DEVELOPMENT AND CHARACTERIZATION OF ANTIMICROBIAL GELATIN AND PECTIN FILMS WITH BORON DERIVATIVES

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DEVELOPMENT AND CHARACTERIZATION OF ANTIMICROBIAL GELATIN AND PECTIN FILMS WITH BORON DERIVATIVES

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ABSTRACT

DEVELOPMENT AND CHARACTERIZATION OF ANTIMICROBIAL GELATIN AND PECTIN FILMS INCORPORATED WITH BORON DERIVATIVES

The main purpose of food packaging is to protect food products from external factors such as oxygen, light, chemical and microbiological contamination. Food packaging technology is continuously developing in order to response changing consumer demands, improve the quality of the preserved food products and mostly to catch today's trends for affecting the consumer perception. Besides the quality and safety issues, environmental concerns have effects on the evolution of food packaging as well. Active packaging and coating is an important innovative packaging technology for increasing the quality and safety of the foodstuffs and also extending the shelf life. Biopolymer based antimicrobial packaging is an important type of active packaging which controls the microbial spoilage of the food by using renewable resources.

Aim of this study was to develop and characterize antimicrobial gelatin and pectin films incorporated with three different boron derivatives (boric acid, disodium octaborate and sodium pentaborate). Gelatin and pectin films were developed with different formulations; effect of type and concentration of each boron derivative on antimicrobial and antifungal activities, and physical, mechanical, chemical properties of the formed films and rheological properties of the film forming solutions were examined with experimental studies. Findings of the study suggest that incorporation of boron derivatives improved antimicrobial and antifungal properties of the films against four of five tested microorganisms and also enhanced the tensile strength of the gelatin and pectin films. The films may be used to develop food packaging or coating material for some certain perishable food products.

ÖZET

BOR TÜREVLERİ İLE BİRLEŞTİRİLMİŞ ANTİMİKROBİYAL JELATİN VE PEKTİN FİLMLERİN GELİŞTİRİLMESİ VE KARAKTERİZASYONU

Gıdaların ambalajlanmasının temel amacı gıda ürünlerini fiziksel, kimyasal ve mikrobiyolojik kontaminasyondan ve diğer dış faktörlerden korumaktır. Gıda ambalajlama teknolojisi, değişen tüketici taleplerini karşılamak, muhafaza edilen gıdaların kalitesini artırmak ve özellikle de tüketici algısını etkilemek için günümüz trendlerini yakalamak amacıyla sürekli gelişim göstermektedir. Kalite ve gıda güvenliğinin yanı sıra, çevresel sorunlar da gıda ambalaj teknolojilerinin gelişiminde etkili olmuştur. Aktif ambalajlama ve kaplama; gıda güvenliği ve kalitesini geliştiren ve gıdanın raf ömrünü uzatan önemli yenilikçi ambalajlama teknolojilerinden biridir. Biyopolimer bazlı antimikrobiyal ambalajlar, gıdaların mikrobiyal bozulmasını kontrol ederken aynı zamanda yenilenebilir hammadde kullanılan önemli bir aktif ambalajlama türüdür.

Bu çalışmanın temel amacı üç farklı bor türevi (borik asit, disodyum oktaborat tetrahidrat ve sodyum pentaborat) kullanılarak antimikrobiyal özellikli jelatin ve pektin filmler geliştirmek ve karakterize etmektir. Jelatin ve pektin filmler farklı formülasyonlarla geliştirilmiş; ve her bor türevi çeşidi ve konsantrasyonunun filmlerin antimikrobiyal ve antifungal aktiviteleri, fiziksel, mekanik ve kimyasal özellikleri üzerine ve film oluşturan solüsyonların reolojik özellikleri üzerine olan etkisi deneysel çalışmalarla incelenmiştir. Çalışma bulguları, bor türevleri eklenmesinin filmlerin antimikrobiyal ve antifungal özelliklerini geliştirdiğini ve aynı zamanda filmlerin çekme direncini artırdığını göstermiştir. Oluşturulan bu filmler bazı kolay bozulabilen gıdalar için ambalajlama veya kaplama materyali geliştirilmesinde kullanılabilir.

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ASTM	American Society for Testing and Materials
FFS	Film forming solution
G'	Storage modulus
G"	Loss modulus
GRAS	Generally recognized as safe
HM	High methoxyl
HSD	Honest significant difference
ISO	International Organization for Standardization
LM	Low methoxyl
MSW	Municipal solid waste
TS	Tensile strength

1. INTRODUCTION

The main purpose of food packaging is to protect food products from external factors such as oxygen, light, moisture, chemical and microbiological contamination and physical damages until they have reached to the final consumers. The food packaging acts as a barrier between the food product and the outer atmosphere. It is also essential for enhancing food quality and safety, extending the shelf life, minimizing food wastage and reducing the usage of preservative food additives in the food products [1].

The most important chemical and biochemical reactions that cause quality loss or food safety problems in food products are enzymatic and non-enzymatic browning reactions, lipid hydrolysis, lipid oxidation, protein denaturation, hydrolysis of oligo and polysaccharides, and degradation of some pigments. In addition to chemical reactions, microbiological deterioration caused by certain bacteria, mold and yeast is another important factor that affects food quality and safety [1], [2].

Food deterioration can arises during production, transportation and storage of the products. Shelf life of a food product depends on every stage in the production process and packaging is one of these stages. In order to determine the shelf life of a food product; first microbiological safety and then chemical and sensorial quality factors should be evaluated. Packaged products are affected from the exogenous factors considerably. By controlling some of these exogenous factors, packaging can be effective on extending the shelf life. Active packaging, smart packaging and modified atmosphere packaging are the most important innovative packaging systems. For this purpose, active packaging systems are commonly used which involve interaction between package and food product or internal atmosphere in the package. These systems prolong shelf life and preserve nutritional value of the food by inhibiting growth of pathogenic microorganisms and preventing migration of potential contaminants. Another important reason for developing these innovative food packaging technologies is to delay food deterioration to provide sufficient food supply for the increasing population globally and minimizing food wastage [3], [4].

Environmental solid waste problems due to use of petrochemical based packaging materials are another issue that promotes the development of the renewable biopolymer based new packaging systems. To decrease these environmental problems caused by packaging waste,

degradation of the packaging material is desired in a reasonable time after usage of the inside product. Here, biodegradable polymers are the promising sources because of their environmental-friendly nature, biodegradable and renewable properties [4].

In this study, active antimicrobial films were developed and characterized with incorporation of different boron derivatives (boric acid, disodium octaborate and sodium pentaborate) into gelatin and pectin films. Boron and pectic polysaccharides are essential elements in plant structure to maintain integrity of the plant cell wall through cross-linking with each other. This affinity between boron and pectin, known antimicrobial properties of boron derivatives, and on the other hand, presence of rich boron reserves in our country (72 percent of the world boron reserves) are the primary reasons behind use of boron derivatives in this study.

Aim of this thesis project was to develop antimicrobial pectin and gelatin films incorporated with boron derivatives as packaging materials. To reach this goal; rheological properties of the film forming solutions were studied and the physical, chemical and microbiological properties of the obtained films were characterized.

1.1. FOOD CONTAMINATION AND SPOILAGE

Food safety is the most important factor in food consumption. Food spoilage is a metabolic process that cause undesirable changes in sensory characteristics of food and make food product unacceptable for human consumption. Food spoilage is classified in four groups; biological (enzymatic) spoilage, microbiological spoilage, chemical spoilage and physical spoilage. [1].

1.1.1. Enzymatic Spoilage

Some enzymes found naturally in raw materials of food products cause some desired or undesired changes in structure, taste, odor, aroma and nutrition value of the food. The undesired changes that are caused by natural enzymes in food is defined as enzymatic spoilage. Enzymatic browning of fruits and vegetables, excess ripening and softening of some fruits, rancidity of lipids by lipase enzyme are the examples of enzymatic spoilage.

Polyphenol oxidase, lipoxygenase, chloropylase and ascorbic acid oxidase are the main enzymes that responsible for enzymatic browning in fruit and vegetables [1],[3].

1.1.2. Microbiological Spoilage

Microbiological spoilage is caused by bacteria, mold and yeasts. Deterioration may occur on texture, odor and aroma of food product as a result of unfavorable enzymatic changes that are caused by microorganisms. Also, they can generate health and quality risks with their toxins or other metabolites. Rancidity of milk by lactic acid bacteria, fruit and vegetable decays, mold growth on bread and deterioration of meat are the examples of microbiological spoilage. Type and number of microorganisms, characteristics of food, processing techniques and ambient conditions affect the degree of microbial deterioration. Oxygen and temperature factors also affect the type and rate of microbial growth depending on the microorganisms [1], [2], [3].

1.1.3. Chemical Spoilage

Generally non-enzymatic browning reactions and lipid oxidation are the main reasons of chemical spoilage. Non-enzymatic browning reaction, Maillard reaction, is generated between carbonyl groups of reducing sugars (glucose, fructose, lactose, etc.) and free amino groups of proteins. As a result of Maillard reactions, brown melanoidin pigments and some non-volatile compound are produced as final products. These reactions are desired for bread crust formation but they are undesired for some other products because of undesired taste and odor formation. There is a directly proportional relationship between water activity of food and rate of Maillard reaction. Lipid oxidation is another important cause of chemical spoilage in foods. Oxygen and light in the ambient conditions are the main factors that triggered lipid oxidation [4], [5].

1.1.4. Physical Spoilage

Physical spoilage is the undesired change on the physical structure of food product. Crystallization, phase separation, melting, drying, moisture loss or gain are the examples of this type of spoilage [2].

Therefore, to produce healthy and safety foods, to minimize the quality losses and to extend the shelf life of food; some preventive processing methods are used like heating, cooling, freezing, freeze drying, drying, salt or sugar addition, fermentation, smoking, oxygen removal and irradiation. But chose of the convenient packaging material and technique is essential for the success of these preventive processes. For the protection of sterile products against all types of contaminations, packaging has very important functions [2], [5].

1.1.5. Factors Affecting Food Quality and Shelf Life

There are many intrinsic and extrinsic factors that may cause deterioration of food products such as water activity, pH, availability of oxygen, redox potential, biochemistry of food, natural microflora of the food, storage temperature, product formulation, relative humidity, exposure to light, composition of gases within the packaging and packaging interactions (migration).

Food packaging have important effect on the extrinsic factors that affect quality of food. Thus, many packaging materials and systems have been developed to reduce impact of these extrinsic factors and extend quality and shelf life of the food. In some cases, packaging may be effective by itself to extend the shelf life or it may be one of the hurdles which are ineffective alone but have effect together on food quality [4].

1.2. FOOD PACKAGING

1.2.1. Conventional Food Packaging

For the conventional food packaging; glass, paper, carton, wood, aluminum, can, plastic and plastic based packaging materials are used. Plastics are the most common used packaging

materials because of their several advantages. Plastics are the low-cost and lightweight materials; their processing and usage is easy; they can be produced as transparent or colorful; they can be firm or flexible according to the purpose; they are resistant against low and higher temperatures; they have moisture and gas permeability in different levels; they are resistant to oils and solvents; and mostly they are chemically inert. Besides all these advantages, their stability in the nature is the most important disadvantage. Plastic production increases yearly worldwide and a considerable amount are used for food packaging such as polyethylene terephthalate (PET), polystyrene (PS) and polyethylene (PE). In the Europe 39% of the produced plastics are used as food packaging materials (Goossens et al.). There are some reasons behind this increase such as the low cost, excellent structural properties, good water and gas barrier properties of plastic materials [8].

Global plastic production has grown from 15 metric tons in 1964 to 311 metric tons in 2014 There is growing concern on excess use of plastics in packaging. Use of the plastics as packaging material in the food industry causes waste contamination and these plastic wastes cause serious environmental problems. Every year, large quantities of plastic waste have been generated and remained in the environment. The disposed plastic waste in landfills decompose releasing both carbon dioxide and methane. In 2008, 20 million tonnes of CO_2 equivalent were released from disposal of solid plastic waste. These CO_2 release also cause change in global climate since CO_2 released from burning plastics trap radiant heat and results in warming of the air [8], [9].

1.2.2. Active Packaging

The active packaging systems have been developed by adding several active components and/or using functional polymers for gaining some functional properties to the packaging beside its barrier role. It is an innovative concept which the packaging material interact with product or ambient atmosphere to extend shelf life or improve sensorial properties of the food. The active agents can be incorporated into the coating materials by different technologies depending on the purpose of migration or stability of the active compound. In the controlled release coating systems, active agent is incorporated into the polymeric matrix in order to migrate and exert its antioxidant, antimicrobial or nutraceutical function to the product [10], [11], [12].

1.2.2.1. Antimicrobial Packaging

Antimicrobial packaging is one of the most important forms of active packaging and designed to control growth of undesired microorganisms. Besides the main purposes of conventional packaging like extending shelf life, preservation of quality and food safety; main purpose of antimicrobial packaging systems is to control microbial growth especially for perishable products that have microbial contamination risks. In the antimicrobial packaging systems, antimicrobial polymers are used directly as packaging material or antimicrobial agents are incorporated into the packaging material in order to inhibit the undesirable microbial growth and reduce the contamination risk created by food pathogens. These systems are used to reduce the microbial growth rate, decrease number of living microorganisms or extend the lag phase period due to antimicrobial activity [13].

Antimicrobial agents are compounds that prevent microbial growth by destructing cell wall or cell membrane, or preventing some important metabolic pathways. Action mechanism of antimicrobial agents used in active packaging differs depending on chemical structure of antimicrobial agent, concentration, treatment time and temperature, pH of the environment, oxygen need of the microorganism (aerobic or anaerobic), structure of the cell wall (Gram (+) or Gram (-)), growth stage (vegetative or spore-forming) and optimum growth temperature (thermophilic, mesophilic or psycrophilic) of the target microorganisms [14].

In the production of antimicrobial packaging materials; different antimicrobials such as weak organic acids (acetic acid, malic acid, lactic acid, citric acid etc.), inorganic acids, bacteriocins (nisin, colicin, pediocin, lacticin, etc.), plant and herb extracts (thyme, rosemary, black pepper, sage, olive leaf extract, grape seed extract, garlic etc.), alcohols, metals and ammonium compounds have been used. Many antimicrobial agents are microbicide at high concentrations and have microbiostatic effect at lower concentrations. There is not a linear and continuous relationship between concentration increase and antimicrobial effect. After a certain concentration, antimicrobial effect of the agent is not changed [14], [15].

The weak acids are the most common used antimicrobials that used in active packaging. Many of these acids are found in fermented products and plants naturally. The weak organic acids penetrate into cell membrane and accumulated in cell cytoplasm and so increase the

acidity in the cell. Bacteriocins are the low molecular weighted cationic polypeptides that produced from bacteria have been also used in antimicrobial packaging. Nisin is the most commonly used bacteriocin that is produced by *Lactococcus lactis subsp*. Nisin exhibits its bactericidal effect on microorganisms by binding and making pores in the bacterial membrane.

In the formation of an antimicrobial packaging system, the antimicrobial agents can be incorporated into the packaging systems by adding vesicles that contain volatile antimicrobial components; by adding directly antimicrobial agents into the polymer; coating the surface of polymer with antimicrobial agents; stabilizing antimicrobials to the polymer with ionic or covalent bonds and using polymers that have already antimicrobial properties [13], [16].

For the antimicrobial packaging, synthetic or naturals polymers can be used as packaging material. Biopolymers that have antimicrobial activity are preferred to be used in antimicrobial packaging and the most common used one is chitosan because of its antifungal properties. Antimicrobial films and coatings have been developed from polymers with antimicrobial properties. These antimicrobial films and coatings, which interact with the food surface directly, reduce number of living microorganisms by lowering the microbial growth rate of some specific microorganisms. Chemical and natural antimicrobial agents can be used in the formation of antimicrobial films and coatings. There are many studies in the literature that shows the effectiveness of antimicrobial film and coatings incorporated with different natural or chemical antimicrobial agents (Table 1.1). In these studies different polymers or biopolymers were used as coating material [14], [16].

Table 1.1. Selected references for biopolymer based antimicrobial films.

Film Material	**Antimicrobial Agent**	**Microorganisms**	**Reference**
Pectin	Papaya puree/cinnamaldehyde	*E. coli, S. enterica, L.monocytogenes, S. Aureus*	17
Gelatin	Silver nanoparticles (AgNPs) and nanoclay	*E. coli, L.monocytogenes*	18
Gelatin	Oregano, lavender essential oils	*E. coli, S. Aureus*	19
Chitosan	Eucalyptus globulus essential oil	*E. coli, S. Aureus, P. Aeruginosa, K. Pneumonia*	20
Gelatin	Zinc oxide nanoparticles (ZnO NPs)	*E. coli, L.monocytogenes*	21
Alginate	Cinnamon bark oil, soybean oil	*E. coli, L.monocytogenes, S.enterica*	22

1.2.2.2. Antioxidant Packaging

Oxidation is one of the main reasons of food deterioration after microbial growth. Especially foods with high lipid content are more sensitive to this type of deterioration. Due to oxidation; nutritional value of food is decreased by destruction of proteins, essential fatty acids and lipid soluble vitamins. It leads to formation of toxic aldehydes. Oxidation causes color change in foods by degrading the color pigments, and also causes formation of undesirable flavor compounds and offensive odors. Thus, oxidative changes affect both chemical, nutritional and sensorial properties of the food products [23].

Generally, antioxidants are added to food directly or suitable packaging systems are used to reduce and minimize the lipid oxidation. Vacuum packaging and modified atmosphere packaging systems are used commonly for controlling oxidation reactions by limiting the oxygen content in the package. But, the food also has some dissolved oxygen and it is very difficult to control and eliminate this interior oxygen completely.

Antioxidant active packaging has been developed by incorporating antioxidant agents into packaging material to retard the lipid oxidation and protein denaturation in foods that are susceptible to oxidation. The active antioxidant agent is integrated in the polymer packaging matrix or on the polymeric surface. Active antioxidant packaging controls oxidation in two ways; by releasing the antioxidant agent directly to the food product and by scavenging the oxygen and undesired compounds from the headspace.

Selection of suitable antioxidant to be used in the packaging material is highly important. The packaging material and antioxidant used should be compatible with each other to obtain a homogeneous material, and also distribution coefficient of the antioxidant should support its release to the food or headspace. Substances with potential antioxidant properties can be obtained from several natural sources. Besides synthetic chemicals, natural antioxidants can be used in the active antioxidant packaging such as green tea extract, rosemary extract, onion extract, ascorbic acid etc. [24].

1.3 BIODEGRADABLE PACKAGING

1.3.1 Biodegradation

Biodegradable (biologically degradable) materials are the materials that can be transformed into carbon dioxide, methane, water, inorganic compounds or biomass by degrading to low molecular weight substances as a result of the activities of bacteria, fungus or algae under proper moisture, oxygen and temperature conditions. Biodegradability of a material can be determined by respirometric analyses such as ASTM D5338 and ISO 15852. By these analysis methods, aerobic biodegradation rate and degree of materials are measured under controllable temperature, moisture and oxygen conditions in laboratory. In these test method, inoculum that obtained by municipal solid waste (MSW) compost is used. Biodegradable materials can be transformed into simple compounds in a shorter time period. In the standard composting conditions, 60-90% of the material should be degraded in between 60 and 180 days. Here, the composting process is the degradation of plant and animal based materials using microorganisms [25].

Biodegradation is composed of two complex stages. The first stage is the physical, thermal or mechanical degradation of the product. This may be occurred by mechanically with

breaking of the material, chemically with ultraviolet radiation, thermally in compost conditions and biologically with the activity of microorganisms or rodents. In this stage product or material is degraded into smaller pieces and become suitable for microbial attacks. The second stage is the biodegradation. In this stage; the material is mineralized by transforming into carbon dioxide, methane, water and biomass with enzymatic activity of microorganisms. Physical and chemical parameters (pH, moisture, temperature), microbiological parameters (microbiological population), structure and characteristics of polymers (size of the chains, crystallinity, moisture and ultraviolet resistance), production processes and thickness of the material affect the biodegradation [25], [26].

1.3.2 Biodegradable Polymers in Packaging

Biodegradation of unnatural packaging materials in the nature will take hundreds of years and especially usage of the non-degradable, petroleum derived synthetic polymers cause environmental pollution. Biopolymers are natural polymers obtained from animal waste or agricultural products. In the recent years, bio-based plastics and biopolymers have been studied as promising alternatives of petroleum-based plastic packaging materials. Feedstocks are renewable for polymeric bio-based materials that include starch, cellulose, protein, fat or natural fibers. Using the natural biopolymers for food packaging has advantages since they are obtained from renewable and sustainable resources; they are biocompatible, biodegradable and ecologically safe [27].

When compared to the conventional petroleum based and non-biodegradable packaging materials, biopolymer based packaging systems can provide solutions to the environmental problems by reducing the need of plastic usage. Their production may be more energy efficient compared to plastic processing.

Monomer structure of biopolymer affects its functions such as thermal stability, barrier properties against gases, moisture resistance and flexibility. Depending on their nature and structure, biopolymer based coatings can be edible and consumed with the food product. They enhance organoleptic properties of food; and act as vehicle for active agents such as antimicrobials, antioxidants, nutraceuticals or colorings [28], [29].

1.3.3. Polysaccharide Films

Polysaccharide films are the carbohydrate based films. Starch and its derivatives, cellulose derivatives, pectin, chitosan, carrageenan, alginate, curdlan, gellan, pullulan, xanthan and some other polysaccharide based gums are used for biodegradable film formation. These polysaccharide raw materials are preferred as raw material due to their high availability, excellent gas permeability and lower cost. But, the lower moisture barrier properties and low elasticity limit their applications because of their hydrophilic structure. Polysaccharide films are formed by first breaking polymer segments and then reforming the polymer chain to develop the film matrix via hydrophilic and hydrogen bonds [29].

1.3.3.1. Pectin

Pectin is a complex anionic polysaccharide and one of the main structural components of the plant cell wall. The plant cell wall is consisted of two different parts named as primary and secondary wall; and pectin is mainly found in the primary cell wall and middle lamellae of plants and fruits. The cell walls are composed of different polysaccharides like pectin, cellulose and hemi-cellulose. These polysaccharides have important roles in the cell structure such as enhancing mechanical strength of the cell wall, contributing the tissue integrity, providing gel formation and adhesion between the cells. Pectin content of the plant depending on tissue, species and environment of the plant [30].

There are various pectic polysaccharides in the structure of plant cell wall. Homogalacturonan (HG), xylogalacturonan (XG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) are the main building blocks in the pectin structure and typically homogalacturonan is the most abundant polysaccharide (Figure 1.1). HG constitutes nearly 65% of pectin, while RG-I constitutes 25-30%, XG and RG-II constitutes less than 10% of the pectin structure. Homogalacturonan is the unbranched backbone chain of the pectin which constitutes the smooth region and contains 1,4-linked α-D-galacturonic acid residues. Rhamnogalacturnan-I and rhamnogalacturonan-II are the branched regions of the pectin backbone [30], [31], [32].

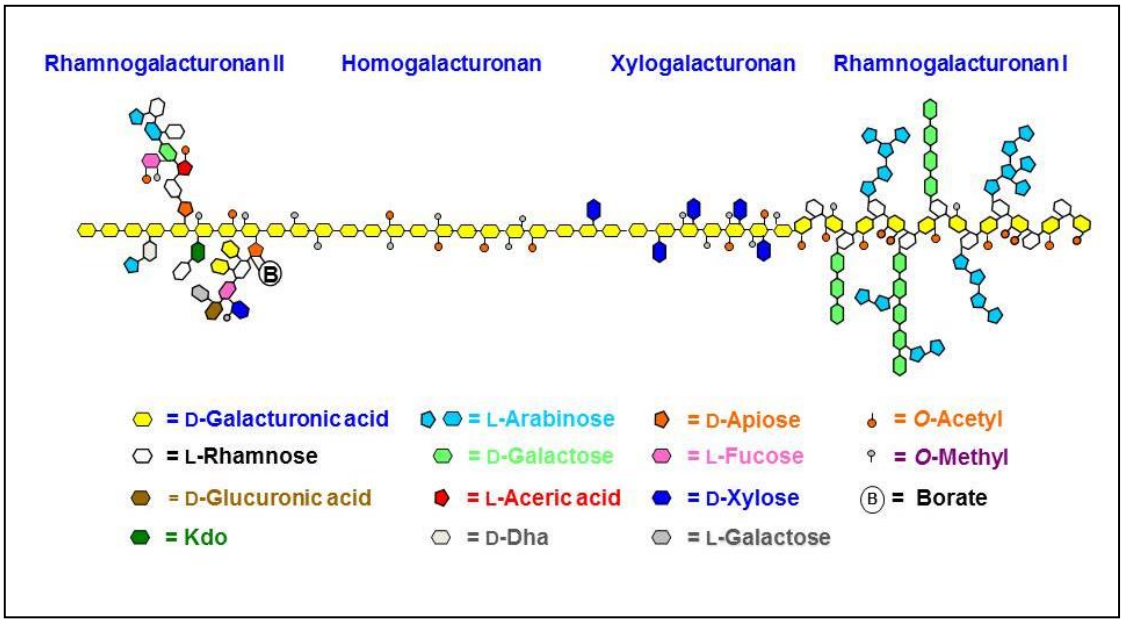


Figure 1.1. Schematic structure of pectin [31].

Pectin is an anionic complex polysaccharide composed of β-1,4-linked D-galacturonic acid residues, where in carboxyl groups of uronic acid are either fully (high methoxyl pectin, DE≥50%) or partially (low methoxyl pectin, DE<50%) methyl esterified [33].

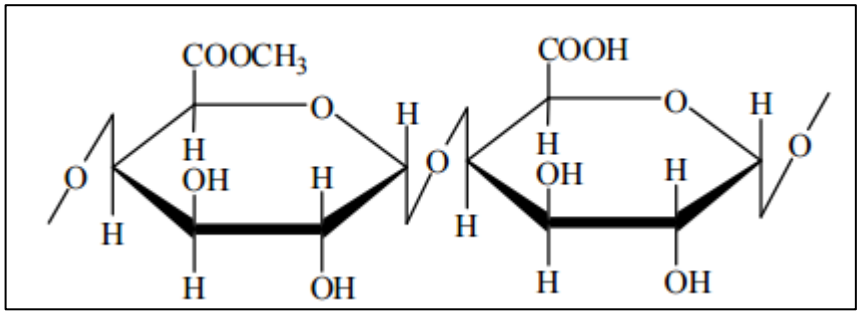


Figure 1.2. Chemical structure of pectin [32].

The most important properties of pectin are degree of esterification, degree of methylation, degree of blockiness, galacturonic acid content, neutral sugar content and molecular weight distribution. Degree of esterification (DE) is the ratio of esterified galacturonic acid groups to total galacturonic acid groups. According to the degree of esterification with methanol, pectin is classified as high methoxyl pectin (HMP) and low methoxyl pectin (LMP). HM pectins have 50% or more galacturonic residues.

Citrus peels and apple pomace obtained as byproducts of fruit juice manufacturing are the major industrial sources for pectin production. Sugar beet waste, mango waste and sunflower heads are also the alternative sources for pectin extraction. Pectin is used as gelling, thickening, stabilizing agent and for coating in the food industry and listed as GRAS (generally recognized as safe) by the Food and Drug Administration. For the gelation of high methoxyl pectins; high sugar content and acidic conditions are needed. Therefore, they are mostly used in confectionary products, jellies, jams, cakes, juices and soft drinks. Low methoxyl pectins are obtained by de-esterification of high methoxyl pectins. LM pectins form gels in the presence of divalent cation in low pH environment. Therefore, they generally used in dairy desserts, fruit gels, syrups of vegetable and fruit canning, low-sugar products and food coatings [34], [35], [36].

Pectins are soluble in pure water and dilute pectin solutions show Newtonian behavior but they exhibit pseudoplastic behavior at certain concentrations. Pectin is used in the food coating and packaging studies due to its biodegradable, biocompatible and nontoxic structure. As a food coating material pectin has good oxygen, carbon dioxide and oil barrier properties, aroma preservation functions. Generally LM pectins are used as food coatings due to their ability to form stable gels at low pH in the presence of calcium and their structural integrity. But at the same time it has low moisture resistance because of its hydrophilic structure. Pectin coating is generally applied on fresh fruits and vegetables like papaya, peach, avocado, berries, apricot, guava, apple and chestnut [37].

Gelation mechanism of pectins is changed depending on their degree of esterification (DE). High methoxyl pectins form gels through hydrophobic interactions under the acidic conditions in the presence of high sugar and aqueous media while low methoxyl pectins form gels through specific ionic interactions between divalent ions and carboxyl groups of pectin [38], [39].

1.3.3.2. Gelation Mechanism of Pectin

Ability to form gels is one of the most important properties of pectin. Low methoxyl pectins form gels by chain-stacking through the specific ionic interactions between divalent ions and carboxyl groups of the polysaccharide macromolecule. The gelation is occurred as a result

of the interactions between blocks of galacturonic acid residues of the pectin backbone and divalent cations such as calcium. [40].

Calcium induced gelation of LM pectins is explained with the egg-box model (Figure 1.3). While the calcium bridges between the carboxyl groups form this well-organized structure; electrostatic interactions, hydrophobic interactions between methoxyl ester groups, hydrogen bonds between carboxyl groups and/or hydroxyl groups and van der Waals interactions stabilize the egg-box structure. During gelation the solvent is confined in a three dimensional network linked by the intermolecular ionic junction zones and life of the junctions depends on the strength of electrostatic bonds [41].

Affinity of pectin chains to the calcium ion increases with decreasing degree of esterification or ionic strength and with increasing polymer concentration. Amidation also enhances the gelling ability of LM pectin and amidated pectins needs less calcium ion to form gel network. Presence of monovalent ions like sodium can affect gel formation of LM pectin since they can reduce cross-linking formation between calcium and carboxyl groups by reacting with free carboxyl groups [42].

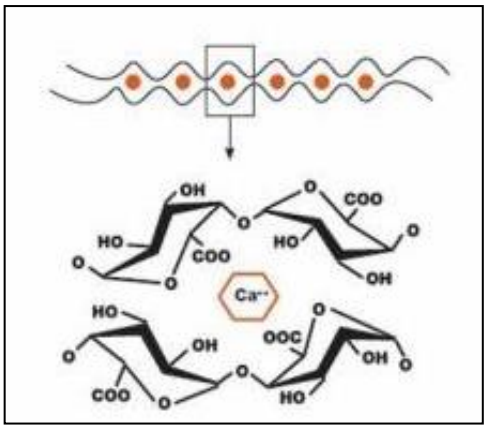


Figure 1.3. Calcium induced gelation mechanism of LM pectin [40].

HM pectins form gels in the presence of an acidic media and soluble solids (typically sucrose) at a concentration above 55%. Hydrophobic interactions and hydrogen bonding are the important forces for aggregation of pectin molecules. During gelation of HM pectin, high sugar level in the solution decreases the water activity and hydration of pectin by competing for water, and so the hydrophobic interactions are occurred between methoxyl groups. Gel

formation induced by hydrogen bonding between free carboxyl groups of pectin molecules and also between hydroxyl groups of adjacent molecules. In the neutral solutions, most of unesterified carboxyl groups are found as partially ionized salts. With the acid addition, carboxyl ions are converted to unionized carboxylic acid groups and so the number of negative charges is decreased. This decrease reduces the attraction between water and pectin molecules and repulsive forces between pectin molecules, and prevents separation of the carboxyl groups. Therefore, these non-separated carboxyl groups can link to the secondary alcohol groups via hydrogen bonding. HM pectins which have higher DE values form gels more rapidly when compared to the HM pectins with low DE values [43].

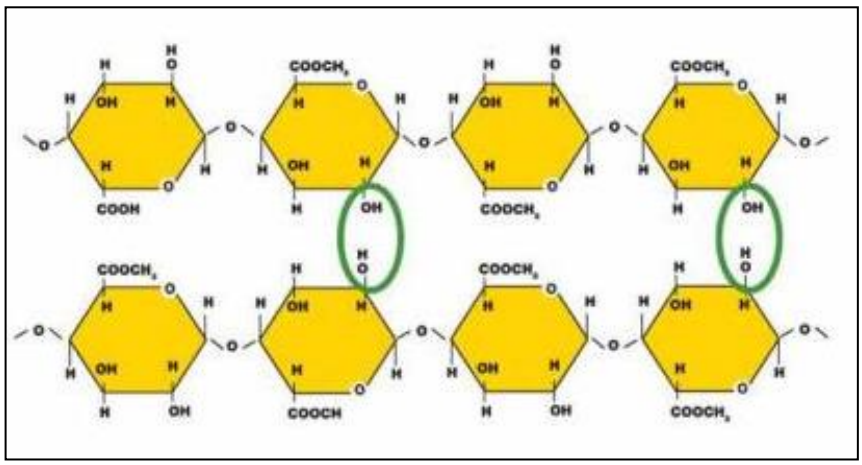


Figure 1.4. Gelation mechanism of HM pectin [42].

1.3.4. Protein Films

Proteins are polymers that are formed by condensation polymerization of different repeated amino acid units. They show differences depending on their source, structure and amino acid composition. Plasticity, elasticity, ability of network formation and high intermolecular binding potential are the advantageous properties of the proteins for packaging applications. Protein based films also have a unique structure that provide wide range of functional properties. They can make bonds at different positions and they have higher potential to form various linkages. When compared to synthetic polymers, lower water vapor resistance and low mechanical strength limit the packaging applications of protein-based polymers [44].

Film forming properties of different proteins has been used in many industrial applications. Casein has been used in glue due to its water resistance property in the ancient civilizations. Corn zein has been used as coating materials for floors, photographic films, wax papers and can linings. Gelatin was one of the first materials that used for polymeric wall formation in microencapsulation. Soy protein, pea protein, corn zein, wheat gluten, rice bran protein and sorghum protein are the common plant based proteins which used for their film forming properties. Besides; gelatin, collagen, casein, whey protein, keratin, egg white and fish myofibrillar proteins are the most common animal based proteins used for biodegradable film formation [44], [45].

1.3.4.1. Gelatin

Gelatin is an animal protein that is derived from partial hydrolysis of collagen found in bones, connective tissues and skin of some animals. Source and type of the collagen for gelatin production is the waste of animal slaughtering and processing. Traditionally, collagen and gelatin are obtained from mammalian sources like cattle and pigs. Besides mammalian gelatin, fish and poultry gelatin are also obtained as by-product of animal processing in the recent years. But fish gelatin production is still limited because of some disadvantages. Since there are many fish species that have different characteristics, different extraction conditions are needed to optimize extraction process and yield. The fish gelatin is mainly used for pharmaceutical and cosmetic industries. Gelatin is accepted as GRAS (generally recognized as safe) by FDA and generally used in the food, pharmaceutical and cosmetic industries. There are many types of gelatin based on the source of collagen, processing method, extraction conditions and thermal history [44].

A collagen is made of right handed triple helix of α-chains and this triple helix is stabilized with hydrogen bonds between the peptide chains. Gelatin is the linear sequence of amino acids. In order to obtain gelatin, collagen is treated chemically to break secondary and higher protein structures. Since gelatin is obtained from partial hydrolysis of collegen, its amino acid composition is similar with the parent collagen. Amino acid sequence and composition of gelatin differ depending on its parent animal and tissues but mainly composed of proline (10-18%), glycine (26-34%) and hydroxyproline (7-15%) amino acids. Another significant

amino acids of gelatin are glutamic acid (10-12%), alanine (8-11%), arginine (8-9%) and aspartic acid (6-7%). Amino acid composition of gelatin affects its chemical properties [45].

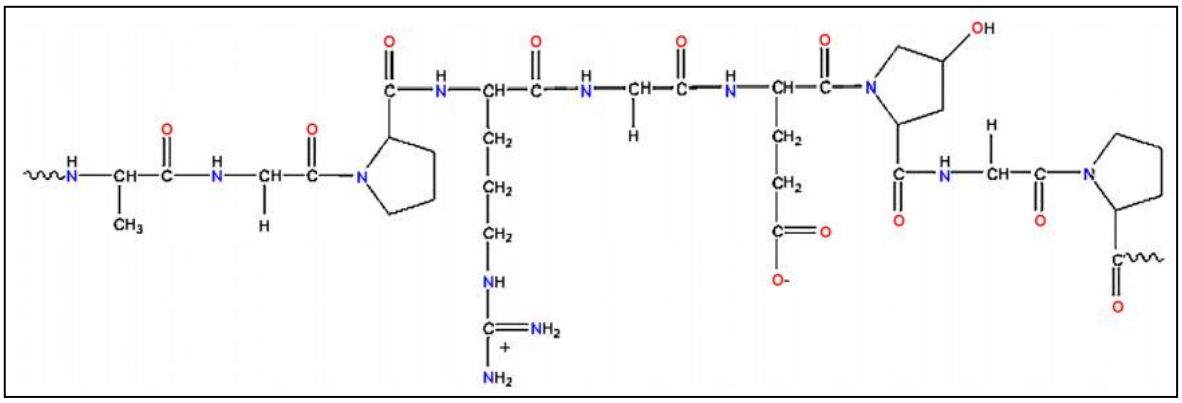


Figure 1.5. Gelatin Structure (-Ala-Gly-Pro-Arg-Gy-Glu-4Hyp-Gly-Pro-) [44].

Gel strength or Bloom value is one of the most important physical properties of gelatin. Commercially gel strength of gelatin is changed between 50 and 300 bloom. The gel strength is associated with α and β chain composition of gelatin and used as a guide for behavior of the gel in food industry. Gelatin has a molecular weight changing between 20,000 and 250,000 g/mol. Gelatin is classified as type A and type B depending on its pretreatment [45].

Gelatin swells in cold water forming visible large particles, and hydrated gelatin particles are dispersed in the solution when heated above its melting temperature. Gelatin is insoluble in alcohol and non-polar solvents like glycerin, sorbitol and mannitol [46].

In the food industry gelatin is mainly used for its gelling, film forming, water binding, thickening, texturizing and stabilizing properties. It is commonly used in desserts, candies, marshmallows, ice cream and dairy products because of its melting property in the mouth. In frozen food products, gelatin prevents crystallization of ice and sugar. In sour cream, it prevents separation of water phase. In marshmallows gelatin keeps the marshmallows soft and elastic by preventing sugar crystallization. Gelatin increases viscosity and stabilizes foam in production [46], [47].

In the pharmaceutical industry, gelatin is used for drug and gene delivery since it is GRAS, has very low antigenicity and its functional groups can be chemically modified easily [48].

1.3.4.2. Gelatin Manufacturing

Gelatin manufacturing process consists of five steps; washing, extraction, purification, concentration and drying. First of all, the raw materials (bones, skin, etc.) are cut into small cubic pieces and these pieces are washed under high-pressure water. Then the washed raw material pieces are degreased in hot water to decrease the fat content to approximately 2%. The degreased bone and skin pieces are dried with hot air at 100^{o}C for about 30 minutes [48].

Collagen dissolves very slowly in water because of the cross-linked nature of collagen. Therefore before the extraction chemical treatment is needed to breakdown the cross linkages in the structure. Partial hydrolysis is occurred with this treatment and the cross-linkages are broken while the protein chains remain intact. Acid or alkali treatment may be used according to the collagen source and age of animal. In addition to chemical treatment, enzymatic treatment (with collagenases) or combination of chemical and enzymatic treatments is used to break crosslinks. For the alkaline process the precut raw materials are treated with alkali in tanks with agitation at a certain temperature and time. At the end of alkali treatment process type B gelatin is obtained and this process is generally applied to bones and hides. Acid treatment is mainly used for pigskin and type A gelatin is obtained in the end. After these treatments, the raw materials are washed to remove excess acid or alkali. The pH which the gelatin solution is neutral is called as isoelectric point (IEP). The isoelectric points of type A and type B gelatin are different. In the alkaline treatment, asparagine and glutamine are converted to aspartic and glutamic acid. For this reason, isoelectric point (IEP) of B type gelatin is 4.8-5.5 when the IEP of B type gelatin is between 8.0 and 9.0. Isoelectric point of type A gelatin is similar with parent collagen [48], [49].

1.3.4.3. Gelation Mechanism of Gelatin

Gelatin forms gels by forming a microstructural network. Thermoreversible gel formation in aqueous solutions is one of gelatin's most important properties. When a heated gelatin solution with a concentration higher than 0.5% is cooled to its gelling temperature (nearly 30-35^{o}C), gelatin forms gels. During the cooling process, first the solution viscosity is increased and then gel formation is occurred as a result of change in molecular conformation

and aggregation of gelatin molecules. If the gelatin gel is heated above its melting temperature again, the gel will melt because of the dissociation of triple helices [50].

Well accepted gelation mechanism of gelatin is random coiled helix reversion. Flexible and disordered coils of gelatin associate into triple helices with hydrogen bonds below the gelling temperature. Amino acid rich parts of different polypeptide chains act as potential junction zones and take up a helical conformation forming the three dimensional gel upon cooling. Ionic bonds and hydrophobic interactions also provide formation of the gel network [51], [52].

The final gel strength depends on the Bloom value of gelatin; concentration, pH and temperature of the gelatin solution and presence of any other substances in the solution.

When the gelation is completed; a big macromolecular gel network is formed, the triple helices form junction zones that bind the gel network and enclose the solvent providing strength and elasticity [52].

1.4 ANTIMICROBIAL FILMS MADE OF PECTIN AND GELATIN

Pectin and gelatin are the major biopolymers that are used as carriers of active agents (such as antimicrobial and antioxidant agents) in the formation of food coating and films. Many studies have been performed to use different antimicrobial agents in gelatin and pectin films. The active agents can be obtained from different sources including bacteria, plants, animals, fungus and byproducts of fruit and vegetable processes. Essential oils are widely used as active agents in the biopolymer films.

Kanmani et al. developed antimicrobial films with gelatin using silver nanoparticles and nanoclay as active agents. Results of the study showed that incorporation of the silver nanoparticles and nanoclay decreased the transparency of the developed nanocomposite films, improved the tensile strength of the films and exhibited significant antimicrobial effect against Gram-positive and Gram-negative food borne pathogens [51]. Similar results were obtained by Shankar et al. when gelatin based composite films were developed with addition of zinc oxide nanoparticles. While the incorporation of zinc oxide decreased the tensile strength of the gelatin films, developed films exhibited significant inhibitory effect on both Gram-negative and Gram-positive food pathogens [21].

Pectin and polylactic acid films incorporated with nisin was developed to inhibit *L. monocytogenes* by Jin et al. The results of the study suggested that the nisin loaded pectin and polylactc acid films inhibited growth of *L. monocytogenes* [53]. Otoni et al. investigated antimicrobial, physical and mechanical properties of the pectin films incorporated with papaya puree and cinnamaldehyde and concluded that cinnamaldehyde added films had inhibitory effect on *S. enterica*, *E. coli*, *L. monocytogenes* and *S. aureus* [17].

Pereda et al. developed composite films by combining chitosan and gelatin, and studied antimicrobial activity and physico-chemical properties of the developed films. With addition of gelatin, they obtained softer and more flexible films when compared to chitosan films. Tensile strength of the composite films also 40% higher than the control chitosan films. Results of the study indicated that the composite films showed inhibitory effect on *E. coli* and *L. monocytogenes* [54].

Zhang et al. investigated antioxidant properties of gelatin films containing different natural antioxidants (green tea extract, grape seed extract, ginger extract, gingko extract) and studied the antioxidant activity, physical and chemical properties of the films. Results of the study suggested that the films containing green tea, grape seed and gingko leave extracts had high antioxidant activity while the addition of the extracts reduce the tensile strength of the films [22].

In this study, antimicrobial pectin and gelatin films were developed via incorporation of the boron derivatives (boric acid, disodium octaborate tetrahydrate and sodium pentaborate) into the films. Boron derivatives were used as active antimicrobial agents.

1.5 BORON

Boron is an essential element for many organisms and higher plants that is commonly found in soil, rocks and water on earth. Boron element is found in 3A group of the periodic table, its atomic number is 5 and atomic weight is 10,81 g/mol. Boron is the 51st element that commonly found in the earth's crust and never found as free form in the nature. Boron is found chemically bound to oxygen except that some boron fluoride minerals. Boron behaves as nonmetal when it reacts with sodium and behaves as metal in the reaction with fluorine. There are approximately 230 kinds of boron minerals in nature. Boron reacts with water at

high temperature to form boric acid and some other byproducts. Due to its bonding tendency with oxygen, there are many different boron-oxygen compounds. General name of boron-oxygen compounds is borate. Compounds of boron and metal or nonmetal elements have different characteristics and this enables the use of boron derivatives in the many fields of industry. Boron behaves nonmetallic in its compounds, but the pure boron is an electrical conductor like carbon. Boron hydrates show similar characteristics with silicon and carbon compounds [55].

Boron and boron compounds are used in many different industries and are known to form complexes rapidly and reversibly in aqueous media with polyhdroxy compounds due to its high affinity to hydroxyl groups. In the recent years, boron is used to improve thermal stability, flexibility, flame retardancy and enhance electrical, mechanical, antibacterial, antifungal properties of polymers by incorporating in the backbone of polymer.

Boron minerals are generally formed between cenozoic sedimentary layers in the earth's crust. Volcanic rocks are also found in the boron containing regions. Boron minerals are generally aqueous borates combined with an alkali cation such as Na^{+}, Ca^{++} and Mg^{++}. Boron minerals contain different ratios of boron oxide (B_2O_3) in their structure.

Although there are many minerals that contain boron, only some of them have commercial value. A borate anion is formed by combining these minerals with a metallic cation or hydrogen and the minerals denominated with name of this metal cation (i.e. sodium borate, calcium borate, etc.). These inorganic minerals are marketed by considering their B_2O_3 content within the international market; it is accepted that the minerals with higher B_2O_3 content are more valuable [56].

Table 1.2. Some of Merchantable Boron Minerals

Name	Formula	B%	B_2O_3 (wt %)
Tincal (Borax)	$Na_2B_4O_7.10H_2O$	11,4	36,5
Colemanite	$Ca_2B_6O_{11}.5H_2O$	15,7	50,8
Ulexite	$NaCaB_5O_9.8H_2O$	13,3	42,9
Kernite (Rasorite)	$Na_2B_4O_7.4H_2O$	15,8	50,9
Priceite (Pandermite)	$CaB_{10}O_{19}.7H_2O$	15,4	49,8
Boracite	$Mg_3B_7O_{13}CI$	19,3	62,2
Sasolite	H_3BO_3	17,5	56,4

After separation from the soil, boron mines are subjected to breaking, sieving, washing and grinding processes respectively for being ready to use in the related industry. The most important boron reserves are found in Turkey, Russia and the USA. World total boron reserve based on B_2O_3 is around $1.2x10^9$ tons. Distributions of the world's boron reserves are shown in the Figure 1.6.

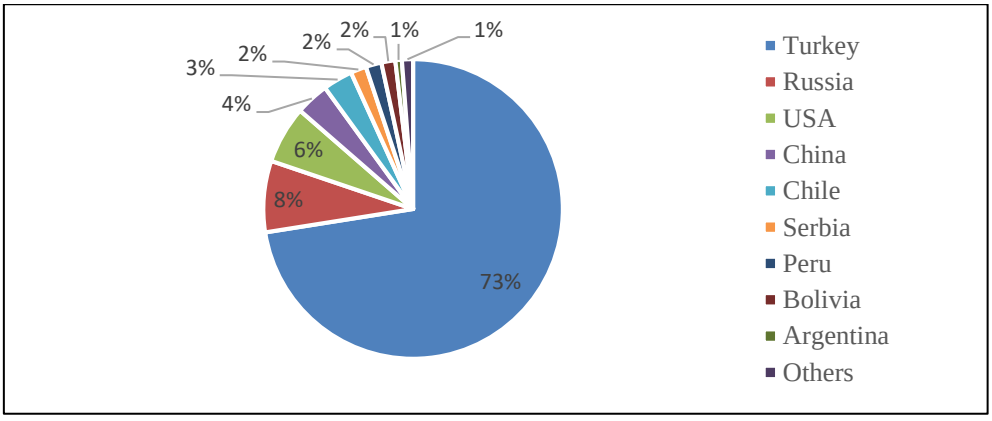


Figure 1.6. Distribution of world boron reserves [56].

When the world boron mine is examined by years, it is seen that most of the production is carried out by two countries. In 1970, 66.4 percent of the world boron production was made by USA, while 15.9 percent was made by Turkey and 17.7 percent was produced in the other countries. In 2012, Turkey was on the first rank with 47 percent share in the regional distribution of boron production on B_2O_3 basis (Figure 1.7).

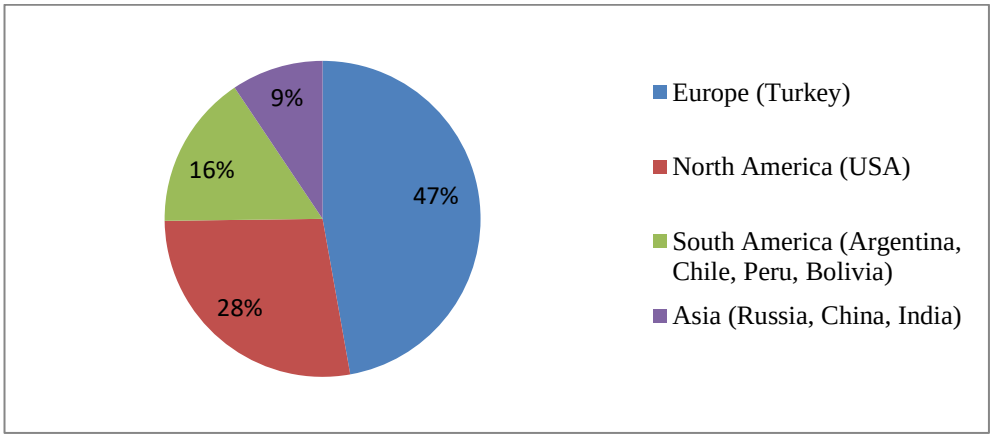


Figure 1.7. Distribution of world's boron production by region [56].

Boron minerals are the natural resources that have great strategic importance for our country. Boron minerals are used in many different industrial areas. Glass and glass fiber, ceramic, agriculture, detergent and bleaching agent industries are the four main industries that constitute 80 percent of the total consumption [56], [57], [58].

1.5.1. Boric Acid (H_3BO_3)

In the environment, boron is mostly present as naturally occurring boric acid. Also other borate compounds can be converted to boric acid. Boric acid is a weak acid containing oxygen, hydrogen and boron elements. Boric acid has been known as a mild antiseptic and accepted in clinical applications for years. Boric acid is also a fungistatic compound that is used for yeast infections. Boric acid is soluble in water and exhibits endothermic dissolution.

Molecular weight of boric acid is 61.83 g/mol, melting point is 169^{o}C, density is 1.43 g/cm^3 and solubility in water at 20^{o}C is 4.7 g boric acid/g solution. Boric acid is one of the refined boron compounds and mainly used in drug, cleaning agent, textile, glass etc. industries [59], [60].

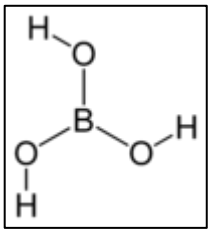


Figure 1.8. Chemical structure of boric acid [59].

1.5.2. Borates

Borates are the salts or esters of boric acid. Boron minerals are classified according to their crystal structure and complex boron-oxygen polyanions in their crystal structure. The boron atom in borates can be either three or four coordinate-bonded to oxygen forming either planar trigonal BO_3 units or negatively charged tetrahedral BO_4^{-1} units.

Disodium octaborate tetrahydrate ($Na_2B_8O_{13}.4H_2O$), has the most abundant usage area among the sodium polyborates. Disodium octaborate is used to compensate boron deficiency of plants by spraying method. It was also used as fertilizer with direct addition to the soil [61].

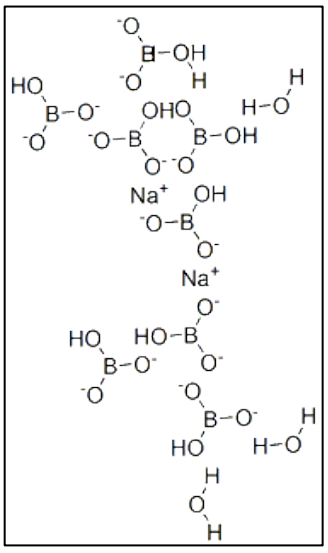


Figure 1.9. Chemical structure of disodium octaborate tetrahydrate [61].

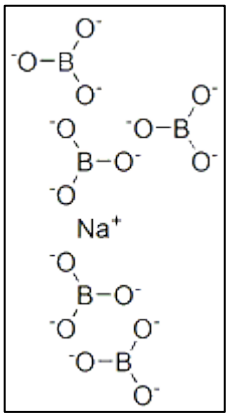


Figure 1.10. Chemical structure of sodium pentaborate [61].

1.5.3. Boron in the Plant Structure

Boron is an essential element for higher plants. Boron is a very important element for plant nutrition and its deficiency causes many physiological problems. Besides that excessive amount may cause toxic effect. Boron has very low toxic effect on people and it can enhance utility of medicines for some diseases [62].

Boron is taken from the soil, moved from the roots and accumulated in the growing parts of plants like leaves and stems. In the leaves and stems, almost 90 percent of this carried boron is localized in the cell wall. During plant growth, primary cell wall is very important for cell size and shape and boron is also very important for integrity of the cell wall. Physical properties of the cell wall are enhanced via cross-linkages between its main components. These main components are cell wall carbohydrates like cellulose and pectic polysaccharides. Borate esters formation between hydroxyl groups of cell wall carbohydrates is responsible for making this type of cross-linkages. Therefore, boron deficiency is responsible for brittleness of the plant leaves, and higher boron levels generated more elastic leaves [63].

In the last years, boron incorporated polymers and their derivatives have gained importance for the scientific studies and industry. Boron incorporation to the polymer backbones improves the mechanical and electrical characteristics, antibacterial and antifungal properties, flexibility and oxidative resistance. Qin et al. studied the inhibition effect of boron against mold (Botrytis cinerea) on table grapes and found that boron inhibited the formation of this type of mold on grapes. According to the study, it was expressed that boron

inhibition may be caused by its disruption effect on cell membrane of the fungal pathogen and loss of cytoplasmic materials [62], [63].

1.5.4. Antimicrobial Effects of Boron Derivatives

In bacteria, boron is the primary part of cell-to-cell communication signal molecules that named as quorum sensing. Many boron containing compounds that have antibacterial, antifungal or antiviral activities are auto-inducers which are regulated and induced by bacterial quorum sensing. Boron is one of the essential parts of signal molecules required by quorum sensing in the bacteria.

Boric acid is a non-polar molecule that is capable of crossing biological membranes. Undissociated form of boric acid can interact with the membrane or pass through the membrane of microbial cells and interfere with many enzymatic processes in the cell [61].

Boron compounds also can react with hydroxyl-rich compounds like phospholipids, lipopolysaccharides and glycoproteins in microbial membranes. These interactions may cause changes in functional activity of membrane and membrane-bound enzymes.

Boric acid shows its antimicrobial effect through two ways; inhibiting the membrane proteins and inhibiting the enzymes in the cells. Inhibition of membrane proteins and membrane enzymes may block transportation of nutrients and slow down the metabolic processes of microorganisms. Inhibition of enzymes in the cell can cause function disorder of metabolic pathways. These processes can cause biostatic effect of boric acid on microorganisms [64].

1.6. CHARACTERIZATON OF FILM FORMING SOLUTIONS AND FILMS

1.6.1. Rheological Characterization

Rheology is defined as the study of flow and deformation of materials under controlled conditions. Response of a material to the applied force (stress) or deformation (strain) can be measured with a rheological study. In another words, rheology measures the relationship between the applied stress on a material and resulting deformation and flow. Rheological

behavior is very important for developing a new product and processing of industrial products especially for food processing. Process parameters should be determined according to rheological properties of the product such as flow and pumping rates through the pipelines. Therefore, rheological properties of the film forming solutions are important to ensure optimum processing requirements.

Rotational and tube type instruments are used for rheological measurements. Rotational rheometers are performed in the oscillatory (dynamic) or steady shear (constant angular velocity) mode. Rotational rheometers can be operated in controlled stress or controlled strain modes [65].

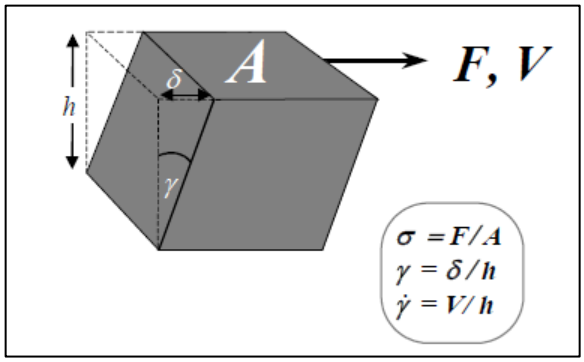


Figure 1.11. Diagram of shear flow [65].

Stress and strain are the main parameters for rheological evaluations. Shear stress is the force per unit area produced by flow, expressed in units of Pascal (N/m^2) and described by σ symbol. Shear strain is the deformation or movement that takes place per length [66].

1.6.2. Viscoelasticity

Viscoelasticity is the property of a material that exhibit both viscous and elastic behaviors when an external stress is applied. Viscoelasticity is a time-dependent response. When an external stress is applied, viscous materials resist shear flow and strain linearly with time. On the other side, elastic materials strain when stretched but return to their initial state quickly when the stress is removed. Viscoelastic materials have both these properties.

Biopolymer gels are viscoelastic materials. The rheological studies provide information on sol-gel and gel-sol transitions and characteristics of the biopolymer gel systems. As a result of the dynamic rheological tests performed in the linear viscoelastic range; storage (elastic) modulus (G') and loss (viscous) modulus (G") parameters are obtained. There are three types of dynamic tests that can be done to achieve gel properties; frequency sweep tests, temperature sweep tests and time sweep tests. G' and G" are determined as a function of frequency (ω) at constant temperatures from frequency sweep studies. With temperature sweep studies, G' and G" are obtained as a function of temperature at fixed frequency. As a result of time sweep tests, the loss and storage modulus are determined as a function of time at constant temperature and frequency [67].

1.6.3. Gels from Food Biopolymers

A gel is a colloid system which the solid phase forms a network by immobilizing the liquid phase inside. This cross-linked gel systems have solid-like properties. Gelation occurs through chemical or physical cross-linkages between polymer chains. In the food systems, mainly proteins and carbohydrates are responsible for the network formation.

There are different biopolymer gel systems and they have different gelation mechanisms. These differences caused by the number and structure of cross-links, attraction and repulsion between framework components and interactions with solvent. In Table 1.3 different gelation mechanisms of some important food gels are given [68]

Table 1.3. Gelation characteristics of important food polymers.

Polymer	Main Factors Promoting Gelation	Mechanism of Gelation
Carrageenans	Cooling; presence of potassium or other gel promoting cations	Association of the molecular chains into double helices followed by aggregation of the ordered domains
Agarose	Cooling	Helix formation followed by aggregation
Alginates	Presence of divalent cations (usually Ca+2)	Specific site binding of calcium with the carboxyl groups of the polyuronic acid residues (mainly the polyguluronic), egg-box model
Pectins		
High-methoxyl pectin	Cooling; low pH and low water activity	Junction zones stabilized by hydrogen bonds and hydrophobic interactions between the ester methyl groups
Low-methoxyl pectin	Presence of divalent cations (usually Ca+2)	Specific site binding of calcium with the carboxyl groups of the polyuronic acid residues, egg-box model
Gellan gum	Cooling; presence of the gel promoting cations	Ion-mediated aggregation of double helices
Starch	Cooling	Composite gels; amylopectin granules threaded by a gelled amylose matrix
Gelatin	Heating solutions followed by cooling	Formation of triple helices randomly distributed in space and separated by chain segments of random-coil conformation
Bovine serum albumin	Heating solutions followed by cooling	Denaturation followed by aggregation, likely by formation of β-sheet regions and other less specific protein-protein associations
β-lactoglobulin	Heating solutions followed by cooling	Denaturation followed by aggregation
Caseins	*Renneted gels: enzymatic action followed by precipitation by Ca+2 *Acid coagulation: acidification and instability of the casein micelles	*Renneted gels: k-casein hydrolysis; instability of casein micelles followed by coagulation, mainly by electrostatic interactions involving Ca+2 *Acid coagulation: Solubilization of the colloidal calcium phosphate and aggregation

1.6.4. Oscillatory Tests

Oscillatory tests are ideal to follow rebuilding of microstructure of sheared systems. In the oscillatory tests, harmonically varying stress or strain is applied on the samples. Oscillatory testing is the most common dynamic method to investigate viscoelastic behavior of materials. In the oscillatory tests, samples are subjected to small sinusoidal strain (γ) or stress [69].

1.6.4.1. Determination of Viscosity

Viscosity (η) is the resistance of a sample against flow and defined as the ratio of shear stress to shear rate. In order to measure viscosity of solutions, shear rate ramp test is performed. In this method, a continuous increase of shear rate is applied on sample. This test shows physical behavior of the sample under increasing shear rate and provides information about flow characteristics (Newtonian or Non-Newtonian) of material [69].

1.6.4.2. Determination of Linear Viscoelastic Region

Before the oscillation tests, Linear Viscoelastic Region (LVER) should be measured for all samples. LVER is the region which the stress/strain ratio and so the modulus is constant. Elastic modulus (G') is the measure of structural integrity of the material, and drop in elastic modulus represents breakdown of material structure. Amplitude sweep test is used for measurement of linear viscoelastic region and so determination a suitable strain value from this linear region. Because when oscillatory measurements are performed in this region, storage and loss modulus are independent from the strain amplitude. To determine the LVER, the storage modulus is plotted against the shear strain [68].

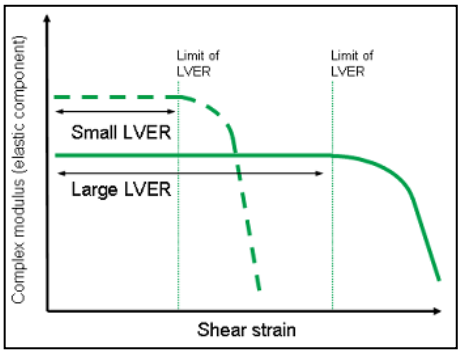


Figure 1.12. Linear viscoelastic region of two samples [68].

1.6.4.3. Determination of Gelling and Melting Points

The gelling and melting points of the gels are determined by measuring storage modulus (G') and loss modulus (G") as a function of time or temperature using oscillation tests. When the liquid-like behavior dominates the system, elastic modulus is smaller than the viscous modulus. The gelation point is the crossover point where G' = G". After the crossover point, storage modulus G' becomes larger than the loss modulus G" and the sample exhibits more solid-like behavior (Figure 1.13).

When the solid-like behavior dominates the system, viscous modulus (G") is lower than the elastic modulus. The melting point is the crossover point where G' = G". After the melting point, G" becomes larger than the elastic modulus and the sample exhibits more liquid-like behavior.

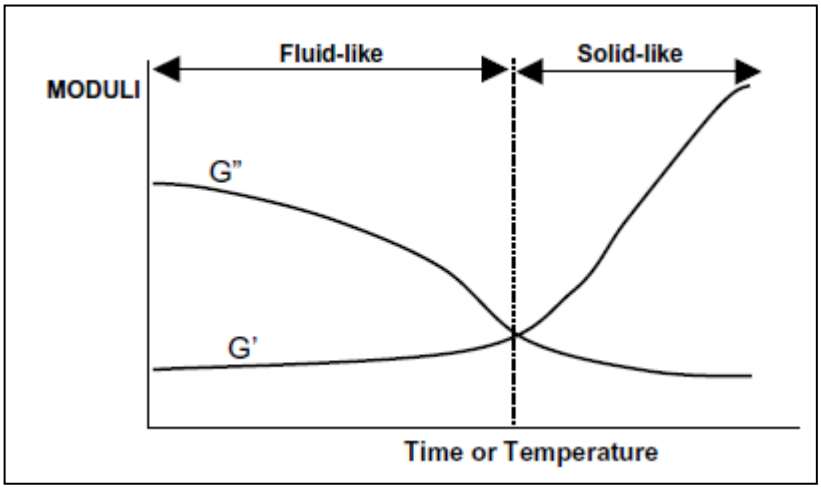


Figure 1.13. Viscoelastic response of a material undergoing gelation [70].

Frequency sweep test is performed to determine viscoelastic response of the sample at different frequencies. During gelation of a biopolymer system, a phase transition is occurred from liquid to gel state. The gel point is the point where G’ and G’’ cross each other at a given frequency [70].

2. MATERIALS AND METHODS

2.1. MATERIALS

Food grade gelatin (type B, 225 Bloom, from bovine skin) was supplied from Sigma-Aldrich and LM pectin (GENU pectin type LM-104 AS-FS, CP Kelco) was kindly provided from Aromsa Inc. Boron derivatives (boric acid, sodium pentaborate and disodium octaborate) were provided from BOREN and Eti Maden. Glycerol was supplied from Merck. For the microbiological studies dextrose starch agar and tyriptic soy agar (Fluka Analytical) were used. Double distilled water was obtained from Milli-Q Academic ultra-pure water system (Millipore Corporation).

2.2. METHODS

2.2.1. Preparation of the Films

Gelatin and low-methoxyl (LM) pectin films were prepared using solvent casting method and the formulations (Table 2.1) were determined according to preliminary studies. Gelatin film forming solutions were prepared by dispersing 3 g glycerol (as plasticizer) and 10 g powdered gelatin in 80 ml double distilled (dd) water at 50^{o}C for 30 min with a continuous stirring at 700 rpm. At the same time, the boron derivative was dissolved in 20 ml dd water at 50^{o}C and this solution was added dropwise to the initial gelatin solution. The final mixture was stirred during 30 minutes, and then the resulting film forming solution (a volume of 20 ml) was poured onto the plastic petri dishes (90 mm in diameter) and dried for 48 hours at ambient temperature until the solvent was evaporated. This method was repeated for all boron derivatives at different concentrations. Samples containing only gelatin and glycerol were considered as control films.

For the preparation of pectin film forming solutions; 3 g of glycerol and 2 g of LM pectin were dissolved in 70 ml dd water at 60^{o}C for 30 min with a continuous stirring at 700 rpm. On the other side, the boron derivative was dissolved in 15 ml dd water at 60^{o}C and this solution was added dropwise to the initial stirring pectin solution. Finally 0.025 g of $CaCl_2$

was dissolved in 15 ml dd-water and this $CaCl_2$ solution was added dropwise to the solution containing pectin and the boron derivative. The final mixture was stirred for 10 minutes, and then the resulting film forming solution (a volume of 20 ml) was poured onto the plastic petri plates and dried for 72 hours at ambient temperature until the solvent was completely evaporated. Same method was repeated for all boron derivatives at different concentrations. Samples containing only pectin, glycerol and $CaCl_2$ were considered as the control films.

The dried gelatin and pectin films were peeled-off the surface of the petri plates and the final film samples were obtained.

Table 2.1. Composition of Film Forming Solutions

	Gelatin (g)	**Pectin (g)**	**Boric Acid (g)**	**Disodium Octaborate (g)**	**Sodium Pentaborate (g)**	**Glycerol (g)**	**$CaCl_2$ (g)**	**Water (ml)**
Sample 1	10	-		-	-	3		100
Sample 2	10	-	0.5	-	-	3		100
Sample 3	10	-	1	-	-	3		100
Sample 4	10	-	1.5	-	-	3		100
Sample 5	10	-	-	0.5	-	3		100
Sample 6	10	-	-	1	-	3		100
Sample 7	10	-	-	1.5	-	3		100
Sample 8	10	-	-	-	0.5	3		100
Sample 9	10	-	-	-	1	3		100
Sample 10	10	-	-	-	1.5	3		100
Sample 11	-	2	-	-	-	3	0.025	100
Sample 12	-	2	0.1	-	-	3	0.025	100
Sample 13	-	2	0.2	-	-	3	0.025	100
Sample 14	-	2	0.3	-	-	3	0.025	100
Sample 15	-	2	-	0.1	-	3	0.025	100
Sample 16	-	2	-	0.2	-	3	0.025	100
Sample 17	-	2	-	0.3	-	3	0.025	100
Sample 18	-	2	-	-	0.1	3	0.025	100
Sample 19	-	2	-	-	0.2	3	0.025	100
Sample 20	-	2	-	-	0.3	3	0.025	100

2.2.2. Determination of Antimicrobial Activity of the Films

Modified agar disc diffusion method was employed to determine antimicrobial activities of the developed gelatin and pectin films. Antibacterial and antifungal activities of the films incorporated with different boron derivatives were tested against Gram-positive bacteria (*Staphylococcus aureus*), two Gram-negative bacteria (*Eshericia coli*, *Pseudomonas aeruginosa*) and two fungal strains (*Candida albicans* and *Aspergillus niger*). In the antimicrobial tests, tyriptic soy agar (TSA) medium was used for the bacterial strains (*E. coli, S. aureus and P. aeruginosa*) and potato dextrose agar (PDA) medium was used for the fungal strains (*C. albicans and A. niger*).

To determine the antimicrobial activity, each of the developed films containing different boron derivatives at three different concentrations and a negative control film were aseptically cut into 1 x 1 cm squares. The aseptically prepared square film samples were placed on the surface of inoculated agar plates with a culture of the target indicator microorganism by using a sterile tweezers. The petri dishes were divided into four sections (Figure 2.1), and different three film samples containing the same concentration of the different boron derivatives (boric acid, disodium octaborate and sodium pentaborate) and a negative control film sample without boron derivatives were placed on pre-defined sections.

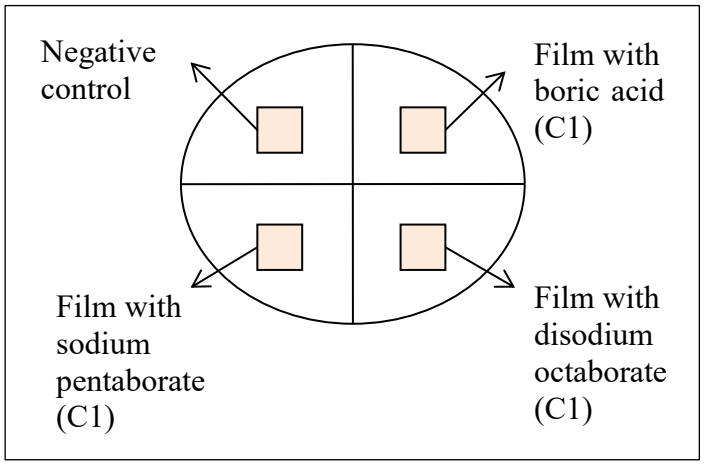


Figure 2.1. Representation of a petri dish.

Petri dishes were sealed with parafilm and left to incubation at 25^{o}C. After 24 hours incubation of bacterial strains and 48 hours incubation of the fungal strains, antimicrobial activities of the films were evaluated measuring the inhibition zone area (colony free area) developed around the film squares with a digital caliper (Mitutoyo Corp). When the inhibition zone was not observed around a film sample, it assumed that the sample does not have inhibitory effect on the target microorganism and zone area was not measured for these samples. The antimicrobial tests were performed in triplicate for each sample.

2.2.3. Rheological Characterization of Film Forming Solutions

Dynamic viscoelasticity and steady state flow measurements were carried out using a controlled strain Kinexus Rheometer (Malvern Instruments Ltd, UK) and the rheological data were obtained from the instrument's software (rSpace for Kinexus). A cone-plate geometry (cone angle 4^{o}, diameter 40 mm) and a cup and bob geometry were used for the rheological tests. Before each analysis, samples were kept on the geometry until it reached to the target temperature.

At first, dynamic measurements of viscoelastic properties of the gelatin and pectin film forming solutions were performed. The amplitude sweep test was applied for each sample in order to measure the linear viscoelastic region (LVER) and to set the upper limit of the LVER. These tests provided information to choose a strain value within the linear region for using in the following oscillation tests. Amplitude sweep tests were performed at a constant frequency of 2 Hz and at 25^{o}C with increasing shear strain from 0.1 to 1000%.

Strain controlled frequency sweep tests were performed for the gelatin and pectin film forming solutions over a frequency range of 0.1-100 Hz at 25^{o}C with 4% shear strain that was chosen from the linear viscoelastic region. Storage modulus was measured as a function of angular frequency and the typical mechanical spectra of the samples was obtained.

Temperature ramp tests were performed within LVER to see the effect of boron incorporation on gelling and melting temperatures of the gelatin gels and film forming solutions by using the cup and bob geometry. Tests were carried out at 4% preselected shear strain and 2 Hz constant frequency. To determine the melting temperature of gelatin gels, the film forming solutions were kept in the rheometer at a temperature of 18^{o}C for 15 minutes

to allow equilibration. Then temperature sweeps were applied on the formed gels at a rate of 1°C/min from 18 to 40°C. To determine the gelling temperatures, gelatin film forming solutions were loaded in geometry that pre-set to 40°C, and during the test temperature was decreased from 40 to 18°C by decreasing at a rate of 1°C/min. Gelling and melting temperatures were determined at temperatures that storage and loss moduli were cross-linked during heating and cooling tests.

Flow curves and viscosities of the gelatin and pectin film forming solutions were obtained from shear rate ramp tests which performed between shear rate range of 1-100 s^{-1} at 25°C using the cone and plate geometry. All rheological measurements were performed in duplicate.

2.2.4. Determination of Physical, Mechanical and Chemical Properties of Films

2.2.4.1. Mechanical Properties of the Films

For determination of the tensile properties of the developed gelatin and pectin films a texture analyzer (TA.XTplus, Stable Micro Systems) was used. Tensile strength (TS) of the films was measured according to the ASTM-D882 standard test method. Tensile grips were attached to base and upper head of texture analyzer and the gap between grips was set to 50 mm. Film specimens (50x20 mm) of each formulation were clamped between tensile grips and the grips were tightened to ensure that the film does not slip out of the grips during the test. When the tensile test was started, the sample was pulled apart at a crosshead speed of 0.5 mm/s until it was broken. A load extension curve was obtained on the Exponent software and the tensile strength was read from the graph. Measurements were done in triplicate for each sample.

2.2.4.2. Morphology of the Films

Morphologies of the pectin and gelatin film samples were analyzed by a scanning electron microscope (EVO 40 series, Carl Zeiss, Germany). Both surface morphology and cross sectional area morphology of the films were examined. Before the SEM imaging, film samples had been kept in a desiccator for 24 hours. Small pieces of film samples were placed

on the SEM specimen holder that is shown in Figure 2.2. Surface of the films were coated with gold at 12-13 nanometer (BAL-TEC SCD 005 Sputter Coater) to enable sample imaging for SEM. Creating this conductive ultra-thin gold layer on samples reduces the thermal damage and increases the signal and surface resolution. SEM micrographs were obtained for all samples.

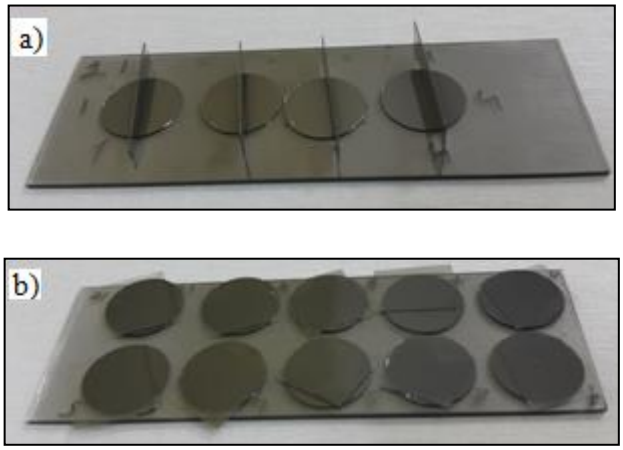


Figure 2.2. Samples for examining a) morphology of cross sectional areas of the films, b) surface morphology of films.

2.2.4.3. Chemical Properties of the Films

The Fourier transform infrared (FT-IR) spectrum of developed gelatin and pectin films were recorded by scanning film samples at wavelengths ranging from 4000 to 600 cm^{-1}, in an infrared spectrometer (FT-IR Nicolet iZ10, Thermo Scientific, USA).

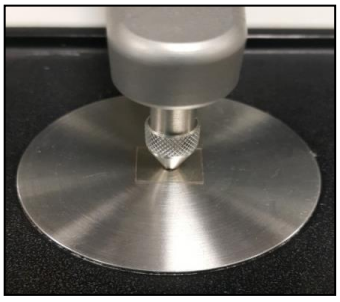


Figure 2.3. Gelatin film sample placed on FT-IR spectrometer

2.2.5. Statistical Analysis

SPSS 20 (SPSS Inc., Chicago, USA) was used for the statistical analyses. The statistical analyses were performed using ANOVA (analysis of variance) and Tukey's test was used. The level of significance was $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial and Antifungal Properties

Antimicrobial and antifungal properties of gelatin and pectin films that incorporated with boric acid, disodium pentaborate and sodium octaborate at different concentrations were evaluated against *Staphylococcus aerus*, *Eshericia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* by using modified disc diffusion method. Films without boron derivatives were used as negative control for the tests.

Antimicrobial and antifungal activity of different boron derivatives (boric acid, disodium pentaborate and sodium octaborate) and the effect of the concentration for each type of boron derivative were assessed with the microbiological studies. As expected, boron-free pectin and gelatin films were not able to create clear zones around the films and they did not have inhibitory effect against tested microorganisms.

Antimicrobial activities of selected boron derivatives against target microorganisms were assessed by modified disc diffusion method are shown in Table 3.1. As seen in the Table 3.1, pectin and gelatin films containing boron derivatives exhibited different effects on the tested microorganisms.

Gelatin films containing different boron derivatives at all concentrations showed inhibitory effect on *S. aureus, P. aeruginosa* and *A. niger* for all film formulations. Except gelatin film containing 5% boric acid, all remaining gelatin films showed antifungal effect against *C. albicans*.

Both gelatin and pectin films could not inhibit the growth of *E. coli*. When the antimicrobial test results of the pectin films were evaluated, it can be seen that only pectin films containing boron derivatives at some certain concentrations were able to create inhibition zones for *S. aureus, P. aeruginosa, A. niger* and *C. albicans.*

Table 3.1. Inhibition effects of film samples incorporated with boron derivatives on bacterial growth.

Bio-polymer	**Anti-microbial agent**	**Antimic. Conc. (based on dry matter)**	***S. aureus***	***P. aeruginosa***	***E. coli***	***A. niger***	***C. albicans***
Gelatin	Control	0%	-	-	-	-	-
	Boric Acid	5%	+	+	-	+	-
		10%	+	+	-	+	+
		15%	+	+	+	+	+
	Disodium Octaborate	5%	+	+	-	+	+
		10%	+	+	-	+	+
		15%	+	+	+	+	+
	Sodium Pentaborate	5%	+	+	-	+	+
		10%	+	+	-	+	+
		15%	+	+	-	+	+
Pectin	Control	0%	-	-	-	-	-
	Boric Acid	5%	-	-	-	+	-
		10%	+	+	-	+	+
		15%	+	+	-	+	-
	Disodium Octaborate	5%	+	-	-	+	-
		10%	+	+	-	+	+
		15%	+	+	-	+	+
	Sodium Pentaborate	5%	+	-	-	+	-
		10%	+	+	-	+	+
		15%	+	+	-	+	+

+: Positive; -: Negative

3.1.1. Antimicrobial and Antifungal Activities of the Pectin Films

Figures 3.1, 3.2, 3.3 and 3.4 show the antimicrobial effectiveness of the pectin films containing boron derivatives through the diameter of inhibition of the microbial growth for tested microorganisms after incubation. The control films (without boron derivatives) were used to compare the microbial growth. When all inhibition zones around the pectin films were evaluated, only pectin films containing boron derivatives at 10% and 15% concentrations have significant inhibitory effect on *S. aureus* and *P. aeruginosa* bacteria ($p<0.05$). On the other hand, *E. coli* was the most resistant of the tested bacteria to the action of boron derivatives and no inhibition zone was observed around the samples against *E. coli* for each type of sample.

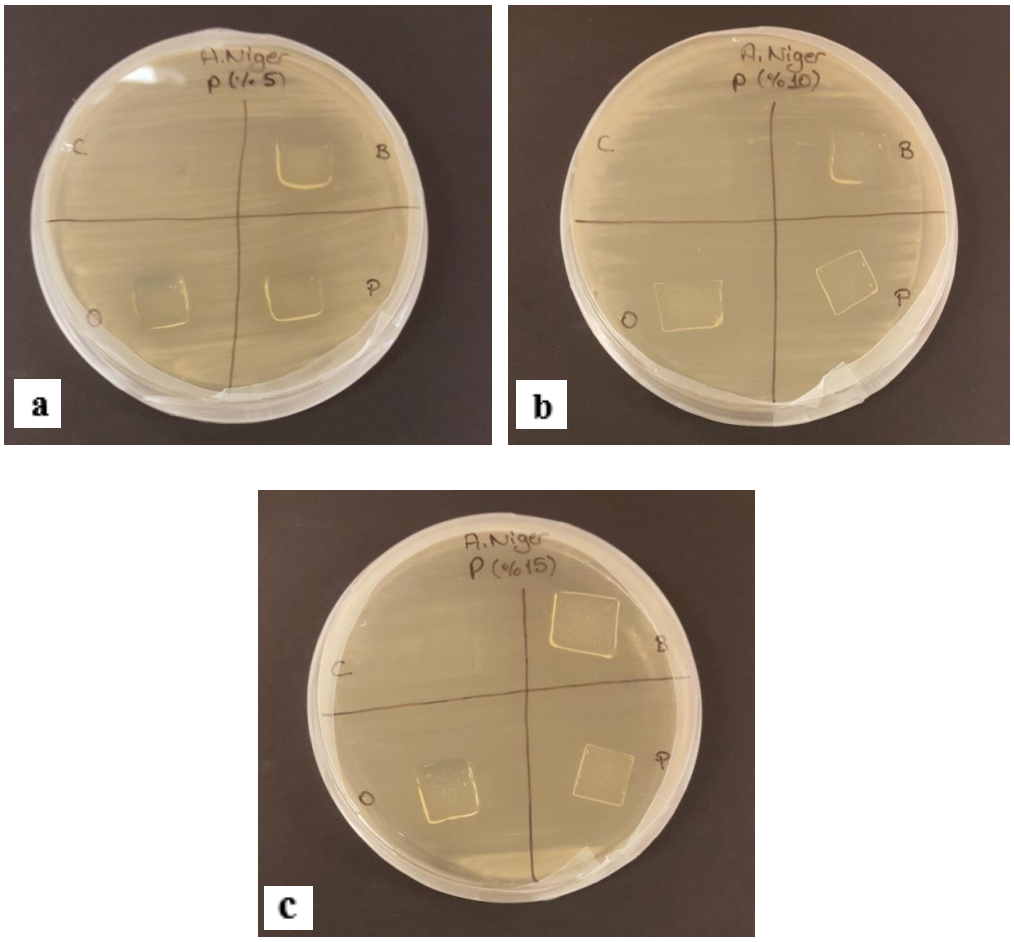


Figure 3.1. Inhibitory effect of pectin films incorporated with boron derivatives against *Aspergillus niger*.

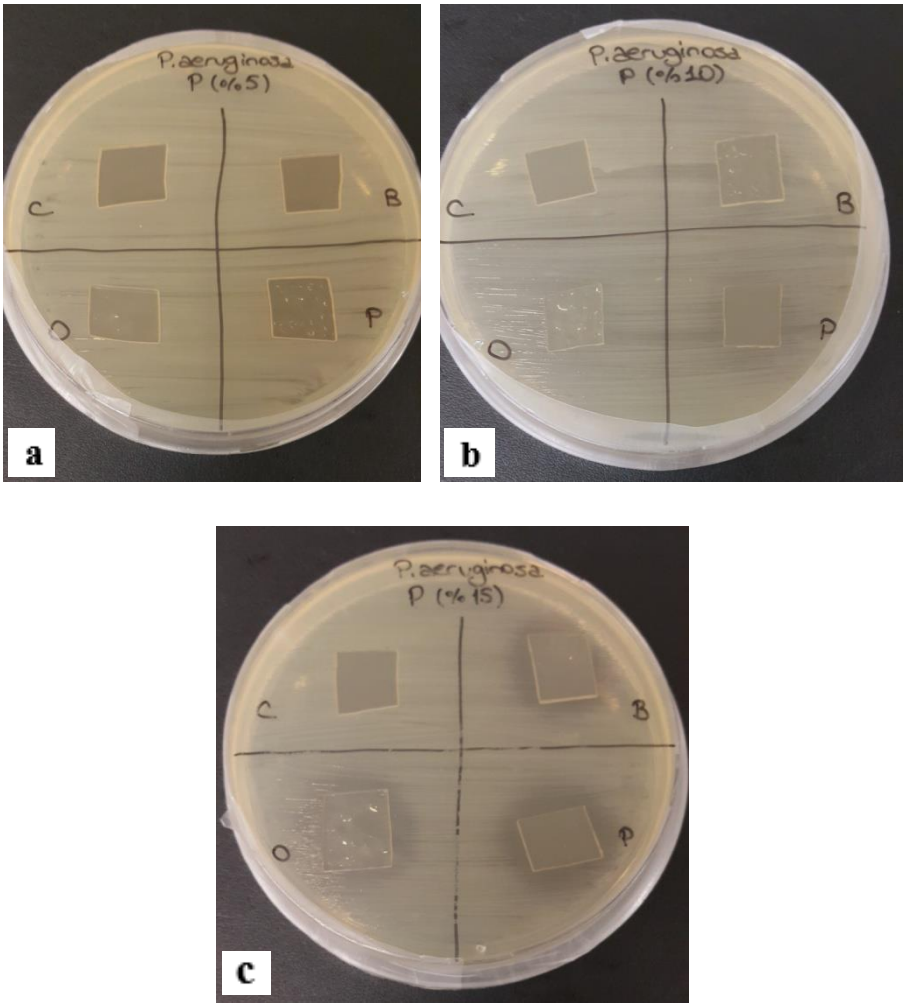
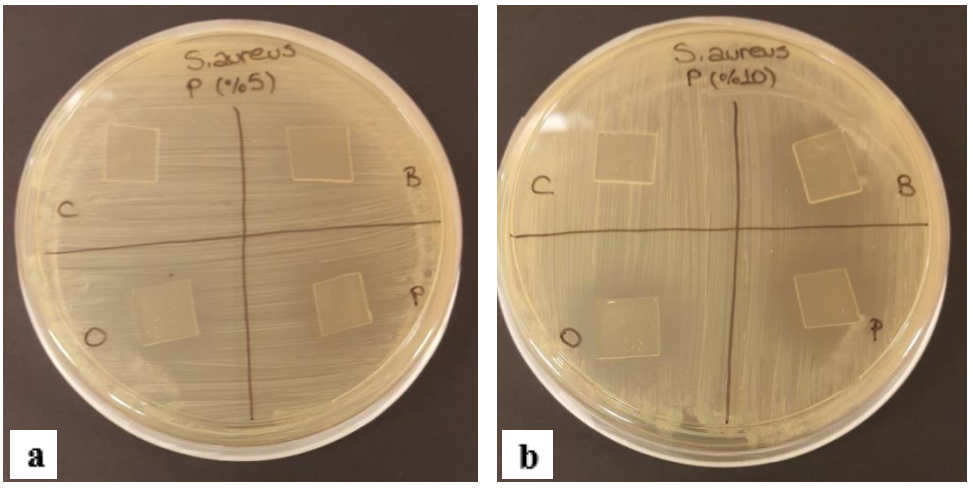


Figure 3.2. Inhibitory effect of pectin films incorporated with boron derivatives against *Pseudomonas aeruginosa*.



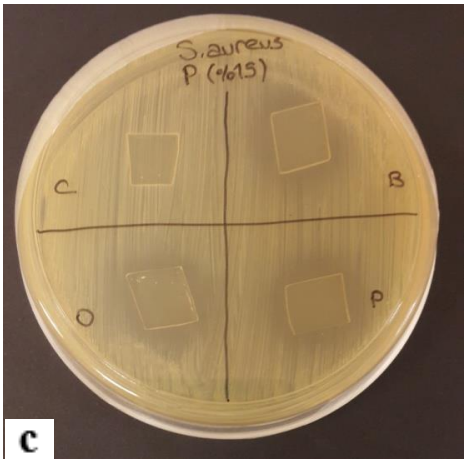


Figure 3.3. Inhibitory effect of pectin films incorporated with boron derivatives against *Staphylococcus aerus.*

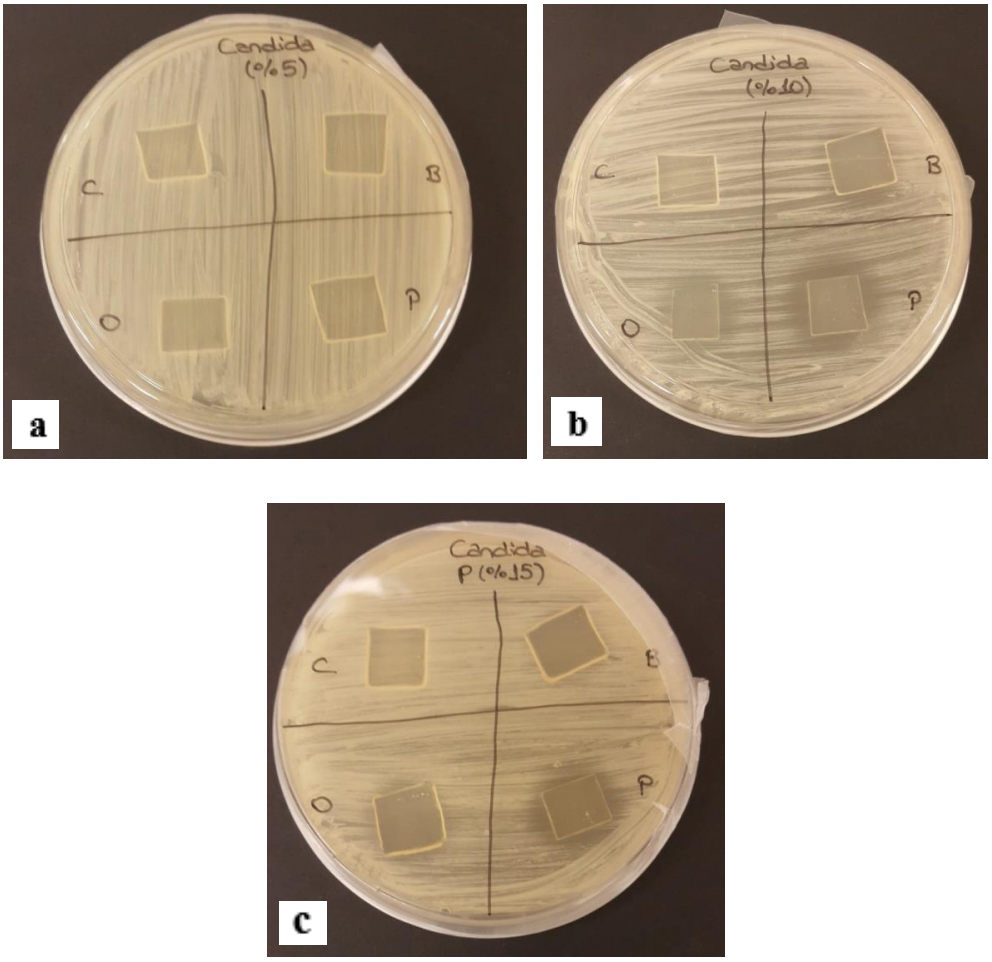


Figure 3.4. Inhibitory effect of pectin films incorporated with boron derivatives against *Candida albicans.*

Table 3.2. Antimicrobial and antifungal activity of pectin films incorporated with boron derivatives at different concentrations.

Conc.	Antimicrobial agent	Inhibition Zones (mm)				
		S. aureus	***P. aeruginosa***	***E. coli***	***A.niger***	***C.albicans***
0%	**--**	0.00 [a]	0.00 [c]	0.00	0.00[f]	0.00[g]
5%	**Boric Acid**	0.00 [a]	0.00 [c]	0.00	7.63[f]	0.00[g]
	Sodium Pentaborate	4.70 [a]	0.00 [c]	0.00	6.43[f]	0.00[g]
	Disodium Octaborate	5.68 [a]	0.00 [c]	0.00	6.06[f]	0.00[g]
10%	**Boric Acid**	17.61 [b]	4.37 [c]	0.00	16.35[f]	4.65[g]
	Sodium Pentaborate	17.76 [b]	14.46 [d]	0.00	17.80[f]	11.49[g]
	Disodium Octaborate	18.01 [b]	13.22 [d]	0.00	16.08[f]	5.15[g]
15%	**Boric Acid**	19.36 [b]	18.39 [e]	0.00	19.30[f]	0.00[g]
	Sodium Pentaborate	19.12 [b]	18.71 [e]	0.00	21.21[f]	11.37[g]
	Disodium Octaborate	19.39 [b]	19.53 [e]	0.00	20.78[f]	5.78[g]

*The mean values with the same letter (within the same column) are not significantly different at P=0.05 level.

3.1.2. Antimicrobial and Antifungal Activities of the Gelatin Films

The inhibitory zones of the gelatin films were shown in Figures 3.5, 3.6, 3.7 and 3.8. Also diameters of the inhibition zones were shown in Table 3.3. Similar to the pectin films, gelatin films containing boron derivatives did not have inhibitory effect on *E. coli;* only slight zones were appeared around the film samples incorporated with boric acid and disodium octaborate at 15% concentration. All formulations of gelatin films containing boron derivatives have significant inhibitory effect on *S. aureus*, *P. aeruginosa* and *A. niger* ($p<0.05$). While gelatin films containing 5% concentration of the each boron derivative did not show significant inhibitory effect on *C. albicans*, films containing all boron derivatives at 10% and 15% concentrations had significant antifungal effect on *C. albicans.*

All gelatin composite films with boron derivatives showed decrease in the cell viabilities of *S. aureus, P. aeruginosa, C. albicans* and *A. niger*. When the pectin and gelatin film samples compared, gelatin based samples had more inhibition effect and formed larger inhibition zones than the pectin based samples. The more improved antimicrobial and antifungal activities of gelatin based samples found in this study were attributable to the larger amounts of boron derivatives in the gelatin based film samples. Because the amount of incorporated boron derivatives were optimized according to the concentration of biopolymer used. Since gelatin and pectin have different characteristics and different optimum concentrations to form a film, used amounts of the boron derivatives were different. Since it was possible to incorporate more boron derivatives into gelatin films due to the higher amount of gelatin used in film forming solutions, antimicrobial and antifungal activity of gelatin films were found to be higher.

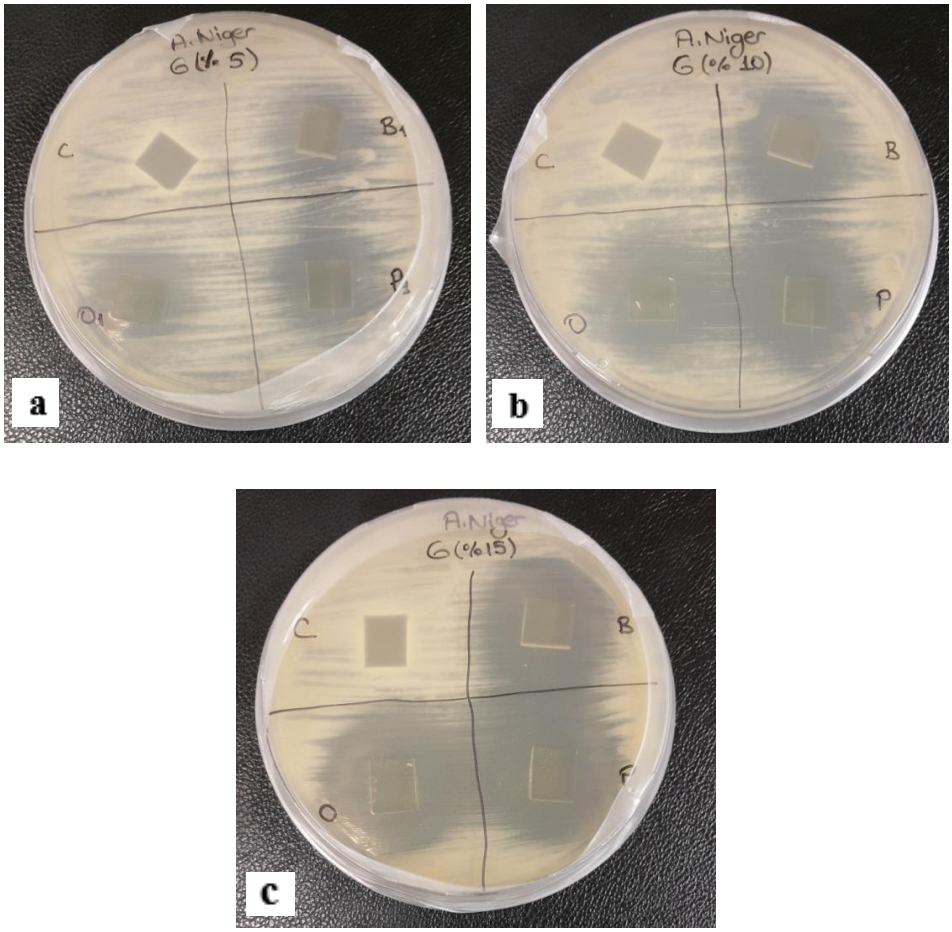


Figure 3.5. Inhibitory effect of gelatin films incorporated with boron derivatives against *Aspergillus niger*.

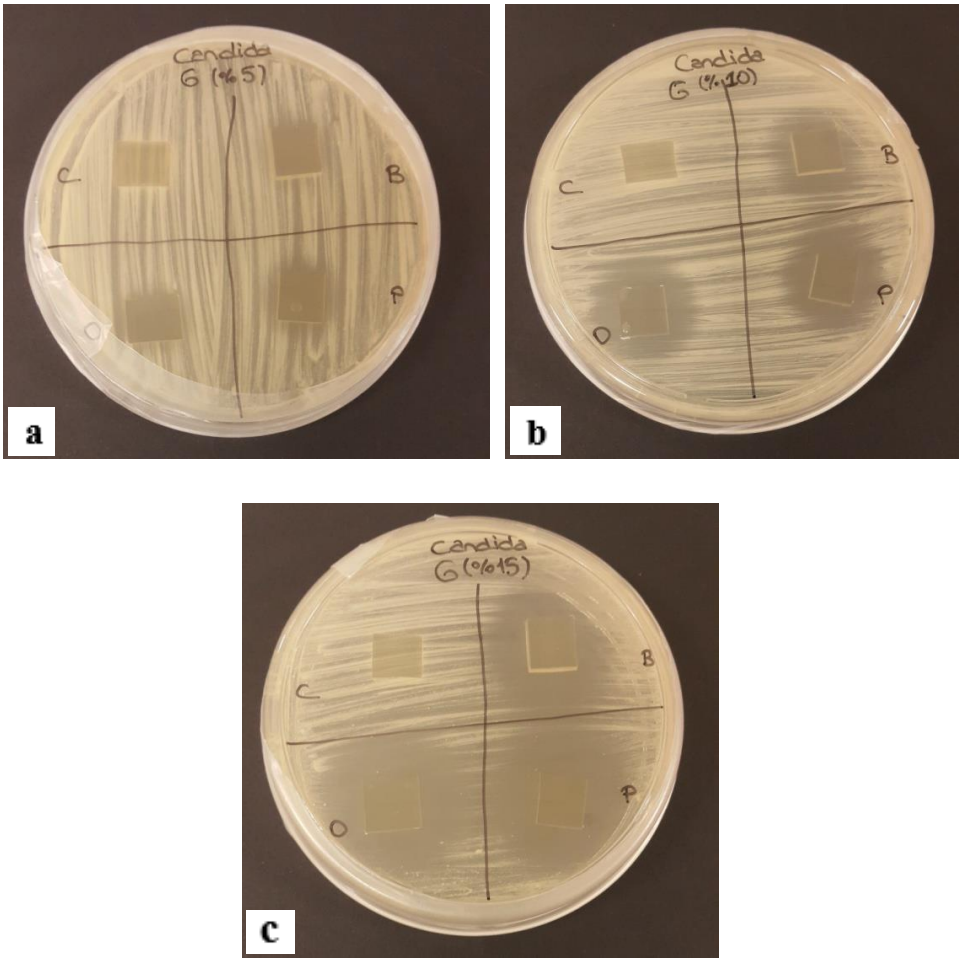
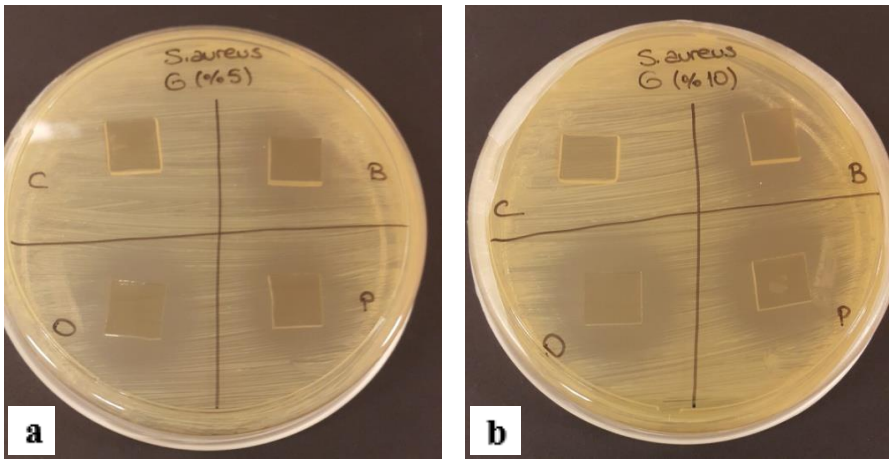


Figure 3.6. Inhibitory effect of gelatin films incorporated with boron derivatives against *Candida albicans.*



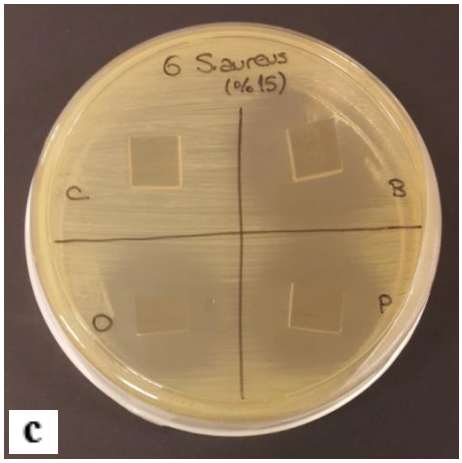


Figure 3.7. Inhibitory effect of gelatin films incorporated with boron derivatives against *Staphylococcus aerus.*

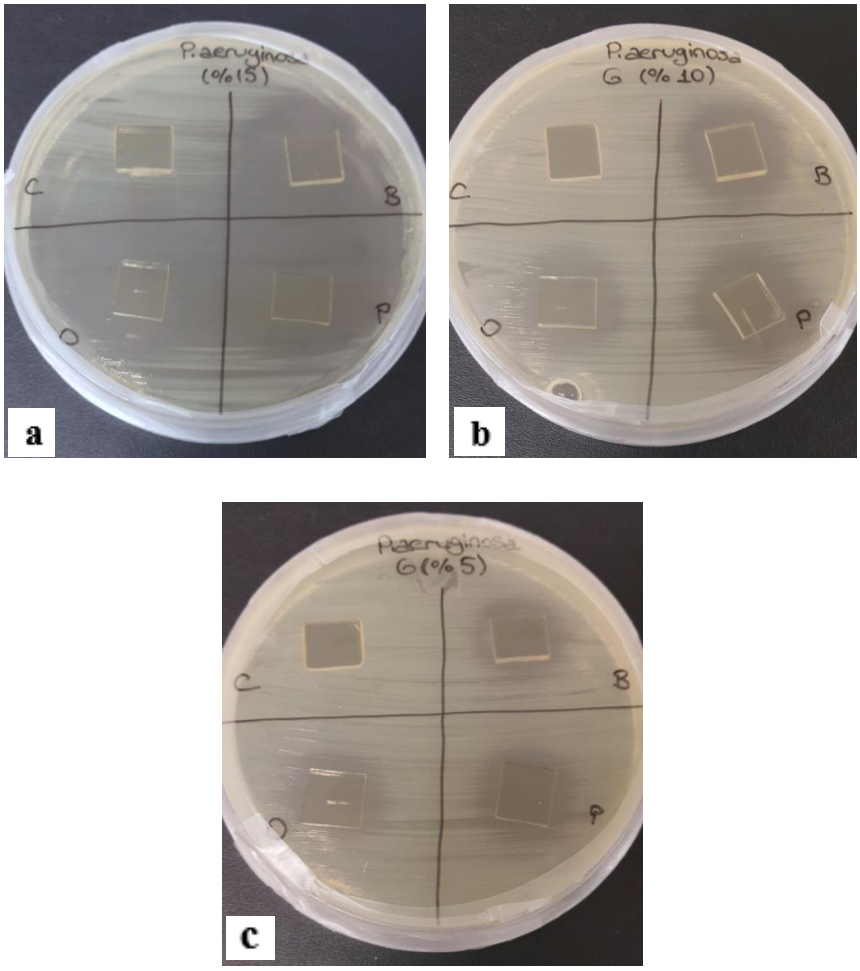


Figure 3.8. Inhibitory effect of gelatin films incorporated with boron derivatives against *Pseudomonas aeruginosa.*

Table 3.3. Antimicrobial and antifungal activity of gelatin films incorporated with boron derivatives at different concentrations.

Conc.	Antimicrobial agent	Inhibition Zones (mm)				
		S. aureus	*P. aeruginosa*	*E. coli*	*A. niger*	*C. albicans*
0%	--	0.00	0.00	0.00	0.00	0.00 [m]
5%	**Boric Acid**	17.61 [a]	14.41	0.00	24.59 [i]	0.00 [m]
	Sodium Pentaborate	17.70 [a]	20.15 [e,f,g]	0.00	25.57 [i,j]	5.17 [m]
	Disodium Octaborate	19.22 [a]	19.02 [e,f]	0.00	26.93 [i,j,l]	4.25 [m]
10%	**Boric Acid**	23.54 [b,c]	19.53 [f]	0.00	29.57 [j,k]	20.44 [n]
	Sodium Pentaborate	22.53 [b]	20.15 [e,f,g]	0.00	29.40 [j,k]	21.05 [n]
	Disodium Octaborate	23.80 [b,c]	19.02 [e,f]	0.00	31.18 [k,l]	21.09 [n]
15%	**Boric Acid**	26.80 [c]	24.79 [g,h]	5.04	32.17 [k]	23.62 [n]
	Sodium Pentaborate	25.90 [b,c]	23.91 [g,h]	0.00	33.56 [k]	22.86 [n]
	Disodium Octaborate	26.79 [c]	25.52 [h]	3.37	33.39 [k]	24.60 [n]

*The mean values with the same letter (within the same column) are not significantly different at P=0.05 level.

3.2. RHEOLOGY OF FILM FORMING SOLUTIONS (FFs)

Storage modulus, loss modulus, viscosity and melting/gelling temperatures of boron derivatives added pectin and gelatin film forming solutions were determined by rheological measurements.

3.2.1. Rheological Characteristics of Pectin FFSs

Steady and dynamic behaviors of the boron derivatives added pectin film forming solutions were examined with rheological tests.

3.2.1.1. Dynamic Behavior

Linear viscoelastic region (LVER) is the region where viscoelastic properties of gels are independent from magnitude of applied strain or stress. In the linear viscoelastic range; storage and loss modulus remain constant while oscillation strain or stress is changing. All rheological tests should be performed in this region to prevent breakdown of sample structure.

To determine the linear viscoelastic region of all samples, freshly prepared pectin film forming solutions were subjected to an amplitude sweep test at 25ºC. In the Figure 3.11, results of the amplitude sweep test were shown. As can be seen in the figure below, the storage modulus of pectin film forming solutions remained constant for a wide range of varying strain and after a strain value the modulus was started to decrease dramatically. Therefore, the strain of 4% was selected within the common linear viscoelastic region of all samples in order to use in the following rheological measurements.

For all boric acid concentrations, the highest G' was observed at concentration of 10%, similarly in sodium octaborate and sodium pentaborate. The storage modulus was decreased by addition of boric acid at 15% concentration, but was almost the same for 5% and 10% concentrations. Disodium octaborate incorporation increased the storage modulus at 5% and 10% concentrations when compared to the control sample, but the modulus is less than the control for 15% concentration. At 10% disodium octaborate concentration, it was observed that storage modulus reached its highest value. For the sodium pentaborate inorporated gels, results of the amplitude sweep test is similar with the results of disodium octaborate incorporated gels. The storage modulus was higher for 10% sodium pentaborate concentration; while control, 5% and 15% concentrations had close modulus values.

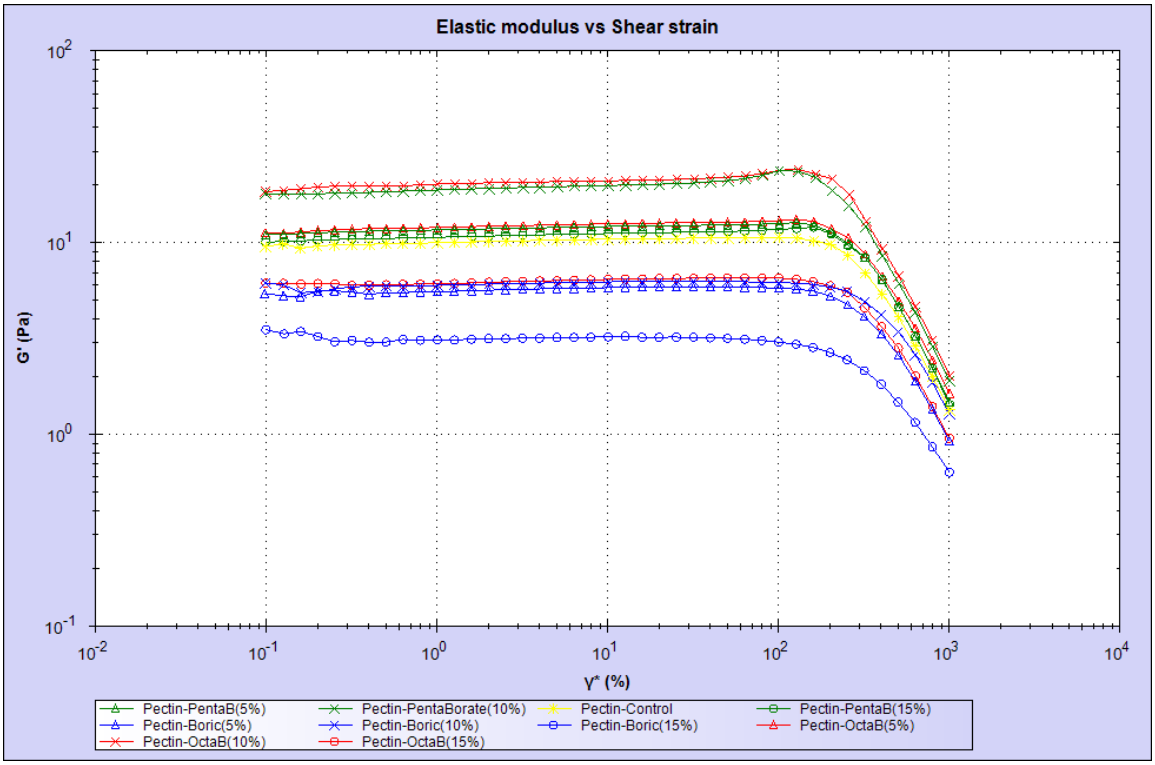


Figure 3.9. Linear viscoelastic region of pectin film forming solutions incorporated with boron derivatives at different concentrations at 25ºC.

Change of storage (G') modulus with frequency is known as the mechanical spectrum of a material. Mechanical spectra within LVER provided information on structure and stability of material at rest and upon during transport. Typical mechanical spectrum of gels has a solid-like behavior predominant over the liquid like behavior and vice versa for solutions.

The frequency sweep tests were carried out at strain of 4% within linear viscoelastic region in the frequency range of 0.1 – 100 Hz to evaluate the effect of the type and concentration of the boron derivatives on dynamic viscoelastic properties of pectin film forming solutions (Figure 3.10). Logarithmic representation of the storage modulus as a function of frequency shows the characteristic spectrum of the pectin FFS. The highest G' value for pectin FFSs was observed at 10% disodium octaborate incorporated solution, 10% boric acid incorporated solution and 15% sodium pentaborate incorporated solution. Solution formulations with boric acid had the smallest G' value compared to the solutions with sodium pentaborate and disodium octaborate. It can be concluded that only addition of disodium octaborate at 10% concentration increased the G' value and the network formation compared to control.

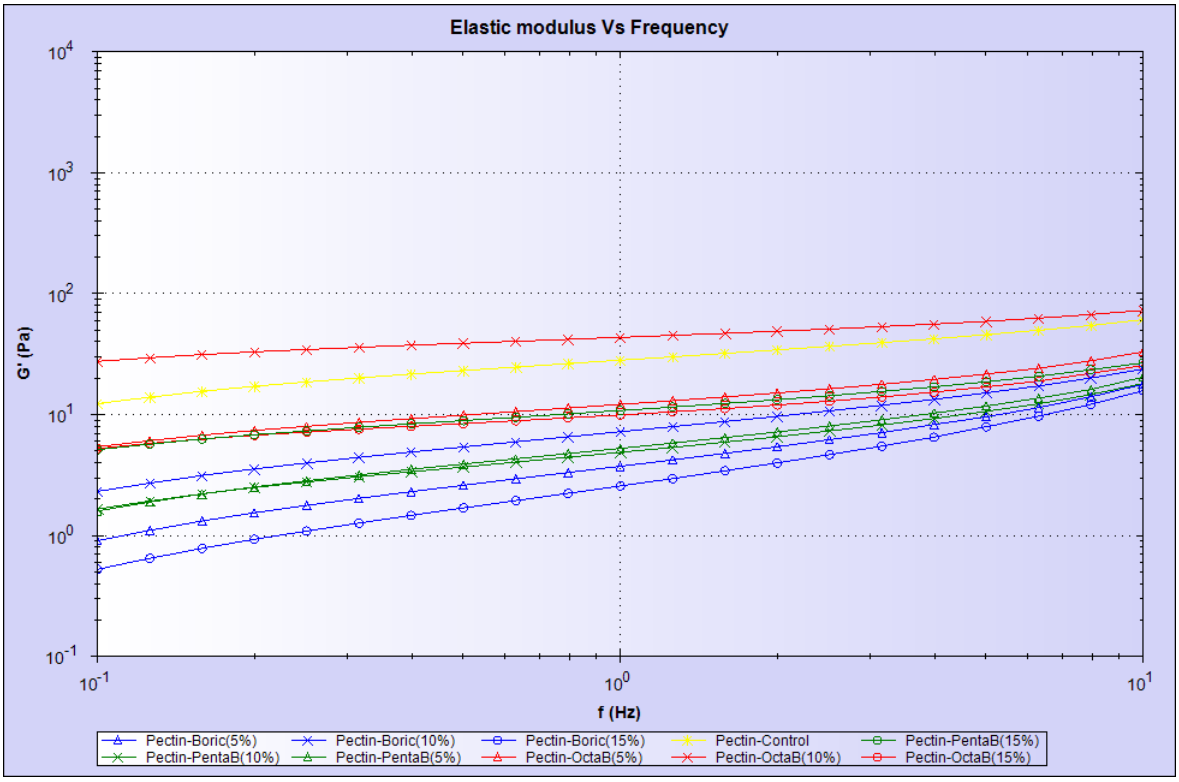


Figure 3.10. Frequency dependence of elastic modulus (G') for pectin FFS with boron derivatives at different concentrations at 25ºC.

3.2.1.2. Steady Shear Behavior

Viscosity-shear rate profiles of pectin film forming solutions incorporated with different boron derivatives (boric acid, sodium pentaborate and disodium octaborate) at different concentrations were plotted in Figure 3.11. It was observed that all pectin FFS presented shear thinning behavior as the viscosity decreased with increasing shear rate. Pectin FFS incorporated with boric acid of 15% concentration yielded the smallest viscosity; while the disodium octaborate (10%) incorporated pectin solution had the maximum viscosity value. As seen on the results of the previous rheological studies (LVER determination and mechanical spectrum analysis), disodium octaborate addition at 10% concentration have the highest improvement on characteristics of pectin FFS.

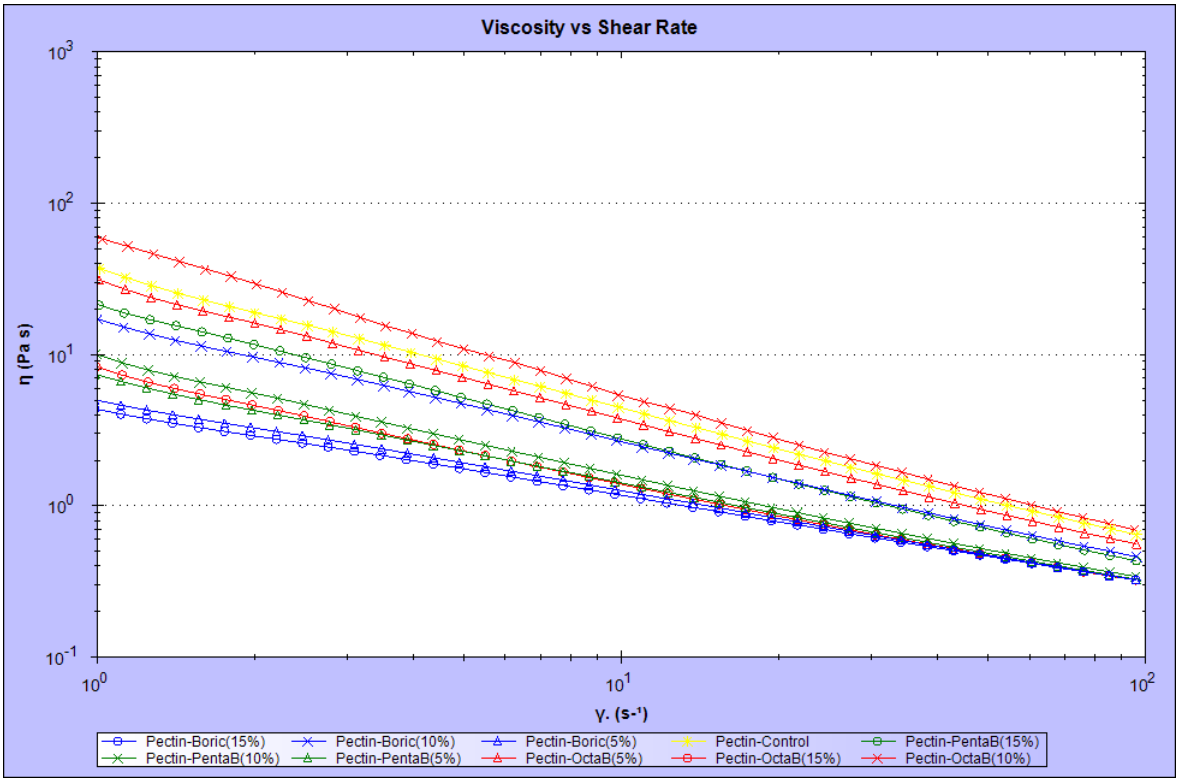


Figure 3.11. Viscosity curve of pectin FFSs incorporated with boron derivatives at 25°C.

3.2.2. Rheological Characteristics of Gelatin FFSs

Steady and dynamic behaviors of the boron derivatives added gelatin film forming solutions were examined with rheological tests.

3.2.2.1. Dynamic Behavior

Dynamic behavior tests study the viscoelastic properties of gelatin FFSs incorporated with boron derivatives at different concentrations. In order to determine the linear viscoelastic region of gelatin FFSs, freshly prepared samples were subjected to an amplitude test at 20°C. In the Figure 3.12, results of the amplitude sweep tests were presented. The storage modulus of gelatin solutions increased throughout the test since gelatin starts to gel at temperatures lower than 35°C. In the range studied, no decrease in the modulus was observed. This means that no structural breakdown realized in the studied shear range. Therefore, similar to pectin 4% strain was also selected for the following oscillation tests.

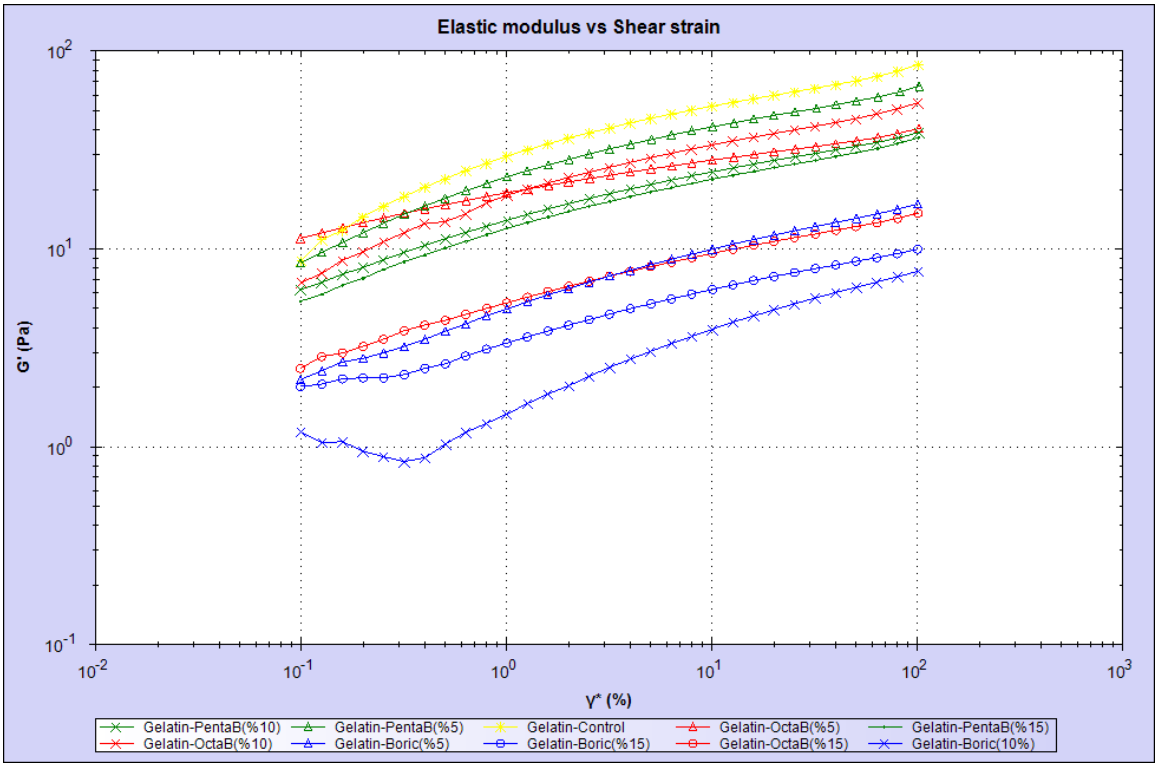


Figure 3.12. Linear viscoelastic regions of gelatin FFSs incorporated with boron derivatives at different concentrations at 20ºC.

The frequency sweep tests were carried out at strain of 4% within linear viscoelastic region in the frequency range of 0.1 – 100 Hz. Effect of the type and concentration of the boron derivatives on dynamic viscoelastic properties of gelatin film forming solutions was studied. Logarithmic representation of the storage modulus as a function of frequency shows the characteristic spectrum of the gelatin FFS (Figure 3.13).

Addition of boron derivatives did not improve the G' values of gelatin gels. On the contrary except the gels with 10% disodium octaborate, the G' values are lower than control. Overall, boric acid incorporated samples have the lowest G' values.

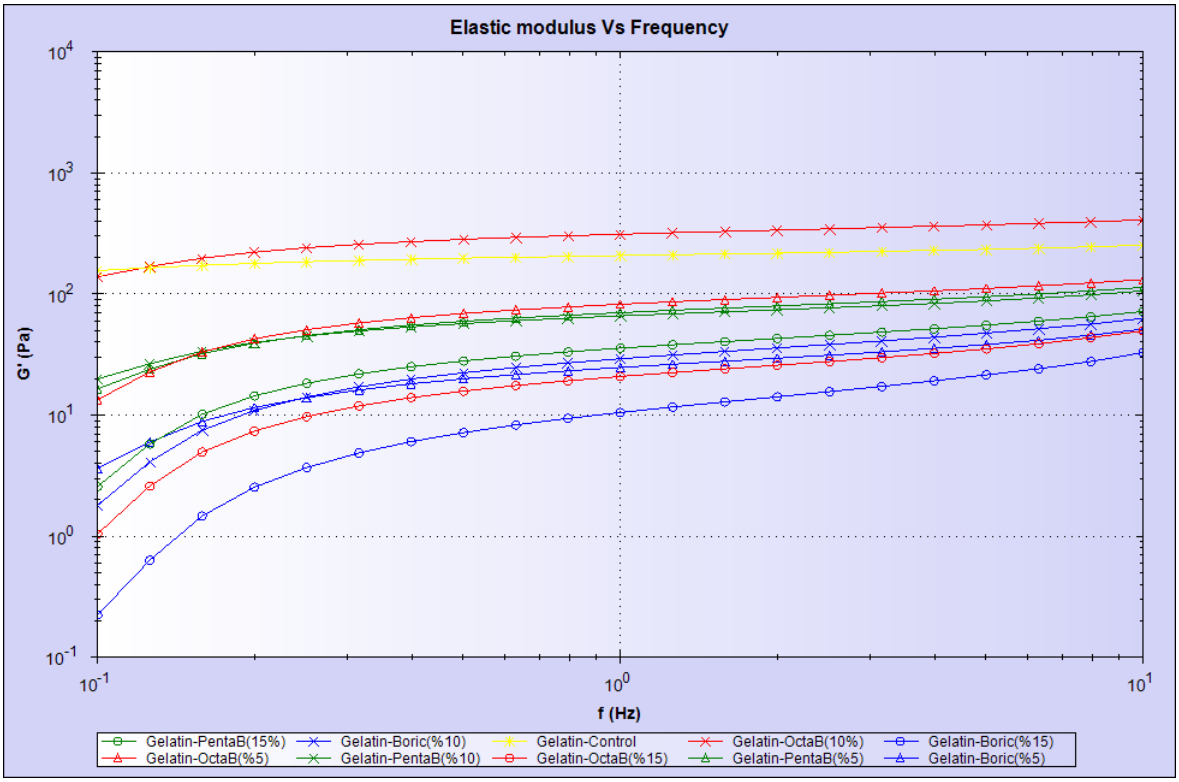


Figure 3.13. Frequency dependence of elastic modulus (G') for gelatin gels with boron derivatives at different concentrations at 25ºC.

Temperature sweep test shows the transitions occurring in the material as well as the changes in the viscoelasticity of the material depending on the temperature. Temperature sweep measurements of gelatin FFSs were conducted with a pre-selected constant strain (4%) and frequency (2 Hz) to observe the structural build up, gelling and melting behaviors with changing temperature.

Both melting and gelling temperatures were measured since gelatin forms thermoreversible gels. To determine the gelling point of gelatin FFSs with different formulations, the samples were subjected to temperature ramp between 20-40ºC. When the gelling and melting points of all gelatin FFSs are compared (Table 3.4), it can be concluded that boron derivatives do not have effect on gelling temperature of gelatin. Similarly, melting temperature of gelatin gels was not affected by the addition of boron derivatives. This shows that the addition of boron derivatives to the gelatin solutions does not interfere with the network formation.

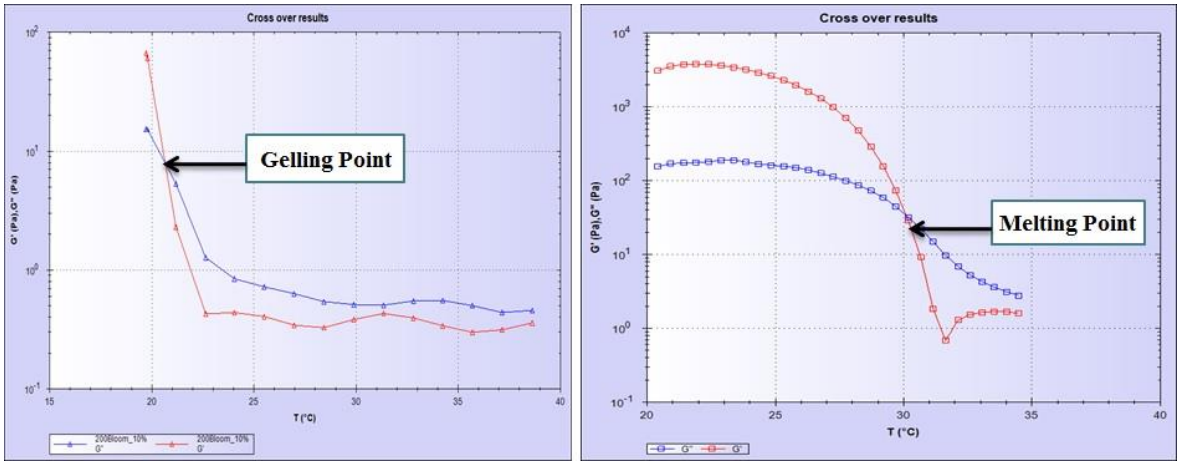


Figure 3.14. Gelling and melting points of the gelatin gels.

Table 3.4. Gelling (T_G) and melting (T_M) points of gelatin FFSs with different formulations.

	Control	**Boric Acid**			**Disodium Octaborate**			**Sodium Pentaborate**		
Conc.	**0%**	**5%**	**10%**	**15%**	**5%**	**10%**	**15%**	**5%**	**10%**	**15%**
T_G	22.7ºC	22.3ºC	22.2ºC	21.4ºC	22.3ºC	21.8ºC	21.4ºC	22.3ºC	21.9ºC	21.4ºC
T_M	30.9ºC	30.7ºC	30.5ºC	30.4ºC	30.9ºC	31.1ºC	30.5ºC	31.1ºC	30.5ºC	30.5ºC

3.2.2.2. Steady Shear Behavior

Viscosity-shear rate profiles of gelatin film forming solutions incorporated with different boron derivatives (boric acid, sodium pentaborate and disodium octaborate) at different concentrations were plotted in Figure 3.15. The viscosities of the gelatin film forming solutions decreased with the shear rate applied. Remarkable decrease in viscosity has been observed in solutions with boric acid and disodium octaborate at 15% concentrations and these FFSs were less dependent on the applied shear rate than other samples.

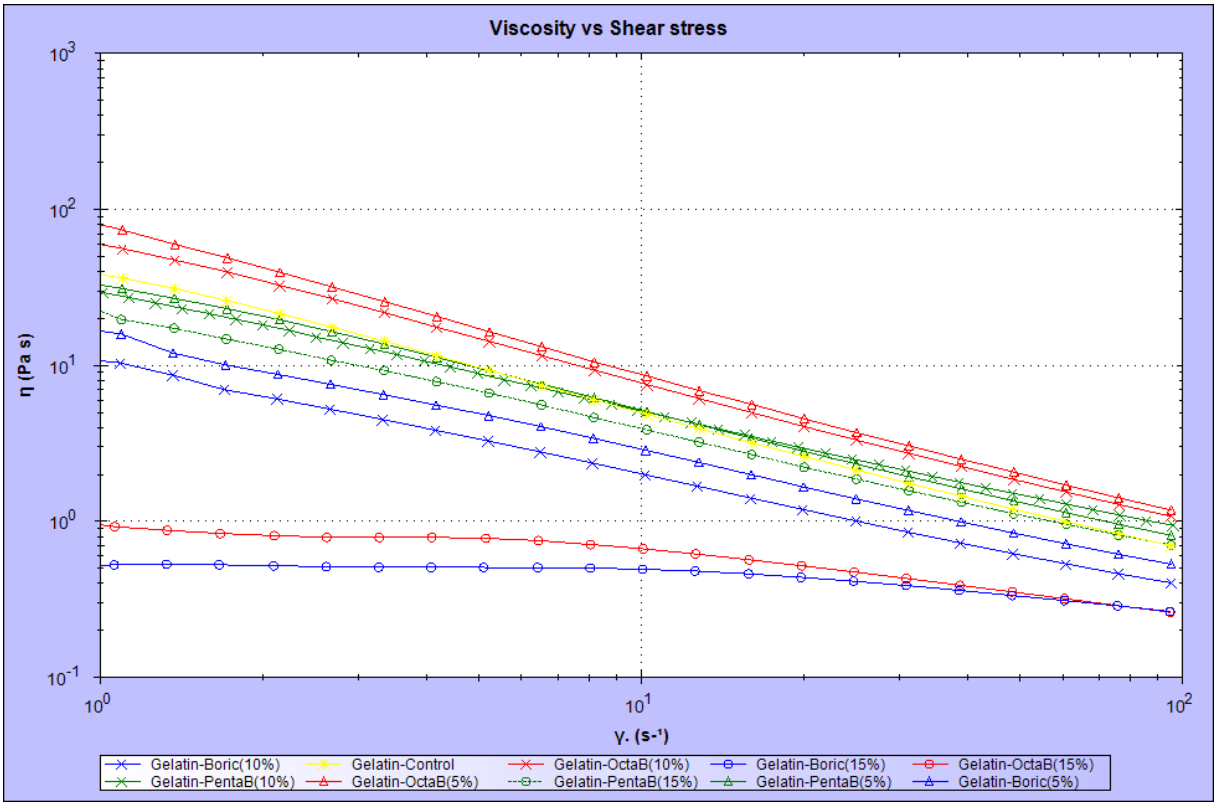


Figure 3.15. Viscosity curve of the gelatin FFSs incorporated with boron derivatives at 25°C.

3.3. PHYSICAL, MECHANICAL AND CHEMICAL PROPERTIES

3.3.1. Physical Properties of the Pectin Films

Physical appearance of a pectin film incorporated with boric acid at 15% concentration obtained by the solvent casting method can be seen in the Figure 3.16. Homogeneous and clear films with completely dissolved boron derivatives can be achieved even at the highest concentration of the derivative used (15%). All pectin films incorporated with different boron derivatives were clear (transparent), smooth, elastic, and easy to peel and handle.

Figure 3.16. Boric acid (15%) incorporated pectin film.

3.3.2. Mechanical Properties of the Pectin Films

Mechanical resistance of a film is expressed as the tensile strength (TS). Mechanical properties of the films are very important for their use in packaging applications. Since the incorporation of boron derivatives might influence mechanical properties of the biopolymer films, the active films were submitted to physical characterization. Tensile strength of the pectin films was shown in Figure 3.17 and it was seen that incorporation of boron derivatives into the pectin films enhanced the tensile strength and the mechanical resistance of the films compared to the control film. While the tensile strength of the control film was 963 g, it was changed between 1070 and 2170 g with addition of boron derivatives and reached to the highest value with sodium pentaborate incorporation at 15% concentration. Except addition of disodium octaborate at 5% concentration, incorporation of the boron derivatives increased the tensile strength values significantly ($p<0.05$). While increase in the sodium pentaborate concentration raise the tensile strength, boric acid and disodium octaborate incorporation did not improve the film strength above concentration of 10%. Potential cross-linkage formation between boron and pectic polysaccharides may be the cause of the change in the tensile strength.

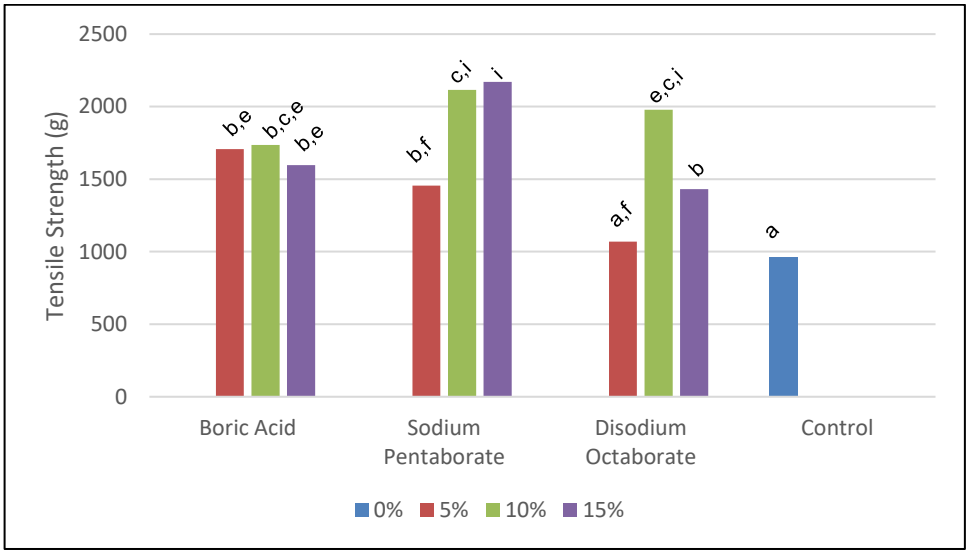


Figure 3.17. Effect of boron derivatives on tensile strength of pectin films (The mean values with the same letter are not significantly different at P=0.05).

Film thickness of the pectin films incorporated with different boron derivatives was shown in Table 3.5. Film thickness varied between the range of 0.14 and 0.21 mm.

Table 3.5. Film thickness of the pectin films.

		Thickness (mm)		Average (mm)	STD
Control	0%	0.2	0.21	0.21	0.01
Boric Acid	5%	0.14	0.13	0.14	0.01
	10%	0.16	0.15	0.16	0.01
	15%	0.14	0.13	0.14	0.01
Sodium Pentaborate	5%	0.18	0.18	0.18	0.00
	10%	0.15	0.15	0.15	0.00
	15%	0.16	0.15	0.16	0.01
Disodium Octaborate	5%	0.18	0.17	0.18	0.01
	10%	0.18	0.18	0.18	0.00
	15%	0.16	0.15	0.16	0.01

3.3.3. Physical Properties of the Gelatin Films

Physical appearance of the gelatin film incorporated with boric acid at 15% concentration obtained by the solvent casting method can be seen in the Figure 3.18. Similar with the pectin films, homogeneous and clear films with completely dissolved boron derivatives were achieved even at the highest concentration of derivative used (15%). Bubble formation was not observed in the gelatin films. Gelatin films have characteristic yellowish color while the pectin sample was completely transparent, and also they were less elastic compared to pectin films.

Figure 3.18. Boric acid (15%) incorporated gelatin film.

3.3.4. Mechanical Properties of the Gelatin Films

Tensile strengths of the gelatin films were shown in Figure 3.19. Incorporation of boron derivatives into the gelatin films enhanced the tensile strength and mechanical resistance of the films compared to the control film. While the average tensile strength of the control film was 9851 g, values of the tensile force ranged from 11375 and 17172 g with changing concentration and type of the boron derivative. Maximum tensile strength of gelatin film was achieved with the incorporation of disodium octaborate at 10% concentration. Similar to the pectin films, incorporation of the boron derivatives increased the tensile strength values significantly ($p<0.05$) except addition of disodium octaborate at 5% concentration. Addition of disodium octaborate at 10% concentration increased the mechanical strength of

gelatin film by 74% percent. While tensile strength increased with increasing boric acid concentration, sodium pentaborate and disodium octaborate incorporation did not improve the film strength above concentration of 10%. The underlying reason might be that the boron derivatives act as fillers between intermolecular and inter-chain spaces preventing the helix formation of gelatin chains after a certain concentration.

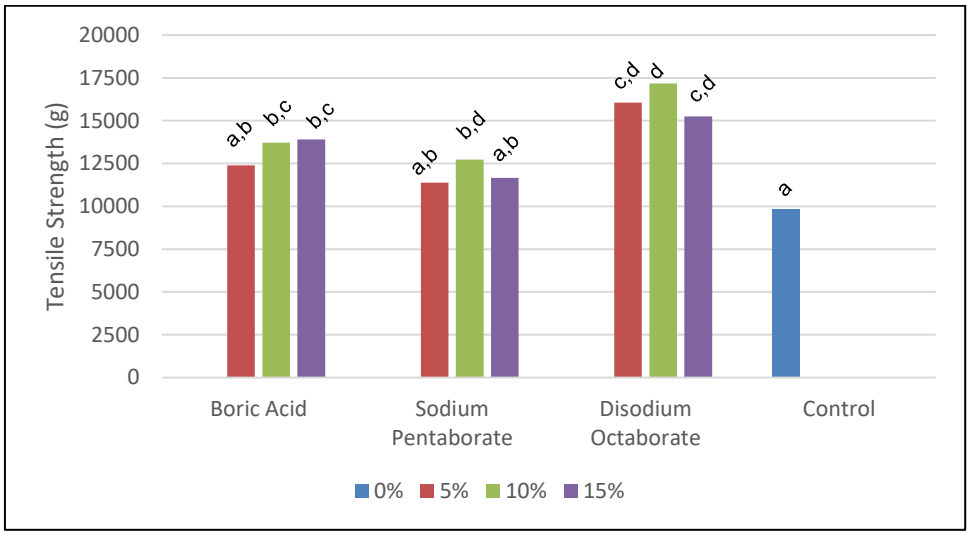


Figure 3.19. Effect of boron derivatives on tensile strength of gelatin films (The mean values with the same letter are not significantly different at P=0.05).

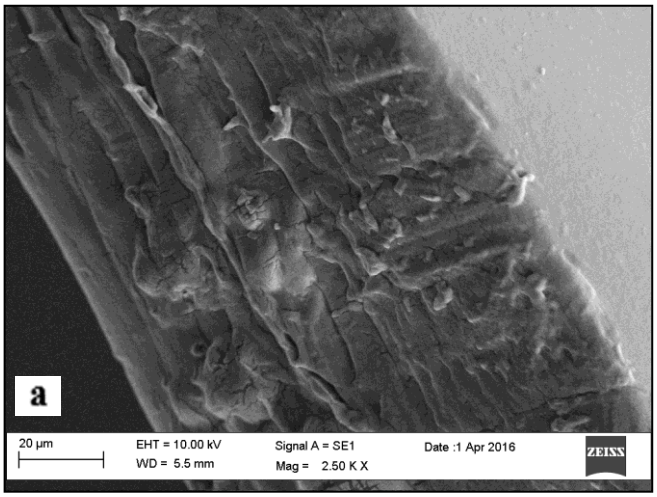
Film thickness of the gelatin films incorporated with different boron derivatives that varied between the range of 0.14 and 0.21 mm was shown in Table 3.6. Different from the pectin films, gelatin films were thicker and harder. This difference was caused by biopolymer concentrations used to form pectin and gelatin films, and may be associated to differences of molecular mass of the biopolymers.

Table 3.6. Film thickness of gelatin films.

		Thickness (mm)		Average (mm)	STD
Control	0%	0.26	0.25	0.26	0.01
Boric Acid	5%	0.22	0.25	0.24	0.02
	10%	0.29	0.28	0.29	0.01
	15%	0.27	0.29	0.28	0.01
Sodium Pentaborate	5%	0.25	0.26	0.26	0.01
	10%	0.26	0.24	0.25	0.01
	15%	0.26	0.26	0.26	0.00
Disodium Octaborate	5%	0.25	0.26	0.26	0.01
	10%	0.28	0.28	0.28	0.00
	15%	0.28	0.28	0.28	0.00

3.3.5. Microstructure of the Pectin Films

Scanning electron microscopy observations were carried out to get a better insight in the homogeneity and the microscopic structure of gelatin and pectin films. Scanning electron micrographs of cross sections of pectin films with different boron derivatives can be seen in Figure 3.20. There is no significant microstructural difference between the cross sectional areas of the pectin films containing boron derivatives and the control film. The film containing boric acid showed more structural discontinuities while the films containing sodium pentaborate and disodium octaborate had more smooth cross sectional regions.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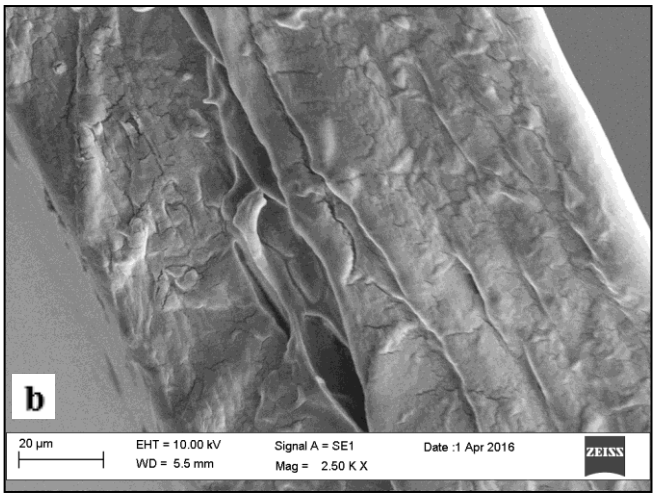
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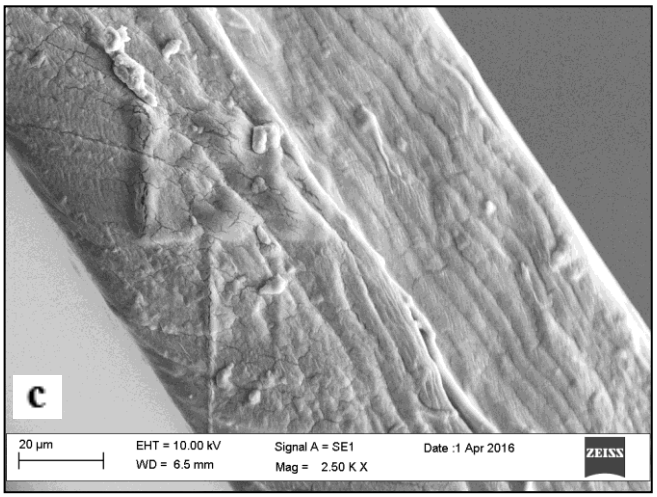
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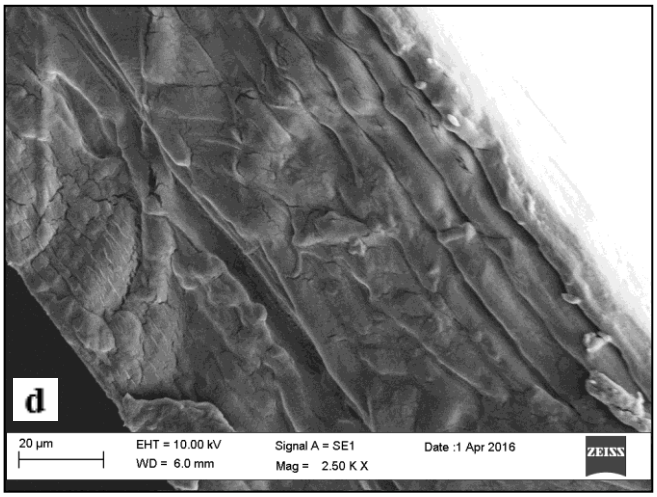
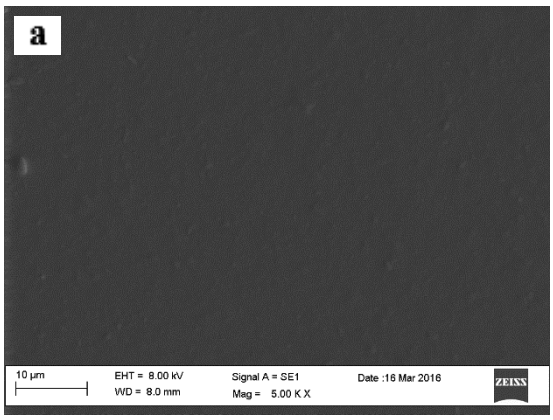
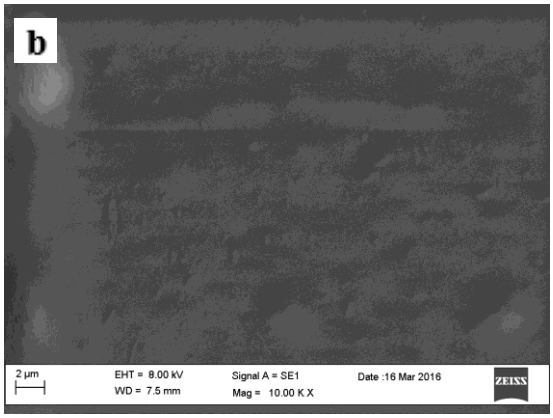


Figure 3.20. Cross-sectional area of a) negative control pectin film, b) pectin film with disodium octaborate of 10% concentration, c) pectin film with sodium pentaborate of 10% concentration, d) pectin film with boric acid of 10% concentration.

Scanning electron micrographs of surface area of pectin films were shown in Figure 3.21. When compared to the negative control, distributed particles were observed under the upper layer of the films. Particles of boron derivatives were relatively well dispersed in the pectin matrix. All of the film samples showed smooth surface structures with compact structural integrity.

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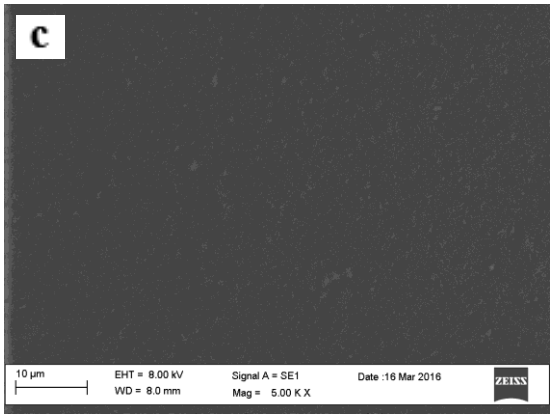
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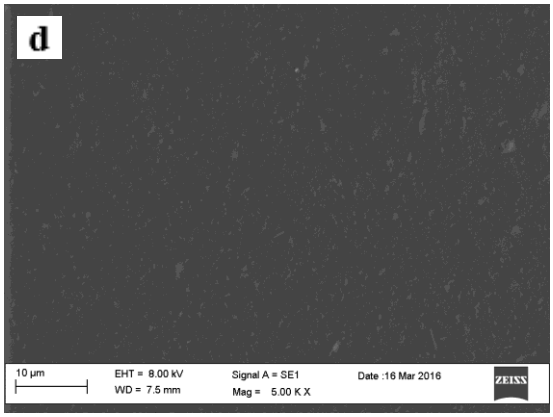
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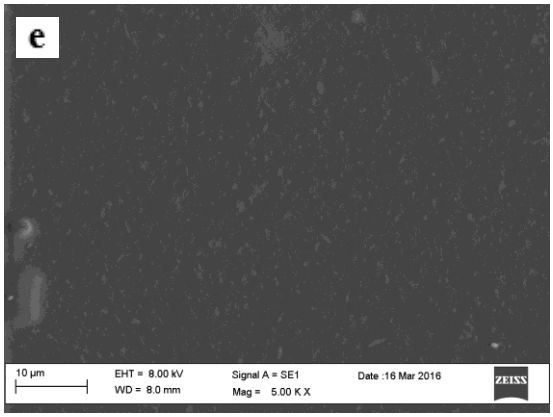
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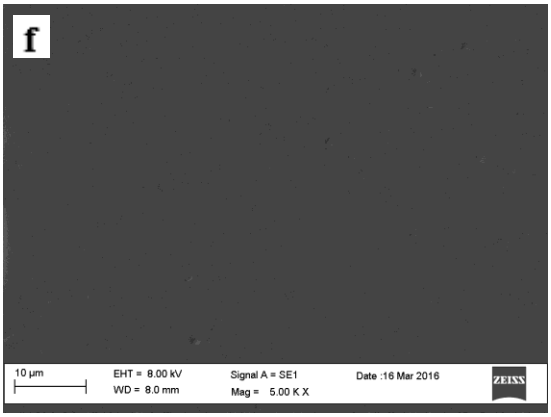
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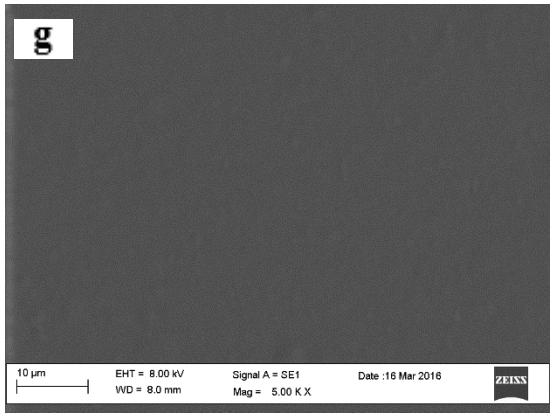
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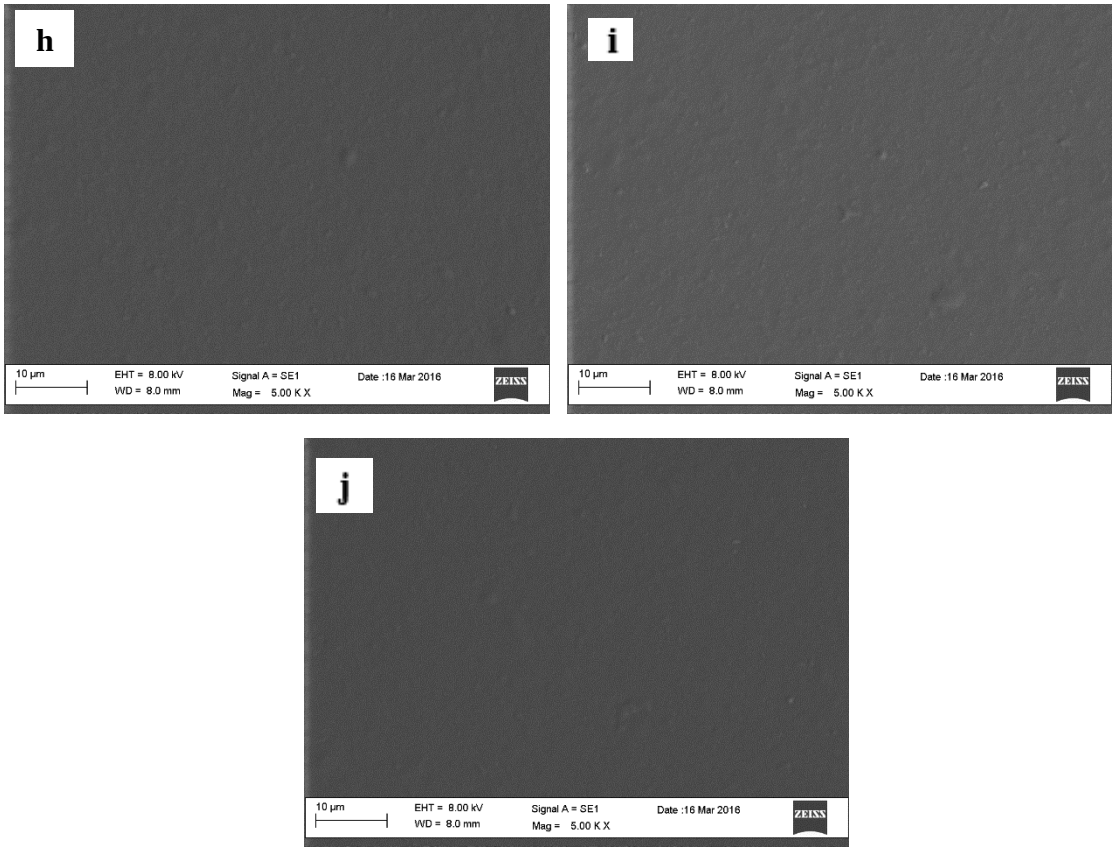
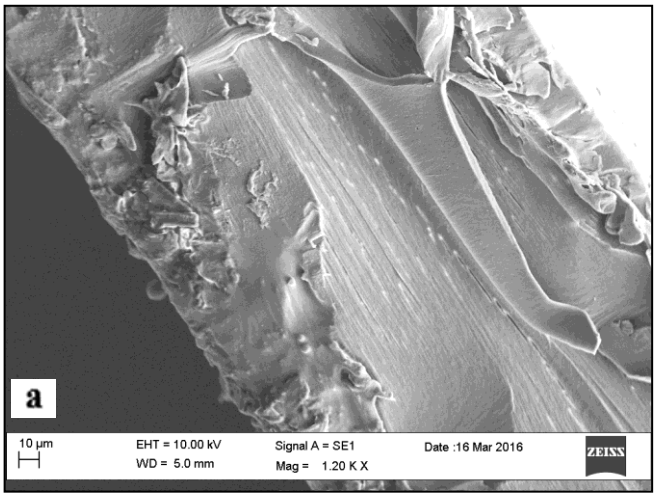


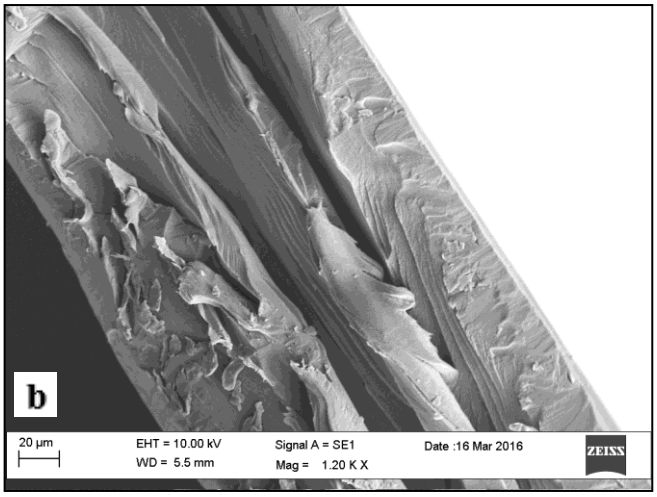
Figure 3.21. Scanning electron micrograph of surface of a) negative control pectin film, b) pectin film containing 5% boric acid, c) pectin film containing 10% boric acid, d) pectin film containing 15% boric acid, e) pectin film cotaining 5% disodium octaborate, f) pectin film cotaining 10% disodium octaborate, g) pectin film cotaining 15% disodium octaborate, h) pectin film containing 5% sodium pentaborate, i) pectin film containing 10% sodium pentaborate, j) pectin film containing 15% sodium pentaborate.

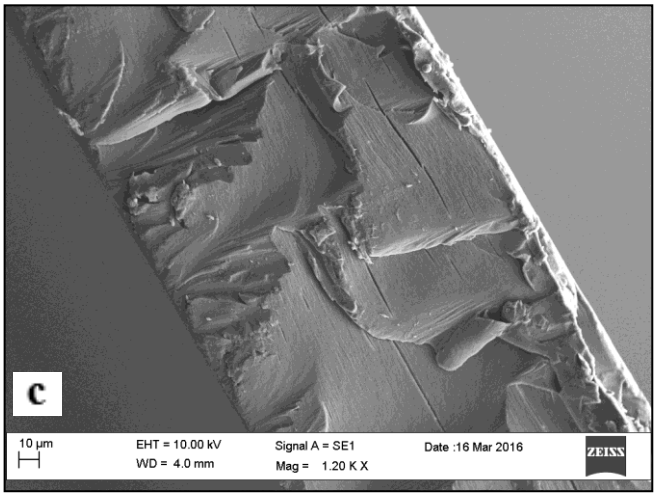
3.3.6. Microstructure of the Gelatin Films

Scanning electron micrographs of cross sections of gelatin films with different boron derivatives can be seen in Figure 3.22. When compared to the negative control, distributed particles were observed under the upper layer of the films. Particles of boron derivatives were relatively well dispersed in the gelatin matrix. All of the film samples showed smooth surface structures with compact structural integrity. Cross sectional areas of the samples did not show any significant microstructural differences between the control composite films.

The micrographs indicated that gelatin based samples have more smooth cross-sectional areas than pectin based samples.







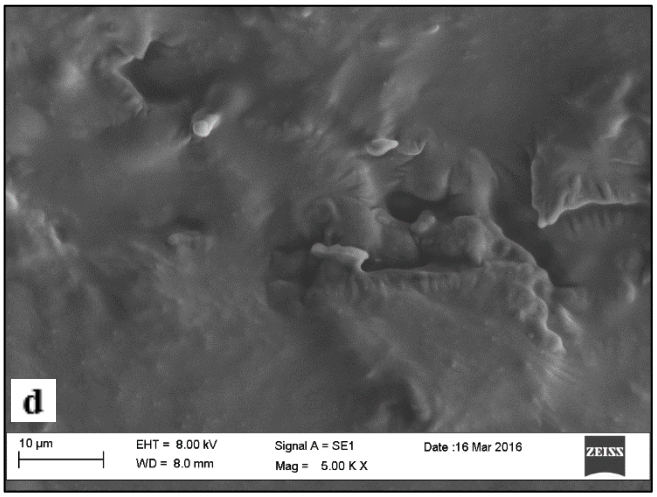
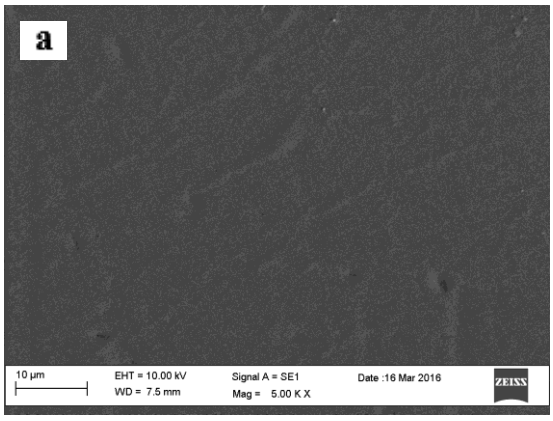
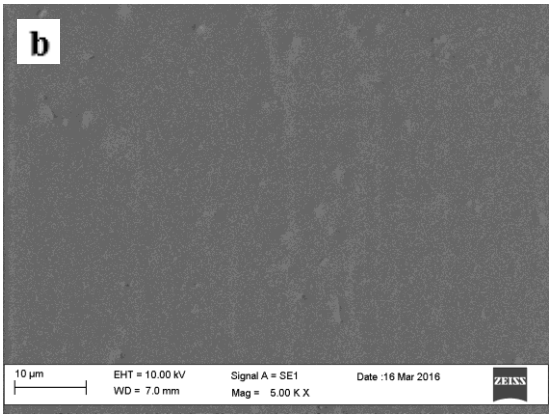


Figure 3.22. Cross-sectional area of a) negative control pectin film, b) gelatin film containing 10% disodium octaborate, c) gelatin film containing 10% sodium pentaborate, d) gelatin film containing 10% boric acid

Scanning electron micrographs of surface area of gelatin films were shown in Figure 3.25. Distributed particles of the boron derivatives can be seen under the upper layer of the gelatin films. The micrographs indicated that gelatin based samples have more smooth cross-sectional areas than pectin based samples.

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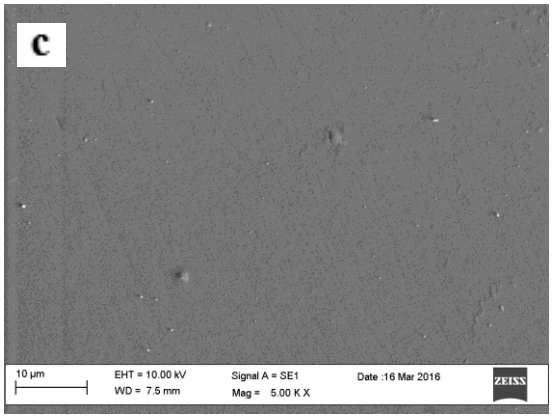
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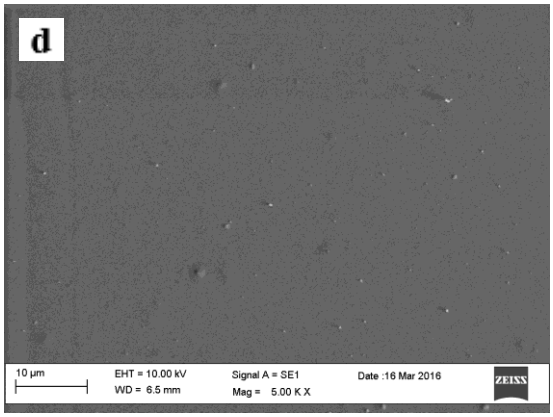
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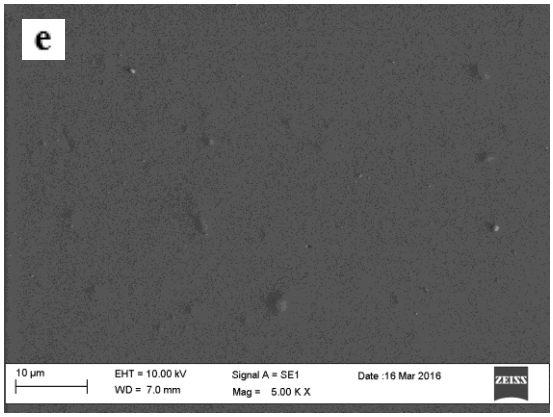
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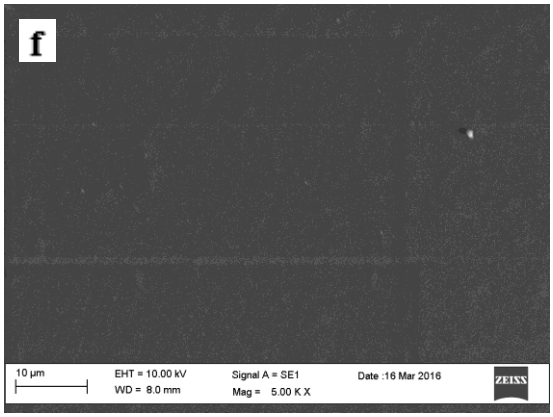
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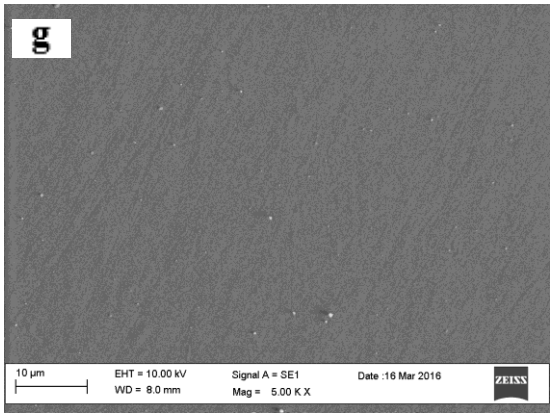
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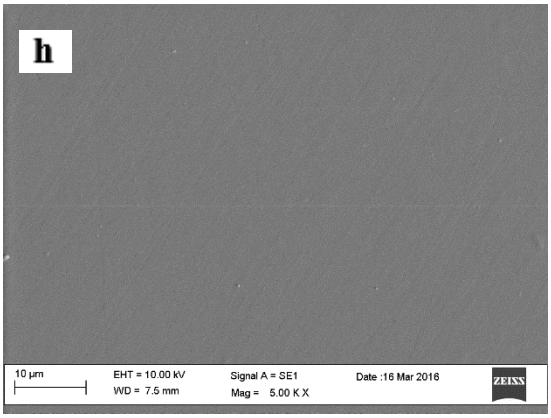
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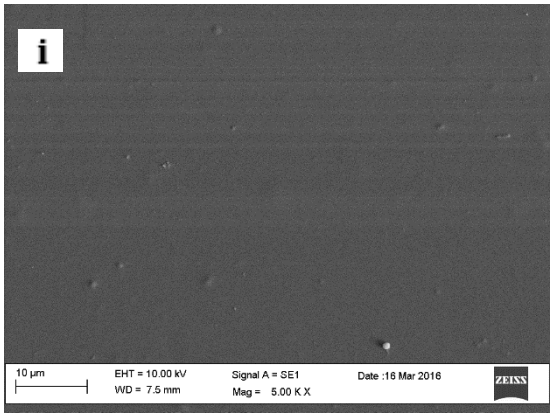
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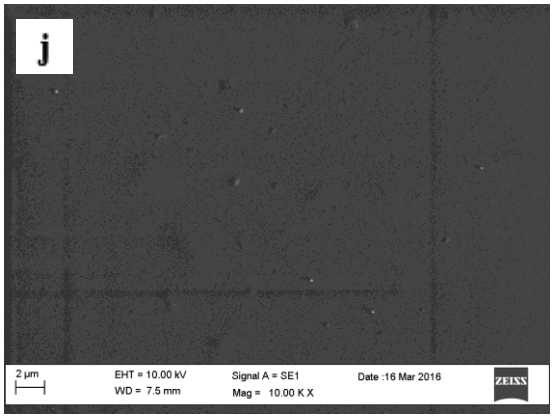

Figure 3.23. Scanning electron micrograph of surface of a) negative control gelatin film, b) gelatin film containing 5% boric acid, c) gelatin film containing 10% boric acid, d) gelatin film containing 15% boric acid, e) gelatin film cotaining 5% disodium octaborate, f) gelatin film cotaining 10% disodium octaborate, g) gelatin film cotaining 15% disodium octaborate, h) gelatin film containing 5% sodium pentaborate, i) gelatin film containing 10% sodium pentaborate, j) gelatin film containing 15% sodium pentaborate.

3.3.7. Chemical Bonds and Interactions in the Network of the Gelatin Films

FT-IR analysis was used to characterize the changes induced by incorporation of boron derivatives into gelatin film matrix by distinguishing the IR bands and vibrational shifts related to boron derivative-film interactions. Figure 3.24 shows the FT-IR spectra of the gelatin and gelatin based composite films. The characteristic absorption peaks appeared at 1629 cm^{-1}, 1546 cm^{-1} and 1238 cm^{-1} which corresponds to C=O stretching (amide-I), N-H stretching (amide-II), C-N and N-H stretching (amide-III) respectively. The peak at 1629 cm^{-1} indicates frequency of carbonyl (C=O) stretching/hydrogen bonding coupled with COO. The characteristic peak at 2922 cm^{-1} corresponds to C-H stretching. All peaks observed on FTIR spectrum of gelatin film and gelatin based films with boron derivatives were similar except peak heights; showing that there is no chemical bonding formation between gelatin and added boron derivatives.

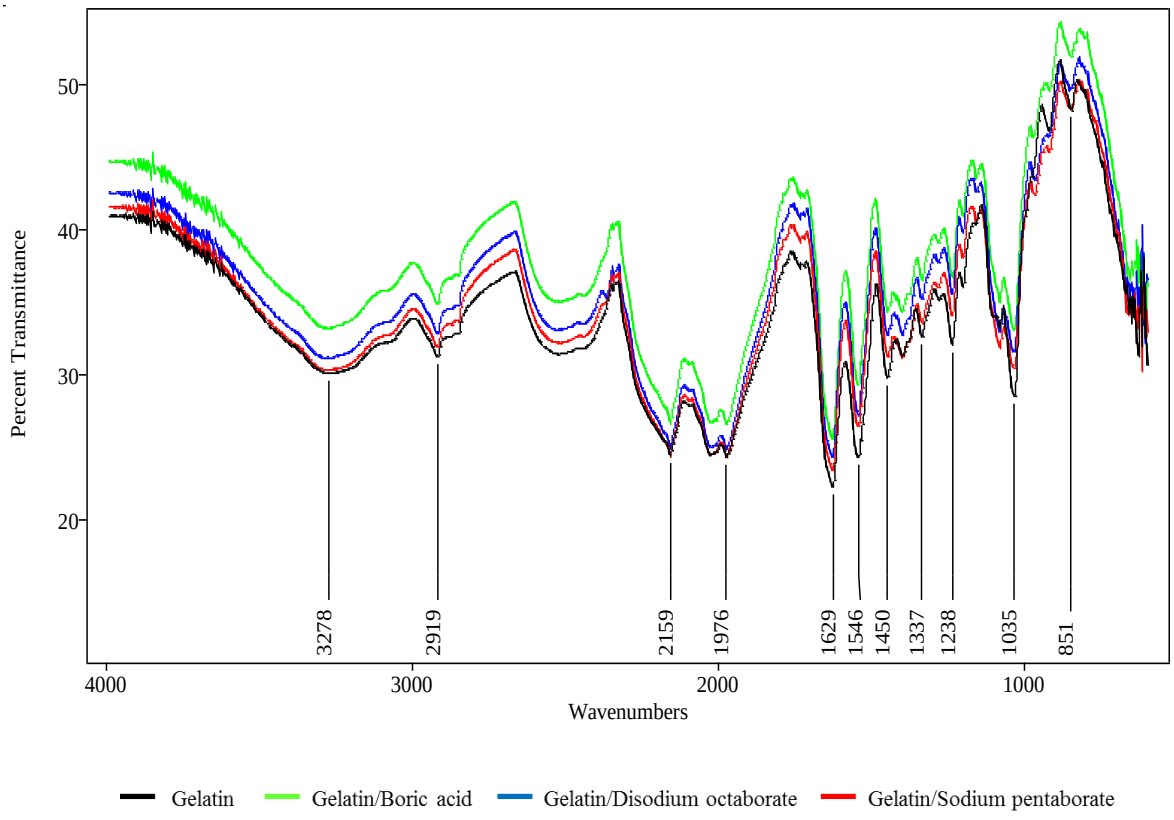

Figure 3.24. FT-IR spectra of gelatin and boron derivative incorporated gelatin films.

3.3.8. Chemical Bonds and Interactions in the Network of the Pectin Films

According to the spectrum of the pectin samples shown in Figure 3.25, it can be observed that FT-IR spectrum of the pectin and pectin/boric acid films are very similar. There was no additional peak formation in the spectrum of pectin/boric acid film, and this shows that there is not a chemical bond formation between boric acid and pectin. On the other side, addition of disodium octaborate and sodium pentaborate caused additional peak formation at 3274, 2935 and 2879 cm^{-1} on the spectrum. Also the peaks at 1647, 1419 and 1036 cm^{-1} are higher for the disodium octaborate and sodium pentaborate spectrums. The broad adsorption peak at 3274 cm^{-1} due to B-OH stretching (bonded –OH groups), and the bands at 2935 cm^{-1} and 2879 cm^{-1} can be attributed to the aliphatic C-H stretching vibration of CH_2 and CH_3 group of methyl ester. The peaks at 2360-2342 cm^{-1} were attributed to –OH groups. The peak at 1647 cm^{-1} suggested C=O stretching vibration peaks. The absorption band at 1419 cm^{-1} and 1332 cm^{-1} represent $–CH_2$ scissoring and B-O stretching vibrations respectively. The

adsorption band at 1036 cm^{-1} represents ether (R-O-R) and cyclic C-C bonds in the ring structure of pectin molecules.

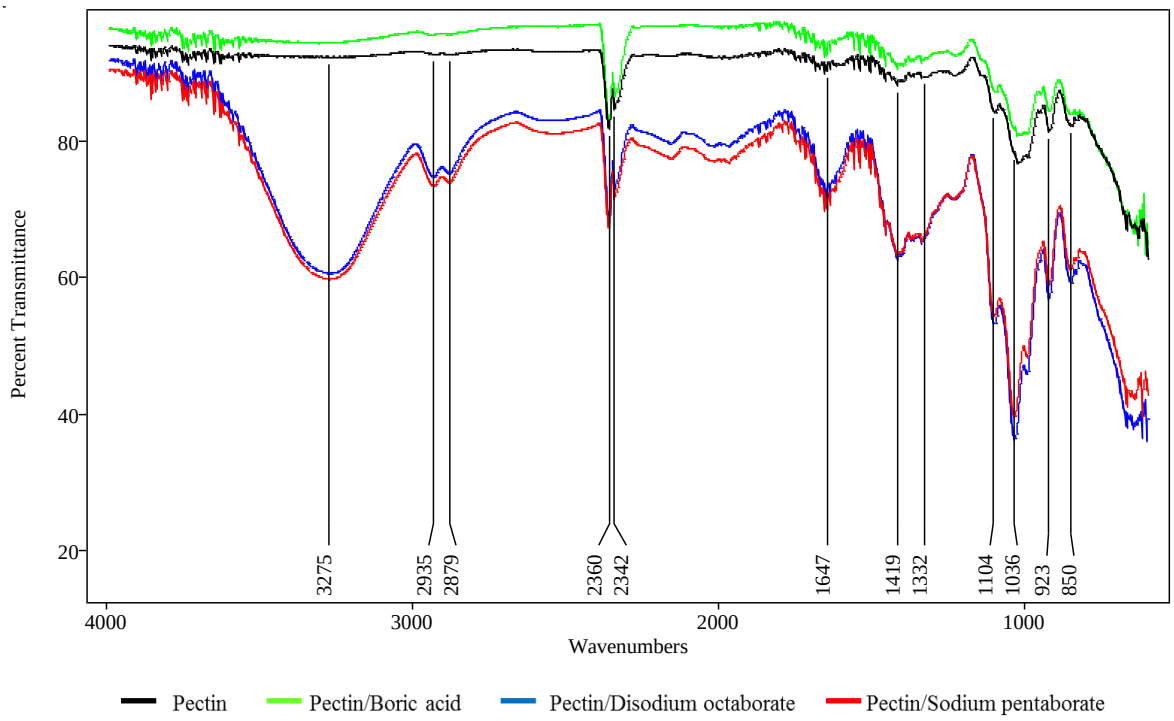

Figure 3.25. FT-IR spectra of pectin and boron derivative incorporated pectin films.

4. CONCLUSION

Gelatin and pectin are the most common biopolymers that are used for food coating and packaging studies. In this work, films were developed from gelatin and pectin biopolymers with antimicrobial and antifungal properties via incorporation of three different boron derivatives. Besides the antimicrobial or antifungal properties; rheological properties of film forming solutions, mechanical and physical characteristics of the developed films were evaluated to ensure that incorporation of these active agents does not have adverse effects on the film formation and film characteristics.

Addition of each boron derivative at different concentrations to the gelatin and pectin films resulted in different antimicrobial and antifungal properties against tested five tested microorganisms. According to the microbiological studies, the modified films that were incorporated with boric acid, disodium octaborate and sodium pentaborate exhibited highest inhibition effect on *A. niger* which is the most common fungi causing food spoilage. Besides, the growth of *S. aerus* that causes staphylococcal food poisoning and the growth of multi-drug resistant *P. aeruginosa* were also inhibited by the developed gelatin and pectin films. Only the pectin films containing 5% boron derivatives were insufficient to show inhibition effect on *P. aeruginosa*. On the other side, growth of the important food pathogen *E.coli* could not be prevented by the gelatin and pectin films. Results of the antimicrobial studies suggest that incorporation of the used boron derivatives improved antimicrobial and antifungal properties of the films significantly.

Furthermore, incorporation of all concentrations of boron derivatives increased the tensile strength of the gelatin and pectin films. Addition of disodium octaborate at 10% concentration has the highest effect on the tensile strength (TS) of gelatin films and increased the TS by 89.2%. Also, addition of sodium pentaborate at 10% concentration increased the TS of pectin film by 100.9%. Surface morphology of the gelatin and pectin films with boron derivatives were similar with the control films and revealed a homogenous structure.

Rheological studies provide information about structural build up, gelation and melting behaviors of the film forming solutions. The tested hydrocolloid dispersions exhibited a typical non-Newtonian behavior in the studied range. Considering the rheological studies performed, it can be concluded that addition of disodium octaborate to gelatin and pectin

solutions improves the solid-like behavior while boric acid addition enhances the liquid-like behavior. This can be explained by the boric acid addition lower the pH of the gelatin based hydrogel solutions and this change in pH could alter the repulsive forces between protein molecules. Behavior of sodium pentaborate incorporated film forming solutions was similar to the control solutions.

The FT-IR spectroscopy results shows the extra bond formation between pectin and the two incorporated boron derivatives (disodium octaborate, sodium pentaborate) when compared to the control pectin samples while boric acid containing pectin films exhibited similar FT-IR spectrum with the control film. FT-IR spectrum of the gelatin films containing boron derivatives were also similar with the spectrum of the control gelatin film and there was no additional peaks observed.

Findings of this study demonstrated that gelatin films can carry high concentrations of boron derivatives as antimicrobial and antifungal agent to develop composite films, and incorporation of the boron derivatives also enhanced mechanical properties of the gelatin films significantly. The gelatin films containing boron derivatives had significant antimicrobial effect against *S. aureus, P. aeruginosa, A. niger* and *C. albicans* and inhibitory effect of the films were improved with increasing concentration of boron derivatives.

Addition of boron derivatives at 10% and 15% concentrations significantly improved antimicrobial properties of the pectin films against *S. aureus* and *P. aeruginosa.* The most resistant pectin films were obtained by addition of sodium pentaborate while FT-IR spectrum of the pectin films were demonstrated extra chemical bond formations in the sodium pentaborate and disodium octaborate containing films.

Finally, this work confirms that boron derivatives (boric acid, disodium octaborate tetrahydrate and sodium pentaborate) can be used to develop antimicrobial gelatin and pectin films effective against *S. aureus, P. aeruginosa, A. niger* and *C. albicans*. The enhanced antimicrobial activities and mechanical strength can extend use of boron compounds in food packaging applications.

REFERENCES

1. Mustafa Üçüncü. *Gıda Ambalajlama Teknolojisi*. Meta Press, İzmir, 2007.

2. S.D.F. Mihindukulasuriya and L.T. Tim. Nanotechnology Development in Food Packaging: A Review. *Trends in Food Science and Technology*, 40:149-167, 2014.

3. G. L. Robertson. *Food Packaging Principles and Practice*. Taylor and Francis, 2006.

4. H. Brown and J. Williams. Packaged Product Quality and Shelf Life. In: R. Coles, D. McDowell and M. J. Kirwan, editors, *Food Packaging Technology*, pages 65-91. Blackwell Publishing, Oxford, 2003.

5. I. S. Arvanitoyannis and G. Oikonomou. Active and Intelligent Packaging. In: I. S. Arvanitoyannis, editor, *Modified Atmosphere and Active Packaging Technologies*, pages 627-662. CRC Press, New York, 2012.

6. D.G. Blevins and K.M. Lukaszewski. Boron in Plant Structure and Function. *Annual Review of Plant Physiology and Plant Molecular Biology*, 49:481–500, 1998.

7. T. D. Tombal, Ş. G. Özkan, İ. K. Ünver and A. E. Osmanoğlu. Bor Bileşiklerinin Özellikleri, Üretimi, Kullanımı ve Nükleer Reaktör Teknolojisinde Önemi. *Journal of Boron*, 2:86-95, 2016.

8. R. Subbaiah and G. V. Prajapati. *Water Management and Climate Smart Agriculture, Gyan Publishing House*, New Delhi, 2015.

9. Association of Plastics Manufacturers, "An Analysis of European Plastics Production, Demand and Waste Data", http://www.corepla.it/documenti/5f2fa32a-7081-416f-8bac-2efff3ff2fbd/Plastics+TheFacts+2015.pdf [retrieved 11 November 2015].

10. P.Prasad and A. Kochhar. Active Packaging in Food Industry: A Review. *IOSR Journal of Environmental Science, Toxicology and Food Technology,* 8:2319-2402, 2014.

11. L.J. Bastarrachea, D.E. Wong, M.J. Roman, Z. Lin and J.M. Goddard. Review Active Packaging Coatings. *Coatings*, 5:771-791, 2015.

12. P. Suppakul, J. Miltz, K. Sonneveld and S. W. Bigger. Active Packaging Technologies with an Emphasis on Antimicrobial Packaging and its Applications. *Journal of Food Science*, 68:408-420, 2003.

13. Z. Assefa and S. Admassu. Development and Characterization of Antimicrobial Packaging Films. *Journal of Food Process Technology*, 4:235, 2013.

14. B. Malhotra, A. Keshwani and H. Kharwal. Antimicrobial Food Packaging: Potential and Pitfalls. *Frontiers in Microbiology*, 6:611, 2015.

15. P. Appendini and J.H. Hotchkiss. Review of Antimicrobial Food Packaging. *Innovative Food Science and Emerging Technologies*, 3:113-126, 2002.

16. P.J. Espitia, R.J. Avena-Bustillos, W.X. Du, R.F. Teofilo, N.F.F Soares and T.H. McHugh. Optimal Antimicrobial Formulation and Physical–Mechanical Properties of Edible Films Based on Açaí and Pectin For Food Preservation. *Food Packaging and Shelf Life*, 2:38-49, 2014.

17. C.G. Otoni, M.R. de Moura, F.A. Aouada, G.P. Camilloto, R.S. Cruz, M.V. Lorevice, N.F.F. Soares and L.H.C. Mattoso. Antimicrobial and Physical-Mechanical Properties of Pectin/Papaya Puree/Cinnamaldehyde Nanoemulsion Edible Edible Composite Films. *Food Hydrocolloids*, 41:188-194, 2014.

18. P. Kanmani and J. W. Rhim. Physical, Mechanical and Antimicrobial Properties of Gelatin Based Active Nanocomposite Films Containing AgNPs and Nanoclay. *Food Hydrocolloids*, 35:644-652, 2014.

19. J.F. Martucci, L.B. Gende, L.M. Neira and R.A. Ruseckaite. Oregano and Lavender Essential Oils as Antioxidant and Antimicrobial Additives of Biogenic Gelatin Films. *Industrial Crops and Products*, 71:205-213, 2015.

20. J.Hafsa, M.A. Smach, M.R.B. Khedher, B. Charfeddine, K. Limem, H. Majdoub and S. Rouatbi. Physical, Antioxidant and Antimicrobial Properties of Chitosan Films Containing Eucalyptus Globulus Essential Oil. *LWT- Food Science and Technology*, 68:356-364, 2016.

21. S. Shankar, X. Teng and G. L. Jong-Whan Rhim. Preparation, Characterization, and Antimicrobial Activity of Gelatin/ZnO Nanocomposite Films. *Food Hydrocolloids*, 45:264-271, 2015.

22. Y. Zhang, Q. Ma, F. Critzer, P. M. Davidson and Q. Zhong. Physical and Antibacterial Properties of Alginate Films Containing Cinnamon Bark Oil and Soybean Oil. *LWT- Food Science and Technology*, 64:423-430, 2015.

23. J. G. Estaca, C. L. Dicastillo, P. H. Munoz, R. Catala and R. Gavara. Advances in Antioxidant Active Food Packaging. *Trends in Food Science and Technology*, 35:42-51, 2014.

24. J. Vartiainen, M. Vähä-Nissi and A. Harlin. Biopolymer Films and Coatings in Packaging Applications-A Review of Recent Developments. *Material Science and Applications*, 5:708-718, 2014.

25. D.Gabor and O. Tita. Biopolymers Used in Food Packaging: A Review. *Acta Universitatis Cibiniensis Series E: Food Technology*, 16, 2012.

26. J. W. Rhim and P. K. W. Ng. Natural Biopolymer-Based Nanocomposite Films for Packaging Applications. *Critical Reviews in Food Science and Nutrition*, 47:411-433, 2007.

27. P.J.P. Espitia, W. Du, R. J. Avena-Bustillos, N.F.F. Soares and T.H. McHugh. Edible Films From Pectin: Physical-Mechanical and Antimicrobial Properties – A Review. *Food Hydrocolloids*, 35:287-296, 2014.

28. A. Penhasi and V.M. Meidan. Preparation and Characterization of in situ Ionic Cross-linked Pectin Films: Unique Biodegradable Polymers. *Carbohydrate Polymers*, 102:254-260, 2014.

29. A.C.K. Bierhalz, M.A. da Silva and T.G. Kieckbusch. Natamycin Release From Alginate/Pectin Films for Food Packaging Applications. *Journal of Food Engineering*, 110:18-25, 2012.

30. V. N. Burgos, A. Jiménez and M.C. Garrigós. Natural Pectin Polysaccharides as Edible Coatings. *Coatings*, 5:865-886, 2015.

31. J. Harholt, A. Suttangkakul and H. V. Scheller. Biosynthesis of Pectin. *Journal of Plant Physiology*, 153:384-395, 2010.

32. S. Galus and A. Lenart. Development and Characterization of Composite Edible Films Based on Sodium Alginate and Pectin. *Journal of Food Engineering*, 115:459-465, 2013.

33. A.S. Raj, S. Rubila, R. Jayabalan and T. V. Ranganathan. A Review on Pectin: Chemistry due to General Properties of Pectin and its Pharmaceutical Uses. *Open Access Scientific Reports*. 1:12, 2012.

34. E. A. Bursali, S. Coskun, M. Kizil and M. Yurdakoc. Synthesis, Characterization and in vitro Antimicrobial Activities of Boron/Starch/Polyvinyl Alcohol Hydrogels. *Carbohydrate Polymers*, 83:1377-1383, 2011.

35. P. Sriamornsak. Chemistry of Pectin and Its Pharmaceutical Uses: A Review. *Silpakorn University International Journal*, 3:206-228, 2003.

36. H.R. Moreira, F. Munarin, R. Gentilini, L. Visai, P. L. Granja, M.C. Tanzi and P. Petrini. Injectable Pectin Hydrogels Produced by Internal Gelation: pH Dependence of Gelling and Rheological Properties. *Carbohydrate Polymers*, 103:339-347, 2014.

37. B. R. Sharma, N.C. Dhuldhoya, S.U. Merchant and U.C.Merchant. An Overview on Pectins. *Times Food Processing Journal*, 44-51, 2006.

38. M.A. da Silva, A.C.K. Bierhalz and T.G. Kieckbusch. Alginate and Pectin Composite Films Crosslinked with Ca^{2+} Ions: Effect of the Plasticizer Concentration. *Carbohydrate Polymers*, 77:736-742, 2009.

39. D.N.A. Zaidel, N.N. Zainudin, Y.M.M. Jusoh and I.I. Muhamad. Extraction and Characterization of Pectin from Sweet Potato (Ipomoea Batatas) Pulp. *Journal of Engineering Science and Technology*, 3:22-29, 2015.

40. D.Lootens, F. Capel, D. Durand, T. Nicolai, P. Boulenguer and V. Langendorff. Influence of pH, Ca Concentration, Temperature and Amidation on the Gelation of Low Methoxyl Pectin. *Food Hydrocolloids*, 17: 237-244, 2003.

41. I. Fraeye, T. Duvetter, E. Doungla, A.V Loey and M. Hendickx. Fine-tuning the Properties of Pectin-Calcium Gels by Control of Pectin Fine Structure, Gel Composition and Environmental Conditions. *Trends in Food Science and Technology*, 21:219-228, 2010.

42. A. R. Nesic. Pectin films for application in food packaging: review. In: P. L. Bush, editor, *Pectin: Chemical Propeties, Uses and Health Benefits*, pages 225-251. Nova Science Publishers, 2014.

43. H. Kastner, U. Einhorn-Stoll and B. Senge. Structure Formation in Sugar Containing Pectin Gels – Influence of Ca^{+2} on the Gelation of Low-methoxylated Pectin at Acidic pH. *Food Hydrocolloids*, 27:42-49, 2012.

44. A.A. Mariod and H.F. Adam. Review: Gelatin, Source, Extraction and Industrial Applications. *Acta Scientiarum Polonorum, Technologia Alimentaria*, 12:135-147, 2013.

45. R. N. Flores, B. Gimenez, F. F. Martin, M. E. Caballero, M. P. Montero and M. C. Guillen. Physical and Functional Characterization of Active Fish Gelatin Films Incorporated with Lignin. *Food Hydrocolloids*, 30:163-172, 2013.

46. K. B. Djagny, Z. Wang and S. Xu. Gelatin: A Valuable Protein for Food and Pharmaceutical Industries: Review. *Critical Reviews in Food Science and Nutrition*, 41:481-492, 2001.

47. Z. A. Hanani, J. A. O'Mahony, Y. H. Roos, P. M. Oliveira and J. P. Kerry. Extrusion of Gelatin-Based Composite Films: Effects of Processing Temperature and pH of Film Forming Solution on Mechanical and Barrier Properties of Manufactured Films. *Food Packaging and Shelf Life*, 2:91-101, 2014.

48. R. Morales, M. J. Martinez and A. M. R. Pilosof. Dynamics of Gelation, Textural and Microstructural Properties of Gelatin Gels in The Presence of Casein Glycomacropeptide. *Food Research International*, 84:102-107, 2016.

49. A. M. Cozmuta, A. Turila, R. Apjok, A. Ciocian, L. M. Cozmuta, A. Peter, C. Nicula, N. Gali and T. Benkovi. Preparation and Characterization of Improved Gelatin Films Incorporating Hemp and Sage Oils. *Food Hydrocolloids*, 49:144-155, 2015.

50. Z.A.N. Hanani, Y.H. Roos and J.P Kerry. Use and Application of Gelatin as Potential Biodegradable Packaging Materials for Food Products. *International Journal of Biological Macromolecules*, 71:94-102, 2014.

51. P. Kanmani and J. W. Rhim. Physicochemical Properties of Gelatin/Silver Nanoparticle Antimicrobial Composite Films. *Food Chemistry*, 148:162-169, 2014.

52. Z. Pang, H. Deeth, P. Sopade, R. Sharma and N. Bansal. Rheology, Texture and Microstructure of Gelatin Gels with and Without Milk Proteins. *Food Hydrocolloids*, 35:484-493, 2014.

53. T. Jin, L. Liu, H. Zhang and K. Hicks. Antimicrobial Activity of Nisin Incorporated in Pectin and Polylactic Acid Composite Films Against *Listeria Monocytogenes*. *International Journal of Food Science and Technology*, 44:322–329, 2009.

54. M. Pereda, A.G. Ponce, N.E. Marcovich, R.A. Ruseckaite and J.F. Martucci. Chitosan-Gelatin Composites and Bi-layer Films with Potential Antimicrobial Activity. *Food Hydrocolloids*, 25:1372-1381, 2011.

55. B. Kuskay and A. N. Bulutcu. Design Parameters of Boric Acid Production Process from Colemanite Ore in the Presence of Propionic Acid. *Chemical Engineering and Processing*, 50:377-383, 2011.

56. Ulusal Bor Araştırma Enstitüsü BOREN, Bor Mineralleri, http://www.boren.gov.tr/tr/bor/bor-mineralleri, [retrieved 1 December 2015].

57. M.T. Yılmaz. Minimum Inhibitory and Minimum Bactericidal Concentrations of Boron Compounds against Several Bacterial Strains. T*urkish Journal of Medical Sciences*, 42:1423-1429, 2012.

58. Z. Sayin, U. S. Ucan and A. Sakmanoglu. Antibacterial and Antibiofilm Effects of Boron on Different Bacteria. *Biological Trace Element Research*, 10:628-637, 2016.

59. V. M. Dembitsky, A. A. Quntar and M. Srebnik. Natural and Synthetic Small Boron-Containing Molecules as Potential Inhibitors of Bacterial and Fungal Quorum Sensing. *Chemical Reviews*, 111:209-237, 2011.

60. P. J. Bailey, G. Cousins, G. A. Snows and J. White. Boron-Containing Antibacterial Agents: Effects on Growth and Morphology of Bacteria under Various Culture Conditions. *Antimicrobial Agents and Chemotherapy*, 17:549-553, 1980.

61. R. D. Houlsby, M. Ghajar and G. O. Chavez. Antimicrobial Activity of Borate-Buffered Solutions. *Antimicrobial Agents and Chemotherapy*, 29: 803-806, 1986.

62. R. Zan, I. Hubbezoglu, A. K. Özdemir, T. Tunc, Z. Sumer and O. Alici. Antibacterial Effect of Different Concentration of Boric Acid against *Enterococcus Faecalis* Biofilms in Root Canal. *Marmara Dental Journal*, 2:76-80, 2013.

63. T. Matoh and M.Kobayashi. Boron and Calcium, Essential Inorganic Constituents of Pectic Polysaccharides in Higher Plant Cell Walls. *Journal of Plant Research*, 111:179-190, 1998.

64. G. Qin, Y. Zong, Q. Chen, D. Hua and S. Tian. Inhibitory Effect of Boron against *Botrytis cinerea* on Table Grapes and its Possible Mechanisms of Action. *International Journal of Food Microbiology*, 138:145-150,2010.

65. A. Ström, E. Schuster and S.M. Goh. Rheological Characterization of Acid Pectin Samples in the Absence and Presence of Monovalent Ions. *Carbohydrate Polymers*, 113:336-343, 2014.

66. M.A. Rao, *Rheology of Fluid and Semisolid Foods: Principles and Applications*, Food Engineering Series, Springer New York, 2007.

67. G. Tabilo-Munigaza and G. V. Barbosa-Canovas, Rheology for the Food Industry. *Journal of Food Engineering*, 67:147-156, 2005.

68. H. A. Barnes, *A Hanbook of Elementary Rheology* Institute of Non-Newtonian Fluid Mechanics,

69. E. A. Foegeding. Rheology and Sensory Texture of Biopolymer Gels. *Current Opinion in Colloid and Interface Science*, 12:242-250, 2007.

70. H. A. Barnes. A *Handbook of Elementary Rheology*. University of Whales, Institute of Non-Newtonian Fluid Mechanics, 2000.

APPENDIX A: RESULTS OF TUKEY'S HSD POST HOC TESTS FOR ANTIMICROBIAL ACTIVITY OF THE FILMS

The data obtained from antimicrobial tests were statistically analyzed by applying Tukey's post-hoc test to compare the groups using SPSS software (version 20). Results of the statistical tests were shown in the figures below.

Table A.1. Results of Tukey's HSD post hoc test for antimicrobial activity of pectin films on *S. aureus*.

(I) Sample		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Boric Acid (5%)	0,0000	1,4762	1,000	-5,228	5,228
	Boric Acid (10%)	-7,6133*	1,4762	,002	-12,841	-2,386
	Boric Acid (15%)	-9,3633*	1,4762	,000	-14,591	-4,136
	Sodium Pentaborate (5%)	-1,3700	1,4762	,993	-6,598	3,858
	Sodium Pentaborate (10%)	-7,7567*	1,4762	,001	-12,984	-2,529
	Sodium Pentaborate (15%)	-9,1167*	1,4762	,000	-14,344	-3,889
	Disodium Octaborate (5%)	-2,3467	1,4762	,838	-7,574	2,881
	Disodium Octaborate (10%)	-8,0100*	1,4762	,001	-13,238	-2,782
	Disodium Octaborate (15%)	-9,3933*	1,4762	,000	-14,621	-4,166
Boric Acid (5%)	Control	0,0000	1,4762	1,000	-5,228	5,228
	Boric Acid (10%)	-7,6133*	1,4762	,002	-12,841	-2,386
	Boric Acid (15%)	-9,3633*	1,4762	,000	-14,591	-4,136
	Sodium Pentaborate (5%)	-1,3700	1,4762	,993	-6,598	3,858
	Sodium Pentaborate (10%)	-7,7567*	1,4762	,001	-12,984	-2,529
	Sodium Pentaborate (15%)	-9,1167*	1,4762	,000	-14,344	-3,889
	Disodium Octaborate (5%)	-2,3467	1,4762	,838	-7,574	2,881
	Disodium Octaborate (10%)	-8,0100*	1,4762	,001	-13,238	-2,782
	Disodium Octaborate (15%)	-9,3933*	1,4762	,000	-14,621	-4,166

Boric Acid (10%)	Control	7,6133*	1,4762	,002	2,386	12,841
	Boric Acid (5%)	7,6133*	1,4762	,002	2,386	12,841
	Boric Acid (15%)	-1,7500	1,4762	,966	-6,978	3,478
	Sodium Pentaborate (5%)	6,2433*	1,4762	,012	1,016	11,471
	Sodium Pentaborate (10%)	-,1433	1,4762	1,000	-5,371	5,084
	Sodium Pentaborate (15%)	-1,5033	1,4762	,987	-6,731	3,724
	Disodium Octaborate (5%)	5,2667*	1,4762	,047	,039	10,494
	Disodium Octaborate (10%)	-,3967	1,4762	1,000	-5,624	4,831
	Disodium Octaborate (15%)	-1,7800	1,4762	,963	-7,008	3,448
Boric Acid (15%)	Control	9,3633*	1,4762	,000	4,136	14,591
	Boric Acid (5%)	9,3633*	1,4762	,000	4,136	14,591
	Boric Acid (10%)	1,7500	1,4762	,966	-3,478	6,978
	Sodium Pentaborate (5%)	7,9933*	1,4762	,001	2,766	13,221
	Sodium Pentaborate (10%)	1,6067	1,4762	,980	-3,621	6,834
	Sodium Pentaborate (15%)	,2467	1,4762	1,000	-4,981	5,474
	Disodium Octaborate (5%)	7,0167*	1,4762	,004	1,789	12,244
	Disodium Octaborate (10%)	1,3533	1,4762	,994	-3,874	6,581
	Disodium Octaborate (15%)	-,0300	1,4762	1,000	-5,258	5,198
Sodium Pentaborate (5%)	Control	1,3700	1,4762	,993	-3,858	6,598
	Boric Acid (5%)	1,3700	1,4762	,993	-3,858	6,598
	Boric Acid (10%)	-6,2433*	1,4762	,012	-11,471	-1,016
	Boric Acid (15%)	-7,9933*	1,4762	,001	-13,221	-2,766
	Sodium Pentaborate (10%)	-6,3867*	1,4762	,010	-11,614	-1,159
	Sodium Pentaborate (15%)	-7,7467*	1,4762	,001	-12,974	-2,519
	Disodium Octaborate (5%)	-,9767	1,4762	,999	-6,204	4,251
	Disodium Octaborate (10%)	-6,6400*	1,4762	,007	-11,868	-1,412
	Disodium Octaborate (15%)	-8,0233*	1,4762	,001	-13,251	-2,796

Sodium Pentaborate (10%)	Control	7,7567*	1,4762	,001	2,529	12,984
	Boric Acid (5%)	7,7567*	1,4762	,001	2,529	12,984
	Boric Acid (10%)	,1433	1,4762	1,000	-5,084	5,371
	Boric Acid (15%)	-1,6067	1,4762	,980	-6,834	3,621
	Sodium Pentaborate (5%)	6,3867*	1,4762	,010	1,159	11,614
	Sodium Pentaborate (15%)	-1,3600	1,4762	,994	-6,588	3,868
	Disodium Octaborate (5%)	5,4100*	1,4762	,039	,182	10,638
	Disodium Octaborate (10%)	-,2533	1,4762	1,000	-5,481	4,974
	Disodium Octaborate (15%)	-1,6367	1,4762	,978	-6,864	3,591
Sodium Pentaborate (15%)	Control	9,1167*	1,4762	,000	3,889	14,344
	Boric Acid (5%)	9,1167*	1,4762	,000	3,889	14,344
	Boric Acid (10%)	1,5033	1,4762	,987	-3,724	6,731
	Boric Acid (15%)	-,2467	1,4762	1,000	-5,474	4,981
	Sodium Pentaborate (5%)	7,7467*	1,4762	,001	2,519	12,974
	Sodium Pentaborate (10%)	1,3600	1,4762	,994	-3,868	6,588
	Disodium Octaborate (5%)	6,7700*	1,4762	,005	1,542	11,998
	Disodium Octaborate (10%)	1,1067	1,4762	,999	-4,121	6,334
	Disodium Octaborate (15%)	-,2767	1,4762	1,000	-5,504	4,951
Disodium Octaborate (5%)	Control	2,3467	1,4762	,838	-2,881	7,574
	Boric Acid (5%)	2,3467	1,4762	,838	-2,881	7,574
	Boric Acid (10%)	-5,2667*	1,4762	,047	-10,494	-,039
	Boric Acid (15%)	-7,0167*	1,4762	,004	-12,244	-1,789
	Sodium Pentaborate (5%)	,9767	1,4762	,999	-4,251	6,204
	Sodium Pentaborate (10%)	-5,4100*	1,4762	,039	-10,638	-,182
	Sodium Pentaborate (15%)	-6,7700*	1,4762	,005	-11,998	-1,542
	Disodium Octaborate (10%)	-5,6633*	1,4762	,027	-10,891	-,436
	Disodium Octaborate (15%)	-7,0467*	1,4762	,004	-12,274	-1,819

Disodium Octaborate (10%)	Control	8,0100*	1,4762	,001	2,782	13,238
	Boric Acid (5%)	8,0100*	1,4762	,001	2,782	13,238
	Boric Acid (10%)	,3967	1,4762	1,000	-4,831	5,624
	Boric Acid (15%)	-1,3533	1,4762	,994	-6,581	3,874
	Sodium Pentaborate (5%)	6,6400*	1,4762	,007	1,412	11,868
	Sodium Pentaborate (10%)	,2533	1,4762	1,000	-4,974	5,481
	Sodium Pentaborate (15%)	-1,1067	1,4762	,999	-6,334	4,121
	Disodium Octaborate (5%)	5,6633*	1,4762	,027	,436	10,891
	Disodium Octaborate (15%)	-1,3833	1,4762	,993	-6,611	3,844
Disodium Octaborate (15%)	Control	9,3933*	1,4762	,000	4,166	14,621
	Boric Acid (5%)	9,3933*	1,4762	,000	4,166	14,621
	Boric Acid (10%)	1,7800	1,4762	,963	-3,448	7,008
	Boric Acid (15%)	,0300	1,4762	1,000	-5,198	5,258
	Sodium Pentaborate (5%)	8,0233*	1,4762	,001	2,796	13,251
	Sodium Pentaborate (10%)	1,6367	1,4762	,978	-3,591	6,864
	Sodium Pentaborate (15%)	,2767	1,4762	1,000	-4,951	5,504
	Disodium Octaborate (5%)	7,0467*	1,4762	,004	1,819	12,274
	Disodium Octaborate (10%)	1,3833	1,4762	,993	-3,844	6,611

*. The mean difference is significant at the 0.05 level.

Table A.2. Results of Tukey's HSD post hoc test for antimicrobial activity of pectin films on *P. aeruginosa*.

(I) Sample		**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Control	Boric Acid (5%)	0,0000	,7596	1,000	-2,690	2,690
	Boric Acid (10%)	-1,0333	,7596	,926	-3,723	1,656
	Boric Acid (15%)	-8,3933*	,7596	,000	-11,083	-5,704
	Sodium Pentaborate (5%)	0,0000	,7596	1,000	-2,690	2,690
	Sodium Pentaborate (10%)	-4,4567*	,7596	,000	-7,146	-1,767
	Sodium Pentaborate (15%)	-8,7133*	,7596	,000	-11,403	-6,024
	Disodium Octaborate (5%)	0,0000	,7596	1,000	-2,690	2,690
	Disodium Octaborate (10%)	-3,2200*	,7596	,012	-5,910	-,530
	Disodium Octaborate (15%)	-9,5267*	,7596	,000	-12,216	-6,837

Boric Acid (5%)	Control	0,0000	,7596	1,000	-2,690	2,690
	Boric Acid (10%)	-1,0333	,7596	,926	-3,723	1,656
	Boric Acid (15%)	-8,3933*	,7596	,000	-11,083	-5,704
	Sodium Pentaborate (5%)	0,0000	,7596	1,000	-2,690	2,690
	Sodium Pentaborate (10%)	-4,4567*	,7596	,000	-7,146	-1,767
	Sodium Pentaborate (15%)	-8,7133*	,7596	,000	-11,403	-6,024
	Disodium Octaborate (5%)	0,0000	,7596	1,000	-2,690	2,690
	Disodium Octaborate (10%)	-3,2200*	,7596	,012	-5,910	-,530
	Disodium Octaborate (15%)	-9,5267*	,7596	,000	-12,216	-6,837
Boric Acid (10%)	Control	1,0333	,7596	,926	-1,656	3,723
	Boric Acid (5%)	1,0333	,7596	,926	-1,656	3,723
	Boric Acid (15%)	-7,3600*	,7596	,000	-10,050	-4,670
	Sodium Pentaborate (5%)	1,0333	,7596	,926	-1,656	3,723
	Sodium Pentaborate (10%)	-3,4233*	,7596	,006	-6,113	-,734
	Sodium Pentaborate (15%)	-7,6800*	,7596	,000	-10,370	-4,990
	Disodium Octaborate (5%)	1,0333	,7596	,926	-1,656	3,723
	Disodium Octaborate (10%)	-2,1867	,7596	,176	-4,876	,503
	Disodium Octaborate (15%)	-8,4933*	,7596	,000	-11,183	-5,804
Boric Acid (15%)	Control	8,3933*	,7596	,000	5,704	11,083
	Boric Acid (5%)	8,3933*	,7596	,000	5,704	11,083
	Boric Acid (10%)	7,3600*	,7596	,000	4,670	10,050
	Sodium Pentaborate (5%)	8,3933*	,7596	,000	5,704	11,083
	Sodium Pentaborate (10%)	3,9367*	,7596	,001	1,247	6,626
	Sodium Pentaborate (15%)	-,3200	,7596	1,000	-3,010	2,370
	Disodium Octaborate (5%)	8,3933*	,7596	,000	5,704	11,083
	Disodium Octaborate (10%)	5,1733*	,7596	,000	2,484	7,863
	Disodium Octaborate (15%)	-1,1333	,7596	,880	-3,823	1,556

Sodium Pentaborate (5%)	Control	0,0000	,7596	1,000		-2,690	2,690
	Boric Acid (5%)	0,0000	,7596	1,000		-2,690	2,690
	Boric Acid (10%)	-1,0333	,7596	,926		-3,723	1,656
	Boric Acid (15%)	-8,3933*	,7596	,000		-11,083	-5,704
	Sodium Pentaborate (10%)	-4,4567*	,7596	,000		-7,146	-1,767
	Sodium Pentaborate (15%)	-8,7133*	,7596	,000		-11,403	-6,024
	Disodium Octaborate (5%)	0,0000	,7596	1,000		-2,690	2,690
	Disodium Octaborate (10%)	-3,2200*	,7596	,012		-5,910	-,530
	Disodium Octaborate (15%)	-9,5267*	,7596	,000		-12,216	-6,837
Sodium Pentaborate (10%)	Control	4,4567*	,7596	,000		1,767	7,146
	Boric Acid (5%)	4,4567*	,7596	,000		1,767	7,146
	Boric Acid (10%)	3,4233*	,7596	,006		,734	6,113
	Boric Acid (15%)	-3,9367*	,7596	,001		-6,626	-1,247
	Sodium Pentaborate (5%)	4,4567*	,7596	,000		1,767	7,146
	Sodium Pentaborate (15%)	-4,2567*	,7596	,001		-6,946	-1,567
	Disodium Octaborate (5%)	4,4567*	,7596	,000		1,767	7,146
	Disodium Octaborate (10%)	1,2367	,7596	,820		-1,453	3,926
	Disodium Octaborate (15%)	-5,0700*	,7596	,000		-7,760	-2,380
Sodium Pentaborate (15%)	Control	8,7133*	,7596	,000		6,024	11,403
	Boric Acid (5%)	8,7133*	,7596	,000		6,024	11,403
	Boric Acid (10%)	7,6800*	,7596	,000		4,990	10,370
	Boric Acid (15%)	,3200	,7596	1,000		-2,370	3,010
	Sodium Pentaborate (5%)	8,7133*	,7596	,000		6,024	11,403
	Sodium Pentaborate (10%)	4,2567*	,7596	,001		1,567	6,946
	Disodium Octaborate (5%)	8,7133*	,7596	,000		6,024	11,403
	Disodium Octaborate (10%)	5,4933*	,7596	,000		2,804	8,183
	Disodium Octaborate (15%)	-,8133	,7596	,982		-3,503	1,876

Disodium Octaborate (5%)	Control	0,0000	,7596	1,000	-2,690	2,690
	Boric Acid (5%)	0,0000	,7596	1,000	-2,690	2,690
	Boric Acid (10%)	-1,0333	,7596	,926	-3,723	1,656
	Boric Acid (15%)	-8,3933*	,7596	,000	-11,083	-5,704
	Sodium Pentaborate (5%)	0,0000	,7596	1,000	-2,690	2,690
	Sodium Pentaborate (10%)	-4,4567*	,7596	,000	-7,146	-1,767
	Sodium Pentaborate (15%)	-8,7133*	,7596	,000	-11,403	-6,024
	Disodium Octaborate (10%)	-3,2200*	,7596	,012	-5,910	-,530
	Disodium Octaborate (15%)	-9,5267*	,7596	,000	-12,216	-6,837
Disodium Octaborate (10%)	Control	3,2200*	,7596	,012	,530	5,910
	Boric Acid (5%)	3,2200*	,7596	,012	,530	5,910
	Boric Acid (10%)	2,1867	,7596	,176	-,503	4,876
	Boric Acid (15%)	-5,1733*	,7596	,000	-7,863	-2,484
	Sodium Pentaborate (5%)	3,2200*	,7596	,012	,530	5,910
	Sodium Pentaborate (10%)	-1,2367	,7596	,820	-3,926	1,453
	Sodium Pentaborate (15%)	-5,4933*	,7596	,000	-8,183	-2,804
	Disodium Octaborate (5%)	3,2200*	,7596	,012	,530	5,910
	Disodium Octaborate (15%)	-6,3067*	,7596	,000	-8,996	-3,617
Disodium Octaborate (15%)	Control	9,5267*	,7596	,000	6,837	12,216
	Boric Acid (5%)	9,5267*	,7596	,000	6,837	12,216
	Boric Acid (10%)	8,4933*	,7596	,000	5,804	11,183
	Boric Acid (15%)	1,1333	,7596	,880	-1,556	3,823
	Sodium Pentaborate (5%)	9,5267*	,7596	,000	6,837	12,216
	Sodium Pentaborate (10%)	5,0700*	,7596	,000	2,380	7,760
	Sodium Pentaborate (15%)	,8133	,7596	,982	-1,876	3,503
	Disodium Octaborate (5%)	9,5267*	,7596	,000	6,837	12,216
	Disodium Octaborate (10%)	6,3067*	,7596	,000	3,617	8,996

*. The mean difference is significant at the 0.05 level.

Table A.3. Results of Tukey's HSD post hoc test for antimicrobial activity of pectin films on *A. niger*.

(I) Sample		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Boric Acid (5%)	-4,3000	7,2802	1,000	-30,080	21,480
	Boric Acid (10%)	-9,6867	7,2802	,934	-35,466	16,093
	Boric Acid (15%)	-12,6333	7,2802	,764	-38,413	13,146
	Sodium Pentaborate (5%)	-3,1000	7,2802	1,000	-28,880	22,680
	Sodium Pentaborate (10%)	-11,1267	7,2802	,865	-36,906	14,653
	Sodium Pentaborate (15%)	-14,5467	7,2802	,610	-40,326	11,233
	Disodium Octaborate (5%)	-2,7333	7,2802	1,000	-28,513	23,046
	Disodium Octaborate (10%)	-9,4100	7,2802	,944	-35,190	16,370
	Disodium Octaborate (15%)	-14,1100	7,2802	,646	-39,890	11,670
Boric Acid (5%)	Control	4,3000	7,2802	1,000	-21,480	30,080
	Boric Acid (10%)	-5,3867	7,2802	,999	-31,166	20,393
	Boric Acid (15%)	-8,3333	7,2802	,973	-34,113	17,446
	Sodium Pentaborate (5%)	1,2000	7,2802	1,000	-24,580	26,980
	Sodium Pentaborate (10%)	-6,8267	7,2802	,993	-32,606	18,953
	Sodium Pentaborate (15%)	-10,2467	7,2802	,911	-36,026	15,533
	Disodium Octaborate (5%)	1,5667	7,2802	1,000	-24,213	27,346
	Disodium Octaborate (10%)	-5,1100	7,2802	,999	-30,890	20,670
	Disodium Octaborate (15%)	-9,8100	7,2802	,930	-35,590	15,970
Boric Acid (10%)	Control	9,6867	7,2802	,934	-16,093	35,466
	Boric Acid (5%)	5,3867	7,2802	,999	-20,393	31,166
	Boric Acid (15%)	-2,9467	7,2802	1,000	-28,726	22,833
	Sodium Pentaborate (5%)	6,5867	7,2802	,994	-19,193	32,366
	Sodium Pentaborate (10%)	-1,4400	7,2802	1,000	-27,220	24,340
	Sodium Pentaborate (15%)	-4,8600	7,2802	,999	-30,640	20,920
	Disodium Octaborate (5%)	6,9533	7,2802	,992	-18,826	32,733
	Disodium Octaborate (10%)	,2767	7,2802	1,000	-25,503	26,056
	Disodium Octaborate (15%)	-4,4233	7,2802	1,000	-30,203	21,356

Boric Acid (15%)	Control	12,6333	7,2802	,764	-13,146	38,413
	Boric Acid (5%)	8,3333	7,2802	,973	-17,446	34,113
	Boric Acid (10%)	2,9467	7,2802	1,000	-22,833	28,726
	Sodium Pentaborate (5%)	9,5333	7,2802	,940	-16,246	35,313
	Sodium Pentaborate (10%)	1,5067	7,2802	1,000	-24,273	27,286
	Sodium Pentaborate (15%)	-1,9133	7,2802	1,000	-27,693	23,866
	Disodium Octaborate (5%)	9,9000	7,2802	,926	-15,880	35,680
	Disodium Octaborate (10%)	3,2233	7,2802	1,000	-22,556	29,003
	Disodium Octaborate (15%)	-1,4767	7,2802	1,000	-27,256	24,303
Sodium Pentaborate (5%)	Control	3,1000	7,2802	1,000	-22,680	28,880
	Boric Acid (5%)	-1,2000	7,2802	1,000	-26,980	24,580
	Boric Acid (10%)	-6,5867	7,2802	,994	-32,366	19,193
	Boric Acid (15%)	-9,5333	7,2802	,940	-35,313	16,246
	Sodium Pentaborate (10%)	-8,0267	7,2802	,979	-33,806	17,753
	Sodium Pentaborate (15%)	-11,4467	7,2802	,846	-37,226	14,333
	Disodium Octaborate (5%)	,3667	7,2802	1,000	-25,413	26,146
	Disodium Octaborate (10%)	-6,3100	7,2802	,996	-32,090	19,470
	Disodium Octaborate (15%)	-11,0100	7,2802	,872	-36,790	14,770
Sodium Pentaborate (10%)	Control	11,1267	7,2802	,865	-14,653	36,906
	Boric Acid (5%)	6,8267	7,2802	,993	-18,953	32,606
	Boric Acid (10%)	1,4400	7,2802	1,000	-24,340	27,220
	Boric Acid (15%)	-1,5067	7,2802	1,000	-27,286	24,273
	Sodium Pentaborate (5%)	8,0267	7,2802	,979	-17,753	33,806
	Sodium Pentaborate (15%)	-3,4200	7,2802	1,000	-29,200	22,360
	Disodium Octaborate (5%)	8,3933	7,2802	,972	-17,386	34,173
	Disodium Octaborate (10%)	1,7167	7,2802	1,000	-24,063	27,496
	Disodium Octaborate (15%)	-2,9833	7,2802	1,000	-28,763	22,796

Sodium Pentaborate (15%)	Control	14,5467	7,2802	,610	-11,233	40,326
	Boric Acid (5%)	10,2467	7,2802	,911	-15,533	36,026
	Boric Acid (10%)	4,8600	7,2802	,999	-20,920	30,640
	Boric Acid (15%)	1,9133	7,2802	1,000	-23,866	27,693
	Sodium Pentaborate (5%)	11,4467	7,2802	,846	-14,333	37,226
	Sodium Pentaborate (10%)	3,4200	7,2802	1,000	-22,360	29,200
	Disodium Octaborate (5%)	11,8133	7,2802	,823	-13,966	37,593
	Disodium Octaborate (10%)	5,1367	7,2802	,999	-20,643	30,916
	Disodium Octaborate (15%)	,4367	7,2802	1,000	-25,343	26,216
Disodium Octaborate (5%)	Control	2,7333	7,2802	1,000	-23,046	28,513
	Boric Acid (5%)	-1,5667	7,2802	1,000	-27,346	24,213
	Boric Acid (10%)	-6,9533	7,2802	,992	-32,733	18,826
	Boric Acid (15%)	-9,9000	7,2802	,926	-35,680	15,880
	Sodium Pentaborate (5%)	-,3667	7,2802	1,000	-26,146	25,413
	Sodium Pentaborate (10%)	-8,3933	7,2802	,972	-34,173	17,386
	Sodium Pentaborate (15%)	-11,8133	7,2802	,823	-37,593	13,966
	Disodium Octaborate (10%)	-6,6767	7,2802	,994	-32,456	19,103
	Disodium Octaborate (15%)	-11,3767	7,2802	,851	-37,156	14,403
Disodium Octaborate (10%)	Control	9,4100	7,2802	,944	-16,370	35,190
	Boric Acid (5%)	5,1100	7,2802	,999	-20,670	30,890
	Boric Acid (10%)	-,2767	7,2802	1,000	-26,056	25,503
	Boric Acid (15%)	-3,2233	7,2802	1,000	-29,003	22,556
	Sodium Pentaborate (5%)	6,3100	7,2802	,996	-19,470	32,090
	Sodium Pentaborate (10%)	-1,7167	7,2802	1,000	-27,496	24,063
	Sodium Pentaborate (15%)	-5,1367	7,2802	,999	-30,916	20,643
	Disodium Octaborate (5%)	6,6767	7,2802	,994	-19,103	32,456
	Disodium Octaborate (15%)	-4,7000	7,2802	1,000	-30,480	21,080

Disodium Octaborate (15%)	Control	14,1100	7,2802	,646	-11,670	39,890
	Boric Acid (5%)	9,8100	7,2802	,930	-15,970	35,590
	Boric Acid (10%)	4,4233	7,2802	1,000	-21,356	30,203
	Boric Acid (15%)	1,4767	7,2802	1,000	-24,303	27,256
	Sodium Pentaborate (5%)	11,0100	7,2802	,872	-14,770	36,790
	Sodium Pentaborate (10%)	2,9833	7,2802	1,000	-22,796	28,763
	Sodium Pentaborate (15%)	-,4367	7,2802	1,000	-26,216	25,343
	Disodium Octaborate (5%)	11,3767	7,2802	,851	-14,403	37,156
	Disodium Octaborate (10%)	4,7000	7,2802	1,000	-21,080	30,480

*. The mean difference is significant at the 0.05 level.

Table A.4. Results of Tukey's HSD post hoc test for antimicrobial activity of pectin films on *C. albicans*.

(I) Sample		**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Control	Boric Acid (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Boric Acid (10%)	-1,3133	2,1825	1,000	-9,042	6,415
	Boric Acid (15%)	0,0000	2,1825	1,000	-7,729	7,729
	Sodium Pentaborate (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Sodium Pentaborate (10%)	-4,8267	2,1825	,480	-12,555	2,902
	Sodium Pentaborate (15%)	-4,7033	2,1825	,514	-12,432	3,025
	Disodium Octaborate (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Disodium Octaborate (10%)	-1,8200	2,1825	,997	-9,549	5,909
	Disodium Octaborate (15%)	-2,4467	2,1825	,976	-10,175	5,282
Boric Acid (5%)	Control	0,0000	2,1825	1,000	-7,729	7,729
	Boric Acid (10%)	-1,3133	2,1825	1,000	-9,042	6,415
	Boric Acid (15%)	0,0000	2,1825	1,000	-7,729	7,729
	Sodium Pentaborate (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Sodium Pentaborate (10%)	-4,8267	2,1825	,480	-12,555	2,902
	Sodium Pentaborate (15%)	-4,7033	2,1825	,514	-12,432	3,025
	Disodium Octaborate (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Disodium Octaborate (10%)	-1,8200	2,1825	,997	-9,549	5,909
	Disodium Octaborate (15%)	-2,4467	2,1825	,976	-10,175	5,282

Boric Acid (10%)	Control	1,3133	2,1825	1,000	-6,415	9,042
	Boric Acid (5%)	1,3133	2,1825	1,000	-6,415	9,042
	Boric Acid (15%)	1,3133	2,1825	1,000	-6,415	9,042
	Sodium Pentaborate (5%)	1,3133	2,1825	1,000	-6,415	9,042
	Sodium Pentaborate (10%)	-3,5133	2,1825	,829	-11,242	4,215
	Sodium Pentaborate (15%)	-3,3900	2,1825	,855	-11,119	4,339
	Disodium Octaborate (5%)	1,3133	2,1825	1,000	-6,415	9,042
	Disodium Octaborate (10%)	-,5067	2,1825	1,000	-8,235	7,222
	Disodium Octaborate (15%)	-1,1333	2,1825	1,000	-8,862	6,595
Boric Acid (15%)	Control	0,0000	2,1825	1,000	-7,729	7,729
	Boric Acid (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Boric Acid (10%)	-1,3133	2,1825	1,000	-9,042	6,415
	Sodium Pentaborate (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Sodium Pentaborate (10%)	-4,8267	2,1825	,480	-12,555	2,902
	Sodium Pentaborate (15%)	-4,7033	2,1825	,514	-12,432	3,025
	Disodium Octaborate (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Disodium Octaborate (10%)	-1,8200	2,1825	,997	-9,549	5,909
	Disodium Octaborate (15%)	-2,4467	2,1825	,976	-10,175	5,282
Sodium Pentaborate (5%)	Control	0,0000	2,1825	1,000	-7,729	7,729
	Boric Acid (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Boric Acid (10%)	-1,3133	2,1825	1,000	-9,042	6,415
	Boric Acid (15%)	0,0000	2,1825	1,000	-7,729	7,729
	Sodium Pentaborate (10%)	-4,8267	2,1825	,480	-12,555	2,902
	Sodium Pentaborate (15%)	-4,7033	2,1825	,514	-12,432	3,025
	Disodium Octaborate (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Disodium Octaborate (10%)	-1,8200	2,1825	,997	-9,549	5,909
	Disodium Octaborate (15%)	-2,4467	2,1825	,976	-10,175	5,282

Sodium Pentaborate (10%)	Control	4,8267	2,1825	,480	-2,902	12,555
	Boric Acid (5%)	4,8267	2,1825	,480	-2,902	12,555
	Boric Acid (10%)	3,5133	2,1825	,829	-4,215	11,242
	Boric Acid (15%)	4,8267	2,1825	,480	-2,902	12,555
	Sodium Pentaborate (5%)	4,8267	2,1825	,480	-2,902	12,555
	Sodium Pentaborate (15%)	,1233	2,1825	1,000	-7,605	7,852
	Disodium Octaborate (5%)	4,8267	2,1825	,480	-2,902	12,555
	Disodium Octaborate (10%)	3,0067	2,1825	,921	-4,722	10,735
	Disodium Octaborate (15%)	2,3800	2,1825	,980	-5,349	10,109
Sodium Pentaborate (15%)	Control	4,7033	2,1825	,514	-3,025	12,432
	Boric Acid (5%)	4,7033	2,1825	,514	-3,025	12,432
	Boric Acid (10%)	3,3900	2,1825	,855	-4,339	11,119
	Boric Acid (15%)	4,7033	2,1825	,514	-3,025	12,432
	Sodium Pentaborate (5%)	4,7033	2,1825	,514	-3,025	12,432
	Sodium Pentaborate (10%)	-,1233	2,1825	1,000	-7,852	7,605
	Disodium Octaborate (5%)	4,7033	2,1825	,514	-3,025	12,432
	Disodium Octaborate (10%)	2,8833	2,1825	,937	-4,845	10,612
	Disodium Octaborate (15%)	2,2567	2,1825	,986	-5,472	9,985
Disodium Octaborate (5%)	Control	0,0000	2,1825	1,000	-7,729	7,729
	Boric Acid (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Boric Acid (10%)	-1,3133	2,1825	1,000	-9,042	6,415
	Boric Acid (15%)	0,0000	2,1825	1,000	-7,729	7,729
	Sodium Pentaborate (5%)	0,0000	2,1825	1,000	-7,729	7,729
	Sodium Pentaborate (10%)	-4,8267	2,1825	,480	-12,555	2,902
	Sodium Pentaborate (15%)	-4,7033	2,1825	,514	-12,432	3,025
	Disodium Octaborate (10%)	-1,8200	2,1825	,997	-9,549	5,909
	Disodium Octaborate (15%)	-2,4467	2,1825	,976	-10,175	5,282

Disodium Octaborate (10%)	Control	1,8200	2,1825	,997	-5,909	9,549
	Boric Acid (5%)	1,8200	2,1825	,997	-5,909	9,549
	Boric Acid (10%)	,5067	2,1825	1,000	-7,222	8,235
	Boric Acid (15%)	1,8200	2,1825	,997	-5,909	9,549
	Sodium Pentaborate (5%)	1,8200	2,1825	,997	-5,909	9,549
	Sodium Pentaborate (10%)	-3,0067	2,1825	,921	-10,735	4,722
	Sodium Pentaborate (15%)	-2,8833	2,1825	,937	-10,612	4,845
	Disodium Octaborate (5%)	1,8200	2,1825	,997	-5,909	9,549
	Disodium Octaborate (15%)	-,6267	2,1825	1,000	-8,355	7,102
Disodium Octaborate (15%)	Control	2,4467	2,1825	,976	-5,282	10,175
	Boric Acid (5%)	2,4467	2,1825	,976	-5,282	10,175
	Boric Acid (10%)	1,1333	2,1825	1,000	-6,595	8,862
	Boric Acid (15%)	2,4467	2,1825	,976	-5,282	10,175
	Sodium Pentaborate (5%)	2,4467	2,1825	,976	-5,282	10,175
	Sodium Pentaborate (10%)	-2,3800	2,1825	,980	-10,109	5,349
	Sodium Pentaborate (15%)	-2,2567	2,1825	,986	-9,985	5,472
	Disodium Octaborate (5%)	2,4467	2,1825	,976	-5,282	10,175
	Disodium Octaborate (10%)	,6267	2,1825	1,000	-7,102	8,355

*. The mean difference is significant at the 0.05 level.

Table A.5. Results of Tukey's HSD post hoc test for antimicrobial activity of gelatin films on *S. aerus*.

(I) Sample		**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Control	Boric Acid (5%)	-7,6133*	1,0185	,000	-11,220	-4,007
	Boric Acid (10%)	-13,5433*	1,0185	,000	-17,150	-9,937
	Boric Acid (15%)	-16,7967*	1,0185	,000	-20,403	-13,190
	Sodium Pentaborate (5%)	-7,6967*	1,0185	,000	-11,303	-4,090
	Sodium Pentaborate (10%)	-12,5300*	1,0185	,000	-16,136	-8,924
	Sodium Pentaborate (15%)	-15,9033*	1,0185	,000	-19,510	-12,297
	Disodium Octaborate (5%)	-9,2233*	1,0185	,000	-12,830	-5,617
	Disodium Octaborate (10%)	-13,8000*	1,0185	,000	-17,406	-10,194
	Disodium Octaborate (15%)	-16,7933*	1,0185	,000	-20,400	-13,187

Boric Acid (5%)	Control	7,6133*	1,0185	,000	4,007	11,220
	Boric Acid (10%)	-5,9300*	1,0185	,000	-9,536	-2,324
	Boric Acid (15%)	-9,1833*	1,0185	,000	-12,790	-5,577
	Sodium Pentaborate (5%)	-,0833	1,0185	1,000	-3,690	3,523
	Sodium Pentaborate (10%)	-4,9167*	1,0185	,003	-8,523	-1,310
	Sodium Pentaborate (15%)	-8,2900*	1,0185	,000	-11,896	-4,684
	Disodium Octaborate (5%)	-1,6100	1,0185	,842	-5,216	1,996
	Disodium Octaborate (10%)	-6,1867*	1,0185	,000	-9,793	-2,580
	Disodium Octaborate (15%)	-9,1800*	1,0185	,000	-12,786	-5,574
Boric Acid (10%)	Control	13,5433*	1,0185	,000	9,937	17,150
	Boric Acid (5%)	5,9300*	1,0185	,000	2,324	9,536
	Boric Acid (15%)	-3,2533	1,0185	,099	-6,860	,353
	Sodium Pentaborate (5%)	5,8467*	1,0185	,000	2,240	9,453
	Sodium Pentaborate (10%)	1,0133	1,0185	,989	-2,593	4,620
	Sodium Pentaborate (15%)	-2,3600	1,0185	,420	-5,966	1,246
	Disodium Octaborate (5%)	4,3200*	1,0185	,011	,714	7,926
	Disodium Octaborate (10%)	-,2567	1,0185	1,000	-3,863	3,350
	Disodium Octaborate (15%)	-3,2500	1,0185	,100	-6,856	,356
Boric Acid (15%)	Control	16,7967*	1,0185	,000	13,190	20,403
	Boric Acid (5%)	9,1833*	1,0185	,000	5,577	12,790
	Boric Acid (10%)	3,2533	1,0185	,099	-,353	6,860
	Sodium Pentaborate (5%)	9,1000*	1,0185	,000	5,494	12,706
	Sodium Pentaborate (10%)	4,2667*	1,0185	,013	,660	7,873
	Sodium Pentaborate (15%)	,8933	1,0185	,996	-2,713	4,500
	Disodium Octaborate (5%)	7,5733*	1,0185	,000	3,967	11,180
	Disodium Octaborate (10%)	2,9967	1,0185	,157	-,610	6,603
	Disodium Octaborate (15%)	,0033	1,0185	1,000	-3,603	3,610

Sodium Pentaborate (5%)	Control	7,6967*	1,0185	,000	4,090	11,303
	Boric Acid (5%)	,0833	1,0185	1,000	-3,523	3,690
	Boric Acid (10%)	-5,8467*	1,0185	,000	-9,453	-2,240
	Boric Acid (15%)	-9,1000*	1,0185	,000	-12,706	-5,494
	Sodium Pentaborate (10%)	-4,8333*	1,0185	,004	-8,440	-1,227
	Sodium Pentaborate (15%)	-8,2067*	1,0185	,000	-11,813	-4,600
	Disodium Octaborate (5%)	-1,5267	1,0185	,877	-5,133	2,080
	Disodium Octaborate (10%)	-6,1033*	1,0185	,000	-9,710	-2,497
	Disodium Octaborate (15%)	-9,0967*	1,0185	,000	-12,703	-5,490
Sodium Pentaborate (10%)	Control	12,5300*	1,0185	,000	8,924	16,136
	Boric Acid (5%)	4,9167*	1,0185	,003	1,310	8,523
	Boric Acid (10%)	-1,0133	1,0185	,989	-4,620	2,593
	Boric Acid (15%)	-4,2667*	1,0185	,013	-7,873	-,660
	Sodium Pentaborate (5%)	4,8333*	1,0185	,004	1,227	8,440
	Sodium Pentaborate (15%)	-3,3733	1,0185	,079	-6,980	,233
	Disodium Octaborate (5%)	3,3067	1,0185	,090	-,300	6,913
	Disodium Octaborate (10%)	-1,2700	1,0185	,955	-4,876	2,336
	Disodium Octaborate (15%)	-4,2633*	1,0185	,013	-7,870	-,657
Sodium Pentaborate (15%)	Control	15,9033*	1,0185	,000	12,297	19,510
	Boric Acid (5%)	8,2900*	1,0185	,000	4,684	11,896
	Boric Acid (10%)	2,3600	1,0185	,420	-1,246	5,966
	Boric Acid (15%)	-,8933	1,0185	,996	-4,500	2,713
	Sodium Pentaborate (5%)	8,2067*	1,0185	,000	4,600	11,813
	Sodium Pentaborate (10%)	3,3733	1,0185	,079	-,233	6,980
	Disodium Octaborate (5%)	6,6800*	1,0185	,000	3,074	10,286
	Disodium Octaborate (10%)	2,1033	1,0185	,568	-1,503	5,710
	Disodium Octaborate (15%)	-,8900	1,0185	,996	-4,496	2,716

Disodium Octaborate (5%)	Control	9,2233*	1,0185	,000	5,617	12,830
	Boric Acid (5%)	1,6100	1,0185	,842	-1,996	5,216
	Boric Acid (10%)	-4,3200*	1,0185	,011	-7,926	-,714
	Boric Acid (15%)	-7,5733*	1,0185	,000	-11,180	-3,967
	Sodium Pentaborate (5%)	1,5267	1,0185	,877	-2,080	5,133
	Sodium Pentaborate (10%)	-3,3067	1,0185	,090	-6,913	,300
	Sodium Pentaborate (15%)	-6,6800*	1,0185	,000	-10,286	-3,074
	Disodium Octaborate (10%)	-4,5767*	1,0185	,007	-8,183	-,970
	Disodium Octaborate (15%)	-7,5700*	1,0185	,000	-11,176	-3,964
Disodium Octaborate (10%)	Control	13,8000*	1,0185	,000	10,194	17,406
	Boric Acid (5%)	6,1867*	1,0185	,000	2,580	9,793
	Boric Acid (10%)	,2567	1,0185	1,000	-3,350	3,863
	Boric Acid (15%)	-2,9967	1,0185	,157	-6,603	,610
	Sodium Pentaborate (5%)	6,1033*	1,0185	,000	2,497	9,710
	Sodium Pentaborate (10%)	1,2700	1,0185	,955	-2,336	4,876
	Sodium Pentaborate (15%)	-2,1033	1,0185	,568	-5,710	1,503
	Disodium Octaborate (5%)	4,5767*	1,0185	,007	,970	8,183
	Disodium Octaborate (15%)	-2,9933	1,0185	,158	-6,600	,613
Disodium Octaborate (15%)	Control	16,7933*	1,0185	,000	13,187	20,400
	Boric Acid (5%)	9,1800*	1,0185	,000	5,574	12,786
	Boric Acid (10%)	3,2500	1,0185	,100	-,356	6,856
	Boric Acid (15%)	-,0033	1,0185	1,000	-3,610	3,603
	Sodium Pentaborate (5%)	9,0967*	1,0185	,000	5,490	12,703
	Sodium Pentaborate (10%)	4,2633*	1,0185	,013	,657	7,870
	Sodium Pentaborate (15%)	,8900	1,0185	,996	-2,716	4,496
	Disodium Octaborate (5%)	7,5700*	1,0185	,000	3,964	11,176
	Disodium Octaborate (10%)	2,9933	1,0185	,158	-,613	6,600

*. The mean difference is significant at the 0.05 level.

Table A.6. Results of Tukey's HSD post hoc test for antimicrobial activity of gelatin films on *P. aeruginosa*.

(I) Sample		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Boric Acid (5%)	-4,4067*	1,1094	,020	-8,335	-,478
	Boric Acid (10%)	-9,5300*	1,1094	,000	-13,459	-5,601
	Boric Acid (15%)	-14,7933*	1,1094	,000	-18,722	-10,865
	Sodium Pentaborate (5%)	-10,1533*	1,1094	,000	-14,082	-6,225
	Sodium Pentaborate (10%)	-10,1533*	1,1094	,000	-14,082	-6,225
	Sodium Pentaborate (15%)	-13,9133*	1,1094	,000	-17,842	-9,985
	Disodium Octaborate (5%)	-9,0200*	1,1094	,000	-12,949	-5,091
	Disodium Octaborate (10%)	-9,0200*	1,1094	,000	-12,949	-5,091
	Disodium Octaborate (15%)	-15,5167*	1,1094	,000	-19,445	-11,588
Boric Acid (5%)	Control	4,4067*	1,1094	,020	,478	8,335
	Boric Acid (10%)	-5,1233*	1,1094	,005	-9,052	-1,195
	Boric Acid (15%)	-10,3867*	1,1094	,000	-14,315	-6,458
	Sodium Pentaborate (5%)	-5,7467*	1,1094	,001	-9,675	-1,818
	Sodium Pentaborate (10%)	-5,7467*	1,1094	,001	-9,675	-1,818
	Sodium Pentaborate (15%)	-9,5067*	1,1094	,000	-13,435	-5,578
	Disodium Octaborate (5%)	-4,6133*	1,1094	,014	-8,542	-,685
	Disodium Octaborate (10%)	-4,6133*	1,1094	,014	-8,542	-,685
	Disodium Octaborate (15%)	-11,1100*	1,1094	,000	-15,039	-7,181
Boric Acid (10%)	Control	9,5300*	1,1094	,000	5,601	13,459
	Boric Acid (5%)	5,1233*	1,1094	,005	1,195	9,052
	Boric Acid (15%)	-5,2633*	1,1094	,004	-9,192	-1,335
	Sodium Pentaborate (5%)	-,6233	1,1094	1,000	-4,552	3,305
	Sodium Pentaborate (10%)	-,6233	1,1094	1,000	-4,552	3,305
	Sodium Pentaborate (15%)	-4,3833*	1,1094	,021	-8,312	-,455
	Disodium Octaborate (5%)	,5100	1,1094	1,000	-3,419	4,439
	Disodium Octaborate (10%)	,5100	1,1094	1,000	-3,419	4,439
	Disodium Octaborate (15%)	-5,9867*	1,1094	,001	-9,915	-2,058

Boric Acid (15%)	Control	14,7933*	1,1094	,000	10,865	18,722
	Boric Acid (5%)	10,3867*	1,1094	,000	6,458	14,315
	Boric Acid (10%)	5,2633*	1,1094	,004	1,335	9,192
	Sodium Pentaborate (5%)	4,6400*	1,1094	,013	,711	8,569
	Sodium Pentaborate (10%)	4,6400*	1,1094	,013	,711	8,569
	Sodium Pentaborate (15%)	,8800	1,1094	,998	-3,049	4,809
	Disodium Octaborate (5%)	5,7733*	1,1094	,001	1,845	9,702
	Disodium Octaborate (10%)	5,7733*	1,1094	,001	1,845	9,702
	Disodium Octaborate (15%)	-,7233	1,1094	1,000	-4,652	3,205
Sodium Pentaborate (5%)	Control	10,1533*	1,1094	,000	6,225	14,082
	Boric Acid (5%)	5,7467*	1,1094	,001	1,818	9,675
	Boric Acid (10%)	,6233	1,1094	1,000	-3,305	4,552
	Boric Acid (15%)	-4,6400*	1,1094	,013	-8,569	-,711
	Sodium Pentaborate (10%)	0,0000	1,1094	1,000	-3,929	3,929
	Sodium Pentaborate (15%)	-3,7600	1,1094	,068	-7,689	,169
	Disodium Octaborate (5%)	1,1333	1,1094	,987	-2,795	5,062
	Disodium Octaborate (10%)	1,1333	1,1094	,987	-2,795	5,062
	Disodium Octaborate (15%)	-5,3633*	1,1094	,003	-9,292	-1,435
Sodium Pentaborate (10%)	Control	10,1533*	1,1094	,000	6,225	14,082
	Boric Acid (5%)	5,7467*	1,1094	,001	1,818	9,675
	Boric Acid (10%)	,6233	1,1094	1,000	-3,305	4,552
	Boric Acid (15%)	-4,6400*	1,1094	,013	-8,569	-,711
	Sodium Pentaborate (5%)	0,0000	1,1094	1,000	-3,929	3,929
	Sodium Pentaborate (15%)	-3,7600	1,1094	,068	-7,689	,169
	Disodium Octaborate (5%)	1,1333	1,1094	,987	-2,795	5,062
	Disodium Octaborate (10%)	1,1333	1,1094	,987	-2,795	5,062
	Disodium Octaborate (15%)	-5,3633*	1,1094	,003	-9,292	-1,435

Sodium Pentaborate (15%)	Control	13,9133*	1,1094	,000	9,985	17,842
	Boric Acid (5%)	9,5067*	1,1094	,000	5,578	13,435
	Boric Acid (10%)	4,3833*	1,1094	,021	,455	8,312
	Boric Acid (15%)	-,8800	1,1094	,998	-4,809	3,049
	Sodium Pentaborate (5%)	3,7600	1,1094	,068	-,169	7,689
	Sodium Pentaborate (10%)	3,7600	1,1094	,068	-,169	7,689
	Disodium Octaborate (5%)	4,8933*	1,1094	,008	,965	8,822
	Disodium Octaborate (10%)	4,8933*	1,1094	,008	,965	8,822
	Disodium Octaborate (15%)	-1,6033	1,1094	,898	-5,532	2,325
Disodium Octaborate (5%)	Control	9,0200*	1,1094	,000	5,091	12,949
	Boric Acid (5%)	4,6133*	1,1094	,014	,685	8,542
	Boric Acid (10%)	-,5100	1,1094	1,000	-4,439	3,419
	Boric Acid (15%)	-5,7733*	1,1094	,001	-9,702	-1,845
	Sodium Pentaborate (5%)	-1,1333	1,1094	,987	-5,062	2,795
	Sodium Pentaborate (10%)	-1,1333	1,1094	,987	-5,062	2,795
	Sodium Pentaborate (15%)	-4,8933*	1,1094	,008	-8,822	-,965
	Disodium Octaborate (10%)	0,0000	1,1094	1,000	-3,929	3,929
	Disodium Octaborate (15%)	-6,4967*	1,1094	,000	-10,425	-2,568
Disodium Octaborate (10%)	Control	9,0200*	1,1094	,000	5,091	12,949
	Boric Acid (5%)	4,6133*	1,1094	,014	,685	8,542
	Boric Acid (10%)	-,5100	1,1094	1,000	-4,439	3,419
	Boric Acid (15%)	-5,7733*	1,1094	,001	-9,702	-1,845
	Sodium Pentaborate (5%)	-1,1333	1,1094	,987	-5,062	2,795
	Sodium Pentaborate (10%)	-1,1333	1,1094	,987	-5,062	2,795
	Sodium Pentaborate (15%)	-4,8933*	1,1094	,008	-8,822	-,965
	Disodium Octaborate (5%)	0,0000	1,1094	1,000	-3,929	3,929
	Disodium Octaborate (15%)	-6,4967*	1,1094	,000	-10,425	-2,568

Disodium Octaborate (15%)	Control	15,5167*	1,1094	,000	11,588	19,445
	Boric Acid (5%)	11,1100*	1,1094	,000	7,181	15,039
	Boric Acid (10%)	5,9867*	1,1094	,001	2,058	9,915
	Boric Acid (15%)	,7233	1,1094	1,000	-3,205	4,652
	Sodium Pentaborate (5%)	5,3633*	1,1094	,003	1,435	9,292
	Sodium Pentaborate (10%)	5,3633*	1,1094	,003	1,435	9,292
	Sodium Pentaborate (15%)	1,6033	1,1094	,898	-2,325	5,532
	Disodium Octaborate (5%)	6,4967*	1,1094	,000	2,568	10,425
	Disodium Octaborate (10%)	6,4967*	1,1094	,000	2,568	10,425

*. The mean difference is significant at the 0.05 level.

Table A.7. Results of Tukey's HSD post hoc test for antimicrobial activity of gelatin films on *A. niger*.

(I) Sample		**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Control	Boric Acid (5%)	-14,5933*	1,2148	,000	-18,895	-10,292
	Boric Acid (10%)	-19,5667*	1,2148	,000	-23,868	-15,265
	Boric Acid (15%)	-22,1700*	1,2148	,000	-26,472	-17,868
	Sodium Pentaborate (5%)	-15,5667*	1,2148	,000	-19,868	-11,265
	Sodium Pentaborate (10%)	-19,4000*	1,2148	,000	-23,702	-15,098
	Sodium Pentaborate (15%)	-23,5567*	1,2148	,000	-27,858	-19,255
	Disodium Octaborate (5%)	-16,9333*	1,2148	,000	-21,235	-12,632
	Disodium Octaborate (10%)	-21,1833*	1,2148	,000	-25,485	-16,882
	Disodium Octaborate (15%)	-23,3933*	1,2148	,000	-27,695	-19,092
Boric Acid (5%)	Control	14,5933*	1,2148	,000	10,292	18,895
	Boric Acid (10%)	-4,9733*	1,2148	,016	-9,275	-,672
	Boric Acid (15%)	-7,5767*	1,2148	,000	-11,878	-3,275
	Sodium Pentaborate (5%)	-,9733	1,2148	,998	-5,275	3,328
	Sodium Pentaborate (10%)	-4,8067*	1,2148	,021	-9,108	-,505
	Sodium Pentaborate (15%)	-8,9633*	1,2148	,000	-13,265	-4,662
	Disodium Octaborate (5%)	-2,3400	1,2148	,653	-6,642	1,962
	Disodium Octaborate (10%)	-6,5900*	1,2148	,001	-10,892	-2,288
	Disodium Octaborate (15%)	-8,8000*	1,2148	,000	-13,102	-4,498

Boric Acid (10%)	Control	19,5667*	1,2148	,000	15,265	23,868
	Boric Acid (5%)	4,9733*	1,2148	,016	,672	9,275
	Boric Acid (15%)	-2,6033	1,2148	,521	-6,905	1,698
	Sodium Pentaborate (5%)	4,0000	1,2148	,082	-,302	8,302
	Sodium Pentaborate (10%)	,1667	1,2148	1,000	-4,135	4,468
	Sodium Pentaborate (15%)	-3,9900	1,2148	,083	-8,292	,312
	Disodium Octaborate (5%)	2,6333	1,2148	,506	-1,668	6,935
	Disodium Octaborate (10%)	-1,6167	1,2148	,934	-5,918	2,685
	Disodium Octaborate (15%)	-3,8267	1,2148	,108	-8,128	,475
Boric Acid (15%)	Control	22,1700*	1,2148	,000	17,868	26,472
	Boric Acid (5%)	7,5767*	1,2148	,000	3,275	11,878
	Boric Acid (10%)	2,6033	1,2148	,521	-1,698	6,905
	Sodium Pentaborate (5%)	6,6033*	1,2148	,001	2,302	10,905
	Sodium Pentaborate (10%)	2,7700	1,2148	,441	-1,532	7,072
	Sodium Pentaborate (15%)	-1,3867	1,2148	,973	-5,688	2,915
	Disodium Octaborate (5%)	5,2367*	1,2148	,010	,935	9,538
	Disodium Octaborate (10%)	,9867	1,2148	,997	-3,315	5,288
	Disodium Octaborate (15%)	-1,2233	1,2148	,988	-5,525	3,078
Sodium Pentaborate (5%)	Control	15,5667*	1,2148	,000	11,265	19,868
	Boric Acid (5%)	,9733	1,2148	,998	-3,328	5,275
	Boric Acid (10%)	-4,0000	1,2148	,082	-8,302	,302
	Boric Acid (15%)	-6,6033*	1,2148	,001	-10,905	-2,302
	Sodium Pentaborate (10%)	-3,8333	1,2148	,106	-8,135	,468
	Sodium Pentaborate (15%)	-7,9900*	1,2148	,000	-12,292	-3,688
	Disodium Octaborate (5%)	-1,3667	1,2148	,976	-5,668	2,935
	Disodium Octaborate (10%)	-5,6167*	1,2148	,005	-9,918	-1,315
	Disodium Octaborate (15%)	-7,8267*	1,2148	,000	-12,128	-3,525

Sodium Pentaborate (10%)	Control	19,4000*	1,2148	,000	15,098	23,702
	Boric Acid (5%)	4,8067*	1,2148	,021	,505	9,108
	Boric Acid (10%)	-,1667	1,2148	1,000	-4,468	4,135
	Boric Acid (15%)	-2,7700	1,2148	,441	-7,072	1,532
	Sodium Pentaborate (5%)	3,8333	1,2148	,106	-,468	8,135
	Sodium Pentaborate (15%)	-4,1567	1,2148	,064	-8,458	,145
	Disodium Octaborate (5%)	2,4667	1,2148	,590	-1,835	6,768
	Disodium Octaborate (10%)	-1,7833	1,2148	,889	-6,085	2,518
	Disodium Octaborate (15%)	-3,9933	1,2148	,083	-8,295	,308
Sodium Pentaborate (15%)	Control	23,5567*	1,2148	,000	19,255	27,858
	Boric Acid (5%)	8,9633*	1,2148	,000	4,662	13,265
	Boric Acid (10%)	3,9900	1,2148	,083	-,312	8,292
	Boric Acid (15%)	1,3867	1,2148	,973	-2,915	5,688
	Sodium Pentaborate (5%)	7,9900*	1,2148	,000	3,688	12,292
	Sodium Pentaborate (10%)	4,1567	1,2148	,064	-,145	8,458
	Disodium Octaborate (5%)	6,6233*	1,2148	,001	2,322	10,925
	Disodium Octaborate (10%)	2,3733	1,2148	,637	-1,928	6,675
	Disodium Octaborate (15%)	,1633	1,2148	1,000	-4,138	4,465
Disodium Octaborate (5%)	Control	16,9333*	1,2148	,000	12,632	21,235
	Boric Acid (5%)	2,3400	1,2148	,653	-1,962	6,642
	Boric Acid (10%)	-2,6333	1,2148	,506	-6,935	1,668
	Boric Acid (15%)	-5,2367*	1,2148	,010	-9,538	-,935
	Sodium Pentaborate (5%)	1,3667	1,2148	,976	-2,935	5,668
	Sodium Pentaborate (10%)	-2,4667	1,2148	,590	-6,768	1,835
	Sodium Pentaborate (15%)	-6,6233*	1,2148	,001	-10,925	-2,322
	Disodium Octaborate (10%)	-4,2500	1,2148	,054	-8,552	,052
	Disodium Octaborate (15%)	-6,4600*	1,2148	,001	-10,762	-2,158

Disodium Octaborate (10%)	Control	21,1833*	1,2148	,000	16,882	25,485
	Boric Acid (5%)	6,5900*	1,2148	,001	2,288	10,892
	Boric Acid (10%)	1,6167	1,2148	,934	-2,685	5,918
	Boric Acid (15%)	-,9867	1,2148	,997	-5,288	3,315
	Sodium Pentaborate (5%)	5,6167*	1,2148	,005	1,315	9,918
	Sodium Pentaborate (10%)	1,7833	1,2148	,889	-2,518	6,085
	Sodium Pentaborate (15%)	-2,3733	1,2148	,637	-6,675	1,928
	Disodium Octaborate (5%)	4,2500	1,2148	,054	-,052	8,552
	Disodium Octaborate (15%)	-2,2100	1,2148	,717	-6,512	2,092
Disodium Octaborate (15%)	Control	23,3933*	1,2148	,000	19,092	27,695
	Boric Acid (5%)	8,8000*	1,2148	,000	4,498	13,102
	Boric Acid (10%)	3,8267	1,2148	,108	-,475	8,128
	Boric Acid (15%)	1,2233	1,2148	,988	-3,078	5,525
	Sodium Pentaborate (5%)	7,8267*	1,2148	,000	3,525	12,128
	Sodium Pentaborate (10%)	3,9933	1,2148	,083	-,308	8,295
	Sodium Pentaborate (15%)	-,1633	1,2148	1,000	-4,465	4,138
	Disodium Octaborate (5%)	6,4600*	1,2148	,001	2,158	10,762
	Disodium Octaborate (10%)	2,2100	1,2148	,717	-2,092	6,512

*. The mean difference is significant at the 0.05 level.

Table A.8. Results of Tukey's HSD post hoc test for antimicrobial activity of gelatin films on *C. albicans*.

(I) Sample		**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Control	Boric Acid (5%)	0,0000	1,8806	1,000	-6,659	6,659
	Boric Acid (10%)	-10,4367*	1,8806	,001	-17,096	-3,777
	Boric Acid (15%)	-13,6167*	1,8806	,000	-20,276	-6,957
	Sodium Pentaborate (5%)	-1,8367	1,8806	,991	-8,496	4,823
	Sodium Pentaborate (10%)	-11,0467*	1,8806	,000	-17,706	-4,387
	Sodium Pentaborate (15%)	-12,8567*	1,8806	,000	-19,516	-6,197
	Disodium Octaborate (5%)	-,9000	1,8806	1,000	-7,559	5,759
	Disodium Octaborate (10%)	-11,0900*	1,8806	,000	-17,749	-4,431
	Disodium Octaborate (15%)	-14,6000*	1,8806	,000	-21,259	-7,941

Boric Acid (5%)	Control	0,0000	1,8806	1,000	-6,659	6,659
	Boric Acid (10%)	-10,4367*	1,8806	,001	-17,096	-3,777
	Boric Acid (15%)	-13,6167*	1,8806	,000	-20,276	-6,957
	Sodium Pentaborate (5%)	-1,8367	1,8806	,991	-8,496	4,823
	Sodium Pentaborate (10%)	-11,0467*	1,8806	,000	-17,706	-4,387
	Sodium Pentaborate (15%)	-12,8567*	1,8806	,000	-19,516	-6,197
	Disodium Octaborate (5%)	-,9000	1,8806	1,000	-7,559	5,759
	Disodium Octaborate (10%)	-11,0900*	1,8806	,000	-17,749	-4,431
	Disodium Octaborate (15%)	-14,6000*	1,8806	,000	-21,259	-7,941
Boric Acid (10%)	Control	10,4367*	1,8806	,001	3,777	17,096
	Boric Acid (5%)	10,4367*	1,8806	,001	3,777	17,096
	Boric Acid (15%)	-3,1800	1,8806	,788	-9,839	3,479
	Sodium Pentaborate (5%)	8,6000*	1,8806	,006	1,941	15,259
	Sodium Pentaborate (10%)	-,6100	1,8806	1,000	-7,269	6,049
	Sodium Pentaborate (15%)	-2,4200	1,8806	,946	-9,079	4,239
	Disodium Octaborate (5%)	9,5367*	1,8806	,002	2,877	16,196
	Disodium Octaborate (10%)	-,6533	1,8806	1,000	-7,313	6,006
	Disodium Octaborate (15%)	-4,1633	1,8806	,479	-10,823	2,496
Boric Acid (15%)	Control	13,6167*	1,8806	,000	6,957	20,276
	Boric Acid (5%)	13,6167*	1,8806	,000	6,957	20,276
	Boric Acid (10%)	3,1800	1,8806	,788	-3,479	9,839
	Sodium Pentaborate (5%)	11,7800*	1,8806	,000	5,121	18,439
	Sodium Pentaborate (10%)	2,5700	1,8806	,924	-4,089	9,229
	Sodium Pentaborate (15%)	,7600	1,8806	1,000	-5,899	7,419
	Disodium Octaborate (5%)	12,7167*	1,8806	,000	6,057	19,376
	Disodium Octaborate (10%)	2,5267	1,8806	,931	-4,133	9,186
	Disodium Octaborate (15%)	-,9833	1,8806	1,000	-7,643	5,676

Sodium Pentaborate (5%)	Control	1,8367	1,8806	,991	-4,823	8,496
	Boric Acid (5%)	1,8367	1,8806	,991	-4,823	8,496
	Boric Acid (10%)	-8,6000*	1,8806	,006	-15,259	-1,941
	Boric Acid (15%)	-11,7800*	1,8806	,000	-18,439	-5,121
	Sodium Pentaborate (10%)	-9,2100*	1,8806	,003	-15,869	-2,551
	Sodium Pentaborate (15%)	-11,0200*	1,8806	,000	-17,679	-4,361
	Disodium Octaborate (5%)	,9367	1,8806	1,000	-5,723	7,596
	Disodium Octaborate (10%)	-9,2533*	1,8806	,003	-15,913	-2,594
	Disodium Octaborate (15%)	-12,7633*	1,8806	,000	-19,423	-6,104
Sodium Pentaborate (10%)	Control	11,0467*	1,8806	,000	4,387	17,706
	Boric Acid (5%)	11,0467*	1,8806	,000	4,387	17,706
	Boric Acid (10%)	,6100	1,8806	1,000	-6,049	7,269
	Boric Acid (15%)	-2,5700	1,8806	,924	-9,229	4,089
	Sodium Pentaborate (5%)	9,2100*	1,8806	,003	2,551	15,869
	Sodium Pentaborate (15%)	-1,8100	1,8806	,991	-8,469	4,849
	Disodium Octaborate (5%)	10,1467*	1,8806	,001	3,487	16,806
	Disodium Octaborate (10%)	-,0433	1,8806	1,000	-6,703	6,616
	Disodium Octaborate (15%)	-3,5533	1,8806	,676	-10,213	3,106
Sodium Pentaborate (15%)	Control	12,8567*	1,8806	,000	6,197	19,516
	Boric Acid (5%)	12,8567*	1,8806	,000	6,197	19,516
	Boric Acid (10%)	2,4200	1,8806	,946	-4,239	9,079
	Boric Acid (15%)	-,7600	1,8806	1,000	-7,419	5,899
	Sodium Pentaborate (5%)	11,0200*	1,8806	,000	4,361	17,679
	Sodium Pentaborate (10%)	1,8100	1,8806	,991	-4,849	8,469
	Disodium Octaborate (5%)	11,9567*	1,8806	,000	5,297	18,616
	Disodium Octaborate (10%)	1,7667	1,8806	,993	-4,893	8,426
	Disodium Octaborate (15%)	-1,7433	1,8806	,993	-8,403	4,916

Disodium Octaborate (5%)	Control	,9000	1,8806	1,000	-5,759	7,559
	Boric Acid (5%)	,9000	1,8806	1,000	-5,759	7,559
	Boric Acid (10%)	-9,5367*	1,8806	,002	-16,196	-2,877
	Boric Acid (15%)	-12,7167*	1,8806	,000	-19,376	-6,057
	Sodium Pentaborate (5%)	-,9367	1,8806	1,000	-7,596	5,723
	Sodium Pentaborate (10%)	-10,1467*	1,8806	,001	-16,806	-3,487
	Sodium Pentaborate (15%)	-11,9567*	1,8806	,000	-18,616	-5,297
	Disodium Octaborate (10%)	-10,1900*	1,8806	,001	-16,849	-3,531
	Disodium Octaborate (15%)	-13,7000*	1,8806	,000	-20,359	-7,041
Disodium Octaborate (10%)	Control	11,0900*	1,8806	,000	4,431	17,749
	Boric Acid (5%)	11,0900*	1,8806	,000	4,431	17,749
	Boric Acid (10%)	,6533	1,8806	1,000	-6,006	7,313
	Boric Acid (15%)	-2,5267	1,8806	,931	-9,186	4,133
	Sodium Pentaborate (5%)	9,2533*	1,8806	,003	2,594	15,913
	Sodium Pentaborate (10%)	,0433	1,8806	1,000	-6,616	6,703
	Sodium Pentaborate (15%)	-1,7667	1,8806	,993	-8,426	4,893
	Disodium Octaborate (5%)	10,1900*	1,8806	,001	3,531	16,849
	Disodium Octaborate (15%)	-3,5100	1,8806	,689	-10,169	3,149
Disodium Octaborate (15%)	Control	14,6000*	1,8806	,000	7,941	21,259
	Boric Acid (5%)	14,6000*	1,8806	,000	7,941	21,259
	Boric Acid (10%)	4,1633	1,8806	,479	-2,496	10,823
	Boric Acid (15%)	,9833	1,8806	1,000	-5,676	7,643
	Sodium Pentaborate (5%)	12,7633*	1,8806	,000	6,104	19,423
	Sodium Pentaborate (10%)	3,5533	1,8806	,676	-3,106	10,213
	Sodium Pentaborate (15%)	1,7433	1,8806	,993	-4,916	8,403
	Disodium Octaborate (5%)	13,7000*	1,8806	,000	7,041	20,359
	Disodium Octaborate (10%)	3,5100	1,8806	,689	-3,149	10,169

*. The mean difference is significant at the 0.05 level.

APPENDIX B: TENSILE STRENGTH OF GELATIN AND PECTIN FILMS

Tensile strengths of the boron derivatives incorporated pectin and gelatin films were shown in Table B.1. and Table B.3. respectively. Tensile strength values of the films were Results of the Tukey's HSD post hoc test for tensile strength of the pectin and gelatin films can be seen in Table B.2. and Table B.4.

Table B.1. Tensile strength of gelatin films incorporated with boron derivatives

Sample	Conc.	Tensile Strength (g)			Average (g)	Std.
Control		8626	9428	11498	9851	1482
Boric Acid	5%	12101	12348	12710	12386	306
	10%	14234	12371	14559	13721	1181
	15%	14483	13633	13601	13906	500
Sodium Pentaborate	5%	11489	11644	10992	11375	341
	10%	12097	12854	13252	12734	587
	15%	11139	11757	12106	11667	490
Disodium Octaborate	5%	15135	16313	16702	16050	816
	10%	17264	17845	16408	17172	723
	15%	16266	16134	13354	15251	1644

Table B.2. Results of Tukey's HSD post hoc test for tensile strength of gelatin films

(I) Sample		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Boric Acid (5%)	-2535,667	753,955	,071	-5205,50	134,17
	Boric Acid (10%)	-3870,667*	753,955	,002	-6540,50	-1200,83
	Boric Acid (15%)	-4055,000*	753,955	,001	-6724,84	-1385,16
	Sodium Pentaborate (5%)	-1524,333	753,955	,595	-4194,17	1145,50
	Sodium Pentaborate (10%)	-2883,667*	753,955	,028	-5553,50	-213,83
	Sodium Pentaborate (15%)	-1816,667	753,955	,370	-4486,50	853,17
	Disodium Octaborate (5%)	-6199,333*	753,955	,000	-8869,17	-3529,50
	Disodium Octaborate (10%)	-7321,667*	753,955	,000	-9991,50	-4651,83
	Disodium Octaborate (15%)	-5400,667*	753,955	,000	-8070,50	-2730,83

Boric Acid (5%)	Control	2535,667	753,955	,071	-134,17	5205,50
	Boric Acid (10%)	-1335,000	753,955	,745	-4004,84	1334,84
	Boric Acid (15%)	-1519,333	753,955	,599	-4189,17	1150,50
	Sodium Pentaborate (5%)	1011,333	753,955	,931	-1658,50	3681,17
	Sodium Pentaborate (10%)	-348,000	753,955	1,000	-3017,84	2321,84
	Sodium Pentaborate (15%)	719,000	753,955	,992	-1950,84	3388,84
	Disodium Octaborate (5%)	-3663,667*	753,955	,003	-6333,50	-993,83
	Disodium Octaborate (10%)	-4786,000*	753,955	,000	-7455,84	-2116,16
	Disodium Octaborate (15%)	-2865,000*	753,955	,029	-5534,84	-195,16
Boric Acid (10%)	Control	3870,667*	753,955	,002	1200,83	6540,50
	Boric Acid (5%)	1335,000	753,955	,745	-1334,84	4004,84
	Boric Acid (15%)	-184,333	753,955	1,000	-2854,17	2485,50
	Sodium Pentaborate (5%)	2346,333	753,955	,116	-323,50	5016,17
	Sodium Pentaborate (10%)	987,000	753,955	,940	-1682,84	3656,84
	Sodium Pentaborate (15%)	2054,000	753,955	,229	-615,84	4723,84
	Disodium Octaborate (5%)	-2328,667	753,955	,121	-4998,50	341,17
	Disodium Octaborate (10%)	-3451,000*	753,955	,006	-6120,84	-781,16
	Disodium Octaborate (15%)	-1530,000	753,955	,590	-4199,84	1139,84
Boric Acid (15%)	Control	4055,000*	753,955	,001	1385,16	6724,84
	Boric Acid (5%)	1519,333	753,955	,599	-1150,50	4189,17
	Boric Acid (10%)	184,333	753,955	1,000	-2485,50	2854,17
	Sodium Pentaborate (5%)	2530,667	753,955	,072	-139,17	5200,50
	Sodium Pentaborate (10%)	1171,333	753,955	,855	-1498,50	3841,17
	Sodium Pentaborate (15%)	2238,333	753,955	,150	-431,50	4908,17
	Disodium Octaborate (5%)	-2144,333	753,955	,187	-4814,17	525,50
	Disodium Octaborate (10%)	-3266,667*	753,955	,009	-5936,50	-596,83
	Disodium Octaborate (15%)	-1345,667	753,955	,737	-4015,50	1324,17

Sodium Pentaborate (5%)	Control	1524,333	753,955	,595	-1145,50	4194,17
	Boric Acid (5%)	-1011,333	753,955	,931	-3681,17	1658,50
	Boric Acid (10%)	-2346,333	753,955	,116	-5016,17	323,50
	Boric Acid (15%)	-2530,667	753,955	,072	-5200,50	139,17
	Sodium Pentaborate (10%)	-1359,333	753,955	,726	-4029,17	1310,50
	Sodium Pentaborate (15%)	-292,333	753,955	1,000	-2962,17	2377,50
	Disodium Octaborate (5%)	-4675,000*	753,955	,000	-7344,84	-2005,16
	Disodium Octaborate (10%)	-5797,333*	753,955	,000	-8467,17	-3127,50
	Disodium Octaborate (15%)	-3876,333*	753,955	,002	-6546,17	-1206,50
Sodium Pentaborate (10%)	Control	2883,667*	753,955	,028	213,83	5553,50
	Boric Acid (5%)	348,000	753,955	1,000	-2321,84	3017,84
	Boric Acid (10%)	-987,000	753,955	,940	-3656,84	1682,84
	Boric Acid (15%)	-1171,333	753,955	,855	-3841,17	1498,50
	Sodium Pentaborate (5%)	1359,333	753,955	,726	-1310,50	4029,17
	Sodium Pentaborate (15%)	1067,000	753,955	,908	-1602,84	3736,84
	Disodium Octaborate (5%)	-3315,667*	753,955	,008	-5985,50	-645,83
	Disodium Octaborate (10%)	-4438,000*	753,955	,000	-7107,84	-1768,16
	Disodium Octaborate (15%)	-2517,000	753,955	,075	-5186,84	152,84
Sodium Pentaborate (15%)	Control	1816,667	753,955	,370	-853,17	4486,50
	Boric Acid (5%)	-719,000	753,955	,992	-3388,84	1950,84
	Boric Acid (10%)	-2054,000	753,955	,229	-4723,84	615,84
	Boric Acid (15%)	-2238,333	753,955	,150	-4908,17	431,50
	Sodium Pentaborate (5%)	292,333	753,955	1,000	-2377,50	2962,17
	Sodium Pentaborate (10%)	-1067,000	753,955	,908	-3736,84	1602,84
	Disodium Octaborate (5%)	-4382,667*	753,955	,000	-7052,50	-1712,83
	Disodium Octaborate (10%)	-5505,000*	753,955	,000	-8174,84	-2835,16
	Disodium Octaborate (15%)	-3584,000*	753,955	,004	-6253,84	-914,16

Disodium Octaborate (5%)	Control	6199,333*	753,955	,000	3529,50	8869,17
	Boric Acid (5%)	3663,667*	753,955	,003	993,83	6333,50
	Boric Acid (10%)	2328,667	753,955	,121	-341,17	4998,50
	Boric Acid (15%)	2144,333	753,955	,187	-525,50	4814,17
	Sodium Pentaborate (5%)	4675,000*	753,955	,000	2005,16	7344,84
	Sodium Pentaborate (10%)	3315,667*	753,955	,008	645,83	5985,50
	Sodium Pentaborate (15%)	4382,667*	753,955	,000	1712,83	7052,50
	Disodium Octaborate (10%)	-1122,333	753,955	,882	-3792,17	1547,50
	Disodium Octaborate (15%)	798,667	753,955	,984	-1871,17	3468,50
Disodium Octaborate (10%)	Control	7321,667*	753,955	,000	4651,83	9991,50
	Boric Acid (5%)	4786,000*	753,955	,000	2116,16	7455,84
	Boric Acid (10%)	3451,000*	753,955	,006	781,16	6120,84
	Boric Acid (15%)	3266,667*	753,955	,009	596,83	5936,50
	Sodium Pentaborate (5%)	5797,333*	753,955	,000	3127,50	8467,17
	Sodium Pentaborate (10%)	4438,000*	753,955	,000	1768,16	7107,84
	Sodium Pentaborate (15%)	5505,000*	753,955	,000	2835,16	8174,84
	Disodium Octaborate (5%)	1122,333	753,955	,882	-1547,50	3792,17
	Disodium Octaborate (15%)	1921,000	753,955	,302	-748,84	4590,84
Disodium Octaborate (15%)	Control	5400,667*	753,955	,000	2730,83	8070,50
	Boric Acid (5%)	2865,000*	753,955	,029	195,16	5534,84
	Boric Acid (10%)	1530,000	753,955	,590	-1139,84	4199,84
	Boric Acid (15%)	1345,667	753,955	,737	-1324,17	4015,50
	Sodium Pentaborate (5%)	3876,333*	753,955	,002	1206,50	6546,17
	Sodium Pentaborate (10%)	2517,000	753,955	,075	-152,84	5186,84
	Sodium Pentaborate (15%)	3584,000*	753,955	,004	914,16	6253,84
	Disodium Octaborate (5%)	-798,667	753,955	,984	-3468,50	1871,17
	Disodium Octaborate (10%)	-1921,000	753,955	,302	-4590,84	748,84

*. The mean difference is significant at the 0.05 level.

Table B.3. Tensile strength of pectin film incorporated with boron derivatives

Sample	Conc.	Tensile Strength (g)			Average (g)	Std.
Control		849	1109	932	963	133
Boric Acid	5%	1623	1649	1853	1708	126
	10%	1672	1712	1825	1736	79
	15%	1603	1601	1588	1597	8
Sodium Pentaborate	5%	1452	1403	1514	1456	56
	10%	1755	2229	2363	2116	319
	15%	2044	2118	2349	2170	159
Disodium Octaborate	5%	1066	1130	1015	1070	58
	10%	1970	2082	1883	1978	100
	15%	1533	1395	1369	1432	88

Table B.4. Results of Tukey's HSD post hoc test for tensile strength of pectin films

(I) Sample		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Boric Acid (5%)	-745,000*	112,948	,000	-1144,96	-345,04
	Boric Acid (10%)	-773,000*	112,948	,000	-1172,96	-373,04
	Boric Acid (15%)	-634,000*	112,948	,001	-1033,96	-234,04
	Sodium Pentaborate (5%)	-493,000*	112,948	,009	-892,96	-93,04
	Sodium Pentaborate (10%)	-1152,333*	112,948	,000	-1552,30	-752,37
	Sodium Pentaborate (15%)	-1207,000*	112,948	,000	-1606,96	-807,04
	Disodium Octaborate (5%)	-107,000	112,948	,992	-506,96	292,96
	Disodium Octaborate (10%)	-1015,000*	112,948	,000	-1414,96	-615,04
	Disodium Octaborate (15%)	-469,000*	112,948	,014	-868,96	-69,04
Boric Acid (5%)	Control	745,000*	112,948	,000	345,04	1144,96
	Boric Acid (10%)	-28,000	112,948	1,000	-427,96	371,96
	Boric Acid (15%)	111,000	112,948	,990	-288,96	510,96
	Sodium Pentaborate (5%)	252,000	112,948	,469	-147,96	651,96
	Sodium Pentaborate (10%)	-407,333*	112,948	,044	-807,30	-7,37
	Sodium Pentaborate (15%)	-462,000*	112,948	,016	-861,96	-62,04
	Disodium Octaborate (5%)	638,000*	112,948	,001	238,04	1037,96
	Disodium Octaborate (10%)	-270,000	112,948	,380	-669,96	129,96
	Disodium Octaborate (15%)	276,000	112,948	,353	-123,96	675,96

Boric Acid (10%)	Control	773,000*	112,948	,000	373,04	1172,96
	Boric Acid (5%)	28,000	112,948	1,000	-371,96	427,96
	Boric Acid (15%)	139,000	112,948	,958	-260,96	538,96
	Sodium Pentaborate (5%)	280,000	112,948	,335	-119,96	679,96
	Sodium Pentaborate (10%)	-379,333	112,948	,072	-779,30	20,63
	Sodium Pentaborate (15%)	-434,000*	112,948	,027	-833,96	-34,04
	Disodium Octaborate (5%)	666,000*	112,948	,000	266,04	1065,96
	Disodium Octaborate (10%)	-242,000	112,948	,521	-641,96	157,96
	Disodium Octaborate (15%)	304,000	112,948	,241	-95,96	703,96
Boric Acid (15%)	Control	634,000*	112,948	,001	234,04	1033,96
	Boric Acid (5%)	-111,000	112,948	,990	-510,96	288,96
	Boric Acid (10%)	-139,000	112,948	,958	-538,96	260,96
	Sodium Pentaborate (5%)	141,000	112,948	,954	-258,96	540,96
	Sodium Pentaborate (10%)	-518,333*	112,948	,005	-918,30	-118,37
	Sodium Pentaborate (15%)	-573,000*	112,948	,002	-972,96	-173,04
	Disodium Octaborate (5%)	527,000*	112,948	,005	127,04	926,96
	Disodium Octaborate (10%)	-381,000	112,948	,070	-780,96	18,96
	Disodium Octaborate (15%)	165,000	112,948	,892	-234,96	564,96
Sodium Pentaborate (5%)	Control	493,000*	112,948	,009	93,04	892,96
	Boric Acid (5%)	-252,000	112,948	,469	-651,96	147,96
	Boric Acid (10%)	-280,000	112,948	,335	-679,96	119,96
	Boric Acid (15%)	-141,000	112,948	,954	-540,96	258,96
	Sodium Pentaborate (10%)	-659,333*	112,948	,000	-1059,30	-259,37
	Sodium Pentaborate (15%)	-714,000*	112,948	,000	-1113,96	-314,04
	Disodium Octaborate (5%)	386,000	112,948	,064	-13,96	785,96
	Disodium Octaborate (10%)	-522,000*	112,948	,005	-921,96	-122,04
	Disodium Octaborate (15%)	24,000	112,948	1,000	-375,96	423,96

Sodium Pentaborate (10%)	Control	1152,333*	112,948	,000	752,37	1552,30
	Boric Acid (5%)	407,333*	112,948	,044	7,37	807,30
	Boric Acid (10%)	379,333	112,948	,072	-20,63	779,30
	Boric Acid (15%)	518,333*	112,948	,005	118,37	918,30
	Sodium Pentaborate (5%)	659,333*	112,948	,000	259,37	1059,30
	Sodium Pentaborate (15%)	-54,667	112,948	1,000	-454,63	345,30
	Disodium Octaborate (5%)	1045,333*	112,948	,000	645,37	1445,30
	Disodium Octaborate (10%)	137,333	112,948	,961	-262,63	537,30
	Disodium Octaborate (15%)	683,333*	112,948	,000	283,37	1083,30
Sodium Pentaborate (15%)	Control	1207,000*	112,948	,000	807,04	1606,96
	Boric Acid (5%)	462,000*	112,948	,016	62,04	861,96
	Boric Acid (10%)	434,000*	112,948	,027	34,04	833,96
	Boric Acid (15%)	573,000*	112,948	,002	173,04	972,96
	Sodium Pentaborate (5%)	714,000*	112,948	,000	314,04	1113,96
	Sodium Pentaborate (10%)	54,667	112,948	1,000	-345,30	454,63
	Disodium Octaborate (5%)	1100,000*	112,948	,000	700,04	1499,96
	Disodium Octaborate (10%)	192,000	112,948	,783	-207,96	591,96
	Disodium Octaborate (15%)	738,000*	112,948	,000	338,04	1137,96
Disodium Octaborate (5%)	Control	107,000	112,948	,992	-292,96	506,96
	Boric Acid (5%)	-638,000*	112,948	,001	-1037,96	-238,04
	Boric Acid (10%)	-666,000*	112,948	,000	-1065,96	-266,04
	Boric Acid (15%)	-527,000*	112,948	,005	-926,96	-127,04
	Sodium Pentaborate (5%)	-386,000	112,948	,064	-785,96	13,96
	Sodium Pentaborate (10%)	-1045,333*	112,948	,000	-1445,30	-645,37
	Sodium Pentaborate (15%)	-1100,000*	112,948	,000	-1499,96	-700,04
	Disodium Octaborate (10%)	-908,000*	112,948	,000	-1307,96	-508,04
	Disodium Octaborate (15%)	-362,000	112,948	,097	-761,96	37,96

Disodium Octaborate (10%)	Control	1015,000*	112,948	,000	615,04	1414,96
	Boric Acid (5%)	270,000	112,948	,380	-129,96	669,96
	Boric Acid (10%)	242,000	112,948	,521	-157,96	641,96
	Boric Acid (15%)	381,000	112,948	,070	-18,96	780,96
	Sodium Pentaborate (5%)	522,000*	112,948	,005	122,04	921,96
	Sodium Pentaborate (10%)	-137,333	112,948	,961	-537,30	262,63
	Sodium Pentaborate (15%)	-192,000	112,948	,783	-591,96	207,96
	Disodium Octaborate (5%)	908,000*	112,948	,000	508,04	1307,96
	Disodium Octaborate (15%)	546,000*	112,948	,003	146,04	945,96
Disodium Octaborate (15%)	Control	469,000*	112,948	,014	69,04	868,96
	Boric Acid (5%)	-276,000	112,948	,353	-675,96	123,96
	Boric Acid (10%)	-304,000	112,948	,241	-703,96	95,96
	Boric Acid (15%)	-165,000	112,948	,892	-564,96	234,96
	Sodium Pentaborate (5%)	-24,000	112,948	1,000	-423,96	375,96
	Sodium Pentaborate (10%)	-683,333*	112,948	,000	-1083,30	-283,37
	Sodium Pentaborate (15%)	-738,000*	112,948	,000	-1137,96	-338,04
	Disodium Octaborate (5%)	362,000	112,948	,097	-37,96	761,96
	Disodium Octaborate (10%)	-546,000*	112,948	,003	-945,96	-146,04

*. The mean difference is significant at the 0.05 level.