile aroma and nonvolatile compounds and may result from the loss of good flavor compounds or from the accumulation of bad flavor compounds (Huxsoll et al. 1989).

Therefore, considering all these mentioned quality deteriorations, the development of new processing techniques is still to be done to overcome adverse effects, and consequently maintain fresh-cut fruit quality at an acceptable level throughout the expected shelf life or prolong the shelf life at least for a while.

### 2.1.4. Microbial contamination

Microbial growth and their activation are significant restrictions of shelf life during the storage of fruits (Putnik et al. 2017). Excessive growth of microorganisms in foods can alter the taste, appearance and texture of fruits and may even become health hazards. The normal micro flora found on the fresh-cut fruit is diverse, bacteria (*Erwinia*, *Enterobacter*, *Pseudomonas* spp., etc.), molds (*Aspergillus*, *Rhizopus*, *Penicillium*), yeasts (*Saccharomyces*, *Zygosaccharomyces*, *Candida* and *Pichia*), or microorganisms breeding during processing due to the high sugar content of the fruit (Corbo et al. 2010, Beaulieu et al. 2001). However, during the production of freshly cut fruit, no process can eliminate the microorganisms that can be found on the surface of the fruit.

Among microorganisms, growth of yeasts and molds, especially yeasts, is easier than bacteria because of their ability to grow at low pH (2.2–5.0), high sugar content of most fresh-cut fruit and high humidity environment during storage (Corbo et al. 2010).

Fruits may become contaminated with pathogenic and spoilage microorganisms by preharvest factors and postharvest factors (Temiz and Ayhan 2017). Several authors showed presence of *Salmonella* spp. and *Escherichia coli* spp.on melons, pears, watermelons, strawberries, mangoes, grapes, causes several outbreaks once they consumed (Ethelberg et al. 2010, Friesema et al. 2007, Harris et al. 2003).

Despite the noticeable growth in marketing and scientific research, microbiological, physical, and chemical spoilage during processing are still limiting the self-life and industry of fresh-cut produce. For this reason, many novel techniques have been carried out in order to develop preservation strategies and to extend the shelf life of such products.

## 2.2. Novel Preservation Technologies

Fresh-cut fruits processing techniques are still under improve due to difficulties in maintaining their qualities for a long time. (Giacalone et al. 2010). To meet consumer and producer expectations has encouraged scientists to develop new technologies on how their quality can be maintained after processing (Chantanawarangoon and Kader 2002).

Recently, different approaches including;

- modified atmosphere packaging (retain proper gas concentration surrounding the cut surface),
- pressurized inert gases (xenon (Xe), neon (Ne), krypton (Kr), argon (Ar) and nitrogen (N2)),
- electron beam irradiation (the exposure of cobalt-60 radioisotopes),
- pulsed light (non-thermal technique),
- ultraviolet light (non-ionizing radiation),
- nanotechnology (nanosized materials),
- ozone (alternative sanitizer to carcinogenic chlorinated compounds)
- biopreservation technologies (bacteriophage, bacteriocins and bioprotective microorganisms),
- and combination of these techniques have been studied (Ma et al. 2017).

Gomes et al. 2012, reported that three equilibrium $O_2$ levels of modified atmosphere packaging (MAP) were not effective in changes of calcium ascorbate treated fresh-cut 'Rocha' pear firmness, titratable acidity, pH and soluble solids after storage at 5 ºC for 20 days. Also, MAP $O_2$ levels were found to have no effect on changes in water activity, ascorbate level and microbial growth.

Oliveira et al. 2014, investigated that the effect of the bacteriophage Listex P100 to control *L. monocytogenes* growth on melon, pear and apple products (juices and slices) stored at 10 ºC. Although there was no effect on apple products, a reduction of *L. monocytogenes* up to 1.50 and 1.00 log cfu $plug^{-1}$ on melon and pear slices treated with the bacteriophage, respectively, was observed after 8 days of storage. In juices, higher reduction was achieved in melons, followed by pear, again unaffected in apple juice. Hence, they suggested the combination the phage application with other technologies to improve its efficacy on high acidity fruits.

## 2.3. The Role of Active Packaging

Year after year, growing the demand for innovative packaging technologies and the driving forces behind the research and development and commercial applications of packaging technologies like active packaging systems are intended to improve quality-safety maintenance and prolong shelf-life of food products (Wilson et al. 2018, Lee 2005). Active packaging can be defined as the packaging in which certain additives, active compounds interact with the perishable product directly into the packaging material or the packaging container for the purpose of enhancing and maintaining shelf life (Han and Floros 2007). The packaging can be called active when it fulfills a desired role, such as antimicrobial and antioxidant activities in the food preservation other than normal packaging functions which provide an inert barrier against external conditions. (Rooney, 1995).

The spectrum of active packaging techniques includes additives that are capable of adsorbing carbon dioxide, scavenging oxygen, releasing antimicrobial, antioxidants, flavorings and/or enzymes, indicating gas and volatile component, and absorbing ethylene (Han and Floros 2007).

Currently, several applications of active packaging technologies have been commercially used in food industry by adding active ingredient in a visible device due to

their simplicity (Wilson et al.2018). However, Aday and Yener 2015 stated that as a result of the survey conducted by Turkish consumers on active food packaging, they do not prefer active substance in sachets because of the possibility of contamination with the product or swallow the active ingredient due to the accidental breakage.

Different techniques such as nanostructured biofilms or coatings have been gaining more and more popularity in recent years by reason of their great potential in packaging, food, medicine and biomedical fields (Cabral et al. 2016).

### 2.3.1. Active packaging containing natural antioxidative or antimicrobial agents

In the packaging industry, companies all over the world are striving to minimize or delay food deterioration in order to provide microbial safety and sufficient shelf-life. As previously described, microbial spoilage and oxidation on surface of perishable packaged foods are the most frequent mechanisms of food deterioration. Modified atmosphere packaging, which was one of the first examples over two decades ago, or oxygen holders are used with gas barrier packaging materials to protect foods from these deteriorations. Even though they work against aerobic microbial growth and oxidative quality changes, they cannot prevent the food deterioration due to anaerobic bacteria. The strategy of active packaging may help to protect the food from oxidation or microbial spoilage (Lee 2005).

Antioxidative packaging is one type of active packaging that has been removal of oxygen by oxygen scavengers or a barrier layer and delivery of antioxidants to the food surface through gradual release from the packaging material would improve the shelf life of food without adding antioxidant into the foods (Dastgerdi et al. 2016). One of the advantages of this technology is to protect food quality parameters such as color or taste by preventing the incorporation of antioxidants into food formulations, and also there is a consumer preference for unacceptability of additives in foods (Realini and Marcos 2014).

Antimicrobial packaging is another type of active packaging designed to release an antimicrobial compound on the food surface to delay microbial growth by using less amounts of active agents. The antimicrobial activity may be obtained by incorporation of

an antimicrobial compound into the packaging polymer or by the use of polymer naturally antimicrobial (e.g. chitosan) (Lee 2005).

In both cases, antioxidant and antimicrobial packaging systems, the antioxidant or antimicrobial substances can be applied in different forms such as incorporated into the polymer matrix, sachets, labels, or coated onto plastic films or paper, multilayer films (Realini and Marcos 2014).

The active compounds in the packaging material are used in a way that to be released into the food gradually to maintain the quality and safety of foods. Although the concept of controlled release is used in the medicine and pharmaceutical industry, the application of this technique in active food packaging is new and limited (Khaneghah et al. 2018). Controlling the release of an active substance into food remains a problem although it seems feasible concept in packaging area from a theoretical point of view, and few studies have attempted to solve this problem (Khaneghah et al. 2018).

In addition, there is a growing potential for the use of natural compounds derived from microbiological, vegetable and animal sources due to consumers' awareness of the potential negative effects of synthetic preservatives on health and environment (Lee 2005).

Perishable foods are susceptible to oxidative and microbial deterioration, so that food safety and quality assurance can be improved by the use of antimicrobial and antioxidative agents.

Lee et al. 2004 fabricated antimicrobial and antioxidant coated-paper using nisin and/or α-tocopherol at a concentration of 3% in a binder medium of vinyl acetate-ethylene copolymer. They examined their migration and potential activities with an emulsion model system in milk cream. The migration of nisin and a-tocopherol from the coating into an oil-in-water emulsion was measured when reached an equilibrium level at about 9% and 6% at 10°C, respectively. In the coating, incorporation of nisin was effective to delay microbial growth, and a-tocopherol to retard lipid oxidation. Therefore, the combination of nisin and α-tocopherol in coated paper could provide both antimicrobial and antioxidative properties without synergic or interactive effects for preserving the perishable foods, and thus, extending their shelf life.

Table 2.3. Some natural antimicrobials and antioxidants that may be suitable for active food packaging

| **Natural Agents** | **Activity** | **References** |
|---|---|---|
| Antimicrobials | | |
| Grape seed extract | Against *E. coli, S.Typhimurium*, and *L. monocytogenes* | Ahn et al. 2004 |
| Citrus oil | Inhibited the growth of Gram-positive and Gram-negative bacteria | Fisher and Phillips 2008 |
| Cinnamon | Inhibited the growth of *A.flavus A. fumigatus A. niger* | Avila-Sosa et al. 2012 |
| Chitosan | Reduced the growth of mesophilic aerobes, yeast and moulds | Krasaekoopt and Mabumrung 2008 |
| Nisin | Against *Clostridium* spp., *L. monocytogenes* | de Arauz et al. 2009 |
| Antioxidants | | |
| Ascorbic acid | Retarding browning | Olivas et al. 2003 |
| Rosemary extract | Antioxidant and antimicrobial effects | Lara et al. 2011 |
| Ziziphus leaves extract | Delay of lipid oxidation | Abdulla et al. 2016 |
| Barberry extracts | Reduces lipid oxidation | Aliakbarlu et al. 2015 |

## 2.4. Biopolymer Coated Active Packaging

Coated packaging films include a unique packaging material category from biodegradable polymers to conventional polymers. They show an alteration according to their functions, mode of formation and also application to foods (Atik 2007).

The most common material has been used in the food packaging industry is plastic materials that are developed for the packaging of fresh quality products (Baner and Piringer 1999, Yam and Lee 1995). Barrier properties are severely important to perishable foods packaged in plastics because of their quality or a reduction in lifetime depend on the permeation of gases (e.g. $O_2$, $CO_2$) and vapors (e.g. $H_2O$, aromas and flavours) through the packages.

Although plastic packaging has beneficial features such as being the most practical, cheapest and useful material, it does not have a positive effect on product life. Plastic food packaging has shown great interest in barrier technology for sustainable solutions to the food protect and also market itself. The biopolymer coated food packaging materials provide barriers towards gases and vapors and/or maintain quality of the food product. Because of strong consumer trends for transparency, convenience food preferences, and the rise of the interest in using natural resources, considerable effort goes into developing barrier coatings made of natural biopolymers to prevent the premature spoilage of food quality due to the oxygen inflow. Various biopolymers have been used totally or partially replace the commercial packaging to obtain good barrier properties with high transparency.

On the other hand, one of the latest investigated approaches to minimize the processing reactions that affect the quality of fresh-cut fruits is edible films and coatings. However, the number of search reported that the use of stand-alone biodegradable materials in food packaging is still limited for packaging material with improved barrier properties due to their great sensitivity to humidity and low mechanical properties (Ayhan 2017, Li et al. 2013).

Thanks to wide range of filmogenic natural biopolymers, such as hydrocolloids (proteins and carbohydrates), and lipids, have been considered as a promising way to create thin layers on plastics or bioderived materials by their deposition. Most research is driven on these polymers by the desire to achieve all the goals of product safety-quality and visibility, low gas permeability, biocompatibility and sustainability. In addition, the

use of biopolymers as carriers of active substances has been proposed as a promising application of active food packaging (Lee 2005).

Table 2.4. Some bio-based materials used for fresh-cut products

| **Fresh-cut product** | **Biodegradable material** | **References** |
|---|---|---|
| Minimally processed lettuce | Polyester-based biodegradable films | Del Nobile et al. 2008a |
| Head lettuce, cut and whole broccoli, tomatoes, and sweet corn | Laminate of chitosan-cellulose/polycaprolactone | Makino and Hirata-1997 |
| Fresh-cut cantaloupe | Chitosan/methyl cellulose film | Sangsuwan et al. 2008 |
| Minimally processed table grapes | Polyester-based biodegradable films | Del Nobile et al. 2008b |
| Ready-to-eat sweet cherries | Coextruded polyester | Conte et al. 2009 |
| Fresh-cut zucchini | Coextruded polyester | Lucera et al. 2010 |
| Asparagus, baby corn, and Chinese cabbage | Banana/chitosan films | Pitak and Rakshit 2011 |

Nanotechnology offers to food scientists various ways to create laminate films consisting of more than two layers of materials with nanoscale sizes that are physically or chemically bonded to each other (Sipahi et al. 2012). One major advantage of these films is that they can be incorporated with active functional agents, antimicrobials, antioxidants, enzymes or probiotics, minerals and vitamins into the film materials. These agents are expected to improve the shelf life and quality of foods they interact with (Leite et al. 2017).

Considering all these, Layer-by-Layer assembly has been used as a good alternative to fabricate of multicomponent films on solid supports to preserve fresh-cut fruit quality and safety by controlled deposition from solutions or dispersions (Leite et al. 2017, Li et al. 2013).

## 2.5. Layer-by-Layer Assembly

The Layer-by-Layer (LbL) deposition technique of molecular and nano objects has been widely attracting interest in recent years due to build multifunctional thin films for technologies, such as chemistry, physics, biology and nanomedicine (Soler 2018).

Langmuir- Blodgett and chemical self-assembly monolayer techniques, ways of fabrication of organic or organic/inorganic multilayers, were mostly known one decade ago (Soler 2018, Choi 2006). The limited compatibility with various materials and the complexity of these two methods for film production had led to the development of the new approach, called Layer-by-Layer self-assembly, is one of the most powerful methods developed by Decher and co-workers (Paul et al. 2014, Fabra et al. 2016). Simple and environmentally friendly LbL method has been used for preparing coated films using electrostatic adsorption between oppositely charged polymers onto solid surfaces. (Paul et al. 2014, Fabra et al. 2016).

In this technique, a wide variety of components are sequentially transferred at the surface of any type of solid support in order to produce functional thin films (Larocca et al. 2018, Paul et al. 2014). In addition, nanometer-thickness, roughness, and porosity of films can be adjusted by selecting the types of the outermost layer, deposition pH, experimental temperature, polyelectrolyte concentration and the number of dipping cycles for different purposes (Gu et al. 2013). The advantages of the LbL technique over many other coating techniques presents many ways to fabricate new films suitable for the use of food product and the packaging applications from the perspective of food scientists (Larocca et al. 2018, Paul et al. 2014, Gu et al. 2013).

### 2.5.1. Multilayer structure of colloidal particles LbL theoretical aspects

In the last few decades, most of studies have been done to understand fundamental mechanism of the formation of multiple layers (Iost et al. 2012).

Irving Langmuir characterized a monolayer adsorption of thorium ions on a monomeric barium stearate layer and showed that this surface was coated with a silica layer. This study had not attracted interest as colloidal adsorption until Iler described the adsorption of colloidal sized particles to the oppositely charged solid surface. Iler used

positive and negative inorganic and organic colloids, cationic polymer and charged protein to fabricate multilayer films, also tested the characterization of multiple layers of colloids as a large micrometer size with light reflection and interference color (Kunitake 2017). In 1991, Decher and Hong has investigated anionic and cationic compounds to make possible sequential electrostatic adsorption on solid surface. This adsorption process reached up to 35 alternative layers (Kunitake, 2017, Choi 2006).

The LbL assembly technique, simply based on the subsequent deposition of oppositely charged polyelectrolytes by electrostatic attraction, which is thought to induce adsorption between ionic charges located on polyelectrolyte molecular structures. In addition to electrostatic interaction, which is the main driving force, hydrogen bonding, coordination bonding, charge transfer, molecular recognition are also necessary forces for fabrication strategy of films (Choi 2006, Iost et al. 2012, Graisuwan et al. 2012).

A number of articles have explored the amount of building water soluble and charged species is dramatically dependent on the pH, ionic strength, polyion concentration, charge density of the polyions, type of polyelectrolyte, rinsing and drying step, as well as the number of depositions. Additionally, these processing factors affect stability and characteristics of the final films. Among the parameters, control of the pH on LbL deposition is particularly essential in weak polyelectrolyte interactions (Choi 2006, Iost et al. 2012, Graisuwan et al. 2012, Antunes et al. 2011).

### 2.5.2. Deposition of multilayer structure LbL assemblies

Among the versatility of LbL deposition methods, recently spin coating and spraying methods have been introduced. During almost 10 years, well known solution-dipping method developed by Decher, have been used to fabricate the films (Michel et al. 2012).

In simplest, LbL is a concept of the alternate adsorption of oppositely charged layers of polyions onto a surface by electrostatic attraction, van der Waals forces and hydrogen bonding. The setup of dipping method is extremely practical.

A schematic representation of an LbL deposition cycle used in this study is shown in Figure 2.1. Practically, under optimum conditions, a suitable charged support is first immersed in an aqueous solution of oppositely charged polyion for a specific time, (Step 1, Figure 2.1), and then, simple rinsing step is applied with pure solvent to the support in

order to remove the excess polymer solution from the surface and dried if required, (Step 2). The sign of the surface charge is reversed, so that a surface-loaded layer is formed for subsequent deposition. Then, the substrate is immersed to a second solution of an oppositely charged molecules (Step 3). The exposure time is the same as in the Step 1. In consequence, the substrate is rinsed and dried again (Step 4). In this way, the substrate is covered with a bilayer LbL film with reversing the sign of the surface charge again. This cycle can be sequentially and alternately repeated to obtain desired thickness or structure of multilayers (Antunes et al. 2011, Choi 2006).

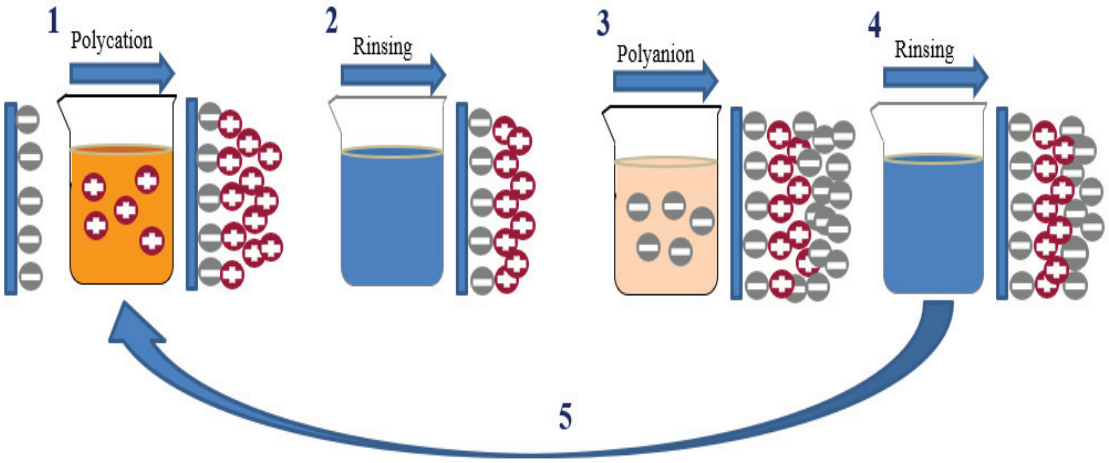


Figure 2.1. Diagram of LbL assembly. (1) deposition of positively charged substrate, (2) rinsing with solvent and dry, (3) deposition of negatively charged substrate, (4) rinsing with solvent and dry, (5) return to step (1)

## 2.6. Polymers for LbL Assembly

The polymers can be classified based on their origin (natural, synthetic, semi-synthetic, structure (linear, branched, network), processing characteristics, polymerization mechanism (addition, condensation), physical properties and applications.

In below, polymers used in this study, which classified according to their origin: synthetic and natural polymers, were discussed in details.

### 2.6.1. Synthetic polymers

Polymers are large molecules, in which consists of repeating monomer molecules and polyethylene terephthalate are more widely used among synthetic polymers.

#### 2.6.1.1. Polyethylene terephthalate

Polyethylene terephthalate (PET), which is a long chain thermoplastic polymer composed of ethylene glycol and terephthalic acid units, has found increasing applications in packaging industry (Pellicer et al. 2017). Its chemical inertness and physical properties such as good barrier properties, being lightweight, tough and transparent, has made it particularly suitable for manufacturing synthetic sheets and films, as well as for food packaging and beverage containers.

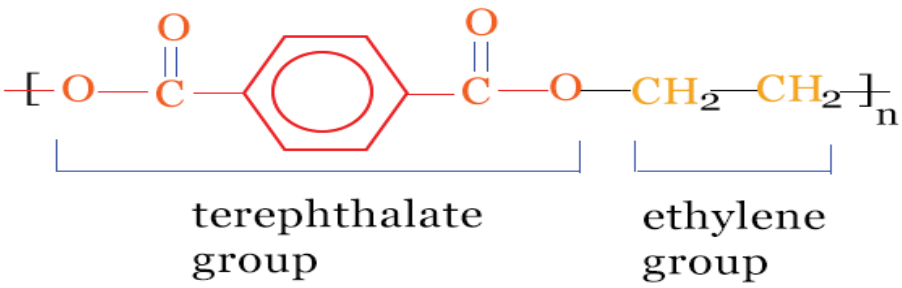


Figure 2.2. The structure of PET (Source: WEB_1 2018)

Since PET is a non-polar polymer causing low surface energy and poor wettability, it is extremely necessary to modify its surface when it is used as substrate for LbL assembly. Although there are several physical (i.e plasma) and chemical (i.e alkaline hydrolysis) modification methods have been used to allow the electrostatic binding to the polyions (Joo et al. 2018), the corona treatment was used in this study. Atmospheric pressure corona treatment is a widely preferred method due to its low cost, in-line and non-vacuum processing, high speed, and the independence of shape of the substrate (Vlaeva et al. 2012).

## 2.6.2. Natural polymers

Natural polymers are obtained and are extracted from nature, usually from plant and animal sources.

### 2.6.2.1. Chitosan/Chitin

Chitosan is a natural polycation polymer (Figure 2.3), β-(1,4)-2-amino-2-deoxy-D-glucoseo, obtained by deacetylation of chitin (a N-acetylglucosamine polymer), which is the major constituent of the exoskeleton of invertebrates and of arthropods (Beverlya et al. 2008, Yuan et al. 2016).

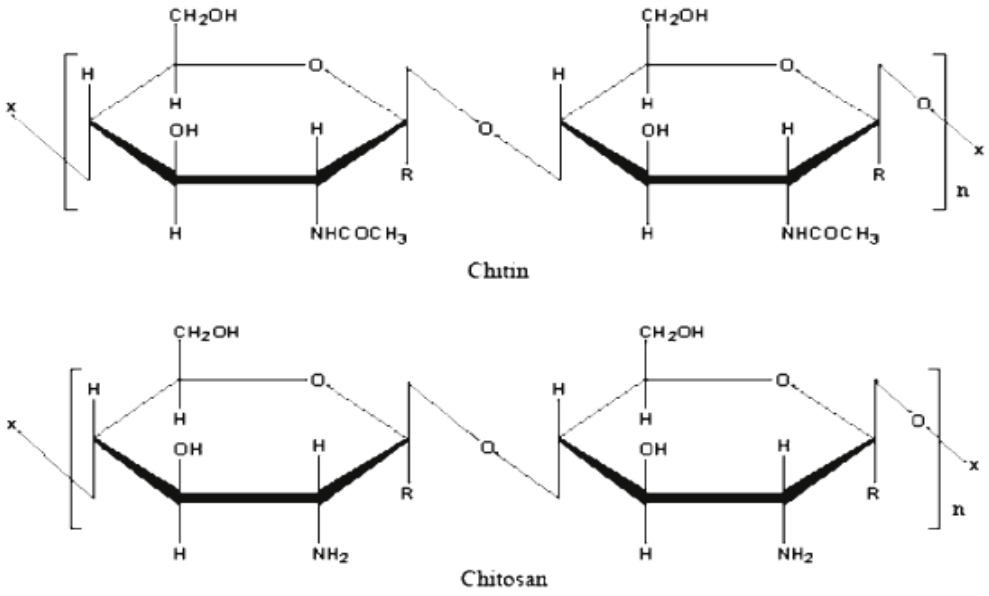


Figure 2.3. Chemical structures of chitin and deacetylated chitosan
(Source: Ghanbarzadeh and Almasi, 2013)

Chitosan is soluble in acidic solvents below pH 6 and it loses its stability above pH 7. To dissolve chitosan, organic acids such as acetic, formic and lactic acids are used, mostly 1% acetic acid solution (Nadarajah, 2005).

The inherent antimicrobial effect of chitosan, which is considered to be a potential food preservative, is occurred due to the fact that the positive charge on the amino group of chitosan to be attracted to negatively charged residues on the microbial cell surface

(Yuan et al. 2016). It is effective in preventing not only the growth of Gram-positive and Gram-negative bacteria but also yeast and molds. Also, it has been accepted as a Generally Recognized as Safe (GARS) food additive by the United States Food and Drug Administration (USFDA) (USFDA, 2013). Besides antimicrobial activities, it is approved that it has been used in food and health related products for its biodegradable, biomedical, biocompatible properties (Nadarajah, 2005).

Table 2.5. Chitosan activity on microorganism (No et al. 2007)

| **Type** | **Name of microorganism** | **Shelf life extention food types** |
|---|---|---|
| Bacteria | *Bacillus cereus* | Fruits and vegetables, meat |
| | *Enrerobacter aeromonas* | Fruits and vegetables |
| | *Listeria monocytogenes* | Fruits and vegetables, kimchi |
| | *Staphylococcus aureus* | Fruits and vegetables, milk, bread, meat |
| Yeast | *Saccharomyces cerevisiae* | Juice, bread, milk |
| | *Zygosaccharomyces bailii* | Juice |
| Mold | *Penicillium digitatum* | Fruits and vegetables |
| | *Penicillium italicum* | Fruits and vegetables |
| | *Rhizopus* sp. | Fruits and vegetables |

In addition, chitosan is considered as an ideal polyelectrolyte for preparing chitosan-based films and coatings due to its desirable properties, good film forming, nontoxicity, as well as strong mechanical properties (Dutta et al. 2009, Graisuwan et al. 2012). Furthermore, thanks to its cationic properties that can supply the electrostatic interaction so that can be easily bonded other anionic compounds and may also be used for production of biocompatible surfaces on other packaging films via multilayer assembly (Srinivasa et al. 2007).

The studies indicated that usage of chitosan by itself has been limited due to its high sensitivity to moisture. For this reason, many different studies have been suggested the combination of chitosan with other biopolymers to make desirable food packaging materials (Park et al. 2001, Suyatma et al. 2004).

### 2.6.2.2. Pectin

Pectin is a complex anionic heteropolysaccharides, which occur widely in the primary cell walls of plants, such as apples, oranges and pears. The linear backbone of pectin is consisting a sequence of 1,4 linked α-D-galactopyranosyluronic acid units and is interrupted with varying frequency by 1,2-linked α-L-rhamnopyranose residues. There is also a large amount of neutral sugars branch from rhamnose portion of the chain.

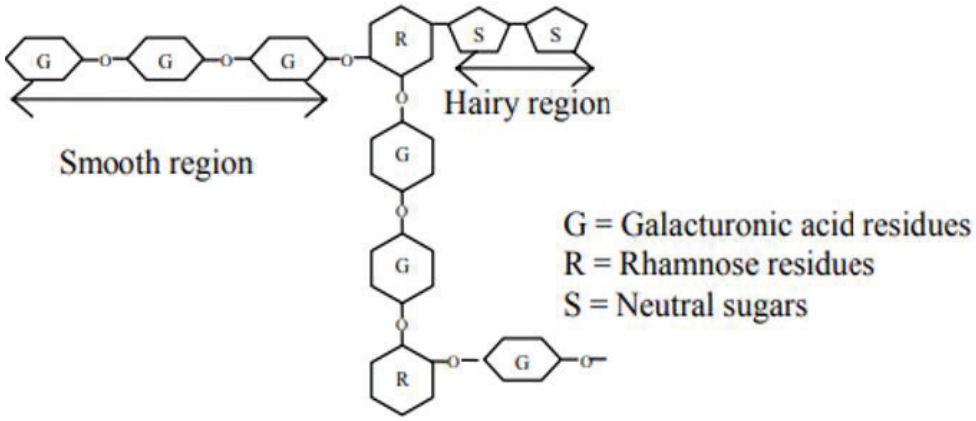


Figure 2.4. Schematic diagram of pectin backbone (Source: WEB_2 2018)

The solubility and gelation of pectins are differentiated by the esterification of galacturonic acid residues with methanol or acetic acid. Methyl esterification is common in pectins and the degree of methylation, which classifies commercial pectins into high methoxyl (>50%) and low-methoxyl (<50%) pectins. Commercial pectins can be amidated which improves the gelling ability of low methoxyl pectins. In some study, it has been proposed to use the low methoxyl pectin substrate as a coating agent for providing an attractive, non-sticky surface to foods.

In the domain of food preservation, pectin as an anionic polyelectrolyte, which is a great impact on its film forming properties, finds application in food packaging and as a carrier molecule for antimicrobials, antioxidants and other compounds (Naqash et al. 2017).

## 2.7. Natural Antioxidant Agents

Antioxidants are substances used to defense against free radical damage to maintain optimum human health and quality of foods. Antioxidant activity is the ability of a bioactive compound due to their capability to retard the oxidation, chelating oxidative metals, inactivation of peroxide, inactivating lipoxygenase and preventing other oxidative damage (Zou et al. 2016, Choe and Min 2006).

A promising trend in recent years include the incorporation of natural antioxidants into packaging materials to prolong the shelf life of foods. In addition, natural antioxidants, which are in the status of GRASS (Generally Recognized as Safe), are easily accepted by consumers. Tocopherols, flavonoids and phenolic acids are the leading natural antioxidants and are found in microorganisms and plants, mostly tea and herbs (Soultani et al. 2014).

### 2.7.1. Green tea extract

Figure 2.5. Illustration of green tea (Source: WEB_3 2018)

Green tea (*Camellia sinensis*) is a widely consumed especially as beverage due to many health benefits such as antioxidant, antimicrobial, anti-carcinogenic and anti-arteriosclerotic properties. Green tea extract (GTE) constitutes an important source of antioxidants, thanks to naturally occurring catechin and polyphenols. In addition to

polyphenols, it contains additional antioxidants such as carotenoids, tocopherols (vitamin E derivatives) and vitamin C.

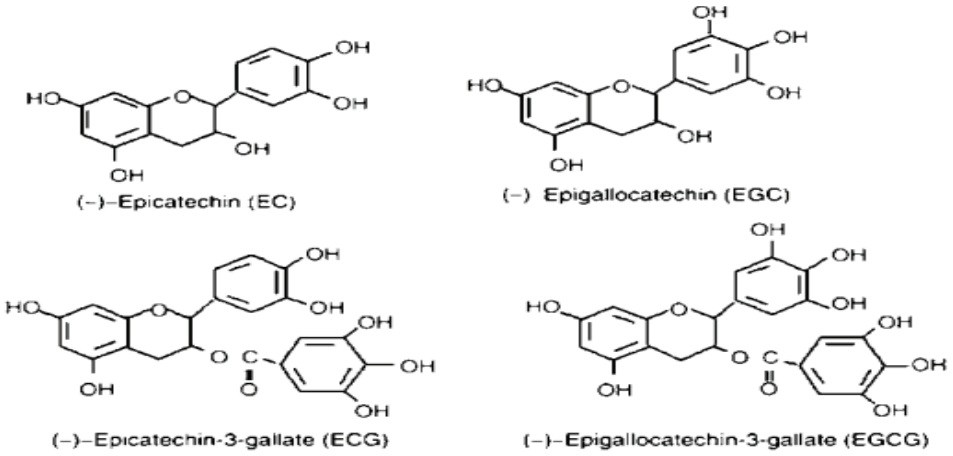


Figure 2.6. Major polyphenols in green tea (Source: WEB_4 2018)

It has been demonstrated that green tea polyphenols structure is primarily responsible for binding and neutralization of lipid free radicals. (Senanayake 2013).

GTE upon direct addition into the oxidation-sensitive food may alter the quality characteristics of the product, and alternatively it can be effectively incorporated into coatings which can be used as active packaging.

## 2.7.2. Oak galls extract

Figure 2.7. Illustration of oak galls (Source: WEB_5 2018)

Galls, in round-shaped, are induced by virus, bacteria, fungi, and even other plants, that resemble tumors in animals (Patel et al. 2018). Oak galls (*Quercus infectoria*) appear on young oak tree branches and are very rich in tannins and their main components are gallotannic acid, gallic acid and ellagic acid, starch and sugar. Galls extracts (GE) has been used to treat many disorders, diseases and symptoms, mainly as astringent and against inflammation. In food related sectors, galls powders and extracts can be used as supplement for bread, coffee substitute, tea or herbal drink (Tayel et al. 2018).

Kaur et al. 2008 reported that the antioxidant activity of ethanolic extract of *Quercus infectoria* galls by testing chemical and biological models. This test exhibited that GE possessed an antioxidant activity containing a large amount of polyphenol. It was concluded that the extract the extract can protect against oxidative damage of lipids and proteins, and also protected a cellular system from oxidative damage. Kaur et al. 2008 showed that the incubation of macrophages with extract protect against oxidative stress, and this antioxidant effect was ascribed to polyphenols.

Tannins, which have important antioxidant activity due to their phenolic nature, are the second most abundant group of polyphenols after lignins. On the basis of their structural characteristics, tannins are divided two major classes (Figure 2.8), namely condensed tannins and hydrolysable tannins.

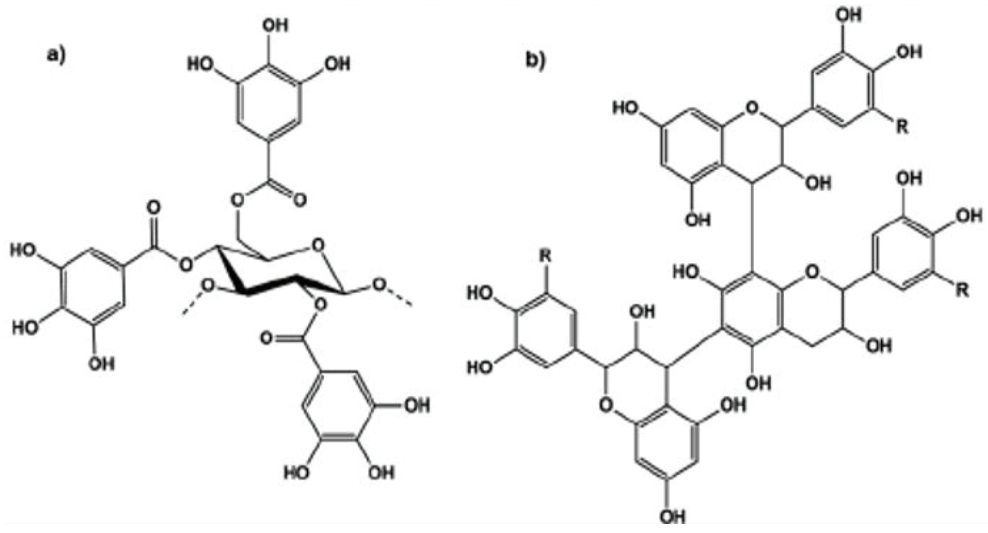


Figure 2.8. Chemical structure of (a) hydrolysable tannin and (b) condensed tannin (Source: WEB_6 2018)

Owing to their antioxidant nature and abilities to form strong complexes with starch, cellulose, protein, minerals and digestive enzymes, tannins are widely used in different fields such as the pharmaceutical, medical and food industries.

Tannins can interact with carbohydrates like pectin, cellulose or dietary fibers and this interaction influences the transport and bioavailability of phenolic compounds. A previous study indicated that the interaction between tannins and pectin may result from hydrogen bonding and hydrophobic interactions; on the contrary, electrostatic interactions, usually containing charged molecules, did not play a dominant role (Mamet et al. 2018).

Soultani et al. 2014 investigated that the effect of pectin on the antioxidant activity and phenolic content of tea (green and black) and herbs (mountain tea and *P. purpureum*) using the methods of Ferric Reducing Antioxidant Power and Folin-Ciocalteu. Study has shown that the addition of pectin did not have negative effect and mask the antioxidant capacity of tea and herbs.

## 2.8. Packaging Applications of Chitosan and Pectin Films

Lehr et al. (1992) revealed that chitosan films can be laminated to pectin films. The electrostatic interactions between carboxyl groups of pectin and amino groups of chitosan can be expected to produce a precipitated or stable membrane between the pectin and chitosan film (Aider 2010).

Martinon et al. 2014 have constructed polysaccharide-based multilayer with antimicrobial agent using chitosan and pectin in order to enhance quality and to extend the shelf life of fresh-cut cantaloupe (*Cucumis melo* L.) stored at 4 ºC. Their work was designed as three different sets of experiments to understand the effect of different concentrations of chitosan (0.5, 1, 2 g/100 g), pectin (0.5, 1, 2 g/100 g), and encapsulated trans-cinnamaldehyde (1, 2, 3 g/100 g) on the quality of selected fruit. Quality changes, namely texture, color, moisture, acidity, and pH were measured. Their recommended coating was composed of 2 g/100 g trans-cinnamaldehyde, 2 g/100 g chitosan and 1 g/100 g pectin to maintain the cantaloupe's quality attributes for 7 - 9 days.

Medeiros et al. 2012, have studied the nanomultilayer coating made of pectin and chitosan on PET as consisting of five nanolayers to characterize in terms of the water vapor, oxygen and carbon dioxide permeabilities and the same coating was applied on

whole mangoes to improve gas barrier and to extend of its shelf-life. After 45 days of storage, coated mangoes presented a lower weight loss and lower total soluble solids compared to the uncoated mangoes. Coated mangoes had also a better external appearance without fungal growth. Therefore, it has been concluded that the nanomultilayer coating of pectin and chitosan has a positive effect as a possible alternative to conventional food coatings on the reduction of gas flow and consequently the longer shelf life of the mangoes.

Sanchís et al. 2016 developed apple pectin coating with incorporation of antioxidants and antimicrobial agents and investigated its effect on enzymatic browning and microbial growth of fresh-cut 'Rojo Brillante' persimmon. Persimmon slices were dipped in potassium sorbate (2 or 4 g/kg), sodium benzoate (4 g/kg), or nisin at 500 (IU/mL) added apple pectin coating, and the aqueous antioxidant solution containing citric acid (10 g/kg) and calcium chloride (10 g/kg). Microbial growth and quality parameters were measured during storage at 5 ºC. Overall, the coatings containing potassium sorbate or sodium benzoate proved to be the most effective to maintain the visual quality of samples and the combination of antioxidants with nisin or sodium benzoate as coating ingredients were the most effective on inhibited the growth of mesophilic aerobics in samples. Results indicated that antimicrobial pectin coatings and antioxidant aqueous solution significantly control enzymatic browning and reduce the total aerobic mesophilic bacteria during 7 days of storage.

Guerreiro et al. 2017 reported that 2% (w/v) pectin edible coating in combination with antibrowning agents (ascorbic and citric acids at 1% (w/v) and sodium chlorite at 0.05% (w/v)) applied to fresh-cut 'Bravo de Esmolfe' apple was effective in reducing the browning index, with ascorbic acid performing best.

# CHAPTER 3

# OBJECTIVE

## 3.1. Aim of the Thesis

The main goal of this research was to develop and evaluate the effectiveness of the multilayered coating process that able to improve the original properties of the flexible plastic materials, using chitosan and pectin in the layer by layer assembly, with galls extract in pectin as natural antioxidant agent. Specific objectives were to:

1. Optimize selection of the pectin-based coating by testing several concentrations of pectin with several pH values.
2. Investigate the changes on conventional packaging material induced by the coating application by different techniques.
3. Determine the effect of different number of layer on the microbiological quality and the appearance of fresh-cut pears shelf-life.
4. Characterize the effectiveness of the optimized coated flexible packaging to enhance product microbial safety and physical, chemical, sensory quality of fresh-cut pears.
5. Examine whether the optimized films retain the incorporated antioxidant agents and gradually release at a level that is high enough to be active during storage of fresh-cut pears.

# CHAPTER 4

# MATERIAL AND METHODS

## 4.1. Materials and Chemicals

The polymer films in this research in order to produce bio-based thin films by the LbL assembly technique were commercial PET had a thickness of 300 nm.

The polymers used in the coating solutions were medium molecular weight chitosan with a degree of deacetylation of 75-85% and acetic acid were supplied by Sigma-Aldrich Chemical Co., LTD. Stabilized High Metoxyl Pectin, Pure High Methoxyl Pectin, Stabilized Amidate Pectin, Pure Amidate Pectin, Stabilized Low Methoxyl Pectin and Pure Low Methoxyl Pectin were obtained from Silvateam S.p.a. As an antioxidative agent, green tea extract (GTE) and oak gall extract (GE) were purchased from Dal Cin Gildo S.p.a, Concorrezzo, Italy. The pH of film solutions was adjusted using sodium hydroxide and hydrochloric acid (Sigma-Aldrich).

The following standards were used for the determination of the total phenolic index of the films: Folin–Ciocalteu reagent (Sigma-Aldrich), sodium carbonate (Sigma-Aldrich) and standard gallic acid (Carlo Erba Reagents S.r.l.).

DPPH radical (2,2-diphenyl-1-picrylhydrazyl) was obtained from Sigma-Aldrich to determine the antioxidant capacity. Methanol and ethanol for the extraction of phenolics were purchased from Sigma-Aldrich.

Microbial growth media - tryptic soy broth (TSB) and malt extract broth (MEB), and pseudomonas agar base were purchased from Merck Millipore, Germany.

The microorganisms, *Pseudomonas putida, Rahnella acqualites, Erwinia persicina, Saccharomyces cerevisiae, Pichia guilliermondii, Candida utilis, Penicillium chrysogenum*, and *Aspergillus niger* were employed in the antimicrobial activity testing and obtained from the culture collection of the University of Milan, Italy.

Pears (*Abate Fetel*) for in vivo applications of the multilayered PET films were supplied by a local distributor and stored at 4 °C prior to processing. Fruits were carefully selected based on size uniformity and peel coloration.

## 4.2. Preliminary Tests

### 4.2.1. Determination of minimum inhibitory concentration of chitosan

The minimum inhibitory concentration of chitosan was evaluated against the growth of bacteria (*Pseudomonas putida, Rahnella acqualites, Erwinia persicina*), yeast (*Saccharomyces cerevisiae, Pichia guilliermondii, Candida utilis*) and molds (*Penicillium chrysogenum, Aspergillus niger*). In this test, one gram of chitosan was dissolved into the 1% acetic acid to obtain the concentration of 1% chitosan solution. The pH of the solution was adjusted at 6.0 using 0.5 M NaOH solution. Then, the different volumes of 1% chitosan samples were added into nutrient agar plate, tryptic soy agar (TSA) or malt extract agar (MEA), (TSB or MEB added with 5 g/L agar). The final concentrations of chitosan samples were 0.2, 0.25, 0.3, 0.35 and 0 % (w/v), respectively. 300 µL of a microbial suspension of bacteria and yeast were inoculated using pipet and molds were inoculated as spores using inoculation loop on each nutrient plate, singularly. The lowest concentrations of chitosan that will inhibit the visible growth of previous microorganisms after incubation at 30 °C for 24 h for bacteria and yeast, and 28 °C for 5 d were defined as the minimum inhibitory concentrations. All assays were performed in duplicate.

### 4.2.2. Determination of antioxidant capacity of GTE and GE powder

The antioxidant capacity of GTE and GE were evaluated by DPPH radical (2,2-diphenyl-1-picrylhydrazyl) scavenging method. The method of Brand-Williams, Cuvelier, and Berset (1995) after a slight adjustment was applied. In brief, 50 µL methanolic solutions of GTE and GE samples were prepared with different concentrations (0–10 g/L) was reacted with 2.45 mL of methanolic DPPH solution (0.1 g/L with the absorbance 1 at 515 nm) in cuvette and shaken properly. These cuvettes were allowed to keep in dark at room temperature for 1 hour. 50 µL methanolic solvent was used as a control was mixed with the same volume of DPPH solution. The absorbance of all samples was measured at a wavelength of 515 nm in a spectrophotometer (L650 with a

150 mm integrating sphere, Perkin-Elmer, Milan, Italy). All assays were done in triplicate.

Afterwards, the same procedure was applied for a calibration curve of Trolox to express the antioxidant capacity of GTE and GE in mg Trolox equivalents per g extract (mg Trolox/g extract) (Kuskoski et al., 2006) and μM Trolox equivalents per g extract (μM Trolox/g extract).

### 4.2.3. Determination of total phenolic index of GTE and GE powder

For total phenolic index examination, the Folin-Ciocalteu method (Singleton and Rossi, 1965) was used. In brief, 0.5 mL of appropriately diluted 50% methanol extracts were added to 2.5 mL 1:10 diluted Folin-Ciocalteu reagent, and 2 mL of sodium carbonate solution (75 g/L) was added to the mixture. After incubation in dark at room temperature for 1 hour, the absorbance of the mixtures was measured at 765 nm versus the related solvent blank. Gallic acid was used for a calibration curve, and the results were expressed as mg/L of gallic acid equivalent. All assays were performed in triplicate.

### 4.2.4. Determination of microbial activity of GTE and GE powder

In order to understand whether GTE and GE powders are effective on prespecified microorganisms or not, the same test of determination of minimum inhibitory concentrations of chitosan was carried out for extracts. One gram of extracts was dissolved separately into the distilled water to obtain the concentration of 1% extract solutions. The pH of the solutions was adjusted at pH 6.0 using 0.1 M NaOH and 0.1 M HCl solutions. Then, the different volumes of 1% extract solutions were added into nutrient agar plate TSA or MEA (TSB or MEB added with 5 g/L agar). The final concentrations of samples were 0.2, 0.25, 0.3, 0.35 and 0% (w/v), respectively. 300 μL of a microbial suspension of bacteria and yeast were inoculated using pipet and molds were inoculated as spores using inoculation loop on each nutrient plate, separately. Inhibition was defined after incubation at 30 °C for 24 h for bacteria and yeast, and 28 °C for 5 d for molds. All assays were performed in duplicate.

### 4.2.5. Determination of zeta potential of pectin

Zeta-potential was determined by using Litesizer™ 500 (Anton Paar, Austria) according to the manufacturer's instructions. The zeta-potential of six types of pectin chains in aqueous solutions was performed to confirm their opposite charge and figure out the most charged one at 25 °C. Changes in the electrical charge of individual biopolymer dispersions, Stabilized High Metoxyl Pectin, Pure High Methoxyl Pectin, Stabilized Amidate Pectin, Pure Amidate Pectin, Stabilized Low Methoxyl Pectin and Pure Low Methoxyl Pectin, were determined against pH and concentration change.

Stock suspensions of pectin (2% w/v) in deionized water were diluted to different concentrations 0.1, 0.2, 0.3 or 0.4% (w/v) and the pH of the specific dispersion was adjusted to pH 6, 7 or 8 by the addition of 0.1 M NaOH and 0.1 M HCl. Twelve separately prepared pectin solutions were loaded into zeta capillary cell to carry out the final zeta-potential values. Three measurements were conducted for each of them and the average results were reported.

## 4.3. Film Preparation and Formulation

### 4.3.1. Preparation of biopolymer coating solutions

Chitosan water dispersion (0.2% (w/v)) was prepared dissolving the powder in acidic water (1.0% v/v glacial acetic acid) while heating and stirring on a plate at 40°C until components completely dissolved. Pure Low Methoxyl Pectin at 0.1% (w/v) and GE at 0.35% (w/v) were weighted and dissolved in distilled water at room temperature under agitation until the solution reached total homogeneity. The pH of solutions was adjusted at 4.0 with HCl and 7.0 NaOH, respectively.

### 4.3.2. LbL assembly on PET

The PET substrates were dipped in polyelectrolyte solutions for deposition the proper coating by dipping LbL. Chitosan/Pectin-GE bilayer film was formed on a transparent PET sheets (18×10 cm) that were firstly rinsed with distilled water, followed

by a thorough rinsing with methanol and distilled water once more. After drying at room temperature to constant weight, both sides of the PET sheets surface were treated by the corona treatment (BD-20 high frequency generator, Electro-Technic Products, Inc., Chicago, IL, USA) just before LbL assembly.

The treated PET sheet was initially dipped into positively charged chitosan solution at pH 4.0 for 1 minute, and one rinse step with distilled water for 15 seconds, the sheet was dried with blowing air. The first layer of chitosan was adsorbed so that the surface charge of the PET sheet was reversed to be positive. Afterwards, the sheet was immersed in negatively charged pectin-GE solution at pH 7.0 for another 1 minute followed by another rinsing and drying cycle. The chitosan and pectin-GE adsorption and rinsing cycles were repeated until the desired number of layers (20-40-60 layers) was obtained, respectively. All coated films were stored in desiccator prior to characterization. A diagram of the coating procedure is represented in Figure 2.1.

## 4.4. Characterization of the Multilayer Film

### 4.4.1. Contact angle analysis

The surface hydrophobicity or wettability studies of the films generally involve measuring the water contact angle as the primary data. The water contact angle of the original PET and LbL coated surface PET were measured using the sessile drop method (Newman and Kwok 1999) by a video contact angle meter (OCA 15 Plus-Data Physics Instruments GmbH, Filderstadt, Germany) with an image analysis software SCA 20 (Data Physics Instruments GmbH, Filderstadt, Germany), in which a 4 ± 0.5 μL droplet of MilliQ water was placed on a horizontal surface with a 500 μL glass syringe (Hamilton, Switzerland). Measurements were made at equilibrium after drop deposition and were performed for each type of surface, two samples were used. For each sample, seven contact angle measurements were carried out at room temperature 25 ± 0.3 ºC.

### 4.4.2. UV-Visible spectrophotometry

To follow the LbL deposition onto PET films was carried out in a simple and easy handling by UV–visible high-performance spectrophotometer (Lambda 650, PerkinElmer, Waltham, MA, USA with a 150 mm integrating sphere, Perkin-Elmer, Milano, Italy). The absorbance of PET films was measured on each subsequent ten layers' surface until acquired the final multilayer film construction. The absorbance was measured at 281 nm on dried films. Control spectra was obtained with original PET. Three measurements were taken.

### 4.4.3. Haze

According to ASTM D 1003 standards (Standard Test Method for Haze and Luminous Transmittance of Transparent Plastics), haze is the fraction of transmitted light that deviates by more than an angle of 2.5º from the direction of the incident beam and it is usually important optical property especially for packaging applications, as being responsible for the reduction in the contrast between objects seen through the specimen. The haze of PET films was measured on the surface of each successive ten layers with a UV–vis high-performance spectrophotometer (Lambda 650, PerkinElmer, Waltham, MA, USA with a 150 mm integrating sphere, Perkin-Elmer, Milano, Italy). Three measurements were considered for each film.

## 4.5. In Vivo Assay

### 4.5.1. Preparation of fresh-cut pear samples

Pears (*Abate Fetel*) were purchased from the local market when they were eating-ripe and selected based on uniformity of size, color, without defects or decay. Fruits were stored at 4 ± 0.5 °C until prepared as fresh-cut. The selected fruits were washed with distilled water and gently dried with paper towels, then, they were randomly divided into four different groups (untreated samples with uncoated PET strips (CTR samples) and treated samples with LbL active coated PET strips (LbL samples) with outermost layer

pectin-GE, 20, 40 and 60 layers). Pears manually cut into the shape of half-moon with 3 cm in diameter and 1.5 cm length using a sterile sharp knife in microbiological safety cabinet. In addition, the pear slices were with peel and without core. Also, the reason for cutting the fruit in this way is to provide similar size and shape in the quality tests. All utensils, which in contact with the fruit were previously sanitized.

In the preliminary in vivo experiment, two slices of pears were set up to be on top of each other in polyethylene terephthalate packages (commercial packages in the form of boxes) of dimensions 19 cm x 12 cm x 4 cm and LbL PET (20, 40 and 60 layers) were placed between each slice at the bottom and top. In untreated samples, CTR PET were placed as described. All packages covered and sealed with stretch film. Finally, one tray of each treatment group was analyzed immediately after preparing (day 0), and after 3, and 5 days of refrigerated storage at 4 ± 0.5 °C to evaluate the effects of LbL active coatings on color and microbiological quality of pears. Results showed that the samples treated with 60 layers LbL PET has better score in terms of color and microbiologically. Therefore, in subsequent in vivo assay 60 layers LbL PET were used. In this case, as distinct from the previously described, four slices of pears were put into packages and at days 0, 3, 5 and 7, samples were evaluated for their physicochemical, microbiological, and sensory evaluation. In addition, in order to find out the release mechanism of active substance from coating to the food, DPPH and TPI assay were applied to LbL PET. For sensory evaluation, specific samples of LbL PET and CTR PET were prepared and monitored during 7 days of storage.

### 4.5.2. Preliminary in vivo assay

In order to understand the effect of number of layers and decide the precise number of layers for in vivo assay, the food contact was performed. In general, spoilage in fruit can be divided into two categories as non-microbial and microbial spoilage (Putnik et al. 2017). Among others, the change of an undesired brown color is one of the main problems of fresh-cut fruits that occurs very early during the storage. This type of non-microbial spoilage is the first attributes that will alert customers when buying a fresh product. (Zambrano-Zaragoza et al. 2014, Putnik et al. 2017). And the other main problems of fresh-cut fruits during the storage is microbial growth (Gómez-López et al. 2007). Presence of intolerable microorganisms injure food quality and pathogen

microorganisms have a negative effect on the safety of food products. From the point of view of quality considerations in fresh-cut fruit, surface discoloration and visible microbial growth are the leading limitations that strongly affecting consumer preference. For this reason, three different number of layers (20-40-60 layers) coated PET films with the outermost layer pectin-GE were studied based on microbial growth and color evaluation as a preliminary shelf-life assay of pears.

### 4.5.3. Color assay

Color of CTR and LbL samples was analyzed using a Minolta CR-300 chromameter (Konica Minolta Sensing, Inc., Japan) calibrated with a standard white plate (Y=93.5, x=0.3114, y=0.3190), where the color changes in the surface of pear samples were measured by CIE system.

To perform the preliminary tests, one box from each treatment group 20-40-60 LbL samples and CTR samples at days 0, 3 and 5 were collected and the box from main experiment, 60 LbL samples and CTR samples, were collected after 0, 3, 5 and 7 days storage. Readings of L* (lightness), a*(green chromaticity), and b* (yellow chromaticity) were performed a total of six measurements for each group of the two experiments. Total color difference delta E* (ΔE) was calculated by:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad (4.1)$$

where $L_0$, $a_0$, $b_0$ are the initial values, obtained in time zero, and L, a, b are the values measured during the experiment.

To standardize differences in different pears' color and get more accurate results, the ΔE of samples were calculated using their correspondent initial values.

### 4.5.4. Microbiological assay

The analysis at 0, 3, and 5 days in the preliminary in vivo, one box for each treatment group of 20-40-60 LbL samples and CTR samples, and in the main in vivo analysis at 0, 3, 5, 7 days one box of 60 LbL samples and CTR samples were separated and sampled for the microbial growth on pears included counts of aerobic mesophilic

microorganisms, and yeasts and molds. For the counts of aerobic mesophilic and molds and yeasts, plate method was performed as below.

Samples were prepared by weighting 10 g of pears that were removed aseptically from each treatment which were then diluted in 90 mL of sterilized saline into a stomacher bag (400 mL PE, Barloworld, France) and homogenized for 1 minute, subsequently, 10-fold dilutions were made in this diluent.

Appropriate dilutions were transferred to the sterilized petri dish, and then TSA (Tryptic Soy Agar) for mesophilics, and MEA (Malt Extract Agar) for yeast and moulds were poured into the dish. All inoculated mesophilics were incubated at 30 °C for 24-72 hours; yeast and moulds were incubated at 28 °C for 48-72 hours. After incubation, colonies were counted and results reported as log cfu/g of pear. The experiments were done in duplicate for each experimental condition.

### 4.5.5. Sensory analysis

The sensory characteristics of pear samples were identified in terms of appearance, color, odor, firmness and overall acceptance by 12 panelists from Food Science and Technology Department of University of Milan, who were familiar with the product and sensory evaluation. The sensory evaluation was performed for 60 LbL samples and CTR samples before treatment (day 0) and after 3, 5 and 7 days of storage at 4±0.5 °C of fruits. In all cases, the samples were left to equilibrate at room temperature, assigned by three-digit codes and presented in white foam tray to the panelists randomly. The panelists were asked to score each sample using a 5 point-scale for answers, (1 = dislike very much to 5 = like very much) (Figure 4.1) without making any comparison between LbL and CTR. The panelists average responses were considered for each attribute.

| **Name Surname:** | | | | | **Date: …./…../…..** |
|---|---|---|---|---|---|
| Please rating the samples according to appearance, color, odor, firmness and overall acceptability. | | | | | |
| **Sample Codes** | **Appearance** | **Color** | **Odor** | **Firmness** | **Overall Acceptability** |
| **572** | | | | | |
| **965** | | | | | |
| **127** | | | | | |

| **SCALE** |
|---|
| 1 – Dislike very much |
| 2 – Dislike moderately |
| 3 – Neutral |
| 4 – Like moderately |
| 5 – Like very much |

Figure 4.1. Sensory attributes score sheet

## 4.6. Studies Related to Release of GE Agent from Films

The release tests were assessed by measuring the residual amounts of GE released from the films on the surface of the PET after the main in vivo assay to understand the kinetics of release. The films after different film-food contact times (3, 5, and 7 days), cut into pieces of $4 \times 4\ cm^2$ square strips were immersed into glass flasks with 4 mL of ethanol:water (50:50%) solution in 1% acetic acid and then incubated for 45 minutes with continuous stirring to get the extract of layers remaining on the PET surfaces. This extraction step was repeated using new solvent until all layers are removed from the surface. It was decided that the entire coating was separated from the surface based on the UV absorbance value prior to processing of PET. The release test solutions were used for subsequent analyses.

### 4.6.1. DPPH assay

GE release activity of the film sample in release medium was measured by DPPH assay according to the method described previously with some modification in section 4.2.2. The ethanol:water (50:50%) solution in 1% acetic acid was used as a solvent. Trolox solutions as standards were also analyzed for a calibration curve and the results were expressed in mg Trolox equivalents per g extract (mg Trolox/g extract) and μM Trolox equivalents per g extract (μM Trolox/g extract). All assays were performed in triplicate.

### 4.6.2. Total phenolic index

The release medium was also used for GE release activity by the method of total phenolic index assay given in section 4.2.3. using the ethanol:water (50:50%) solution in 1% acetic acid as a solvent. Gallic acid was used as the standard for a calibration curve, and the results were expressed as mg/L of gallic acid equivalent. All assays were performed in triplicate.

## 4.7. Statistical analysis

All experimental measurements were repeated at least three times and the results expressed as mean value ± standard deviation in the present study. Data were analyzed by the SPSS 20.0 software package. Statistical analysis was calculated by one-way analysis of variance (ANOVA) and differences were considered to be statistically significant with $p < 0.05$.

# CHAPTER 5

# RESULTS and DISCUSSION

## 5.1. Preliminary Tests

### 5.1.1. Antimicrobial activity of chitosan

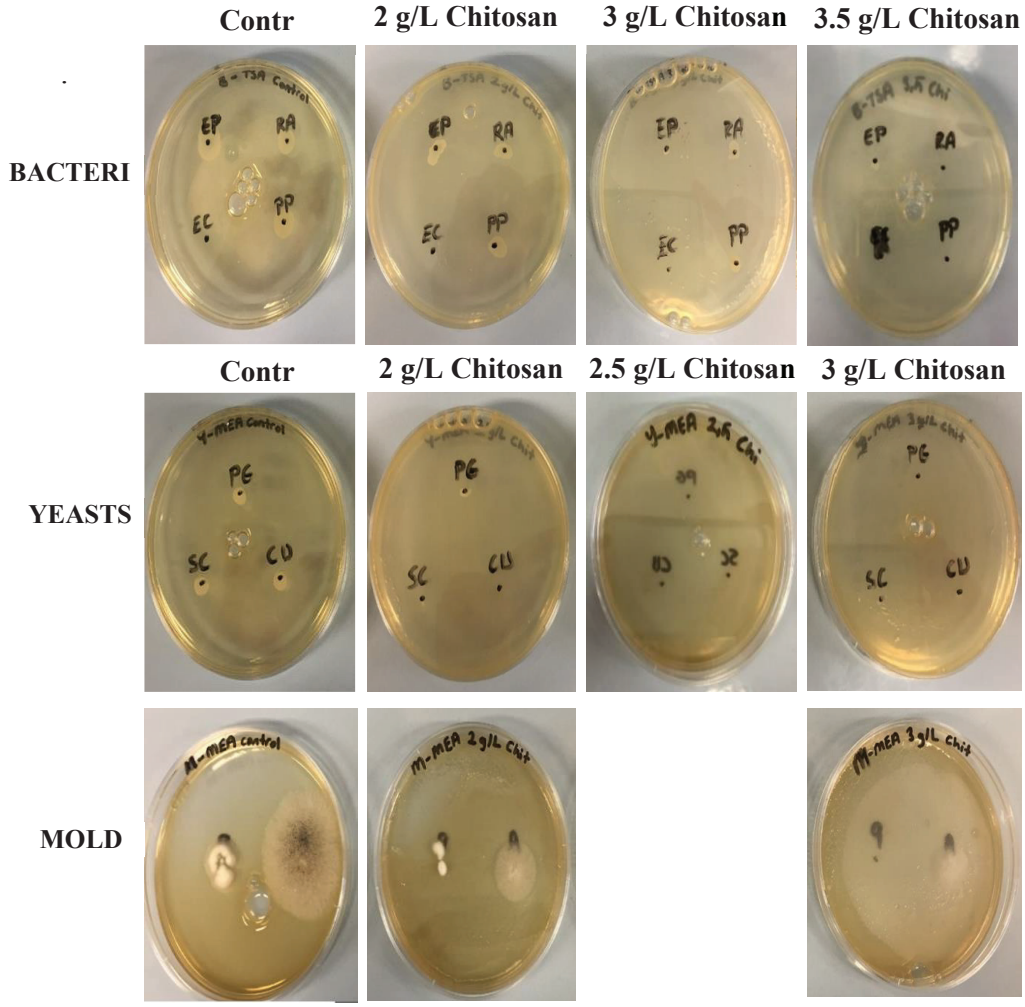


Figure 5.1. Inhibition zones of various concentrations of chitosan against common fruits and vegetables microorganisms in diffusion antimicrobial tests.

*PP: *Pseudomonas putida*, RA: *Rahnella acqualites*, EP: *Erwinia persicina*, SC: *Saccharomyces cerevisiae*, PG: *Pichia guilliermondii*, CU: *Candida utilis*, PC: *Penicillium chrysogenum,* AN: *Aspergillus niger*.

The antimicrobial activity of different concentration of chitosan solutions against Pseudomonas putida, Rahnella acqualites, Erwinia persicina, Saccharomyces cerevisiae, Pichia guilliermondii, Candida utilis, Penicillium chrysogenum, and Aspergillus niger was investigated by qualitative analysis (Figure 5.1).

Several researchers have observed that chitosan has an antimicrobial activity against various of microorganisms, including fungi, algae, and some bacteria. As a result of this, it is taken into account as one of the well-recognized antimicrobial substances (Rabea et al. 2003, Durango et al. 2006). However, the antimicrobial action is influenced by various factors that are known microbial factors, intrinsic factors of chitosan, and the environmental factors (Ma et al. 2017).

This test was conducted with final chitosan concentrations of: 0%, 0.2%, 0.25%, 0.3% and 0.35% at pH 6.0 in order to not to prevent the growth of microorganisms. These concentrations were selected due to the result of previous studies that 0.2% was enough to completely eliminate *E. coli* O157:H7 (Jeon et al. 2014) and *S. cerevisiae* (Elmacı et al. 2014). All tested microbial strains were susceptible to all concentrations of chitosan used. Chitosan exhibited complete inhibition against all studied yeasts at 2.5 g $L^{-1}$. In addition, there was a correlation between the inhibition of microorganism and the increasing concentration of chitosan. E. persicina was less growing but there is an inhibition on R. acqualitis and P. putida with 3.5 g $L^{-1}$. More effective inhibition on selected bacteria and molds was observed 3 g $L^{-1}$, but yeasts 2.5 g $L^{-1}$.

Moreover, it has been shown that pH plays an important role in the antimicrobial activity of chitosan and chitosan-based films that increases by decreasing pH. This effect may be due to the fact that the positive charge of amino groups at pH values lower than the pKa of chitosan (pH < 6.3-6.5) at which this functional group binds to microbial cell wall through electrostatic interaction and may cause a leakage of the cell (Ma et al. 2017, Rabea et al. 2003).

Therefore, based on chitosan's noticeable antimicrobial effect even at low concentration with studied pH and the purpose of building-up nanostructure on PET surfaces, 2 g $L^{-1}$ chitosan concentration was selected to fabricate active packaging material.

### 5.1.2. Total phenolic index and antioxidant activity of GTE and GE powder

Foline-Ciocalteu phenol reagent is used to acquire an estimation of the amounts of phenolic groups existing in the GTE and GE. Phenolic compounds in plant extract react with phosphotungstic and phosphomolybdic acids in the Foline Ciocalteu reagent to a blue complex. The color development relies on the transfer of electrons in alkaline medium to reduce the phosphomolybdic/phosphotungstic acid complexes (Curcioetal 2009). Total phenolic content of the extracts was expressed as gallic acid exultance (GAE) in mg (the standard curve equation: $y = 0.045x + 0.0231$, $R^2 = 0.9997$).

The DPPH scavenging assay, which is popular for the study of natural antioxidants (Villano et al., 2007), was used to evaluate antioxidant activity of extract powders. This assay is based on the absorbance properties a stable color-free radical, DPPH, that can be quenched, and thereby the absorbance values decrease when the radical is reduced by antioxidants resulting in a reduction in absorbance (Diouf, Stevanovic, & Cloutier, 2009). Also, the antioxidant activity of the studied compounds expressed in µM Trolox (the standard curve equation: $y = 0.0103x + 0.0403$, $R^2 = 0.9996$).

All the experiments showed that GE statistically exhibited the highest total phenolic index and antioxidant activities than GTE ($p < 0.05$). All comparisons were based on the same concentration of the components, 50 mg/L for TPI and 125 mg/L for DPPH assay. The total phenolic compound of GTE and GE found to be 842 mg GAE/g of extract and 1065 mg GAE/g of extract, respectively. The scavenging activity on DPPH radical of extracts was 9.3 µM Trolox/g of extract for GTE and 12.1 µM Trolox/g of extract for GE. The obtained results are shown in Table 5.1. It has been shown that GE has good antioxidant activity due to its ability to reduce the DPPH radical with less concentration. These results suggested that the use of the GE in the pectin coating solution could have a potential antioxidative effect of active packaging.

Moreover, literature has showed that pectin has no significant effect on the phenolic content and antioxidant activity of the teas and herbs extract, on the contrary it can be useful to preserve polyphenol because it acts as a cooperative hydrogen bonding between the oxygen atom of the carbohydrate and the phenolic hydroxyl group (Soultani et al. 2014, Vernhet et al. 1996).

Table 5.1. Total phenolic index (TPI) and scavenging effect on DPPH radical of extracts

| Extracts | TPI (mg GAE/g of extract) | DPPH (μM Trolox/g of extract) |
|---|---|---|
| GTE | 842 ± 20 | 9.3 ± 0.14 |
| GE | 1065 ± 50 | 12.1 ± 0.2 |

*Each value is the average of three analyses ± standard deviation.

## 5.1.3. Antimicrobial activity of GTE and GE powder

The antimicrobial activity of different concentrations of GTE and GE powder solutions against *Pseudomonas putida*, *Rahnella acqualites*, *Erwinia persicina*, *Saccharomyces cerevisiae*, *Pichia guilliermondii*, *Candida utilis*, *Penicillium chrysogenum*, and *Aspergillus niger* was investigated by visual inspection for the presence or absence of microbial growth.

The antioxidant activity results of GTE and GE were different, but they showed a similar tendency in the antimicrobial activities. Our results revealed that all of the tested microorganisms were less sensitive to the exposure of extracts. In addition, the resistance of the microorganisms was independent of the extract at the different concentrations tested. In Figures 5.2 and 5.3, there are visually shown the antimicrobial activities of the extracts. Complete inhibition of microorganisms was not achieved with both extracts and their different concentrations. Our results comply with the findings of earlier studies that have demonstrated no activity of tea extracts against Gram-negative *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*.

The reduction in growth of microorganism comparison with control is probably due to the polyphenols extracts contain. It has been reported that catechins, which are the major compounds of green tea, has antimicrobial activities against several pathogens (Gordon and Wareham 2010). The absence of the antimicrobial effect in this study of the samples emphasizes the disparity of the exact mode of action of natural extracts due to differences in microbial strains used, to the damage of their cell membrane, and to the differences in types and concentrations of extracts used by many research laboratories.

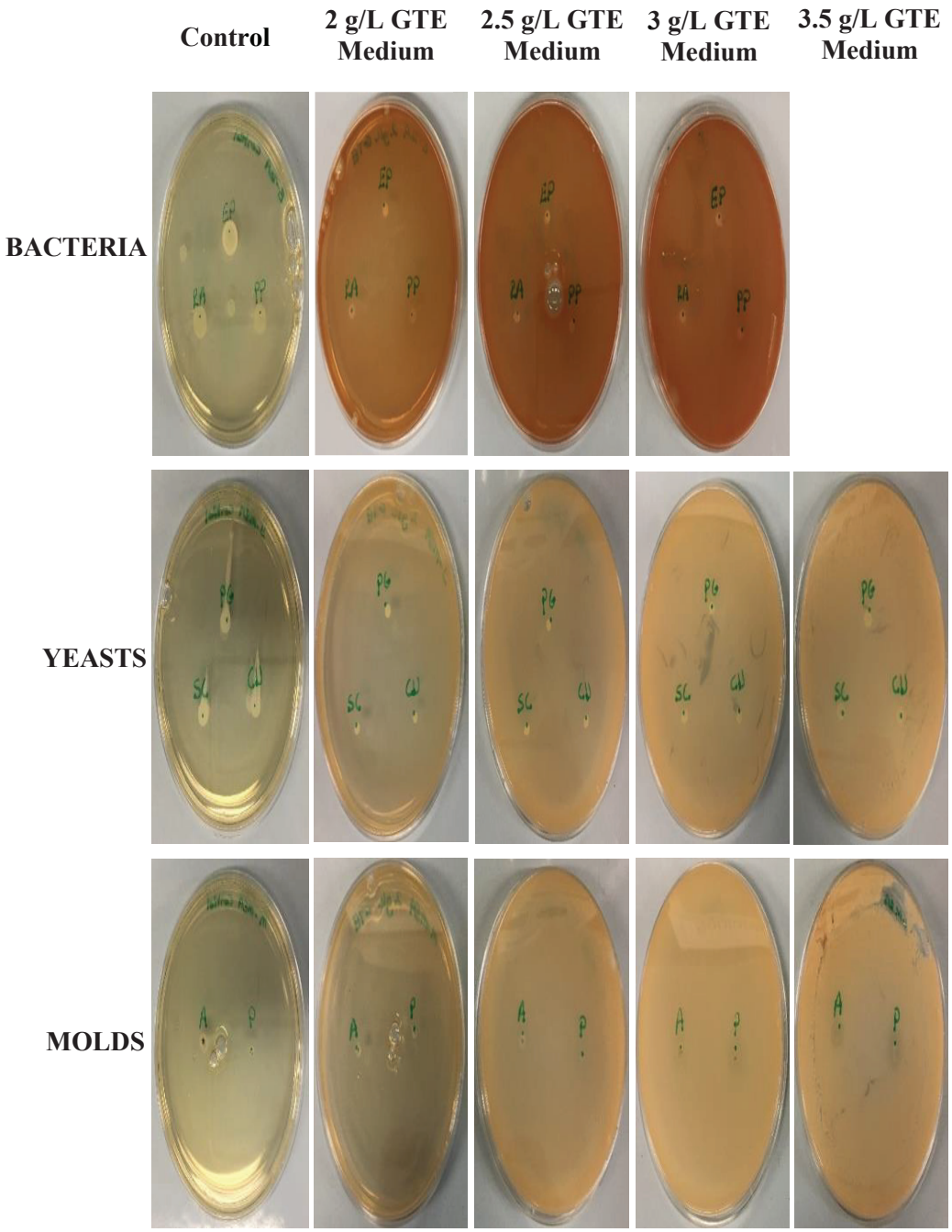


Figure 5.2. Inhibition zones of various concentrations of GTE against common fruits and vegetables microorganisms in diffusion antimicrobial tests.

*PP: *Pseudomonas putida*, RA: *Rahnella acqualites*, EP: *Erwinia persicina*, SC: *Saccharomyces cerevisiae*, PG: *Pichia guilliermondii*, CU: *Candida utilis*, PC: *Penicillium chrysogenum,* AN: *Aspergillus niger*.

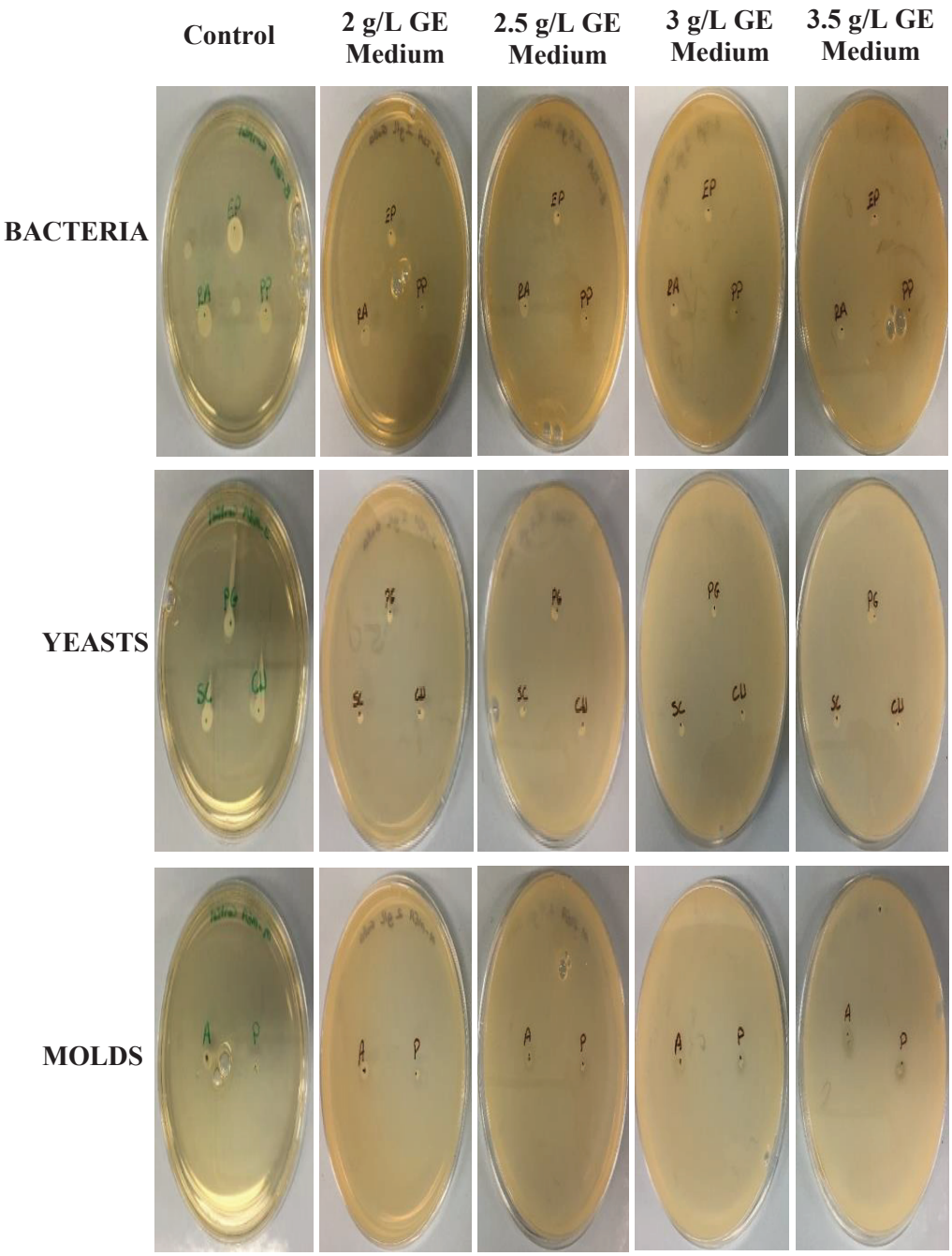


Figure 5.3. Inhibition zones of various concentrations of GE against common fruits and vegetables microorganisms in diffusion antimicrobial tests.

*PP: *Pseudomonas putida*, RA: *Rahnella acqualites*, EP: *Erwinia persicina*, SC: *Saccharomyces cerevisiae*, PG: *Pichia guilliermondii*, CU: *Candida utilis*, PC: *Penicillium chrysogenum,* AN: *Aspergillus niger.*

### 5.1.4. Zeta potential of pectin

The zeta-potential is an important parameter of the potential difference across phase boundaries of solid/liquid and liquid/gaseous (Salopek et al. 1992). It is a measurement of the electrical charge, a property that all materials possess, or acquire, when suspended in a fluid and the charge is usually negative than positive.

When measuring the zeta-potential, the main focus is on the magnitude of it, is measured in millivolts (mV), and not whether it is positive or negative. Because the magnitude is related to nanoparticle stability or aggregation in solution. Generally, the larger magnitude of the zeta-potential brings about less coagulation due to more repulsion the phases. A well-accepted line that divides stability and instability is at +30 mV or -30 mV. The higher magnitudes beyond those values means the more stable colloidal system (Liang 2006).

In this study, preexisting different pectin samples in the laboratory was measured in terms of pectin concentration (0.1 - 0.4%) and pH (6.0 - 8.0) of the aqueous phase in order to choose the proper pectin could have a strong interact with chitosan through opposite charge interactions. Since the pectin polymers are formed from galacturonic acid units that can be the methyl esterified at the C-6 carboxyl group, these carboxylic groups can be negatively charged depending on the pH of the continuous phase (Verkempink et al. 2018).

Table 5.2. presented zeta-potential of pectin solutions. As shown, the zeta-potential values for all concentration and pH studied, the pectin solutions had negative charges indicating that all samples are anionic as was expected. The effect of all tested pectin pH was limited on the charge of pectin, the charge did not significantly change. On the other hand, the zeta-potential of all kind of pectin increased with decreasing concentration of solutions.

Based on 0.1% (w/v) pectin concentration, the highest zeta-potential value obtained for PLM pectin solutions was found to be -52.87 ± 0.44 mV at pH 7.0. Therefore, it was chosen to conduct the following experiments.

Table 5.2. Zeta potential (mV) of different pectin corresponding to various concentration and pH

| | | **Zeta potential (mV) of Types of Pectin** | | | | | |
|---|---|---|---|---|---|---|---|
| Concentration (%w/v) | pH | SHM | PHM | SA | PA | SLM | PLM |
| 0.1 | 6 | -40.87 ± 0.31 | -38.8 ± 0.10 | -48.67 ± 1.15 | -47 ± 0.53 | -50.47 ± 0.40 | -52.2 ± 0.44 |
| | 7 | -42.87 ± 0.65 | -40.63 ± 0.90 | -50.1 ± 1.05 | -46.97 ± 0.65 | -51.17 ± 0.60 | -52.87 ± 0.21 |
| | 8 | -41.77 ± 0.23 | -39.77 ± 0.25 | -49.27 ± 0.90 | -47.27 ± 0.57 | -51.17 ± 0.35 | -52.83 ± 0.75 |
| 0.2 | 6 | -39.3 ± 0.66 | -36.5 ± 0.85 | -44.87 ± 0.40 | -42.97 ± 0.31 | -50.23 ± 0.15 | -48.7 ± 0.44 |
| | 7 | -39.9 ± 0.70 | -37.03 ± 0.80 | -43.47 ± 1.36 | -43.53 ± 0.51 | -51.13 ± 0.97 | -49.57 ± 1.11 |
| | 8 | -39.17 ± 0.21 | -36.93 ± 0.06 | -45.23 ± 0.21 | -43.17 ± 0.15 | -50.33 ± 0.31 | -49.97 ± 0.75 |
| 0.3 | 6 | -36.33 ± 1.10 | -34.87 ± 0.40 | -42.67 ± 0.81 | -39.1 ± 2.34 | -48.63 ± 0.59 | -47.47 ± 0.57 |
| | 7 | -37.8 ± 0.30 | -34.37 ± 0.35 | -42.1 ± 0.36 | -40.7 ± 0.17 | -49.37 ± 0.46 | -47.17 ± 0.15 |
| | 8 | -38.23 ± 0.90 | -34.97 ± 0.45 | -42.87 ± 0.21 | -40.8 ± 0.44 | -49.5 ± 0.95 | -46.93 ± 0.35 |
| 0.4 | 6 | -34.43 ± 0.46 | -32.67 ± 0.45 | -40.6 ± 0.44 | -39.03 ± 0.40 | -45.77 ± 0.85 | -46.4 ± 0.60 |
| | 7 | -36.37 ± 0.49 | -33.37 ± 0.29 | -40.4 ± 0.36 | -38.77 ± 0.40 | -45.87 ± 0.76 | -46.17 ± 0.67 |
| | 8 | -35.27 ± 0.49 | -33.03 ± 0.23 | -41.23 ± 0.38 | -39.57 ± 1.08 | -45.53 ± 0.15 | -44.87 ± 1.32 |

*SHM: Stabilized High Metoxyl, PHM: Pure High Methoxyl, SA: Stabilized Amidate, PA: Pure Amidate, SLM: Stabilized Low Methoxyl, PLM: Pure Low Methoxyl

## 5.2. Characterization of the LbL coating on PET

### 5.2.1. Contact angle

To confirm change on the surface wettability of polymers after surface treatments and to have knowledge about the developed coatings, the contact angle technique is one of the most practical methods. The water contact angle indicates the degree of hydrophilicity of films, lower values correspond to higher hydrophilicity, on the contrary, the hydrophobic surfaces show high values (Munhuweyi et al. 2017, Fabra et al. 2016). In this way, the deposition of the successive layers on the PET can be easily followed owing to the different wettability properties of the electrolyte solutions used to fabricate of multilayered films by contact angle measurements.

The observed contact angle values of the original PET, the corona treated PET and after successive layers were added are displayed in Figure 5.4. Each data point is an average of seven measurements and the error bars represent the standard deviation. The contact angle on the original PET film was found to be 69.9 ± 0.59º and significantly ($p < 0.05$) decreased to 51.1 ± 1.75º after treated to corona discharge treatment (0 layer deposition). This behavior is because lower contact angle values show the presence of multiple hydrophilic groups on the surface and the results confirmed the effectiveness of corona treatment of the original PET surface.

In this work, odd number of layers coincide with chitosan assembled at pH 4.0, while even number of layers correspond to pectin-GE assembled at pH 7.0. The contact angle was followed alternative adsorption of chitosan and pectin-GE one by one until 10th layer, and then after subsequent five layers until 60th layer. The angles with significantly ($p < 0.05$) higher magnitude were obtained for the chitosan layers and low angles were obtained for the pectin-GE layers. It could be seen that the contact angle values which is depicted Figure 5.4. periodically varied between above 21º to 53º from 20th to 60th layer. The fluctuation of the contact angles with the same outermost layer until to be fixed might be a consequence of the interpenetration between neighboring chitosan and pectin-GE layers, and also would be a penetration of polyelectrolytes into the pores of the support surface.

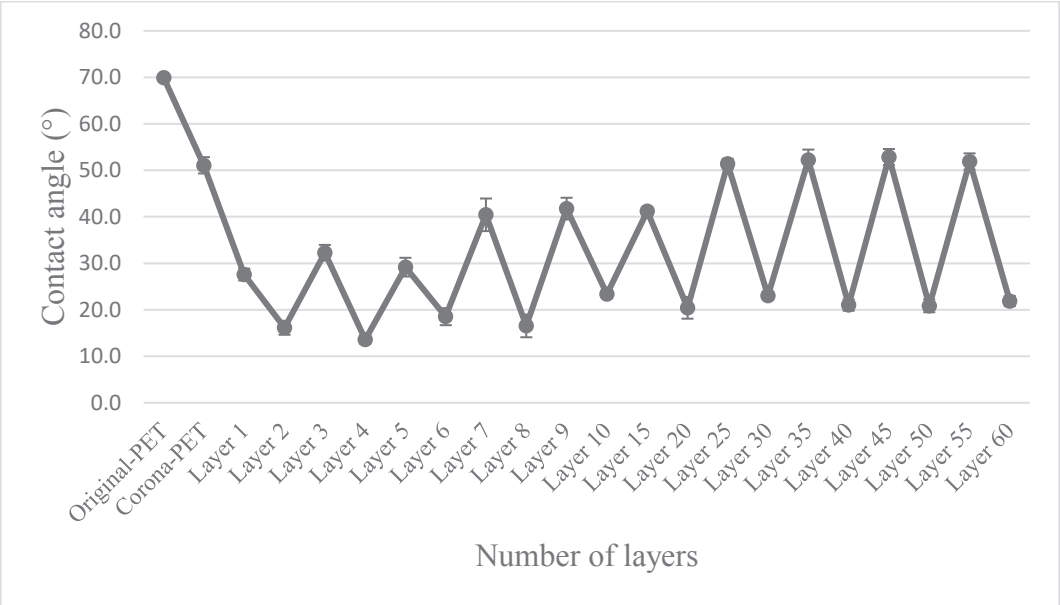


Figure 5.4. Contact angles results on Original-PET, on Corona-PET, on the ten successive layers and after every five successive layers until 60$^{th}$ layer

Table 5.3. Contact angle values on the ten successive layers and after every five successive layers until 60$^{th}$ layer

| Number of Layer | Water Contact Angle (º) | Number of Layer | Water Contact Angle (º) |
|---|---|---|---|
| 1 | 27.6 ± 1.28 | 15 | 41.2 ± 0.94 |
| 2 | 16.1 ± 1.50 | 20 | 20.4 ± 2.34 |
| 3 | 32.3 ± 1.68 | 25 | 51.4 ± 1.08 |
| 4 | 13.6 ± 0.91 | 30 | 23.0 ± 0.85 |
| 5 | 29.2 ± 2.01 | 35 | 52.2 ± 2.24 |
| 6 | 18.5 ± 1.83 | 40 | 21.0 ± 1.22 |
| 7 | 40.4 ± 3.50 | 45 | 52.9 ± 1.73 |
| 8 | 16.5 ± 2.44 | 50 | 20.8 ± 1.38 |
| 9 | 41.8 ± 2.34 | 55 | 51.9 ± 1.76 |
| 10 | 23.4 ± 0.91 | 60 | 21.9 ± 1.10 |

*Each data point is the average of seven measurements with the standard deviation.

The obtained contact angle values of PET films after each surface treatment are given in Table 5.3. As shown, after the deposition of the 15th layer, chitosan contact angle measurements started to be fixed around 51.4 ± 1.08° while for pectin-GE around 21.0 ± 1.22° with a significant difference ($p < 0.05$). After 20th layer, there was no particular difference ($p > 0.05$) between the odd number of layers and between the even number of layers. It can be therefore deduced that the changing of surface hydrophilicity confirmed

that the film was deposited by sequential adsorption of these two polyelectrolytes, chitosan and pectin-GE on the PET films (Figure 5.4). The contact angle values with similar magnitude and behavior were also observed by Medeiros et al. 2012 for a multilayer film of chitosan and pectin assembled at pH 3.0 and pH 7.0 on PET, respectively.

### 5.2.2. UV-visible

The successful buildup of each five bilayers, being the outermost layer pectin-GE, of chitosan/pectin-GE on the PET substrates in the cyclic dipping procedure was monitored by UV-visible spectroscopy at 281 nm that was a well-appeared absorption peak between 230-500 nm and the pretreatment result of absorbance value of different concentrations of chitosan and pectin solutions in the range of 200-400 nm. The absorbance of PET films increased as a function of the number of layers deposited, thus confirming the sequential formation of layers after an assembly process. As shown in Figure 5.5, the film absorbance (from about 1.91 ± 0.051 to 2.35 ± 0.064) showed a linear growth with the increase of chitosan/pectin-GE bilayer numbers, which was common for many LbL films. The linear increase of absorbance indicated a regular and uniform deposition on PET films, thus stating a uniform LbL assembly process.

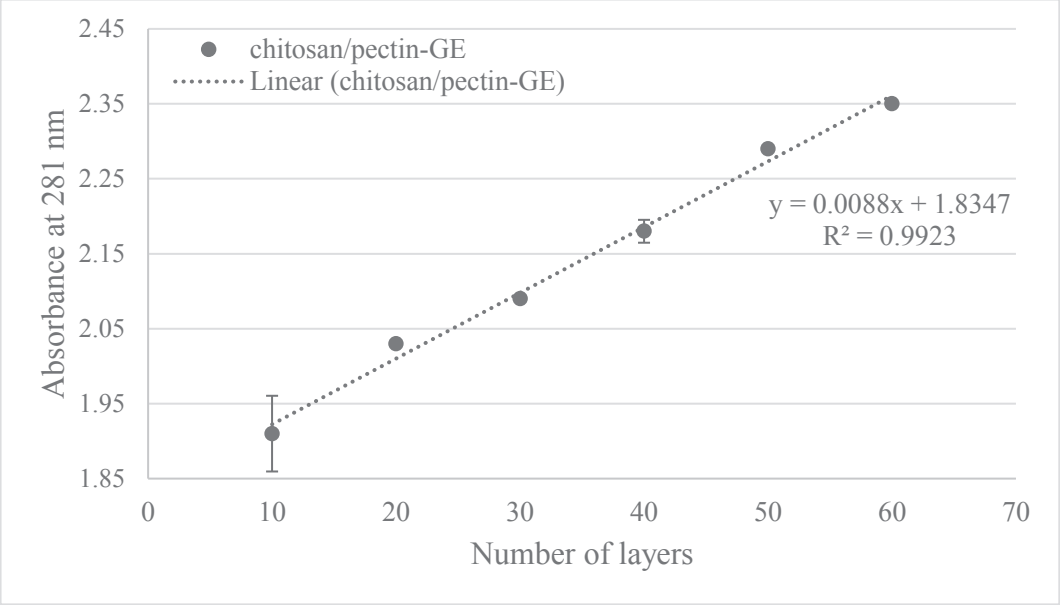


Figure 5.5. The absorbance as a function of the number of layers deposited

Although the mass adsorbed cannot be quantified with UV-Vis spectra, information about the growth regime of multilayers can be obtained (Acevedo-Fani et al. 2017). It demonstrated that the thickness of every new chitosan/pectin-GE bilayer increased linearly with the increase of the layers. Therefore, it suggests that the chitosan and pectin solutions are prepared successfully in order to ensure the same adsorption quantity of each layer during the LbL assembly process.

### 5.2.3. Haze

Recent research has suggested that the controlling the surface layer composition and hence measurements of the optical haze of the surface is a very important step in achieving the high clarity materials. It is the ratio of forward scattered light through the transparent material to the total transmitted light (Sibin et al. 2017). Figure 5.6. shows the measured haze values for the original PET and the chitosan/pectin-GE PET films.

The uncoated films exhibited an average haze of 9.09 ± 0.097%, and the haze linearly increased with increasing number of layers deposited onto the PET films. The corresponding haze value of 60$^{th}$ layer reached 10.78 ± 0.029%. This slightly increases make contribution to the chitosan/pectin-GE PET films for its packaging applications where the food product must be clearly seen through package by consumers.

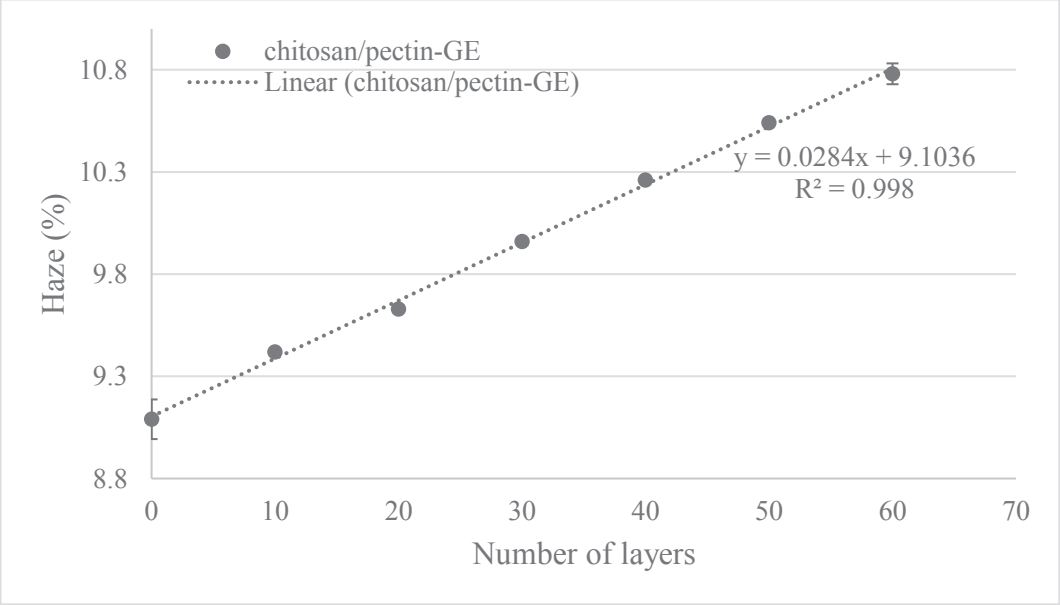


Figure 5.6. Haze measurements as a function of number of layers

## 5.3. In Vivo

### 5.3.1. Color

The color of the pear is an important parameter and browning of fresh-cut pears is one of the major disorders during storage, since color relates directly to the acceptance of quality by the consumers (Larrigaudière et al. 2004). Being the pear color important attribute for consumer acceptance, it was one of the main quality indicator parameters chosen in this work, to evaluate the potential of the multilayered films to maintain the quality parameters and to extend shelf life of the cut pears.

The effect of different number of layers of coated films on color of cut pears was analyzed in order to decide precise number of layer for the main in vivo assay. Table 5.4 present the average values of L*, a*, and b* of the cut surface of control and treated fresh-cut pear samples for 5 days of storage at 4 ºC as a preliminary shelf-life test and apparent color is documented in Figure 5.7. The total color difference (ΔE*) was measured to monitor changes in color (Figure 5.10).

Table 5.4. Color measurements of control pear and pears treated with 20, 40 and 60 LbL PET films during 5 days

| Sample | Color | Storage time (days) | | |
|---|---|---|---|---|
| | | 0 | 3 | 5 |
| Control | L* | 77.61 ± 1.13 | 58.93 ± 0.85 | 57.53 ± 0.41 |
| | a* | 2.03 ± 0.09 | 3.77 ± 0.11 | 3.82 ± 0.17 |
| | b* | 10.91 ± 0.59 | 15.12 ± 0.61 | 15.45 ± 0.34 |
| 20 LbL | L* | 78.00 ± 0.93 | 70.50 ± 0.75 | 68.1 ± 0.17 |
| | a* | 2.00 ± 0.07 | 3.10 ± 0.23 | 3.10 ± 0.26 |
| | b* | 10.60 ± 0.38 | 15.00 ± 0.70 | 15.50 ± 0.33 |
| 40 LbL | L* | 78.24 ± 0.42 | 71.50 ± 1.38 | 70.23 ± 1.19 |
| | a* | 1.54 ± 0.15 | 2.25 ± 0.11 | 2.31 ± 0.14 |
| | b* | 11.23 ± 0.15 | 13.50 ± 2.11 | 12.68 ± 0.80 |
| 60 LbL | L* | 78.48 ± 0.72 | 71.26 ± 0.66 | 70.78 ± 0.80 |
| | a* | 2.00 ± 0.17 | 2.71 ± 0.26 | 3.45 ± 0.30 |
| | b* | 10.82 ± 0.60 | 11.67 ± 0.43 | 12.33 ± 0.53 |

Values for L* (Figure 5.7) varied significantly ($p < 0.05$) with a trend towards lower values through time (Table 5.3) for treated and control pears. However, this trend was found severely for control samples. No particular reduction was found among samples contact with different coated layers. On day 3 and 5, there was no significant ($p > 0.05$) difference of lightness values within the samples with LbL coated films, however, control samples showed significantly difference ($p < 0.05$) in the L* values when compared to that of LbL treated fruits. During the 5 days of storage, there were not significant differences ($p > 0.05$) for samples treated with 40 LbL coated films, on the contrary, 20 LbL treated samples had a significant difference ($p < 0.05$) of L* values. When control and 60 LbL samples compared with their own initial L* values it can be seen that there is a significant decrease until the 3rd day, even though there was no significant difference between 3rd and 5th day in lightness. Overall, the control samples throughout the storage had significant ($p < 0.05$) lower values of lightness (darker samples).

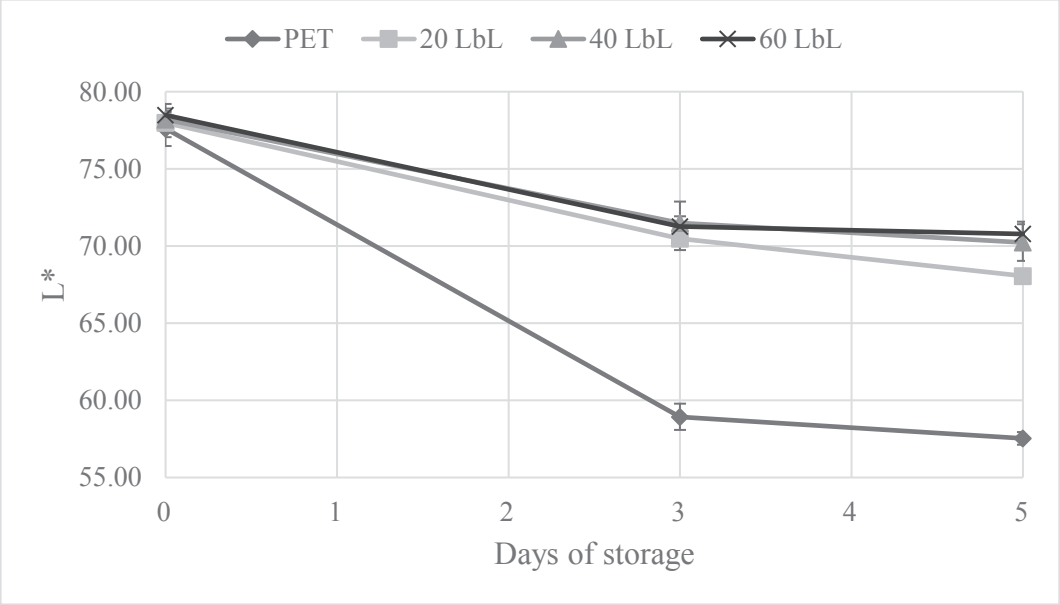


Figure 5.7. L* values of pear slices subjected to four treatments.

In accordance to the L* values which indicated that the control samples turned darker as the shelf life progressed, the a* value indicated an increase in red color intensity after day 0 (Figure 5.8). The a* values increased for the days of analyses, with significant differences ($p < 0.05$) in both control and treated samples. Among the fruits with three

different LbL films showed no significant difference ($p > 0.05$) of the red color intensity change, while the significant difference ($p < 0.05$) observed when the control samples compared to that of all LbL treated samples on day 3. There was no significant difference ($p < 0.05$) of the a* values among the samples of control and 20 and 40 LbL treated from day 3 to day 5. In terms of trends, control samples had higher values of redness from 2.05 ± 0.38 to 4.15 ± 0.89 when compared with the LbL samples throughout storage, while the 40 LbL samples present an increase from 1.25 ± 0.36 to 2.56 ± 0.37. Overall, application of the LbL had a positive effect on the degree of redness of fresh-cut pears.

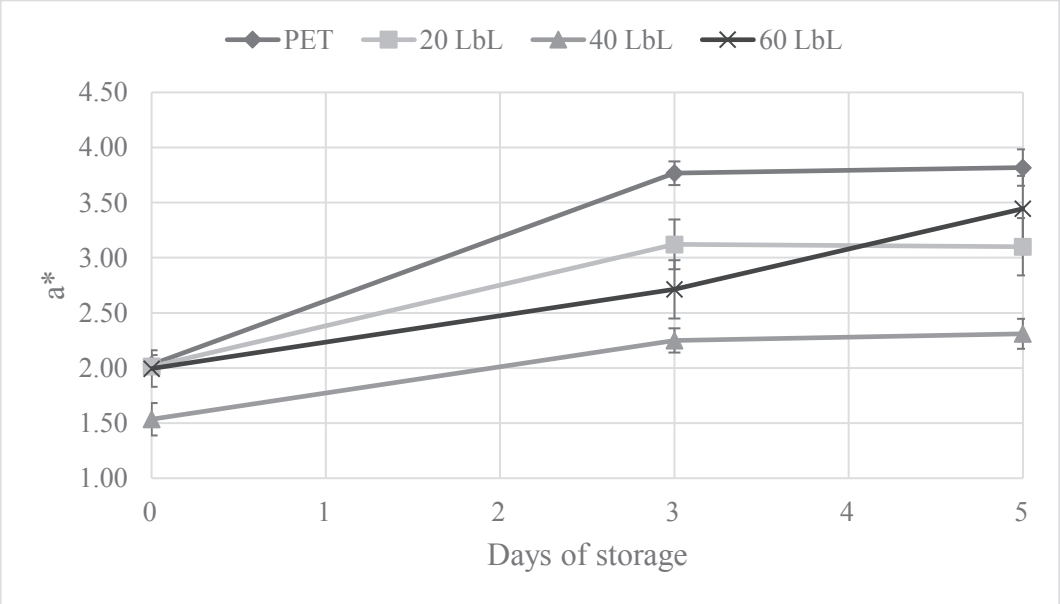


Figure 5.8. a* values of pear slices subjected to four treatments

Values of b* (Figure 5.9) present a significantly differences ($p > 0.05$) between four groups during the storage time, tending to increase toward the end of the storage period in yellowness from 12.77 ± 3.27 to 14.52 ± 2.12 and from 10.19 ± 0.86 to 15.94 ± 0.81 for the control and 20 LbL pears, respectively, however there was a fluctuation in the values of b* during storage for 40 LbL samples. The increase in the values of a* of 40 LbL samples, as well as reduction in the values of b* may indicate an oxidative browning (Freitas et al. 2013). On day 5, statistically there was no significant difference ($p < 0.05$) between 40 LbL and 60 LbL pears for b* values. What seemingly different from day 0 to day 3 in Figure 5.9 was found to be relatively constant from day 3 to day 5 when the data were statistically analyzed. In other words, between each LbL treated fruit,

there was no effect of different LbL films treatments on the change of the yellow color from day 3 to day 5.

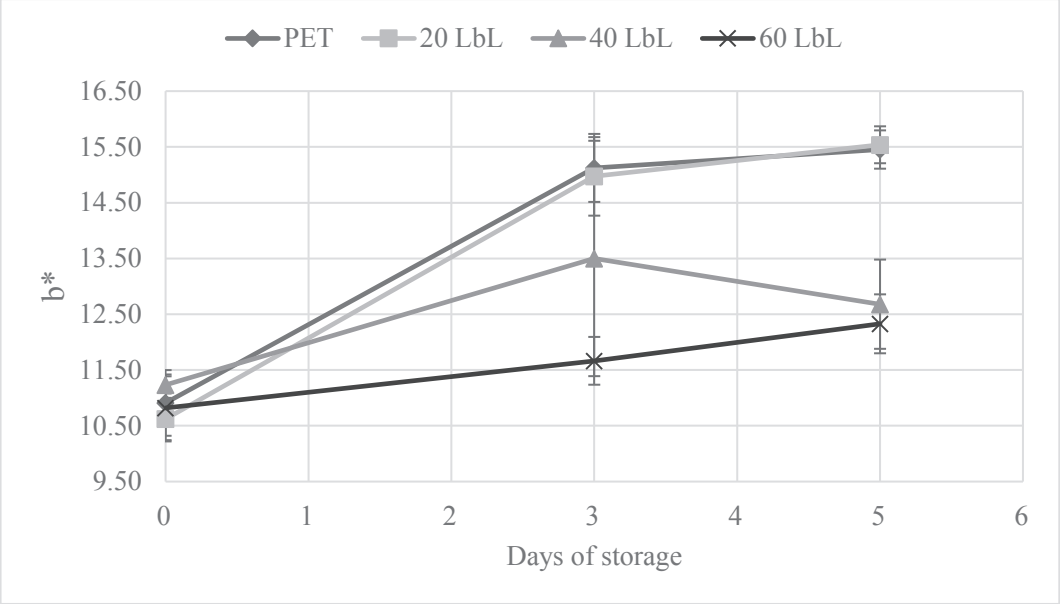


Figure 5.9. b* values of pear slices subjected to four treatments

The ΔE* (Figure 5.10) between four groups, there was an significantly ($p < 0.05$) increase of control samples during the storage time, and because the change of L* values, the ΔE of control samples of 18.64 ± 3.81 was almost twofold of that in 20-40-60 LbL samples, 10.60 ± 1.62, 8.91 ± 2.23 and 8.54 ± 1.32, respectively. As can be seen in Figure 5.10, the ΔE* values of LbL samples are quite low than control samples, concluding that the colors are very similar for the different treatments over time. The different number of layer coated films do not significantly ($p < 0.05$) change the initial color of pears. These results indicate that all tested number of layers films helped to prevent drastic color changes on the fruits in color because the barrier may delay oxygen interchanges (Rojas-Graü et al., 2009), so that the LbL contact samples showed more whiteness at the end of storage compared to the control. Overall, the coating with 60 LbL samples showed better results in retention of fruit lightness.

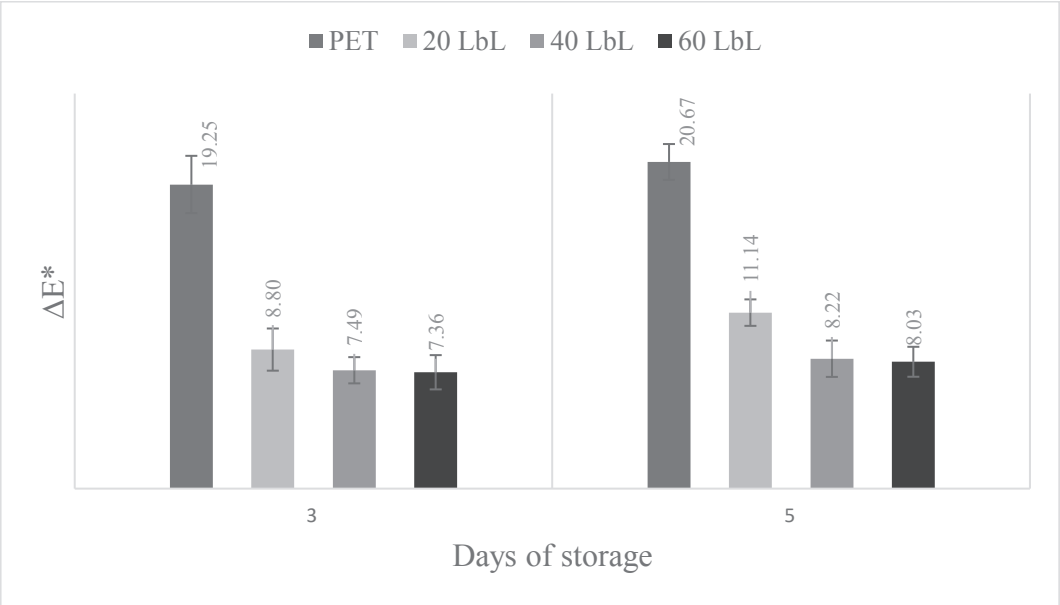


Figure 5.10. ΔE* values of pear slices subjected to four treatments

It can be said that the pears treated with LbL films showed lower color changes during storage compared to the control. This can be attributed to the presence of GE in the coating that it is known by its antioxidant activity described previously. In addition, the coating has an ability to act as a barrier to oxygen necessary for browning reactions. As parallel, as stated by many works, chitosan may delay color changes in fruits similar and it can be explained by reduction of the respiration rate of fruits. Several researches have reported the effectiveness of application of coating formulations incorporating with antioxidant agents to control the browning of fresh-cut fruits. In this conducted preliminary work, a pectin-based coating including 3.5 g/L GE has shown to be effective in controlling the browning of fresh-cut pears. Being in agreement with a study realized by Oms-Oliu et al. (2008), where the browning of fresh-cut pears treated pectin-based coatings with N-acetylcysteine and glutathione added as antioxidants were significantly less than that untreated pears.

During storage, the 60 LbL had a decrease of the whiteness of 9.8%, while the control had a reduction of 23.4%, and moreover it had the least total color difference among the others. Since the fruits treated with 60 LbL resulted in a better alternative, it was decided to use 60 LbL films for the main shelf life test, which includes the evaluation of the release of the antioxidant agent, so that the color of fresh cut pears can be maintained for a long period of time. Because many studies reported the effects and releases of antimicrobial and antioxidant agents from multilayer films vary depending on

the number of layers and several layers of films provide a prolonged release of the active component (Mlalila et al. 2018). This result can be attributed to the more accumulation of the active agents in the packaging materials when the number of layers on the surface of substrates increase.

As shown in Table 5.5, in the main in vivo application have involved 60 LbL active coated films on cut pear samples for 7 days of storage at 4 ºC, L* values of all samples decreased from 0 to 7 days. Although the trend towards lower values ($p < 0.05$) was again found regarding retention of fruit lightness, the samples processed coated with the multilayer films remained lighter. L* values of control samples decreased ($p < 0.05$) during the storage and showed higher values on day 7, whereas no significant differences ($p > 0.05$) were observed among fruit treated with 60 LbL from day 3 to day 7. This occurrence may be explained by the fact that the GE being an antioxidant compound, helps to maintain stability and conservation of compounds present in pear cuts, and consequently, retaining their color.

Table 5.5. Color measurements of control pear and pears treated with 60 LbL PET films during 7 days

| **Sample** | **Color** | **Storage time (days)** | | | |
|---|---|---|---|---|---|
| | | 0 | 3 | 5 | 7 |
| Control | L* | 77.28 ± 0.15 | 65.80 ± 0.43 | 65.07 ± 0.57 | 62.74 ± 0.78 |
| | a* | 2.15 ± 0.03 | 3.08 ± 0.24 | 3.95 ± 0.16 | 4.29 ± 0.14 |
| | b* | 10.53 ± 0.09 | 13.13 ± 0.64 | 14.62 ± 0.26 | 16.48 ± 0.49 |
| 60 LbL | L* | 76.62 ± 0.08 | 68.34 ± 0.46 | 68.74 ± 0.74 | 69.11 ± 0.74 |
| | a* | 2.47 ± 0.08 | 3.60 ± 0.15 | 3.87 ± 0.13 | 3.27 ± 0.17 |
| | b* | 10.48 ± 0.21 | 16.18 ± 0.74 | 13.53 ± 0.45 | 13.18 ± 0.69 |

The ΔE* (Figure 5.11) between the control and treated samples, there was an increase during the storage time and by the day 7, the ΔE* was found 15.86 ± 0.63 for control samples and 8.04 ± 0.84 for treated samples that was almost twofold like previous color analysis. The visual observation and ΔE* values showed that in the control fruits browning took place on the first day. There were significant differences in ΔE* values

during the 7 days storage for fruits control and treated with 60 LbL. Indeed, the ΔE* remained stable during storage time for active packaged fruits, similar to the study by Trevino-Garza et al. (2017). The color measurements of the main in vivo assay showed that chitosan/pectin-GE multilayered films can be successfully utilized and repeated for packaging applications because of the presenting similar results with the preliminary assay.

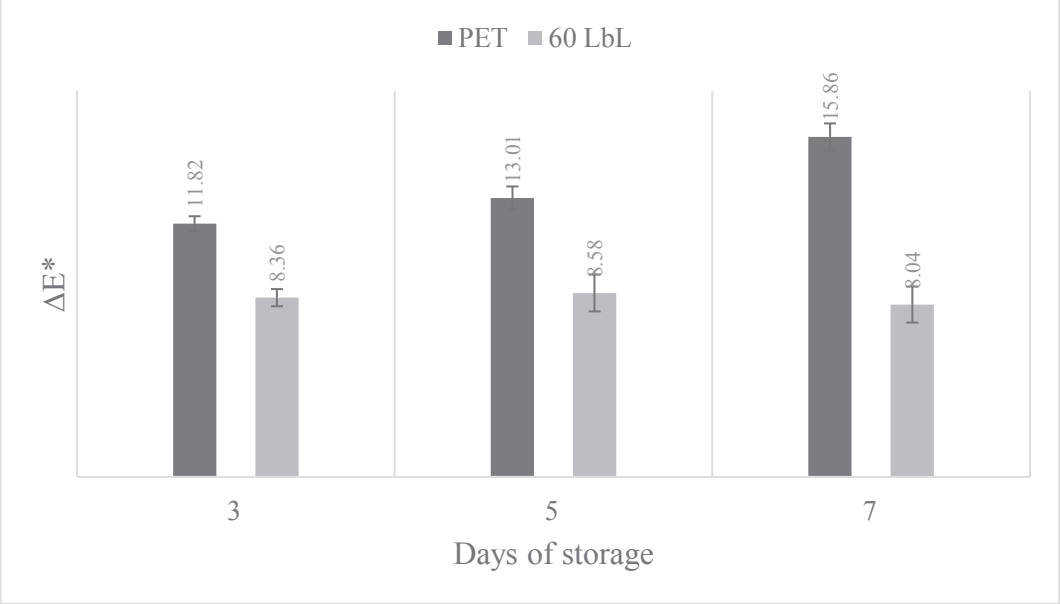


Figure 5.11. ΔE* values of pear slices subjected to 60 LbL coated films and control

Overall, these results indicate that all tested number of layers films helped to prevent drastic color changes on the fruits because the barrier may delay oxygen interchanges (Rojas-Graü et al., 2009), so that the LbL contact samples showed more whiteness at the end of storage compared to the control. However, while GE increased with increasing number of layers, it did slow changing of original color of pears by preventing enzymatic or oxidative browning.

As a result, LbL active coating on substrate is suggested to remain the color of fresh-cut fruits to a certain degree and to sustain the visual quality of the fruits.

## 5.3.2. Microbial quality

Microbiological analysis results are presented in Figures 5.12 and 5.15 for mesophilics and yeast and moulds counts, respectively. Counts of mesophilic, yeast and molds in non-inoculated pear pieces trays were evaluated at 0, 3, and 5 days of storage at 4 ºC for preliminary in vivo assay in order to reveal the effect of the different number of layers and to test for antimicrobial activity of coated films, and after deciding precise number of layers they were evaluated on the days 0, 3, 5 and 7 at same storage conditions for the main in vivo assay. A pair of randomly chosen pears of each treatment was conducted in duplicated at each time.

In the preliminary microbial analysis, mesophilic microorganism results are shown in Figure 5.12. The application of LbL significantly ($p < 0.05$) maintained or reduced the growth the initial mesophilic counts that were approximately 2.7 log cfu/g on just processed fresh-cut pears. 60 LbL coated films demonstrated to be highly effective in the reduction of the microbial population until 3rd day. A faster mesophilic microorganisms growth was obtained in control samples in comparison with that all LbL coated films. 60 LbL films were significantly ($p < 0.05$) reduced the growth of mesophilic microorganisms by approximately 0.71 log cfu/g compared to the control films (~ 4 log cfu/g) by the end of storage. Previous studies showed that the microbial count on pear slices coated with xanthan gum alone was same with uncoated samples, whereas the incorporation of cinnamic acid into the xanthan gum coating formulation has been effective in reducing the growth of total mesophilic bacteria approximately by 0.25 log cfu/g during 8 days of storage time (Sharma et al. 2015). Same results were revealed by Oms-Oliu et al. (2008) who reported that different polysaccharide (alginate, gellan, pectin) based coatings were not different from uncoated samples, however the addition of N-acetylcysteine and glutathione into coating materials were effective to control microbial counts in fresh-cut pears.

Yeast and molds growth on fresh-cut pears treated and untreated with LbL films is shown in Figure 5.13. The observed trend in the evolution of yeast and molds was unlike to that above-described for mesophilic, however LbL films again showed significant ($p < 0.05$) effect on microorganism with respect to control films. Initial population of yeast and molds of fresh-cut pears were approximately 2.24 log cfu/g. In the entire period of storage yeast and molds counts on control samples showed

significantly ($p < 0.05$) highest counts by reaching 4.70 log cfu/g on day 7. The samples with 60 LbL treated samples had significantly ($p < 0.05$) lowest counts (4.14 log cfu/g), followed by those with the 40 LbL treated samples by 4.35 log cfu/g and then 20 LbL treated samples by 4.42 log cfu/g.

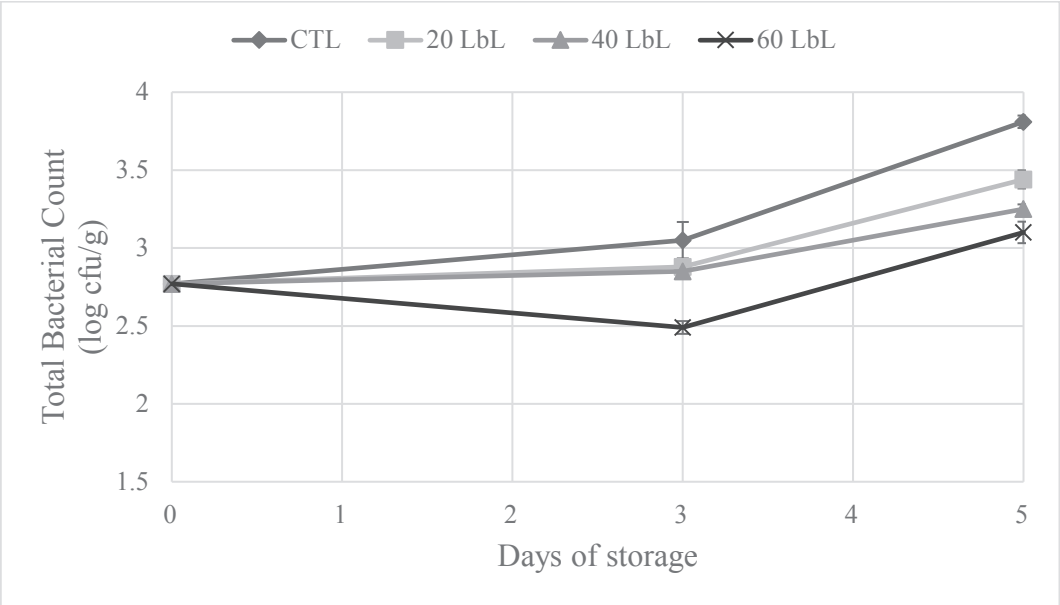


Figure 5.12. Changes in the total mesophilic bacteria of pears as affected by 20, 40 and 60 LbL treatments during 5 days

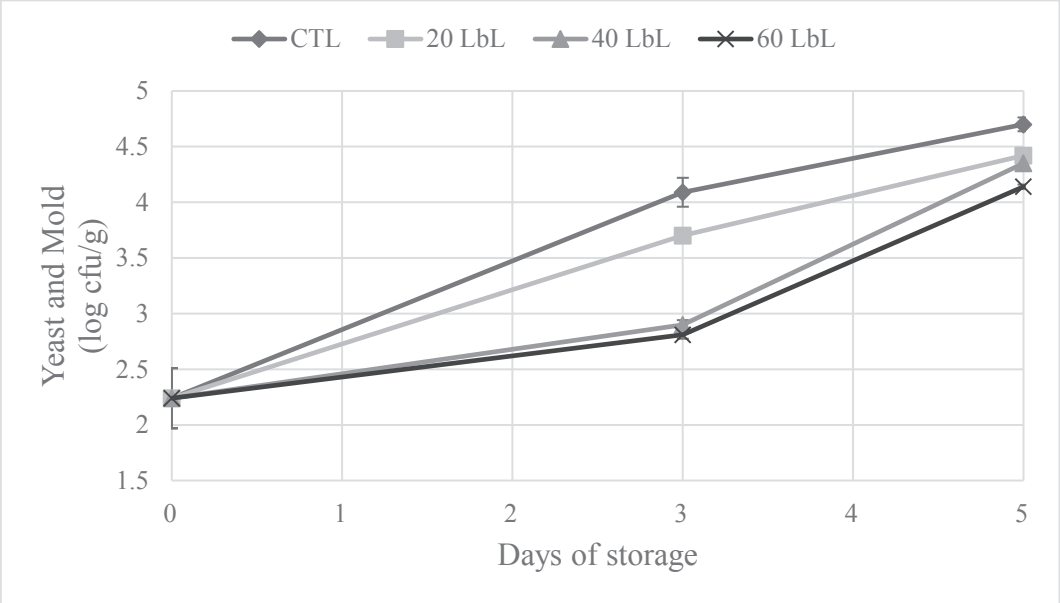


Figure 5.13. Changes in the yeast and molds of pears as affected by 20, 40 and 60 LbL treatments during 5 days

All LbL coated films were significantly ($p < .05$) effective to inhibit the growth throughout the storage time. However, 60 LbL films were more effective in reducing growth of mesophilic microorganisms and yeast and molds in fresh-cut pears when it compared to 20 LbL, 40 LbL and uncoated films because there were significant differences ($p < 0.05$) between the counts of those microorganism. In addition, 60 LbL films was not only effective to inhibit the growth of mesophilic microorganisms through the time, but also effective in reducing their levels. Because of these reasons, 60 LbL films were chosen to be used in the main shelf-life assay in order to provide extended storage period to cut pears and to evaluate release of active agents.

In the main shelf-life experiment (Figure 5.14), there was a significant difference ($p < 0.05$) observed once more in the population of mesophilic microorganisms between control and pears treated with 60 LbL films. Although no significant differences ($p > 0.05$) were found on day 3, the population of total mesophilic microbes of control fruits increased steadily after day 3, which was relatively low for 60 LbL, compared to populations on pears receiving the control treatments. In addition, 60 LbL demonstrated nearly straight line from day 5 to day 7, in which indicated LbL treatment did make significant difference on the growing population of mesophilic microorganisms of the fruits. The total mesophilic counts of the untreated prear samples ranged from 2.27 to 5.57 log cfu/g throughout the entire 7 days of storage, whereas the population of these microbes on pears treated with 60 LbL had a relatively low microbial load (3.59 log cfu/g) compared to control treatments, as expected from preliminary experiments.

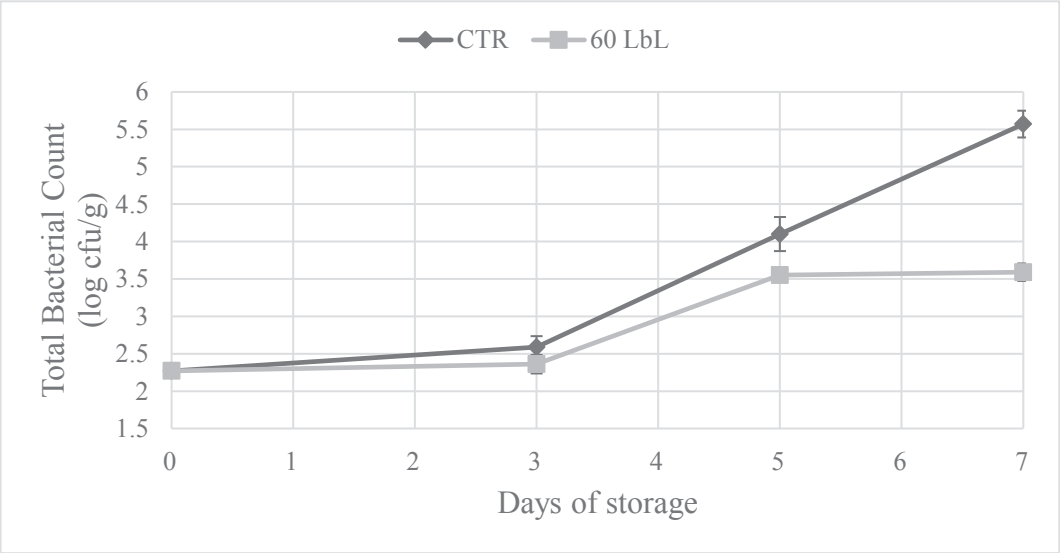


Figure 5.14. Changes in the yeast and molds of pears as affected by 60 LbL treatments during 7 days

The yeast and mold count also showed LbL films antimicrobial effect on the samples once again (Figure 5.15). The control and treated samples had an increasing count on yeast and mold from day to day up to the highest 5.3 log cfu/g and 4.41 log cfu/g on day 7, respectively. However, 60 LbL showed a limited increase in the count on treated samples that were maintained significantly ($p < 0.05$) lower by about 0.89 log cfu/g from that compared to the control samples.

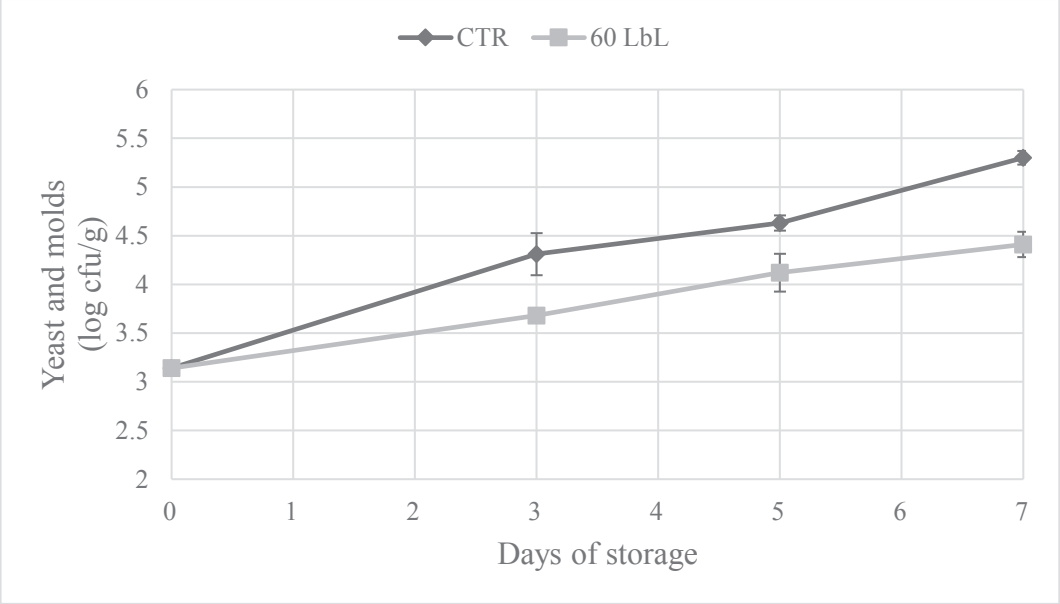


Figure 5.15. Changes in the yeast and molds of pears as affected by 60 LbL treatments during 7 days

According to this study, the use of LbL active coating seems to be a suitable choice in terms of microbial growth while maintaining sensory attributes of fresh-cut fruit. Overall, for both experiments, preliminary and main in vivo assays, a similar trend in growth of microorganisms on pears occurred and the different number of layer of active coated films in the treatment influenced the effectiveness in controlling mesophilic and yeast and mold growth significantly.

### 5.3.3. Sensory evaluation

The sensory analysis of both control and treated with 60 LbL cut pear samples was carried out during 7 days of storage. The parameters such as appearence, color, odor, firmness and overall acceptability were considered to study the effect of LbL on the

sensory attribute of the pear fruits. The results of the sensory test of fresh-cut pears untreated and treated are listed in Table 5.6. Initial (day 0) sensory properties of those fresh-cut fruit presented scores by around 3.1 – 3.7 (Table 5.6). After 7 days of storage in terms of appearance scores, 60 LbL treatments received higher scrores (>3 in a scale of 1-dislike very much to 5-like very much), while control was not appropriate for consumer acceptance (Table 5.6). The results indicate that the application of LbL with GE has no negative effect on pear color and odor during 7 days. Throughout storage, in terms of color scores of all treated and untreated samples decreased, especially in control fruit ($p < 0.05$), and by day 7, treated samples presented the higher scores ($p < 0.05$) when compared to control. These results demonstrate the helpful effect of LbL in maintaining color of fruits due to acting as a barrier. Firmness scores of control samples of pear drastically declined during the storage and presented the lower scores ($p < 0.05$), while treated samples did not change significantly and maintained almost constant in terms of firmness.

Finally, overall acceptance scores, the LbL treated fresh-cut pear was indicated significant preference by consumers during 7 days ($p < 0.05$) compared to the control samples.

## 5.4. Monitoring of GE total phenolic index and antioxidant activity of chitosan/pectin-GE LbL Films

The oxidation is a limitative key factor that causes a particularly undesirable number of changes in the sensory properties of the product, which is responsible for the shelf-life, quality degradation and economic losses of food products (Gramza and Korczak 2005). Incorporation of antioxidants into food packaging materials may contribute to the preservation of the quality of food products (Portes et al. 2009), the protection of antioxidant agents from the external environment and control of their release for effective activity.

In this study, in order to prove the release of antioxidant substances from the release test solution of retained GE in 60 LbL films after the main in vivo assay was determined during the storage time by total phenolic index and DPPH experiments. The films obtained from food contact at day 0, 3, 5 and 7 were used for release tests. It should be noted that the control film was not analyzed because it did not coat with any agent.

Table 5.6. Sensory evaluation of fresh-cut pears treated with 60 LbL films during 7 days

| | **Quality Parameters** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Appearance** | | **Color** | | **Odor** | | **Firmness** | | **Overall Acceptability** | |
| Days | CTR | 60 LbL | CTR | 60 LbL | CTR | 60 LbL | CTR | 60 LbL | CTR | 60 LbL |
| 0 | 3.5 | 3.1 | 3.3 | 3.3 | 3.7 | 3.3 | 3.4 | 3.6 | 3.7 | 3.2 |
| 3 | 1.9 | 3.0 | 1.9 | 3.0 | 2.6 | 3.7 | 2.3 | 3.2 | 2.2 | 3.1 |
| 5 | 2.4 | 3.1 | 2.4 | 2.9 | 3.1 | 3.2 | 2.5 | 3.5 | 2.7 | 3.3 |
| 7 | 2.2 | 2.5 | 2.1 | 2.8 | 2.3 | 3.3 | 2.0 | 3.3 | 2.2 | 3.0 |

*Values represent the mean of 12 replicates

Although these methods might not provide absolute values for GE concentrations, it is sufficient to indicate the relative concentrations of GE among the samples for comparison purpose day to day.

The total phenolic index of the film was expressed as µg gallic acid/cm$^2$ by considering the standard gallic acid exultance under the same conditions. As seen in Figure 5.16, the depletion of the initial total phenolic index of 60 LbL films (616.17 ± 27.9 µg gallic acid/cm$^2$) was significantly occurred from day 0 to day 7 and final total phenolic index was observed of 138.7 ± 29.5 µg gallic acid/cm$^2$.

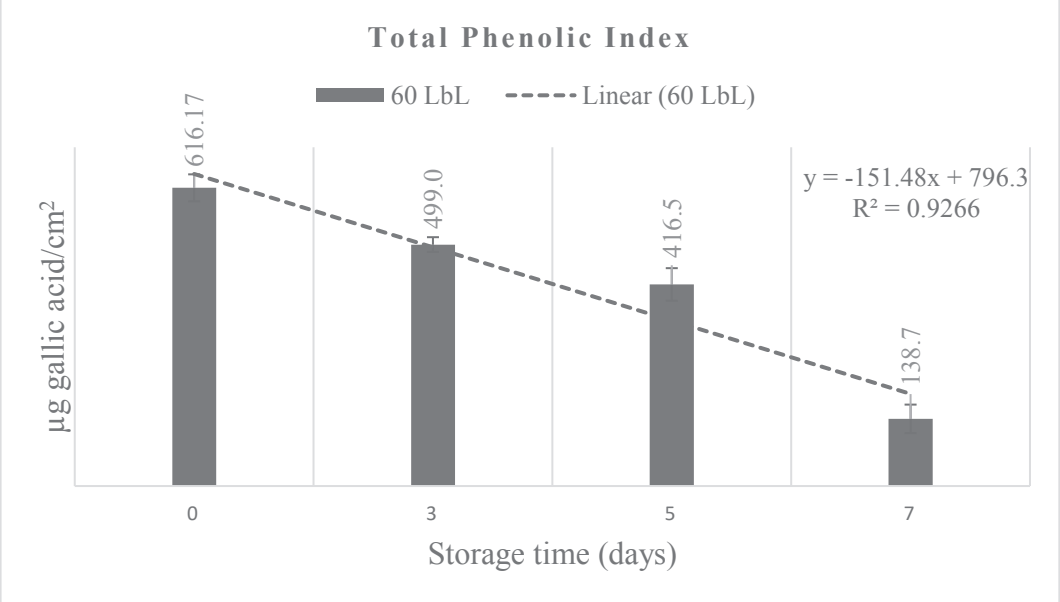


Figure 5.16. Releasing GE from 60 LbL films via total phenolic index assay

Experimental data of the consumption of DPPH from LbL films versus time plotted in Figure 5.17. The antioxidant activity released from the films at the end of release test was calculated as µM Trolox/cm2 by considering the standard Trolox tested under the same conditions. Similar trend was also observed in the release test solution that was statistically exhibited reduction of antioxidant activities (Figure 5.16) day by day ($p < 0.05$). The scavenging activity on DPPH radical of GE in 60 LbL films was initially 11.8 ± 1.04 µM Trolox/cm$^2$ and decreased at 2.34 ± 0.18 µM Trolox/ cm$^2$ by entire storage time.

In this work, as expected, the total phenolic index and antioxidant activity were observed for chitosan/pectin-GE films. The release of antioxidant polyphenols was found

to be gradually reduce during the storage time, so the results simply indicate that a slightly lower amount of GE release occurred from LbL surface of the film. In addition, almost linearity of released active agent from LbL surfaces were also observed for both experiments of release test. This may be due to direct contact of film with food as in real solid medium.

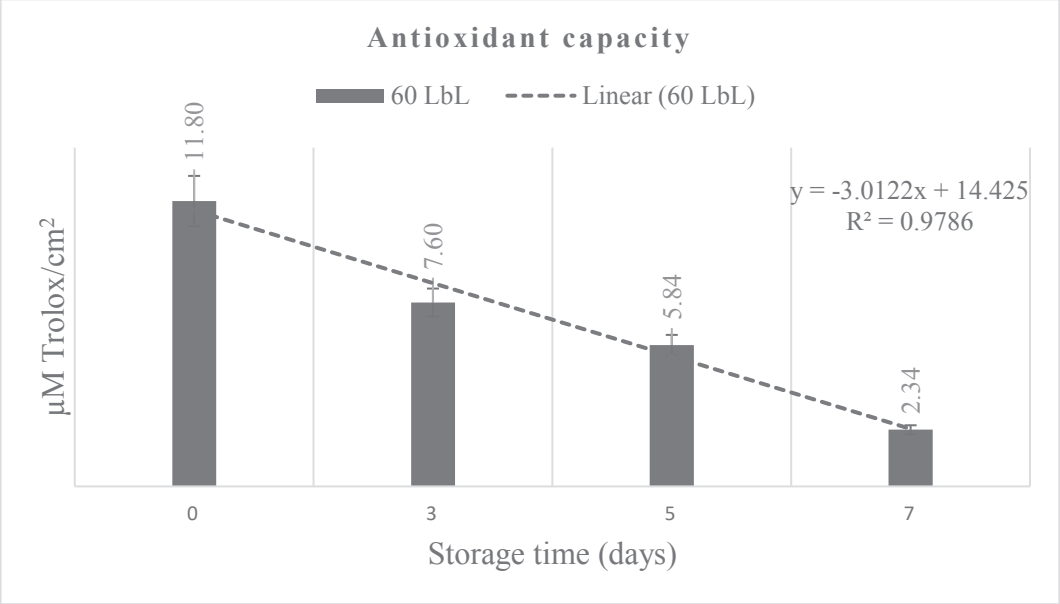


Figure 5.17. Releasing GE results from 60 LbL films via DPPH assay

The total phenolic index and antioxidant activity of LbL films were attributed to the pectin-GE based films. Because in the study, Souza et al. 2015 asses the antioxidant activity of chitosan-based film at 1% (w/v) and found that the films did not display antioxidant activity. A similar result was observed by Portes et al. (2009) using 2% (w/v) chitosan films.

# CHAPTER 6

# CONCLUSION

In this study, the chitosan/pectin-GE multilayer-coated PET films were prepared using LbL assembly technique. The development and application of LbL coated PET films were investigated their surface and optical properties as affected by deposited number of layer and its effectiveness on the quality and shelf-life of fresh-cut pears as a selected food system were evaluated. For investigation of changes of film structure, three different number of layers (20, 40, and 60 layers) deposition on PET were tested. The concentrations of chitosan, pectin and GE powder were kept constant for all treatments (0.2% (w/v), 0.1% (w/v) and 0.35% (w/v), respectively). Fresh-cut pears color and sensory attributes were evaluated in a shelf-life study at 4 ºC during 5 and 7 days. Microbiological analyses were conducted to determine the antimicrobial functions of LbL coated films in microbial growth of fresh-cut pears. In addition, the gradual release of GE powder in the pectin layers during shelf life study was carried out from food contact LbL coated films.

The enhancement of successful bio-based polymer construction on PET film surface was confirmed by the contact angle and UV–vis absorbance measurements. The contact angle measurements of coatings prepared by sequential accumulation of biopolymers behaved markedly in surface interactions with water that it helped to have knowledge about the surface modification after processing.

The color of pears throughout storage was highly improved by the 20 - 40 and 60 layers coated films, although different number of layers had no particular trend on color. Differences in total color were significant after day 3 of evaluation, when uncoated samples started to lose their initial color while the coated fruits kept the same color for longer, most likely due to the release of the active agent (GE) in the pectin layers. Microbiology analyses demonstrated that the effectiveness of the LbL films against microbial growth and increased the effectiveness of films with higher number of layers on substrate as well. Moreover, sensory tests showed overall acceptance of the LbL films treated pears when compared to the controls throughout storage. The release test of

remaining GE on LbL films after food contact evidenced that release from the films during storage was significantly showed decrease trend comparing to the initial value.

In summary, the obtained results suggested that the deposition of biopolymers on PET films can successfully improve the performance of flexible food packaging films. Furthermore, taking into account the general characteristics of the developed multilayered films, those prepared with depositing layers of chitosan and pectin, can be considered promising for food packaging applications to improve the shelf-life of fresh-cut commodities due to the carrier properties for active agents and antimicrobial activity. The developed LbL films provide an insight for the use of LbL assembly and bio-based polymers for food, medical, and industrial fields.

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