PRODUCTION AND CHARACTERIZATION OF CHICKPEA FLOUR BASED BIOFILMS CONTAINING GALLIC ACID

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

PRODUCTION AND CHARACTERIZATION OF CHICKPEA FLOUR BASED BIOFILMS CONTAINING GALLIC ACID

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Chickpea is an easily accessible legume which has beneficial effects on human health. On the other hand, gallic acid is a widespread phenolic compound found in various plants. In this study, it was aimed to obtain chickpea flour based active films and to characterize this films. The effects of pH (9 and 11), glycerol concentration (1% and 3% w/v of film solution) and gallic acid concentration (5% and 10% w/w of total solid) on color, opacity, mechanical strength, water vapor permeability (WVP), microstructure, chemical characteristic, antioxidant activity and phenolic content of films were investigated. At pH 11, films were stronger, had lower lightness value and had higher antioxidant activity. On the other hand, higher glycerol concentration made the films more flexible but less strong and more hydrophilic which resulted in higher WVP. There was no effect of glycerol on color and phenolic content of the films. Higher gallic acid concentration made great contribution to the chickpea flour based films by decreasing WVP and by increasing opacity, antioxidant activity, phenolic content, lightness and elongation. In this study, combination of chickpea flour and gallic acid as an antioxidant agent which has glycerol concentration of 1% w/v of film solution and gallic acid concentration of 10% w/w of total solid at pH 11 was found to be a great combination in order to obtain antioxidant rich edible composite films.

Keywords: Chickpea Flour, Biodegradable Film, Gallic Acid, Active Film

ÖZ

GALİK ASİT İÇEREN VE NOHUT UNUNDAN ELDE EDİLEN BİYOFİLMLERİN ÜRETİLMESİ VE KARAKTERİZASYONU

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Nohut unu besleyici, ucuz ve herkes tarafından kolay erişilebilir bir bakliyattır. Galik asit ise pek çok bitkide bulunan yaygın bir bileşendir. Bu çalışmada, galik asit içeren nohut unundan yapılmış aktif paketleme filmi eldesi ve bu filmlerin karakterizasyonunun yapılması amaçlanmıştır. Renk, opaklık, mekanik direnç, su buharı geçirgenliği, mikroyapı, kimyasal yapı, antioksidan aktivitesi ve toplam fenolik miktarı üzerinde pH (9 ve 11); gliserol konsantrasyonunun (%1 ve %3) ve galik asit konsantrasyonunun (%5 ve %10) etkisi araştırılmıştır. Filmler pH 11 değerinde daha sağlam, koyu renkte ve yüksek antioksidan aktivitesine sahip bulunmuştur. Diğer yandan yüksek gliserol konsantrasyonu filmlerin elastikiyetini artırırken mekanik dayanımını azaltmış ve su buharı geçirgenliğini arttırmıştır. Renk ve toplam fenolik üzerinde ise istatistiksel olarak bir etkisi olmamıştır. Yüksek konsantrasyonda eklenen galik asit ise su buharı geçirgenliğini azaltarak iyi bir gaz bariyeri sağlamıştır. Ayrıca opaklık, antioksidan aktivitesi, elastikiyet ve toplam fenolik miktarını arttırmıştır. Sonuç olarak, bu çalışmada pH 11 değerinde %1 gliserol ve %10 galik asit ile hazırlanan nohut unu filmlerinin antioksidan açısından zengin aktif paketleme filmi oluşturmak için iyi bir kombinasyon olduğu görülmüştür.

Anahtar Kelimeler: Aktif Paketleme, Nohut Unu, Biyobozunur Film, Galik Asit

To my family

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INTRODUCTION

1.1. Raw Material

1.1.1. Chickpea Flour

Chickpea (Cicer arietinum L) named also as Garbanzo bean or Bengal gram is the most abundant crop that is grown in India, Middle East, southern Europe, North Africa, continents of America and Australia constituting over 50 countries (Jukanti et al., 2012). The annual production of chickpea flour in the world is 10.1 million tons which takes the third rank among the pulse crop. Consistence increment has been observing since 1961. India is the dominant producer with 70% of total chickpea production of worldwide. It is followed by Pakistan and Iran with 10% and 5% production, respectively. Turkey comes as other major country producing chickpea with 4% (Muehlbauer & Sarker, 2017).

Chickpea has two types named as Kabuli and Desi. Kabuli is identified with yellow cothyledon and thin coat on small seeds. Desi is described with color range from light tan to black small seeds and have pink flowers. Color changes with anthocyanin pigmentation on stems. Weight of the seeds for Kabuli is around 0.2-0.6 g and around 0.1-0.3 g for Desi (Jukanti et al., 2012). In general, Desi type chickpea overrides Kabuli types with the 80% of production globally (Muehlbauer & Sarker, 2017).

Starch is the main constituent of the chickpea flour with composition of 48%. Chickpea is a highly nutritional crop with its high protein amount. Protein takes the second rank in chickpea flour composition with 26.3 %. Fat, dietary fibers, ash and moisture of the chickpea flour are 6, 11, 2.4 and 10.6% respectively (Emami & Tabil, 2008). In Table 1.1, nutritional properties of chickpea flour are given.

Table 1.1. *Chemical composition of chickpea flour (Wani & Kumar, 2014)*

Parameters	*Composition (%)*
Moisture	8.40 ± 0.50
Ash	2.79 ± 0.19
Crude protein	24.67 ± 1.37
Crude fat	4.64 ± 0.36
Crude fiber	1.75 ± 0.36
Total carbohydrate	57.78 ± 1.08

In addition to nutritional properties, chickpea flour has functional properties as a natural consequence of starch and protein content. Water absorbing capacity of chickpea flour is directly affected by processing properties. Proteins and polysaccharides found in chickpea flour have a hydrophilic nature and determine the water absorption capacity. Water absorption capacity of chickpea flour is lower than that of pinto bean, black bean, mung bean and lentil flours while it is higher than lima bean (1.17 g/g) and black eye bean (1.12 g/g) flours (Du et al., 2014).

Chickpea flour has significant amount of starch in nutritional composition which gives pasting ability. Pasting temperature of chickpea flour is 73.6^{0}C and peak viscosity is 704 cp. In the light of a previous study, retrogradation of starch component of chickpea flour was found to be difficult due to its high fat content. Inhibition of directional arrangement of dispersed molecular chain of starch conduces to difficult retrogradation (Wani & Kumar, 2014). Besides, chickpea flour shows the highest protein solubility at pH 2-3 and pH 7-10 while the lowest values are observed at pH 4-6. Protein-protein interactions at isoelectric point are low which lead to the lowest solubility. At pH 7, solubility of proteins differs from 69.6 % to 73.6 %. At pH 10, the maximum value of solubility was observed as 96.2% (Sreerama et al., 2012).

1.1.2. Glycerol

Glycerol having chemical name as 1,2,3-propanetriol is a colorless and odorless substance. It is obtained from petrochemical and natural feedstock. Glycerol has three hydroxyl groups that provide the solubility in water and hygroscopic nature. There are 126 conformers in glycerol giving flexible structure. Glycerol acts as a humectant and provides softness (Pagliaro & Rossi, 2008). Due to its hygroscopic nature, vapor pressure and volatility of it is low. The change in vapor pressure of glycerol can be regarded as too little between 0 and 70^0C. Glycerol is viscous product that remains liquid without crystallization at 100% concentration (Hasebe et al., 2004).

Glycerol is mostly used as plasticizer in edible film studies in order to improve mechanical and physical properties of films. Generally, addition of glycerol increased the elongation of the films. In a study, different concentrations of glycerol have been used in whey protein isolate based films. It was observed that different amount of glycerol affected water vapor permeability, wetting and thermal properties of films. Water vapor permeability was increased by glycerol addition. Because of that, barrier properties of film had the better values at the lowest glycerol concentration. In addition to barrier properties, thermal properties of film were affected from glycerol which resulted the increase of the degradation temperature (Kokoszka et al., 2010). Effect of glycerol concentration has also been studied in cress seed carbohydrate gum film with three concentrations of glycerol which are 25, 35, 50%. When glycerol concentration was increased from 25% to 50%, it was observed that flexibility of film was improved but water vapor barrier properties decreased (Jouki et al., 2013). Another study was conducted in order to investigate the effect of plasticizer on sage seed gum based film. Glycerol and sorbitol at different concentrations (20, 40, 60, 80, 100 w/w %) were used. According to the results, glycerol provided plasticization at higher efficiency than sorbitol. Glycerol increased moisture content and solubility of the films. As a result of that situation, it showed slightly higher water vapor permeability when compared to sorbitol. On the other hand, sorbitol caused weaker mechanical strength to the film than glycerol did (Mohammad et al., 2015).

1.1.3. Gallic Acid

Gallic acid has a chemical name as 3,4,5-trihydroxybenzoic acid. Food, pigment and pharmaceutical industries use gallic acid and its esters extensively (Lu & Lu, 2007). It is naturally found in pomegranate husk, oat, green, black tea and grape. Gallic acid is obtained from hydrolyzed tannins. It draws attention with its antioxidant, antimicrobial, anti-inflammatory and antifungal properties (Daneshfar et al., 2008).

Stability of the gallic acid is influenced from the temperature. Gallic acid is less stable natural antioxidant when compared to α-tocopherol, caffeic acid and ferulic acid. The initial decomposition temperature of gallic acid is 68^0C and at that point 9% of mass loss occurs (Santos et al., 2012). Moreover, extraction yield of gallic acid are affected from the temperature. Studies showed that extraction of gallic acid increased with increasing temperature up to 50^0C with ultrasound assisted system (Salehan et al., 2016). In light protected system, initial degradation temperature of gallic acid have been observed at 60^0C with 12 % degradation (Setyaningsih et al., 2016).

Solubility of gallic acid is important for industrial and pharmaceutical applications. Solubility of gallic acid was measured at temperatures between 25^0C and 60^0C in different solvents which are methanol, ethanol, water and ethyl acetate. In water, solubility of gallic acid increased with increased temperature from 25^0C to 60^0C and the maximum solubility of 7.378 w/w was obtained at 60^0C (Daneshfar et al., 2008).

Gallic acid is the powerful antioxidant substance. For this reason, several film studies were conducted with gallic acid on the purpose of adding antimicrobial and antioxidant property. Besides, gallic acid was used on the purpose of improving mechanical properties. In order to investigate the effect of addition of gallic acid on mechanical properties of film, a study has been conducted with wheat gluten films. Addition of 1-2 % w/w gallic acid decreased the elongation while addition of 5-10 % w/w increased the elongation (Hager et al., 2012). In addition to antioxidant property, gallic acid has also the antimicrobial property. In a study, the effect of different amount of gallic acid addition on *Campylobacter jejuni* has been investigated in zein and zein-wax films. Gallic acid was added in order to get 1.25, 2.5, 5, 10 mg gallic acid/ cm^2 of dried films. When antimicrobial activity of film on *C. jejuni* was analyzed, 2-fold increment of gallic acid concentration provided the increase in almost 3-fold antimicrobial activity. Release test in water and solid agar was also conducted. Both zein and zein-wax films showed similar release with the concentration of 2.5 mg/cm^2 gallic acid. However, at higher amount of gallic acid zein-wax films showed faster release than zein films. When mechanical properties were analyzed, addition of gallic acid increased elongation. Moreover, zein films were found to be more flexible than zein-wax films. Addition of wax prevented the interaction with gallic acid. Due to this blockage, the lower elongation in zein-wax films was observed (Alkan et al., 2011).

1.2. Edible Films

Different kind of materials can be used as material of the film solution according to their film forming properties. Materials can be categorized into four components including proteins, polysaccharides, lipids and composites (Figure 1.1).

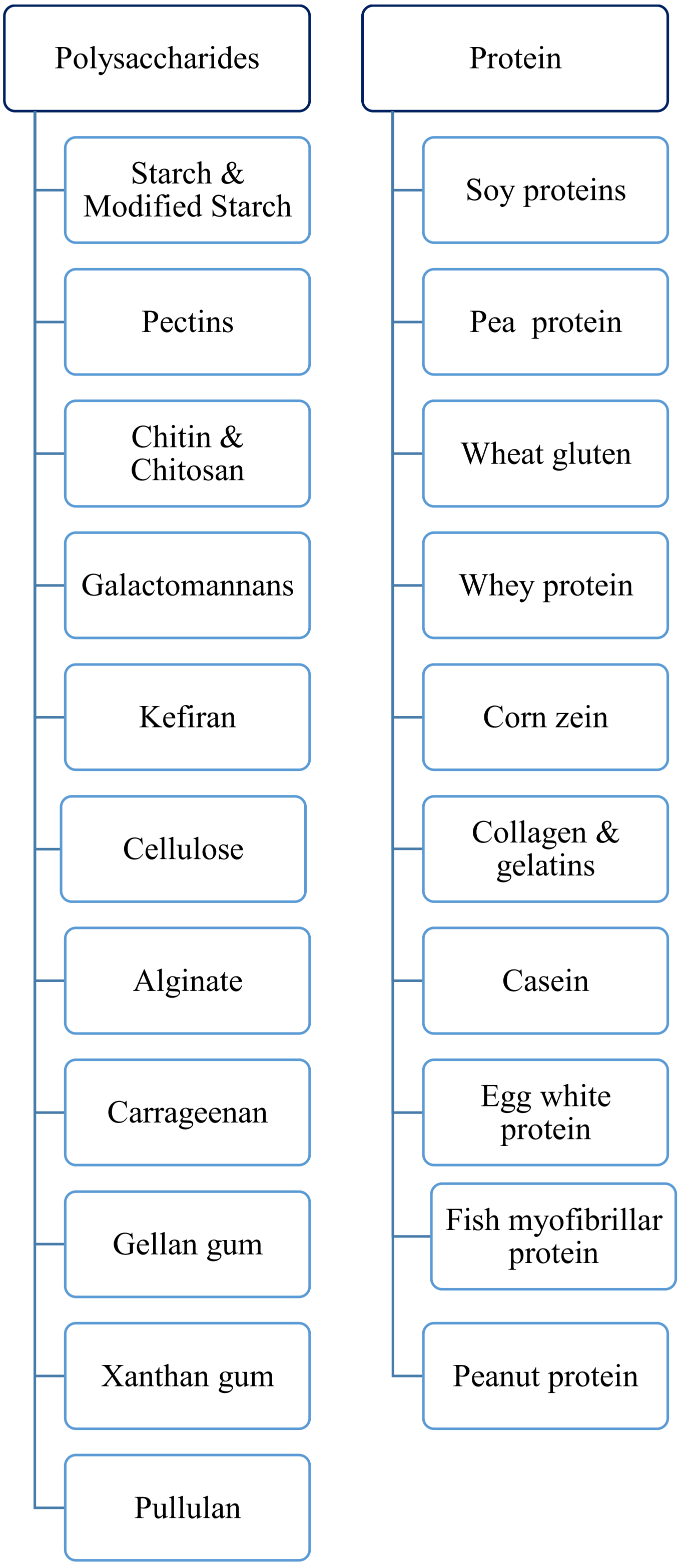

Figure 1.1. Sources of biofilms

1.2.1. Protein Based Films

Proteins are classified as fibrous and globular proteins. Fibrous proteins has the parallel structure by making hydrogen bonds, while globular protein has the complex spherical structure through hydrogen, hydrophobic, ionic and disulfide bonds. Properties of protein films show difference according to which bonds are dominant in the structure. In general, proteins are denatured by heat, base or solvent to obtain film structure. There are ample source of proteins in order to form film including gelatin, corn zein, wheat gluten, soy protein, collagen, mung bean protein and casein (Bourtoom, 2008a). In addition to the structure of the protein, electrostatic charge, amphyphylic properties and aminoacid composition are effective factors on properties of protein based edible films. Enzymatic, chemical and physical treatment provide cross linking which result with stronger mechanical and gas barrier properties (Bourtoom, 2008b).

Generally, protein based films are obtained from solutions which consist of water, ethanol or ethanol/water mixture. Film is formed through evaporation of solvent. Stretched bonds of the film matrix are enabled by denaturation of proteins with the help of acid, base, heat or solvent. Interactions of protein-protein are related to amino acid composition and extension of the chain. Ionic and hydrogen bonding in film matrix make the protein films good oxygen barrier while it makes films prone to permeable to water vapor (Wittaya, 2012).

Several edible film studies were conducted with plant based and animal based protein source. Legume proteins were studied in edible film studies. Different sources of proteins give the films different mechanical properties. In a study, mechanical properties of lentil, pea, whey and soy protein films were compared (Bamdad et al., 2006). Soy protein isolate films with %30 glycerol at pH 10 showed highest tensile strength (8.5 ±0.5 MPa) while lentil protein films containing % 50 glycerol level at pH 11 showed highest elongation (58.22 ± 12.8 %) (Bamdad et al., 2006). In a study, native and heat treated whey protein films were prepared at different pH values ranged from 3 to 8. Water vapor permeabilities of heat treated whey protein films were found to be similar with native whey protein films at pH 6, 7 and 8. However, at pH 4 and 5 heat denatured whey protein films had higher WVP than native ones. Because heat treated whey protein film solution was viscous, it was hard to remove the air bubbles from the solution at pH 4 and 5. On the other hand, heat denatured whey protein films were found to be stronger and more flexible than native whey protein films at all adjusted pH values. It seemed that covalent cross-linking occured by heat treatment strengthen the mechanical properties of films (Nadaud & Krochta, 1999). The similar result in terms of mechanical properties was found in pea protein isolate films. After denaturation of protein by heat, tensile strength and elongation of the pea protein isolate films increased. As a result of intra molecular bonds between proteins, films were stronger than native pea protein isolate films (Won-Seok & Yung, 2001).

In another study, physical and mechanical properties of peanut protein films were studied. Film solution was treated with heat or chemicals including formaldehyde, glutaraldehyde, succinic anhydride and acetic anhydrite. Formaldehyde and glutaraldehyde supported the cross-linking of covalent bonds of protein which made the films stronger and less permeable to water vapor. Heat treatment at different temperatures (60, 70, 80, 90^0C) did not affect the elongation of the films. However, temperatures higher than 60^0C provided the higher tensile strength and Young's modulus without a significant differences between them (Liu et al., 2004).

1.2.2. Polysaccharide Based Films

Polysaccharide based films can be composed of agar, alginate, carrageenan, cellulose, chitosan, gums, pectin and starch (Skurtys et al., 2010). Polysaccharide based films slow down the moisture loss of the food. However, hydrophilic nature of polysaccharides generally makes the film weaker against water vapor and gases. In general, hydrocolloid films shows good mechanical and structural properties with weaker gas barrier properties (Bourtoom, 2008b).

Chitosan which is an abundant polysaccharide is one of the mostly studied polysaccharide based film. Chitosan is enabled by deacetylation of chitin in alkaline media. It has also antifungal and antimicrobial properties that take the attention as edible film source. Chitosan based films have good mechanical strength but higher water vapor permeability. Cross-linking agents, lipids, fatty acid waxes and clay were added to improve water vapor barrier properties by increasing hydrophobicity. Moreover, irradiation and ultrasonic treatment were used to provide water vapor barrier (Elsabee & Abdou, 2013).

The other abundant polysaccharide based films consist of starch. Potato, corn, wheat, rice and bean were the main sources of the starch in film studies. Starch granule including size, shape and chemical properties vary according to sources of the plant. Starch granules involves amylose which is linear chain and amylopectin consisting of branched chain. In cold water, starch granules are insoluble due to hydrogen bonds. By the help of heating, hydroxyl group and water interact due to broken crystaline structure. Heating of starch suspension in excess water at 65-90 ^{0}C lead to irreversable gelatinization (Cazón et al., 2017).

Hydroxypropyl cellulose (HPC), carboxymethylcellulose (CMC), hydroxypropyl methylcellulose (HPMC) or methylcellulose (MC) which is cellulose derivatives are generally used in edible film studies. Cellulose based films had moderate tensile strength and oxygen barrier with colorless, tasteless and flexible structure. However, water vapor permeability of cellulose based films was found to be high due to its hydrophilic nature. Among the cellulose derivatives, methylcellulose had the best water vapor barrier properties (Shit & Shah 2014). Lipid and other hydrocolloid substances were used in cellulose-derivatives based films in order to improve water barrier properties. In a study, oleic acid was added to carboxymethylcellulose (CMC) based films by emulsification method. Addition of higher concentration of oleic acid lowered the water vapor permeability of CMC film. However, it led to a decrease in ultimate tensile strength but an increase in elongation which was explained with increment of discontinuities. Moreover, oleic acid addition gave the films hazy appearance due to the presence of lipid droplets (Ghanbarzadeh & Almasi 2011).

1.2.3. Lipid Based Films

Lipid based films have the great water vapor barrier properties because of their hydrophobic characteristic. On the contrary, hydrophobicity of the lipid makes the films more brittle and thicker. Generally, lipid based films are combined with polysaccharide or protein composites in order to strengthen the films. Water vapor permeabilities of lipid based films are low which are close to low density polyethylene and polyvinyl chloride. Moreover, films containing solid fat rather than lipid fat have good water vapor barrier propeties. On the contrary, lipid based films show brittle structure. There are some techniques to obtain lipid based films. Casting of emulsion which is composed of lipid and hydrocolloid and accumulation of lipid as layer on hydrocolloid film as mechanical support are the two techniques of lipid based films (Bourtoom, 2008b).

Waxes, vegetable oils and fatty acids can be used in film solutions. Lauric, miristic, palmitic, stearic and oleic acids were used in hydroxypropyl-methylcellulose film. According to result, size of the lipid molecules in micelles affected the WVP of the films (Jiménez et al., 2010). In another study, stearic and oleic acid were used in blue shark skin gelatin film. It was shown that oleic acid had higher plasticizing effect than plasticizing effect of stearic acid. However, addition of stearic acid into gelatin film decreased WVP more than same amount of oleic acid. Authors explained that melting point of the fatty acid was an important factor for emulsion film containing fatty acids. As melting point of fatty acid increased, WVP of films decreased (Limpisophon et al., 2010).

1.2.4. Composite Films

Composite films are composed of two compounds from hydrocolloids, lipids or both of them (Skurtys et al., 2010). Composite based films are generally formed in order to strengthen the structure. In this context, flour based edible films are also considered as composite films. In the previous studies, amaranth, blue corn, achira, rice, red rice, lentil, plantain, semolina, peanut and wheat flour were studied for flour based edible films (Andrade-Mahecha, et al., 2012; Aydogdu et al., 2018; Dias et al., 2010; Gutiérrez et al., 2016; Hager et al., 2012; Jafarzadeh et al., 2018; Riveros et al., 2017; Tapia-Blácido et al., 2007; Valderrama Solano & Rojas de Gante, 2014; Vargas et al., 2017).

Mechanical properties and water vapor permeabilities of achira, amaranth, pinhao, banana, quinoa, rice and lentil flour based films were compared. Pinhao flour based film including 20% glycerol was shown to have the highest tensile strength with the value of 13.1 MPa while amaranth flour film involving 22.5% glycerol was shown to have the lowest tensile strength with the value of 1.50 MPa. In adition to tensile strength, elongation was found to be highest (88.4 %) in quinoa films involving 21% glycerol and the lowest (2.50) in pinhao films. On the other hand, water vapor barrier property was found to be the best in quinoa film (0.06 ng $m^{-1}s^{-1}Pa^{-1}$) but the worst in achira flour film (0.53 ng $m^{-1}s^{-1}Pa^{-1}$). Chemical composition of flours, glycerol concentration used in film solution and preparation methods were shown to be important on properties of films. For instance, lentil flour based films were treated with heat (70 and 90^0C) and three different concentration of glycerol (1, 1.5, 2% w/v) was used. Addition of higher concentration of glycerol slightly increased WVP. However, heat treatment at 90^0C lowered the WVP significantly when compared with films at 70^0C. Moreover, higher temperature improved the tensile strengh of the films. Authors explained that gelatinization was completed easier at higher temperatures. Amylose moved from flour to solution and promote denser matrix which made the film stronger but less flexible (Aydogdu et al., 2018).

Semolina flour was also used in edible film study. Compact and semi-crystalline structure was observed in semolina flour based films due to its high gluten composition. This compact structure enabled the films barrier to the water vapor. Moreover, semolina flour based film was found to be less permeable to water vapor than achira flour and amaranth flour based films (Jafarzadeh et al., 2018).

In another study, amaranth flour film was compared with amaranth protein-lipid and amaranth protein films. It was observed that there was no significant difference between mechanical properties of amarant flour based film and amaranth protein based film. Amaranth flour and amaranth protein based films were stronger and more flexible than amaranth protein-lipid films. Lipids blocked the interaction of proteins and made the films weaker. However, lipids in flour film did not affect mechanical properties negatively. It provided plasticization and prevented brittleness which was caused by starch. On the other hand, amaranth flour based films had the lower WVP than amaranth protein and amaranth lipid-protein based films had. Interactions of lipid and proteins distributed homogenously and hydrophobic zones were created. In addition, amaranth flour films had the lowest oxygen permeability. Lipid composition increased the hydrophobicity of the films (Tapia-Blácido et al., 2007).

Several studies were conducted on composite films with at least one hydrocolloid and one lipid based material or two hydrocolloid materials. For instance, cassava starch and stearic acid composite films were studied. As an expected result, addition of stearic acid decreased the water vapor permeability of the starch film. However, it decreased the tensile strength of the film. It was explained that partial incorporation of lipid molecules and interruption with lipid-lipid interaction made the films weaker (Schmidt et al., 2013). Composite films including two hydrocolloid films were exemplified with starch-kefiran film study. In film matrix, increased amount of starch from 0% to 50% decreased water vapor permeability while higher level of addition of starch from 50% resulted in an increase of water vapor permeability. Hydrophilicity of starch was higher than kefiran that made the films open to the higher water vapor diffusion. Additionally, tensile strength of the films increased with addition of starch from 0 to 50% and decreased after addition of starch from 50 to 100%. Excess addition of starch favored the starch intramolecular hydrogen bonds instead of starch-kefiran intermolecular bonds which lowered the tensile strength (Motedayen et al., 2013).

In the study which was conducted with pea starch and peanut protein isolate, the increase in protein isolate ratio decreased the tensile strength significantly while protein isolate addition led to an increase of elongation. Moreover, addition of protein isolate into pea starch film solution at 40% level decreased the water vapor permeability and water vapor transmission rate. In 100% pea starch films, swollen starch was observed with uniform structure. In 100% peanut protein isolate films, rough surface with pores was observed while 40% addition of protein isolate to film solution made film rougher and more flexible. It was shown that addition of protein isolate into starch based films had an impact on improvement of film characteristics (Sun et al., 2015).

1.3. Active Films

Active packaging is the new packaging system that maintains the food safety and extend the shelf life. According to definition of European Commission, active packages provide releasing of active substances from packages or absorbing substances from or into the packaged food or the surrounding environment in order to extend the shelf-life and maintain or improve the quality of packaged food. In this context, there have been various types of active packaging systems. Oxygen scavengers, moisture absorbing, ethanol absorbing, antioxidant releasing, anti-fogging and light absorbing are the examples of active packaging systems (Restuccia et al., 2010).

Edible films are also active packages that have various functions which can be preferred as regards to desired property. Edible films provides to barrier to environment, moisture, oxygen, aroma, oil and mass transfer between different compounds in food. Moreover, films can be carrier of the antimicrobial or antioxidant agents, dispenser of food ingredients, enhancer of appearance and enhancer of characteristic of handling (Janjarasskul & Krochta, 2010).

One of the main reasons of active packaging is to prevent or to delay the lipid oxidation. Particularly high lipid foods with high unsaturated fatty acids are prone to lipid oxidation. Nuts, oils, fishery products, meat products are the possible susceptible foods. Hence, antioxidant active packages started to take into consideration. Antioxidant active packaging has two mechanisms. The first one is releasing of antioxidant compound from the package to the food and the surrounding and scavenging the oxygen or free radical substances. Selection of the antioxidant material has great importance because antioxidant substance and packaging material must be coherent and homogeneous. Solubility of antioxidant which specifies the efficiency must be suitable to food material. Hydrophilic free radical scavengers have shown better results in bulk oils whereas hydrophobic ones have better results in emulsified oils. The second one is the reduction of oxygen transmission by scavenging the oxygen from the surrounding of the food. This system involves edible films and coatings. Some synthetic antioxidants including polyphenols, organophosphate and thioester compounds have been used in packaging system. However, toxic substance migration from package to food material has shown argumentative results. This toxicity risk have canalizes the researches of natural antioxidants (Gómez-Estaca et al., 2014).

In the literature, antioxidant property has been tried in films from different sources. Vitamin E and alpha tocopherol have been used in hydroxypropyl cellulose, carboxymethyl cellulose and whey protein films for application to nuts. Oregano, rosemary essential oils, sodium ascorbate, citric acid, fish oil, vitamin E and ferulic acid have been used in milk protein, chitosan, gelatin and soy protein based films in order to be used for meat and fish products. Nuts, meat and fish products are prone to lipid oxidation due to presence of unsaturated fatty acids. For this reason, addition of antioxidant substance into films makes the films proper for usage in these products. Ascorbic acid, citric acid, and oleoresins have been added to chitosan, alginate, and whey protein concentrate based films for application to fruit and vegetable products (Bonilla et al., 2012).

In previous film studies, gallic acid was used as antioxidant substances. Effects of antioxidants involving gallic acid were investigated in rice flour and cassava starch composite film study (Rachtanapun & Tongdeesoontorn, 2009). Antioxidant activity of zein/chitosan composite films incorporated with dicarboxylic acids was studied (Cheng et al., 2015). In another study conducted with chitosan/gelatin composite film, gallic acid conjugated and incorporated films were compared (Rui et al., 2017). In addition to antioxidant property, antimicrobial property of gallic acid on biofilms was investigated. Antimicrobial activity of gallic acid in zein based film on *Campylobacter jejuni* was studied (Alkan et al., 2011). Other study on antimicrobial activity of gallic acid in biofilms was chitosan based film (Sun et al., 2014).

1.4. Factors Affecting the Film Forming Ability

There are several factors that affect the film forming ability concerning the formulation of films. The most important factors for composite films are pH, temperature of film forming solution, type of plasticizer used in film solution and drying temperature of the film solution.

1.4.1. pH

Biodegradable films consisting of protein are affected from the pH. pH influences the solubility of the proteins in film solution. The protein solubility is maximized when pH is away from the isoelectric point of protein. The soluble molecules consist of charged particles which affect the functionality of the polymer. The same charged group repels each other by providing stretching in the polymer chain. By the help of ionization of protein molecules, polymer matrix is formed with hydrophobic interaction of solvent (Wittaya, 2012).

Effect of pH on different protein based films was studied. In pea protein isolate film, three pH values of 7, 9 and 11 were studied with and without heat treatment. WVP of films were not affected from pH regardless of heat treatment. However, solubility of heat treated film solution increased with increasing pH from 7 to 11 (Kowalczyk & Baraniak, 2011). In chicken breast protein films, acidic and alkaline pH values were used in order to investigate the pH effect. Film solubility was lower in film prepared at acidic pH than that was prepared at alkaline pH. Authors explained this situation with shorter chain length of protein that resulted lower interaction and cross-linking. Moreover, opacity of the films was found higher for film prepared at pH 3 due to lower solubility (Nemet et al., 2010).

Alkaline pH values were studied in films containing zein and wheat gluten. Elongation of the films increased up to pH 10 and decreased from pH 11 to pH 13, while tensile strength of the films increased with the increased pH. On the other hand, WVP of the films decreased up to pH 11 and then increased after pH 11. It was explained that increased pH of the solution could favor the formation of S-S disulfide bonds which provided the strong matrix. At higher pH values than pH 11 repulsion of the molecules increased and structural form could be relaxed. This caused the higher water vapor permeability (Guo et al., 2012).

1.4.2. Temperature

Temperature is the most important parameter for film forming ability. Denaturation of proteins and gelatinizations of starch occurs with heat treatment which was directly related to film formation. In cuttlefish skin gelatin film study, the effect of different temperatures (40, 50, 60, 70, 80, 90^{0}C) on film properties was investigated. It was observed that tensile strength of the cuttlefish skin gelatin based films was found maximum at 60 and 70^{0}C. After 70^{0}C, tensile strength of the films decreased while elongation increased. This situation was explained by the degradation of gelatin and shorter chain that prevented the formation of strong film matrix. Although negative effect of higher heat treatment on cuttlefish skin gelatin based films was observed, at 90^{0}C lowest water vapor permeability was obtained (Hoque et al., 2010). In another study, zein and wheat gluten film solution was treated at different temperatures including 40, 50, 60, 70 and 80^0C. Tensile strength of the films increased with increased temperature and elongation increased up to 50^0C while water vapor permeability decreased. Authors explained that denaturation occurred with the unfolding of three dimensional structures of proteins. Intermolecular matrix could be formed through S-S interchain reactions, S-H molecular forces and hydrophobic bonds (Guo et al., 2012).

In general manner, heat treatment on protein based edible film solution provides good water vapor barrier properties. Heat treatment unfolds the polypeptide chain and intermolecular forces formed. Hydrophobic interactions and S-S covalent bonds occurred between unfolded proteins give coherence which gives good water vapor barrier (Kowalczyk & Baraniak, 2011).

1.4.3. Type of Plasticizer

Plasticizer addition is an important parameter in order to obtain flexible edible films. Plasticizers provide flexible property and mobility to films by decreasing the intermolecular forces in film. However, water vapor permeation can increase with decreased cohesion of film matrix. Proper plasticizer can show differences concerning with type of the edible films including protein based, polysaccharide based or lipid based. For instance, glycerol and sorbitol was found as the best choice for cassava starch while mannose, glucose, fructose, glycerol and sorbitol were the preferences for pea starch in a study (Vieira et al., 2011). Other examples can be oleic and linoleic acids for zein films and fatty acids for fish skin proteins. These plasticizers affect the characteristic of the films differently. For this reason, the effect of different plasticizer is studied in several researches (Vieira et al., 2011).

In common, monosaccharides, disaccharides, oligosaccharides like fructose-glucose syrups and sucrose, polyols like glycerol and sorbitol, lipids and derivatives like phospholipids, fatty acids, and surfactants are used in film formation. Addition amount of plasticizer is related to stiffness of used polymers but generally 10-60 % of plasticizer is used in dry basis. There are two plasticization theories for edible films.

The first theory is gel theory including attachment of plasticizer polymer chain by disturbing polymer-polymer attachments. This causes the gel flexibility. Moreover, gel flexibility is also provided with unattached aggregated plasticizer which increases the mobility of polymers. The second theory is free volume theory consisting of four approaches (Sothornvit & Krochta, 2005).

Remarkable film studies were conducted in order to investigate the type of plasticizer and concentration on film characteristics. For instance, a study has been conducted in order to investigate effect of plasticizer on sage seed gum based film. Glycerol and sorbitol at different concentration (20, 40, 60, 80, 100 w/w %) were used. According to results, glycerol provided plasticization at higher efficiency than sorbitol. Glycerol gave the film high moisture content, high solubility and stronger mechanical properties than sorbitol (Mohammad et al., 2015). In gelatin film study, the effects of different kind of plasticizer was investigated on film characteristics. For this purpose, four group of plasticizer was used and their effects on films were compared. Mannitol and sorbitol were the first group, ethylene glycol (EG), diethylene glycol (DEG), triethylene glycol (TEG), ethanolamine (EA), diethanolamine (DEA), triethanolamine (TEA) were the second group, polyethylene glycols (PEG) with different molecular weights (300, 400, 600, 800, 1500, 4000, 10 000, 20 000) were the third group and sucrose, oleic acid, citric acid, tartaric acid, malic acid were the last group. Malic acid, PEG300, sorbitol, EG, DEG, TEG, EA, DEA and TEA were found the best plasticizer in terms of WVP and water content (Cao et al., 2009). The effect of plasticizer involving glycerol, ethyleneglycol, diethyleneglycol and polypropyleneglycol was investigated on moisture content of gelatin film. It was found that glycerol had the greatest hygroscopicity than others in gelatin film (Bergo et al., 2013). In sugar palm starch film study, the effects of different plasticizer type on mechanical properties were analyzed. Glycerol, sorbitol and glycerol-sorbitol combination were used in the study. At the same concentrations, films including sorbitol had higher tensile strength than sorbitol-glycerol combination and glycerol while the opposite results were obtained in elongation (Sanyang et al., 2015).

1.4.4. Drying Temperature

Drying temperature is one of the important factor that affects the film characteristic. Color, water vapor permeability and mechanical properties are affected from different drying temperatures. The effect of different drying temperatures (70, 80, 90 ^{0}C) on cassava starch -chitosan biocomposite films were investigated. It was observed that drying temperature affected the color of the films. At 70 ^{0}C with the duration of three hours, films were brownish, while at 80 and 90 ^{0}C with same duration color was found to be transparent white (Wahyuni & Arifan, 2018).

The effect of drying temperature was also investigated in emulsion films. Whey protein-lipid emulsion film was used in order to determine the effect of drying temperature at three different temperature (25, 40, 80^{0}C) and 40% relative humidity. Lipid distribution and film appeareance showed differences with drying temperature. At 25^{0}C, films appeared homogeneous while at 40 and 80^{0}C lipid molecules accumulated at the edges of the casted surface. This can be explained by the fact that at higher drying temperatures, films begin to dry from the edges and lipid migrates to hydrophobic side. For whey protein isolate based films water vapor permeability did not differ when drying occured at 40 and 80 ^{0}C. However, for emulsion film water vapor permeability decreased with increasing drying temperature. It was explained that lipid reached to melting point and passed to more stable morphology with cooling through crystallization. As a result, formation of lipid crystals increased molecular density and strengthened barrier properties to water vapor. On the other hand, drying temperature did not affect the mechanical properties of emulsion film (Pérez-Gago & Krochta, 2001).

Another study was conducted on konjac flour film with different drying temperatures. Konjac flour films were dried at 45, 50, 55 ^{0}C in heat pump drier. Results showed that increasing drying temperature increased tensile strength but it decreased elongation. On the other hand, water vapor transmission rate was found to be significantly lowest at 50^{0}C and solubility was the highest at 50^{0}C (Jomlapeeratikul et al., 2017).

1.5. Objectives of the Study

Synthetic packaging materials are not desired due to the rising awareness of environmental concern of the consumers. In this context, many studies have been conducted about the usage of biodegradable materials in food packaging. However, there is no study in literature about production of chickpea flour based films. Chickpea is easily accessible legume which has protein, starch and lipid composition. Due to its multi-composition, chickpea flour has a great potential to make biodegradable composite films. Protein and polysaccharide could give stronger mechanical barrier while lipid does not. On the other hand, lipid could support the barrier properties of films which could not be provided with polysaccharides and proteins.

Antioxidant active biodegradable films also attract attention due to the ability of extending shelf life and improving food quality. Antioxidant films are promising especially for food products which are susceptible to oxidation. They could be a solution for protection of highly oxidative foods such as nuts, chips from oxygen. In the previous studies, many antioxidant substances were used to provide antioxidant activity to the biodegradable films. However, the studies about addition of gallic acid to the film to provide antioxidant ability are limited. Thus, the objectives of the study are to obtain chickpea flour based active films containing gallic acid and to evaluate the physical, morphological and antioxidant properties of these films with respect to the parameters of pH, glycerol concentration and gallic acid concentration.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Chickpea flour was supplied from Aro Gıda (İzmir, Turkey). Folin Ciocalteu's phenol reagent, acetic acid, ethanol, sodium carbonate, sodium hydroxide, magnesium nitrate and 2,2-Diphenyl-1- picrylhydrazyl were provided from Sigma-Aldrich Chemie Gmbh (Darmstadt, Germany). Gallic acid and glycerol were purchased from Merck.

2.2. Preparation of Films

Chickpea flour film forming solution was prepared at concentration of 7.5 % (w/v) in 100 ml distilled water. Solution was stirred by magnetic stirrer at 1000 rpm for 20 min. After adjustment of pH to 9 and 11 with 1N NaOH, solution was placed into water bath at 90^0C for 30 min. The solution was allowed to be cooled down to 45-50^0C at room temperature. Then, gallic acid was added as antioxidant agent to the solution at concentration of 5% and 10% w/w of the total solid (chickpea flour and gallic acid) while stirring at constant speed (500 rpm). According to the preliminary experiments, it was observed that gallic acid at 2.5% w/w of the total solid had a negative impact on color of the solution and films. Moreover, films containing 2.5% w/w gallic acid of total solid had brittle texture. Therefore, gallic acid concentration was chosen as 5% and 10% w/w. Glycerol was added at the concentration of 1% and 3% w/v of solution while stirring at 500 rpm. High speed homogenizer (IKA T18 Digital Ultra Turrax, Staufen, Germany) was used in order to homogenize the gallic acid and glycerol in the solution and to provide efficient mixture. Solutions were homogenized at 10000 rpm for 3 min. High spinning of solution caused formation of air bubbles which had an effect on mechanical properties of film. Therefore, ultrasonic water bath (Lab Companion, Jeiotech, Seoul, Korea) was used in order to eliminate air bubbles at 40 kHz for 30 min. Solutions of 6 g were poured into LDPE Petri plates. Petri plates were put into oven (Binder ED 115, Tuttlingen, Germany) at 30 ^{0}C for 18 hours. After 18 hours, plates were removed from the oven and put into desiccator which was equilibrated to 52% RH with magnesium nitrate solution for 24 hours. Dried films were peeled off and kept in a desiccator prior to analysis. Films were symbolized according to pH, glycerol and gallic acid concentrations. For example, P9-G1-GA5 denotes film with pH=9, 1% (w/v) glycerol and 5% (w/w) gallic acid (Table 2.1).

Table 2.1. *Nomenclature of the films*

Nomenclature	*pH*	*Glycerol (w/v %)*	*Gallic Acid (w/w %)*
P9-G1-GA5	9	1	5
P9-G1-GA10	9	1	10
P9-G3-GA5	9	3	5
P9-G3-GA10	9	3	10
P11-G1-GA5	11	1	5
P11-G1-GA10	11	1	10
P11-G3-GA5	11	3	5
P11-G3-GA10	11	3	10

2.3. Characterization of Films

Films were characterized in terms of color, opacity, total phenolic content, antioxidant activity, mechanical property, SEM image, water vapor permeability and FTIR spectra.

2.3.1. Color Measurements

Color of the films was measured by Spectrophotometer (Konica Minolta CM5, Japan). CIE L*, a^* and b^* color scale was used. The L^* value represents lightness ranging from 0 (black) to 100 (white). The a^* value represents redness or greenness ranging from

+60 (red) to –60 (green). The b^* value represents yellowness or blueness, which ranges from +60 (yellow) to –60 (blue). Total color change was calculated with Equation (1):

$$\Delta E^* = \sqrt{\left(L^*_{film} - L^*_{standard}\right)^2 + \left(a^*_{film} - a^*_{standard}\right)^2 + \left(b^*_{film} - b^*_{standard}\right)^2} \quad (1)$$

For white calibration, $L^*_{standard} = 100, a^*_{standard} = 0, b^*_{standard} = 0.$

Measurements were performed for two different samples prepared in each condition. In addition, data were taken on three random places on each film.

2.3.2. Opacity

Opacity values of the films were determined by the method stated by Tonyalı et al. (2018). Films were cut in 3 × 1 cm and placed in macro cuvettes. Absorbance values of films were read at 600 nm by UV-VIS Spectrophotometer (UV 1700, Shimadzu). Opacity values were calculated using Equation (2):

$$Opacity = \frac{A_{600}}{x} \quad (2)$$

where A_{600} was the Absorbance of films at 600 nm and x (mm) was the thickness of the films.

Five films prepared in the same condition were used for opacity measurement and samples were taken from two different position of each film. Thicknesses of films were measured with digital micrometer (LOYKA 5202, Loyka, Ankara, Turkey) on three different places.

2.3.3. Total Phenolic Content

Total phenolic content was determined by Folin- Ciocalteau method as described by Cilek et al. (2012). Film samples were weighed as 100 mg and treated with 3 mL acetic acid: ethanol: water (8:50:42 v/v). Mixture was mixed using vortex for 1 min. After that, it was centrifuged by centrifuge (MIKRO 220R Hettich Zentrifugen, Tuttlingen, Germany) at 6000 rpm for 30 min. After centrifugation, supernatant was passed filtered using 500 μm syringe filter in order to eliminate the undissolved particles. Solution was diluted 50 times with acetic acid: ethanol: water (8:50:42 v/v) mixture. Solution of 0.1 mL was taken to glass tubes. Blank solution was prepared with only addition of 0.1 mL acetic acid:ethanol:water (8:50:42 v/v). Folin Ciocalteau reagent 0.2 N of 2.5 mL was added to the mixtures and mixed by vortex. Thereafter, mixtures were kept at dark for 5 min. Then, 4.5 mL sodium carbonate solution (7.5 %w/v) was added to the solutions and kept for 1 hour at dark. After 1 hour, solutions were poured into spectrophotometer cuvettes and absorbance of solutions were measured at 760 nm by T70 UV-VIS Spectrophotometer (PG Instruments LTD, UK). Calibration curve was prepared with different amount of gallic acid (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 ppm) in acetic acid: ethanol: water (8:50:42 v/v/v) solution. Total phenolic content was represented as mg gallic acid equivalent per g dry film weight using Eq. (3)

$$Total\ Phenolic\ Content\left(\frac{mg\ GAE}{g\ dry\ film\ weight}\right)=\frac{A\times L\ of\ solution\times dilution\ rate}{g\ of\ dry\ film\ weight} \tag{3}$$

where A is the read value from calibration curve in mg gallic acid equivalent/ L using absorbance value of solutions. Calibration curve was given in Appendix (Table A.1).

2.3.4. Antioxidant Activity

Antioxidant activity of films were determined by DPPH$^{\cdot}$ method (Jouki et al., 2014). Methanol solution containing DPPH$^{\cdot}$ in 1 mM concentration was prepared. Film samples of 5 mg and 5 mL of acetic acid:ethanol:water (8:50:42 v/v) solution were put into glass tubes. Tubes were placed in centrifuge (MIKRO 220R Hettich Zentrifugen, Tuttlingen, Germany) and centrifuged at 6000 rpm for 15 min. Syringe filter of 500 μm was used to separate insoluble part of the solution from the soluble part. Solution of 0.1 mL was taken with micro pipette and 3.9 mL of 0.1 mM DPPH$^{\cdot}$ solution was added to the film solution in glass tubes. Solutions were kept at ambient temperature for 30 min. After 30 min, macro cuvettes were filled with solutions and absorbance of solutions were measured at 517 nm by UV-VIS Spectrophotometer. Methanol was used as blank. Calibration curve was prepared with different concentrations of DPPH$^{\cdot}$ in methanol (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 ppm) curve (Appendix Table A.1). Total antioxidant activity was expressed as mg DPPH$^{\cdot}$/ g dry film weight and calculated by Equation (4).

$$\text{Antioxidant Activity}\left(\frac{mg\ DPPH}{g\ dry\ film\ weight}\right)=\frac{A\times L\ solution\times dilution\ rate}{g\ of\ dry\ film\ weight} \tag{4}$$

where A is the read value from calibration curve as mg DPPH$^{\cdot}$ equivalent/ L using absorbance value of solutions. Calibration curve was given in Appendix (Table A.2).

2.3.5. Mechanical Properties

The tensile strength, Young's modulus and percentage elongation at break of the films were determined by universal testing machine (Z250, Zwick/Roell, Ulm, Germany). Mechanical properties of films were performed with 0.1N pre-load at speed of 25 mm/min. Films were placed between grips and grip to grip separation at the start position was adjusted as 25 mm. Measurements were replicated twice at room temperature and 48-50% RH.

2.3.6. SEM

Morphological properties of films were analyzed with a Nova NanoSEM 430 electron microscope (FEI, Oregon). Samples 20×20 mm2 in size were prepared by coating with 10 nm gold layer. Magnification of $1000 \times$ was used to take the image of cross-sectional micrograph of film samples.

2.3.7. Water Vapor Permeability

Water Vapor Permeability test was performed using the method described by Aydogdu et al. (2018). Permeability cups were filled with 35 mL of water to provide 100% RH. Film samples were cut in 3.5 cm diameter and thickness was recorded at 6 random points using digital micrometer. Then, cup was covered with film as the shiny side of the film was up. The cups were put into the desiccator containing saturated solution of magnesium nitrate providing 52% RH at 25^0C. During 10 hours, cups were weighed using an analytical balance with the 1 h intervals. Water vapor permeability rate was calculated from weight loss during 10 hours. Water vapor permeability rate (ng s^{-1} m^{-2}) and water vapor permeability (ng s^{-1} m^{-1} Pa^{-1}) were calculated with eq. (5)

$$WVP = \frac{\Delta m \times x}{A \times \Delta t \times \Delta P} \tag{5}$$

where Δm (ng) was the weight loss during time of permeation Δt (s); x (m) was the thickness of film; A was permeation area and ΔP (Pa) was the difference of partial pressure of water vapor inside and outside of the cup. Water vapor transmission rates of films representing the weight loss during time of permeation were given in Appendix B.

2.3.8. FTIR

Fourier Transform Infrared Spectroscopy analysis was performed to analyze the chemical interaction of gallic acid, glycerol and chickpea flour. Film samples which have dimensions of 20×20 mm^2 were analyzed with FTIR instrument (IR-Affinity1, Shimadzu, Kyoto, Japan). In analysis, 32 number of scan was used with the range of wave number 600-4000 cm^{-1}. Measurements were replicated twice.

2.3.9. Statistical Analysis

ANOVA was used to decide the significant difference between independent variables $(p \leq 0.05)$. For variables including significant differences, Tukey's test was used for comparison. Statistical analysis was performed by using Minitab 17 (State College, PA, USA).

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1. Color

Color of the packaging films is important to attract the attention of consumers. Darker film color might be unattractive for consumers as wrapping of foods. However, darker color is advantageous for food products which are prone to deterioration with light.

Figure 3.1 shows the effects of gallic acid, glycerol concentration and pH on L^* values of films. According to ANOVA, L^* values representing the lightness changed significantly with pH values and gallic acid concentrations (Table C.1). Glycerol was a colorless substance so it did not affect the color parameters of chickpea flour based films containing gallic acid. Addition of higher concentration of gallic acid increased the lightness of the chickpea flour based films. The analogous results were found in antioxidant active methylcellulose films. In methylcellulose film study, addition of α-tocopherol increased the lightness of film (Noronha et al., 2014).

In addition to concentration of gallic acid, it seems that pH made a contribution on lightness of the films significantly while concentration of glycerol did not create any significant effect. The same result was observed in faba bean protein isolate films. Faba bean protein isolate films were found darker at pH 12 than at pH 9. Moreover, there was no significant effect of plasticizer on color of films. It was explained that more pigments are extracted at alkaline pH. As a result of more pigment extraction, color of the films was darker (Saremnezhad et al., 2011). In chickpea, β-carotene, cryptoxanthin, lutein, and zeaxanthin which are the carotenoids was found as color pigments (Yildirim & Öner, 2015). At more alkali pH, color pigments of chickpea were extracted that caused darker films. On the other hand, glycerol was a colorless substance that did not affect the lightness of chickpea flour based films significantly.

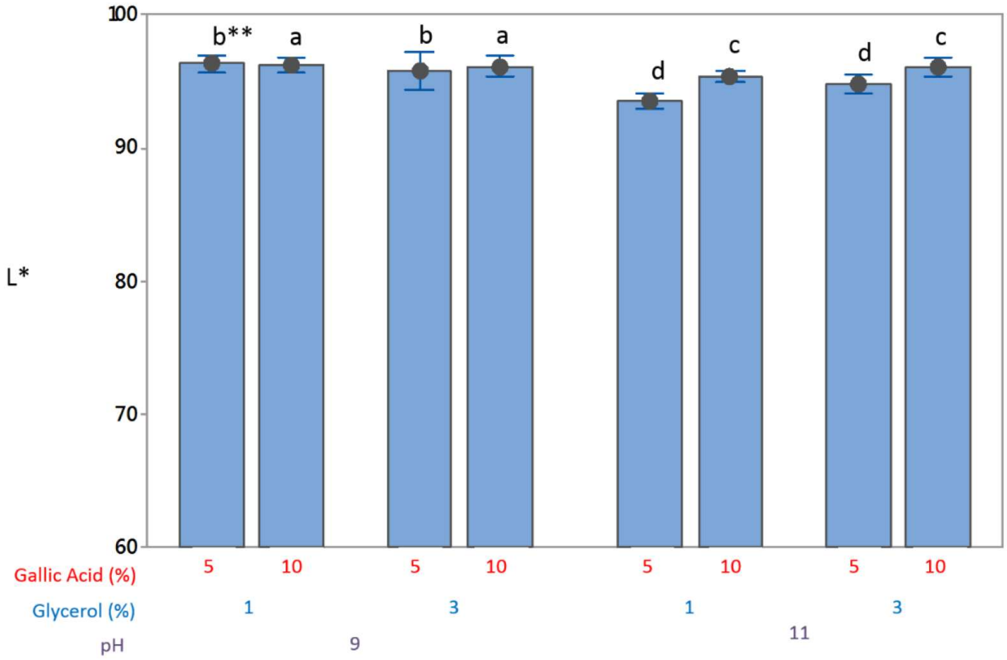

Figure 3.1. The effects of gallic acid and glycerol concentration and pH on L* values of films

**Different letters on the bars show significant difference ($p \leq 0.05$)

Table 3.1 represents a* and b* values of the films. The significant effect on a* and b* values of the films was observed by changing pH value. At more alkaline pH which was 11 in this study, chickpea flour based films had the highest a* and b* values. The results showed similarity with soy protein films. Soy protein films were shown to have higher a*, b* and ΔE* at pH 11 as compared to those at pH 8 (Mauri & Añón, 2008). The a* and b* value of chickpea was 2.96 and 19.87 respectively (Piñeros-Hernandez et al., 2017). Natural color of chickpea flour was yellowish. Due to higher solubility of proteins at higher alkaline pH, the films were observed to be more yellowish and reddish than at pH 11 than at pH 9.

Table 3.1. *a* and b* values of films*

Films	a^*	b^*
P9-G1-GA5[**]	-0.12 ±0.11^{b***}	5.49 ± 1.06^b
P9-G1-GA10	-0.04 ± 0.0^b	6.11 ± 0.64^b
P9-G3-GA5	-0.09 ± 0.09^b	5.32 ± 0.76^b
P9-G3-GA10	0.03 ± 0.06^b	6.55 ± 0.86^b
P11-G1-GA5	0.21 ± 0.06^a	8.58 ± 1.30^a
P11-G1-GA10	0.05 ± 0.04^a	5.76 ± 0.77^a
P11-G3-GA5	0.06 ± 0.09^a	6.39 ± 0.93^a
P11-G3-GA10	0.08 ± 0.07^a	7.16 ± 0.65^a

[**] P denotes pH, G denotes glycerol (%), GA denotes gallic acid (%)

[***] Different letters in the same column represent significant difference ($p \leq 0.05$)

ΔE^* value of chickpea flour based films was shown in Figure 3.2. Only pH created a significant difference between films on the degree of total color change of films. Since proteins became more soluble at pH 11, interactions of proteins with phenolic compounds might be easier. At the end of this interaction, ΔE^* values of chickpea flour based films increased.

Moreover, Maillard reaction was effective on color properties of the films. Chickpea flour included protein and starch in its composite matrix. In film preparation, heat treatment, drying and pH promoted the Maillard reaction. Therefore, at pH 11 films had higher redness and yellowness values while lightness values of the films were found to be lower. Additionally, total color difference of the films became higher with the improving of Maillard reaction. In red tilapia muscle protein film study, it was observed that at alkaline pH (11) b^* and ΔE value of the films were found to be higher as compared to pH at 3. According to authors, alkaline pH promoted the Maillard reaction by providing carbonyl group and formation of reductone which caused changing in color (Tongnuanchan et al., 2011). In another film study conducted with chicken breast protein at acidic and alkaline pH (3, 11), similar results were obtained.

The a* and b* values were found to be higher while lightness of the films was observed darker at alkaline pH. Higher yellowness of the films was explained with Maillard reaction. At alkaline pH, nucleophilicity was increased due to deprotonation of amino groups. As a result, Maillard reaction occurred when the carbonyl group provided by reduced sugar during drying of the films (Nemet et al., 2010).

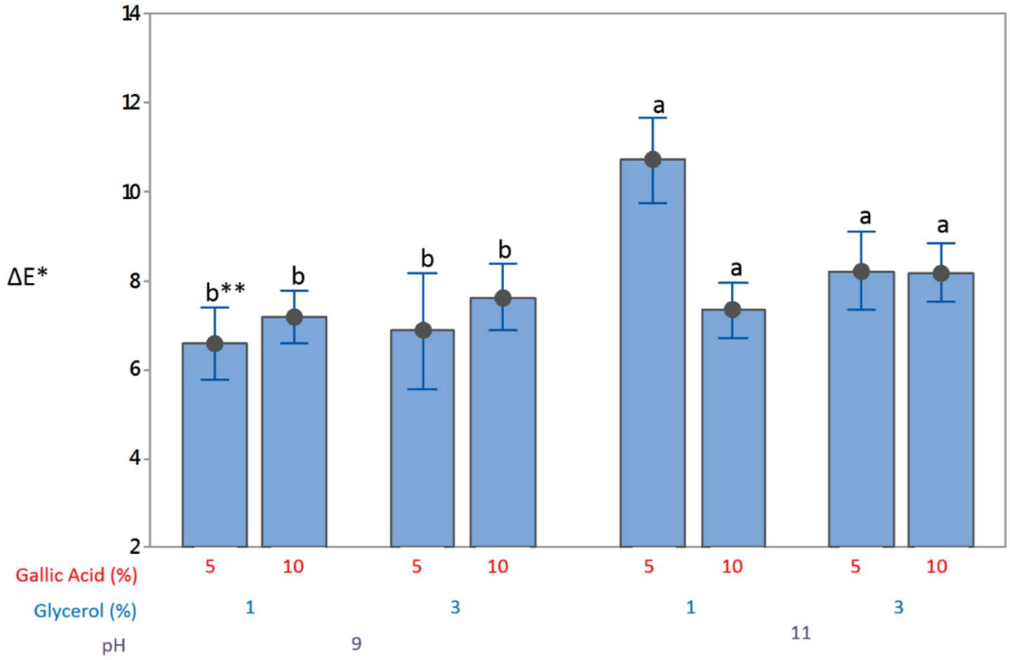

Figure 3.2. The effects of gallic acid and glycerol concentration and pH on ΔE values of films

**Different letters on the bars show significant difference ($p \leq 0.05$)

3.2. Opacity

Table 3.2 denotes the opacity of the chickpea flour based films containing gallic acid. It was observed that gallic acid concentration affected the opacity of the films significantly (Table C.5). Higher level of gallic acid concentration which was 10% (w/w of total solid) showed the highest opacity. It was determined that aromatic amino acids in protein structure and antioxidant compounds made a contribution to the opacity of the films. By the mechanism of absorbing light, those compounds gave the more opaque color to the films (Vargas et al., 2017). Since protein based films have great absorbing ability, films containing protein might be better barrier for UV than other films. By this way, protein based films become more talented to prevent the food from UV as compared to plastic films (Jafarzadeh et al., 2018). In addition to protein content, addition of antioxidant compound to the film influenced the opacity. In a study conducted by Zataria, antioxidant addition resulted in increased opacity values of multiflora essential oil and gelatin based films (Kavoosi et al., 2014). In the study, similar results were obtained that gallic acid concentration in chickpea flour films had an effect on opacity. Incorporation of higher concentration of gallic acid made the films opaquer. On the other hand, glycerol as a colorless substance did not affect the opacity. In previous study conducted with gelatin based films, addition of glycerol at different concentrations (10, 15, 20, 25, 30 g/ 100 g of gelatin) did not create a significant difference on opacity value of gelatin based films (Vanin et al., 2005).

Table 3.2. *Opacity values of films*

Films	*Opacity*
P9-G1-GA5*	5.63 ± 0.49^{b**}
P9-G1-GA10	8.99 ± 0.77^{a}
P9-G3-GA5	6.59 ± 0.48^{b}
P9-G3-GA10	7.31 ± 0.55^{a}
P11-G1-GA5	5.44 ± 0.80^{b}
P11-G1-GA10	7.64 ± 0.76^{a}
P11-G3-GA5	7.72 ± 0.60^{b}
P11-G3-GA10	7.53 ± 0.41^{a}

*P denotes pH, G denotes glycerol (%), GA denotes gallic acid (%)

**Different letters in the same column represent significant difference (p≤ 0.05)

3.3. Total Phenolic Content

Folin-Ciocalteu reagent was used to determine the total phenolic content. It involves phosphotungstic and phosphomolybdic acids. Transfer of electron between those acids and phenolic compounds occurs. By this way, change in the color can be measured

(Siripatrawan & Harte, 2010). In previous studies, chickpea flour has been investigated for obtaining total phenolic content. Total phenolic content of defatted chickpea flour has been found as 14.3 mg GAE/ g flour on dry weight basis (Sreerama et al., 2012). In this study, total phenolic content of the films included both total phenols of chickpea flour and gallic acid. Figure 3.3 shows that only gallic acid concentration among the variables led to significant differences on total phenolic content of films. High level of gallic acid concentration induced to increase of total phenolic content dramatically. It was observed that glycerol and pH had no contribution on total phenolic content of films significantly (Figure 3.4).

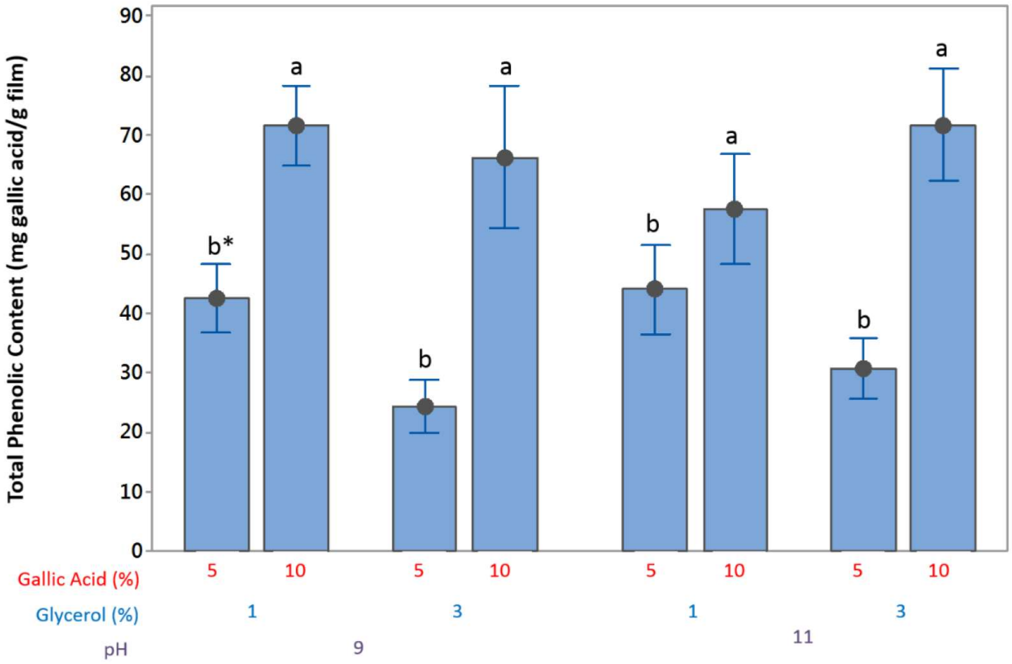

Figure 3.3. The effects of gallic acid and glycerol concentration and pH on total phenolic content of films

*Different letters on the bars show significant difference ($p \leq 0.05$)

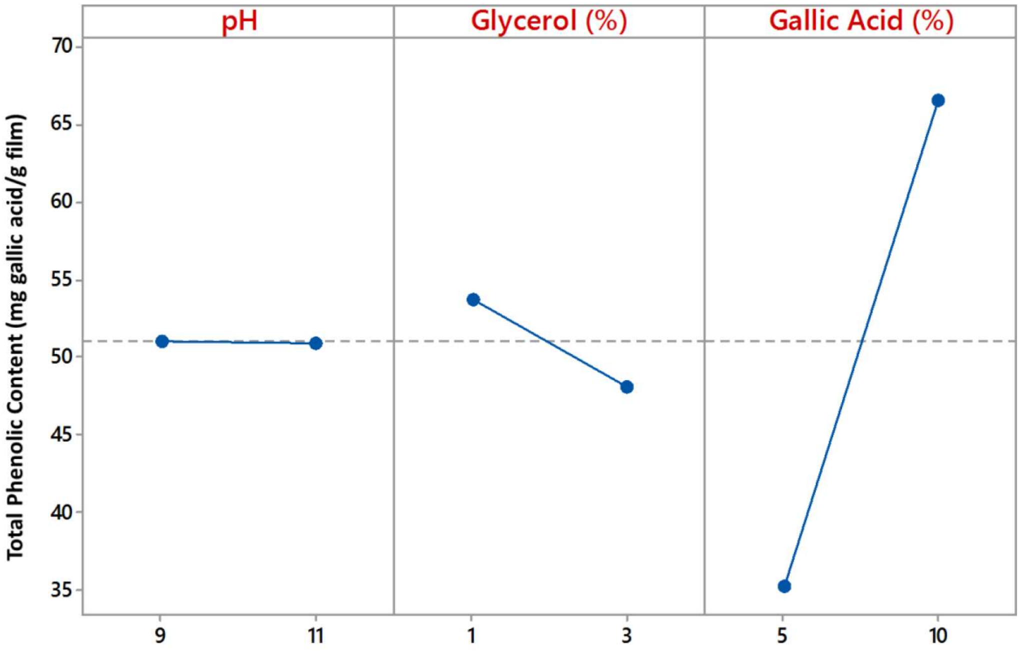

Figure 3.4. Main Effects Plot for Total Phenolic Content of Films

3.4. Antioxidant Activity

DPPH˙ method used in analysis of antioxidant activity is based on free radical scavenging mechanism. Antioxidant substances scavenges the nitrogen free radical mentioned as DPPH˙ and forms a stable molecule (Ruiz-Navajas et al., 2013).

Figure 3.5 shows the antioxidant activity of chickpea flour based active films. All factors that were used in this study created significant difference on antioxidant activity of films independently. As an expected effect, higher concentration of gallic acid made a great contribution to the films by increasing antioxidant activity. The same correlation has been observed in a study conducted with chitosan/gelatin composite films incorporated gallic acid. The increased concentration of gallic acid resulted an increased antioxidant activity of films (Rui et al., 2017).

Furthermore, antioxidant activity of chickpea flour based films showed significant differences with changing concentration of glycerol and pH value. At pH 11, chickpea flour based films had higher antioxidant activity. Since pH influenced the ionization of functional groups in phenolic compounds. pH value of the films had an impact on antioxidant activity. The highest antioxidant activity is obtained at closer pK_a value (Amorati et al., 2006). pK_a values has been found as 4, 8.2, 10.7 and 13.1 for gallic acid (Abbasi et al., 2011). The closer pH value to the pK_a values of gallic acid was pH 11 in this study. For this reason, at pH 11 antioxidant activity of the films was found to be higher than at pH 9.

Moreover, concentration of glycerol had also a significant impact on antioxidant activity of films. Higher concentration of glycerol led to a decrease of antioxidant activity of films. Glycerol might enhance the hydrophilicity of the films. However, previous studies revealed that hydrophobicity increased the antioxidant activity of peptides (Li et al., 2008; Mendis et al., 2005). Due to increased hydrophilicity with increased glycerol concentration, antioxidant activity of chickpea flour based films decreased. The highest antioxidant activity was obtained at pH 11 with the glycerol concentration of 1% and gallic acid concentration of 10%.

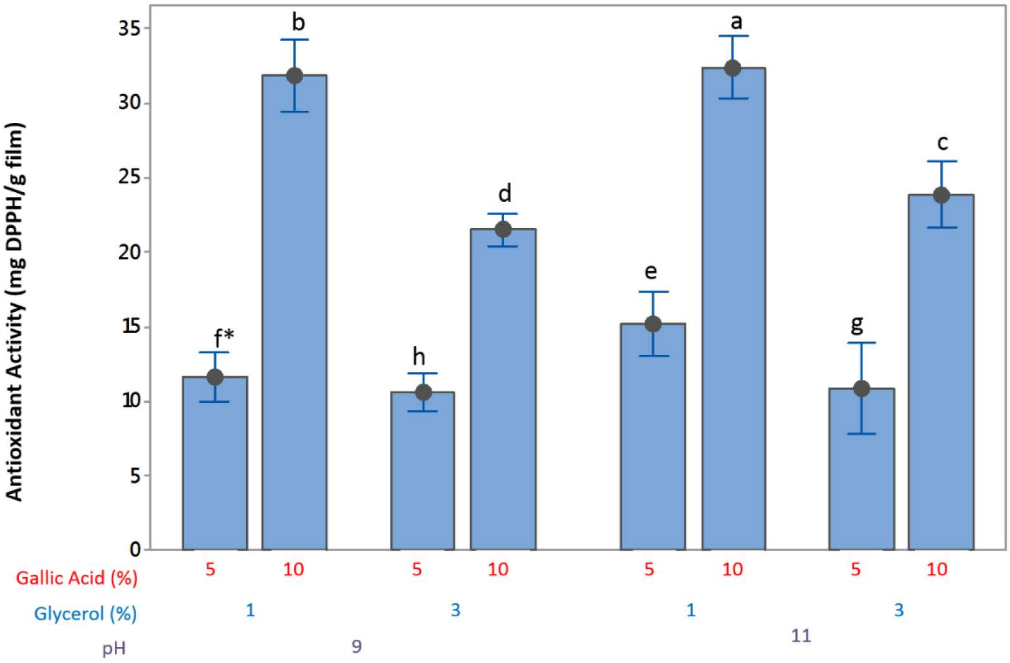

Figure 3.5. The Effects of Gallic Acid and Glycerol Concentration and pH on Antioxidant Activities of Films

[*]Different letters on the bars show significant difference ($p \leq 0.05$)

3.5. Mechanical Properties

Table 3.3 shows the tensile strength, Young's modulus and elongation at break of chickpea flour based active films. It was found that pH and concentration of glycerol had significant effects on tensile strength (Table C.8) and Young's Modulus (Table C.9). Films prepared at pH 11 had higher tensile strength than films prepared at pH 9. Since pH 11 was more distant from the isoelectric point of proteins as compared to pH 9, hydrophobic interactions increased while ionic interactions decreased (Hamaguchi et al., 2007). Proteins were unfolded and solubilized. Stretching occurred in the polymer chain due to repelling of the same charged groups and formed a confined structure. The stronger hydrophobic interactions improved the film tensile properties. However, elongation of films did not show differences with changing pH which was similar to the results obtained in the study of fish myofibrillar protein based films (Shiku et al., 2003).

Table 3.3. Mechanical properties of films

Films	*Tensile Strength (MPa)*	*Young's Modulus (MPa)*	*Elongation at Break (%)*
P9-G1-GA5[*]	$0.47 \pm 0.15^{c**}$	14.20 ± 4.67^{c}	5.95 ± 0.07^{d}
P9-G1-GA10	0.46 ± 0.28^{c}	8.32 ± 4.21^{c}	9.25 ± 0.49^{c}
P9-G3-GA5	0.52 ± 0.25^{d}	7.37 ± 4.14^{d}	15.00 ± 1.41^{b}
P9-G3-GA10	0.43 ± 0.15^{d}	6.71 ± 2.35^{d}	14.00 ± 0.00^{a}
P11-G1-GA5	1.63 ± 0.13^{a}	56.65 ± 11.38^{a}	5.70 ± 0.42^{d}
P11-G1-GA10	1.86 ± 0.48^{a}	42.55 ± 10.04^{a}	14.00 ± 1.41^{c}
P11-G3-GA5	0.75 ± 0.36^{b}	14.45 ± 6.15^{b}	11.00 ± 0.00^{b}
P11-G3-GA10	0.67 ± 0.13^{b}	8.46 ± 2.88^{b}	19.50 ± 2.12^{a}

[*]P denotes pH, G denotes glycerol (%), GA denotes gallic acid (%)

[**]Different letters on the same column represent significant difference ($p \leq 0.05$).

Concentration of glycerol had a significant impact on tensile strength, Young's modulus and elongation of the films. Higher concentration of glycerol (3% w/v) led to a decrease in tensile strength (Table 3.3 & Figure 3.6) and Young's modulus (Table

3.3 & Figure 3.7) while elongation was increased by higher concentration of glycerol (Table 3.3 & Figure 3.8). The same correlation between glycerol and mechanical properties (tensile strength and elongation) was found in tensile properties of achira flour film. In general manner, molecular mobility is increased and intermolecular forces like hydrogen bonding between protein decreases with the addition of glycerol (Andrade-Mahecha et al., 2012). As a result of this mobility, tensile strength of films decreased while elongation increased with addition of higher glycerol concentration.

Gallic acid had a significant effect on elongation at break. Elongation values increased with increasing gallic acid concentration from 5% to 10%. In addition to glycerol, plasticizing effect was also gained with gallic acid. Similar results were observed in wheat gluten films and zein based films which were incorporated with gallic acid (Alkan et al., 2011; Hager et al., 2012). On the other hand, there was no significant effect of gallic acid concentration on tensile strength and Young's modulus which was similar to the results of zein based films containing gallic acid (Alkan et al., 2011).

Chickpea flour based active films had lower tensile strength as compared to that of some other flour based films like achira (7 MPa containing 17% glycerol) and lentil (7.46 MPa containing 20% glycerol) (Andrade-Mahecha et al. 2012; Aydogdu et al. 2018). It can be explained by the effect of addition of gallic acid. Tensile strength of chickpea flour based active films ranged 0.46-1.86 MPa (Table 3.3). These values were similar to the results of previous studies in which galic acid was incorporated. For example, wheat gluten films containing 10% gallic acid had 0.53 MPa tensile strength (Hager et al., 2012).

The other reason of lower tensile properties of chickpea flour based active films as compared to some other flour films might be due to their higher lipid composition. Oil content of chickpea flour is 6.63 g/100g in dry basis while lentil, mung bean, black bean and red kidney bean flour are 1.14, 1.28, 1.90 and 1.56 g/100g in dry basis respectively (Du et al., 2014). Higher level of lipids caused the discontinuous and non-cohesive matrix with some irregularities. Moreover, for starch and protein rich compounds water is an influential plasticizer which decreases tensile strength while increases elongation of films (Dias et al., 2010).

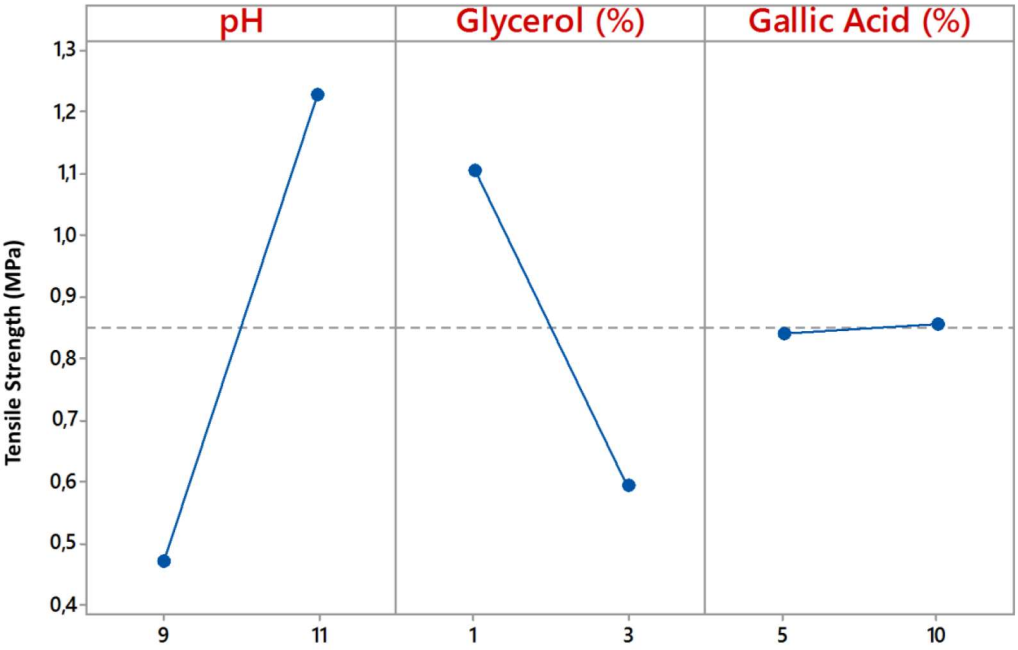

Figure 3.6. Main effects plot for tensile strength of films

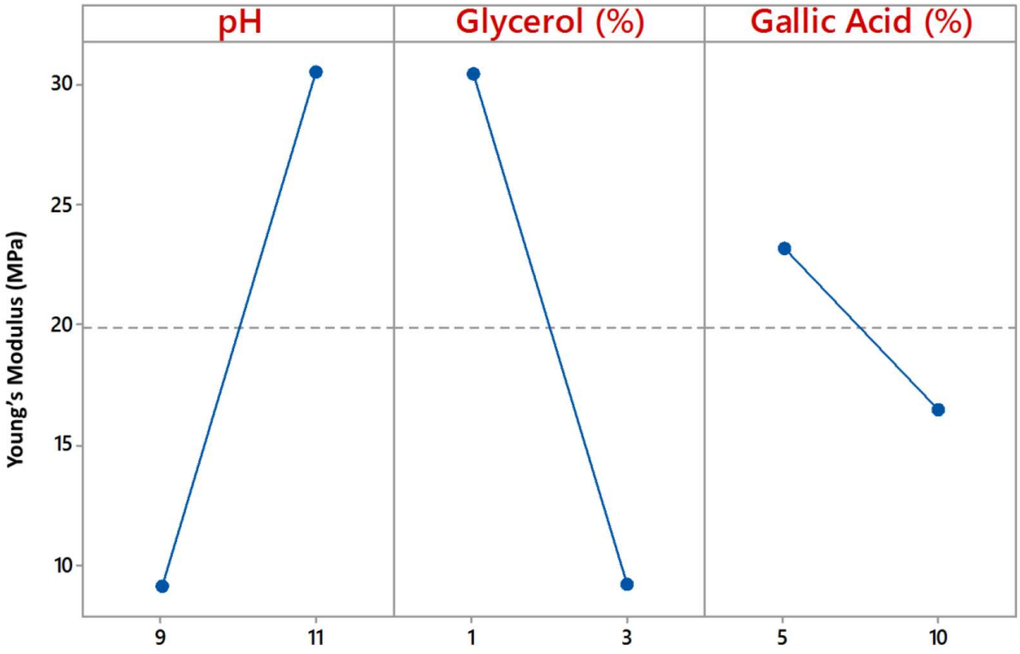

Figure 3.7. Main effects plot for Young's modulus of films

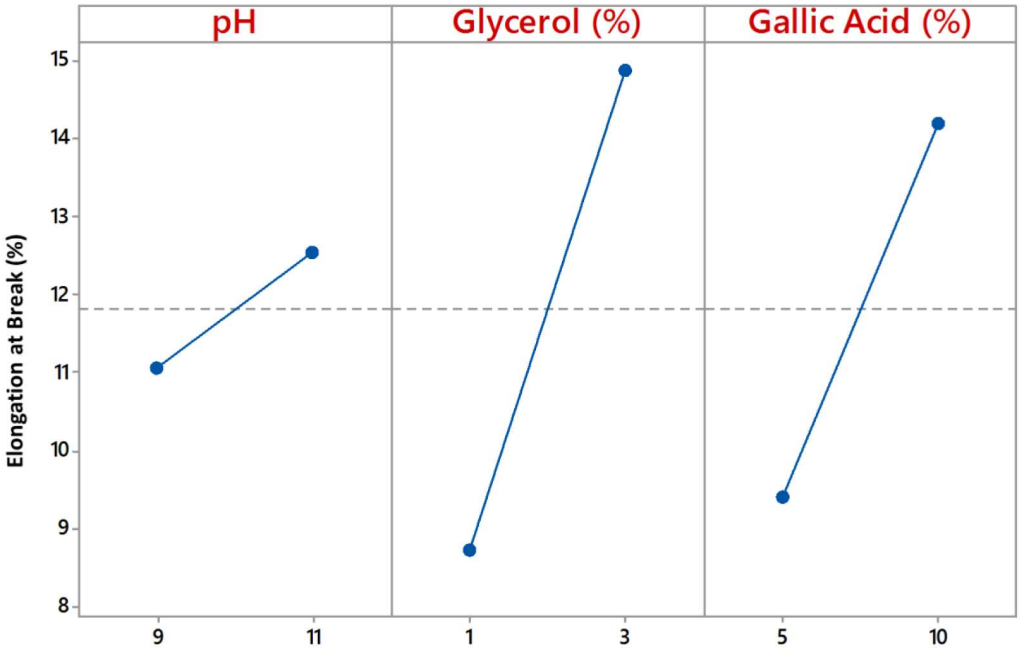

Figure 3.8. Main effects plot for elongation of films

3.6. SEM

Figure 3.9 shows the cross-sectional SEM images of chickpea flour based films containing gallic acid. In images, some irregularities and nonhomogenenities were observed. Cross sectional images were found to be similar with a study which has been conducted with lentil flour (Aydogdu et al., 2018). Flours contain a composite matrix including lipid, starch, protein and fiber. This composite matrix caused some nonhomogenities and irregularities. Authors explained this situation with blockage of lipid-amylose and lipid- protein composites formation due to existence of starch matrix (Dias et al., 2010). There were also some spherical aggregations on cross sections of films. Ungelatinized starch particles because of inadequate applied temperature or time could be the reason of forming of these spherical aggregations. On the other hand, conditioning of films can give the time or situation for recrystallization of starch.

Different concentration of glycerol used in films caused the differences on microstructure of films. Higher concentration of glycerol increased the heterogeneities of films. With regard to pH, pH 11 provided more homogeneous and compact structure due to stronger interaction. The images of the films showed parallel interpretation with tensile strength of the films. On the other hand, it seems that gallic acid had a contribution on microstructure of the films. Addition of higher concentration of gallic acid provided more compact structure and gave the films structural coherence.

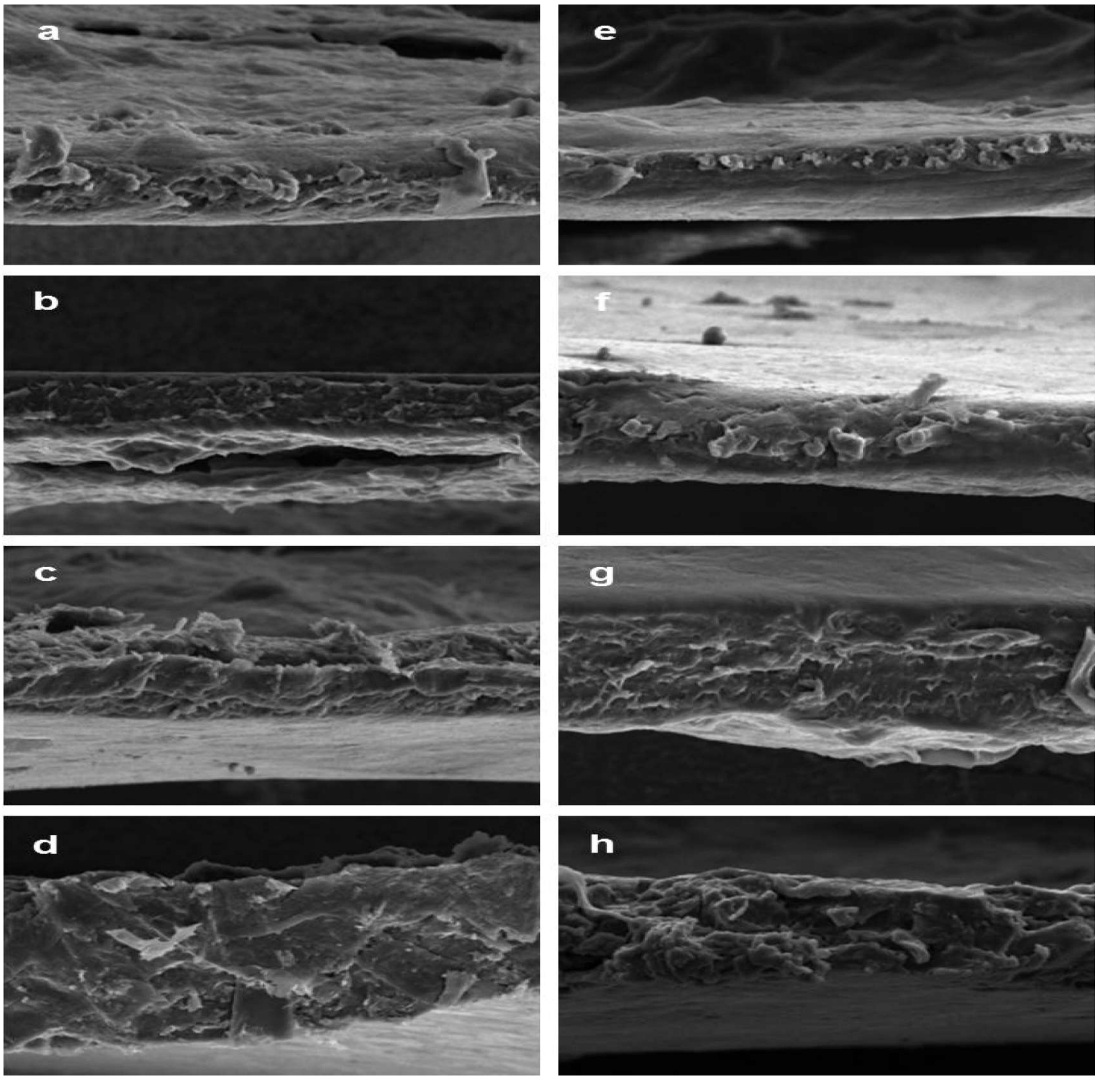

Figure 3.9. Cross sectional images of SEM micrographs (x1000 magnification): a) P9-G1-GA5, b) P9-G1-GA10, c) P9-G3-GA5, d) P9-G3-GA10, e) P11-G1-GA5, f) P11-G1-GA10, g) P11-G3-GA5 and h)P11-G3-GA10

3.7. Water Vapor Permeability

Table 3.4 shows the water vapor permeability (WVP) of the chickpea flour based films. Results show that higher glycerol concentration created a significant increase on water vapor permeability of the films. The same effect of glycerol concentration was seen in cress seed carbohydrate gum based film. The main reason of increased WVP with increased glycerol concentration might be the hydrophilicity of glycerol. Moreover, glycerol might lead to the decrease in intermolecular forces. By this way, free volume was created in film matrix and molecular mobility increased. As a result of mobility, water vapor could move easily in film matrix (Jouki et al., 2013b). With regard to pH, there was no significant effect on WVP of chickpea flour based films. The analogous result has been obtained in soy protein based films at two alkali pH (8 and 11) (Mauri & Añón, 2008).

Table 3.4. *Water vapor permeabilities of films*

Films	*WVP* $(\mathrm{ng} \times \mathrm{m}^{-1} \times \mathrm{s}^{-1} \times \mathrm{Pa}^{-1})$
P9-G1-GA5*	$0.2736 \pm 0.0005^{c**}$
P9-G1-GA10	0.2040 ± 0.0199^{d}
P9-G3-GA5	0.2787 ± 0.0011^{b}
P9-G3-GA10	0.2929 ± 0.0073^{a}
P11-G1-GA5	0.2736 ± 0.0086^{c}
P11-G1-GA10	0.2137 ± 0.0071^{d}
P11-G3-GA5	0.2570 ± 0.0216^{b}
P11-G3-GA10	0.2527 ± 0.0256^{a}

*P denotes pH, G denotes glycerol (%), GA denotes gallic acid (%)

**Different letters in the same column represent significant difference ($p \leq 0.05$).

On the other hand, addition of higher concentration of gallic acid decreased WVP of chickpea flour based films. Thus, addition of 10% (w/w of total solid) gallic acid lowered the WVP. Similar relation was observed in chitosan/gelatin composite films incorporated with gallic acid. Cross-linking between composite film matrix, glycerol and gallic acid interfere the interaction with water molecules by reduced free volume in film matrix (Rui et al., 2017). In another film study which was conducted with gluten films, addition of gallic acid up to concentration of 2% (%w/w gluten) increased WVP. But, it was seen that gallic acid concentration higher than 2% decreased the WVP. Free volume in film matrix was filled with excess gallic acid addition. Due to decrease in free volume, WVP of gluten films decreased (Hager et al., 2012). Results of WVP of chickpea flour based films showed that higher concentration of gallic acid enabled more compact structure as it can be seen from images of SEM in Figure 3.9. When compared to some other flour based films, achira flour based films with 17% glycerol and lentil flour based films with 20% glycerol had higher WVP which were 0.53 ± 0.02 $ng \times m^{-1} \times s^{-1} \times Pa^{-1}$ and 0.25 ± 0.04 $ng \times m^{-1} \times s^{-1} \times Pa^{-1}$, respectively. The promising results were observed for the combination of gallic acid and chickpea flour films especially in terms of low WVP. Moreover, in a study conducted with chickpea protein hydrolysate, it has been observed that the highest hydrophobicity was obtained with the group having highest antioxidant activity. There was a strong relation among the hydrophobicity and antioxidant activity (Li et al., 2008). In our film study, there was a correlation among the WVP and antioxidant activity of the films. Films which had low WVP showed high antioxidant activity.

3.8. FTIR

Figure 3.10 and Figure 3.11 show the FTIR spectra of chickpea flour based films containing gallic acid. Films at different pH and involving different concentrations of gallic acid and glycerol had similar major peaks but with different amplitudes. Three bands exist in FTIR spectra are the demonstration of existence of starch. The C-O stretching around 990-1160, the C-O-H stretching around 1150-1080 and anhydroglucose ring O-C stretch around 990-1030. Starch is one of the main constituent of chickpea flour based films. For this framework, the bands around 990-1160 were seen in the spectra. Moreover, there was 0a peak around 923-927 pointed to glycosidic linkages (Valderrama Solano & Rojas de Gante, 2014).

Other remarkable peaks are Amide A ascribable to N-H stretching around 3275. Amide I around 1653 representing C=O. Amide II 1541 representing C-N stretching and N-H angular deformation. Interactions between phenolic compound and protein resulted shift of the Amide A and Amide B peaks. In gelatin and green tea extract film study, peaks of Amide A (from 3317.60 to 3302.15) and Amide B (2938.31 to

2925.65) shifted lower wavenumbers with the addition of 0.7% green tea extract (Bitencourt et al., 2014). In our study, gallic acid showed similar shifts in Amide A and Amide B. Peaks at Amide A were shifted from 3275 to 3273 with the increasing gallic acid concentration from 5% to 10% (w/w of total solid). Furthermore, changing of gallic acid concentration from 5% to 10% resulted to a shift at Amide B peak from 2926 to 2920 in films containing 1% gallic acid and at pH 9 and from 2922 to 2918 in containing 1% gallic acid and at pH 11.

Incorporation of phenolic substances can be recognized from peak at 1653 which is responsible for C=O stretching within carboxylic group and peak at 1541 representing C=C stretching within aromatic ring. Rise of amplitude at peaks around 1653, 1541 and decline of amplitude at peak around 3273-3275 was shown that there was interaction with hydroxyl group and amino group between gallic acid. The peaks and changing behavior of amplitudes at peaks were found to be similar with gallic acid/fish gelatin film (Limpisophon & Schleining, 2017). By the interaction with gallic acid and chickpea based flour, formation of new hydrogen and covalent bonds lowered free hydrogen group. By this way, formation of hydrophilic interaction with water could be interfered.

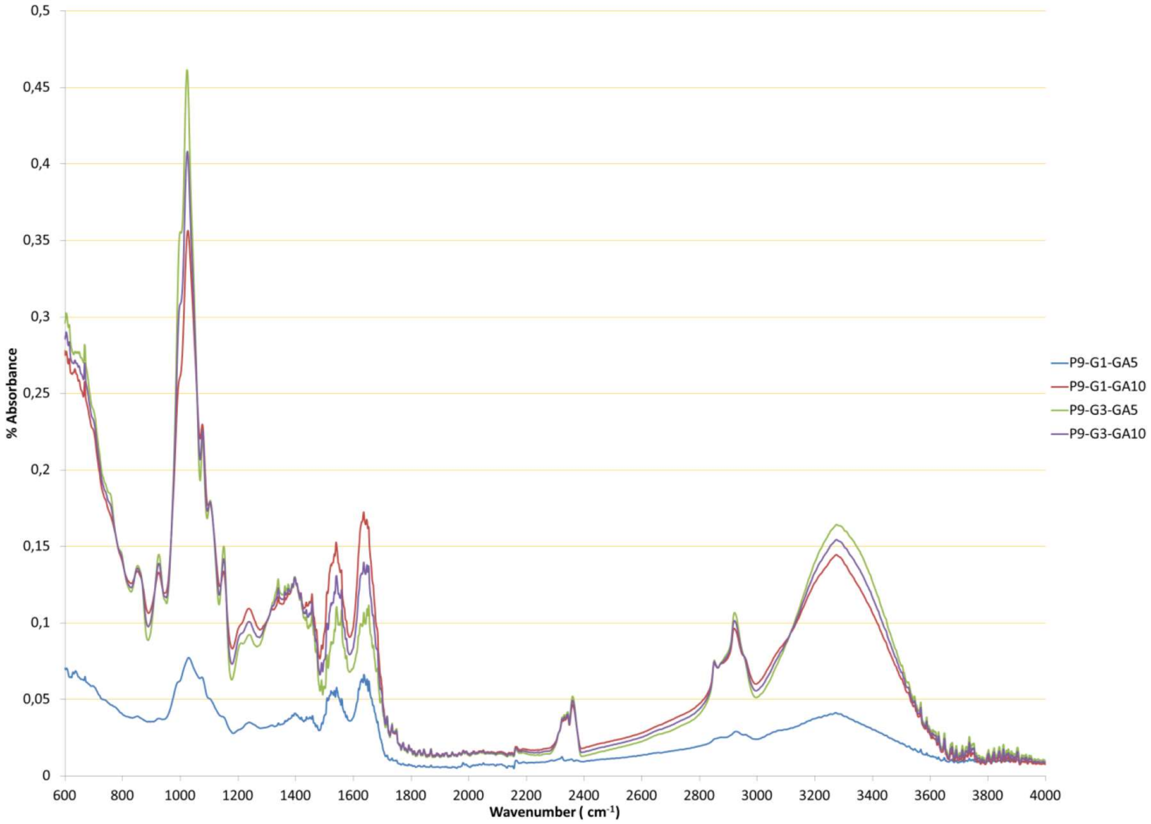

Figure 3.10. FTIR spectra of chickpea flour based films containing gallic acid at pH 9

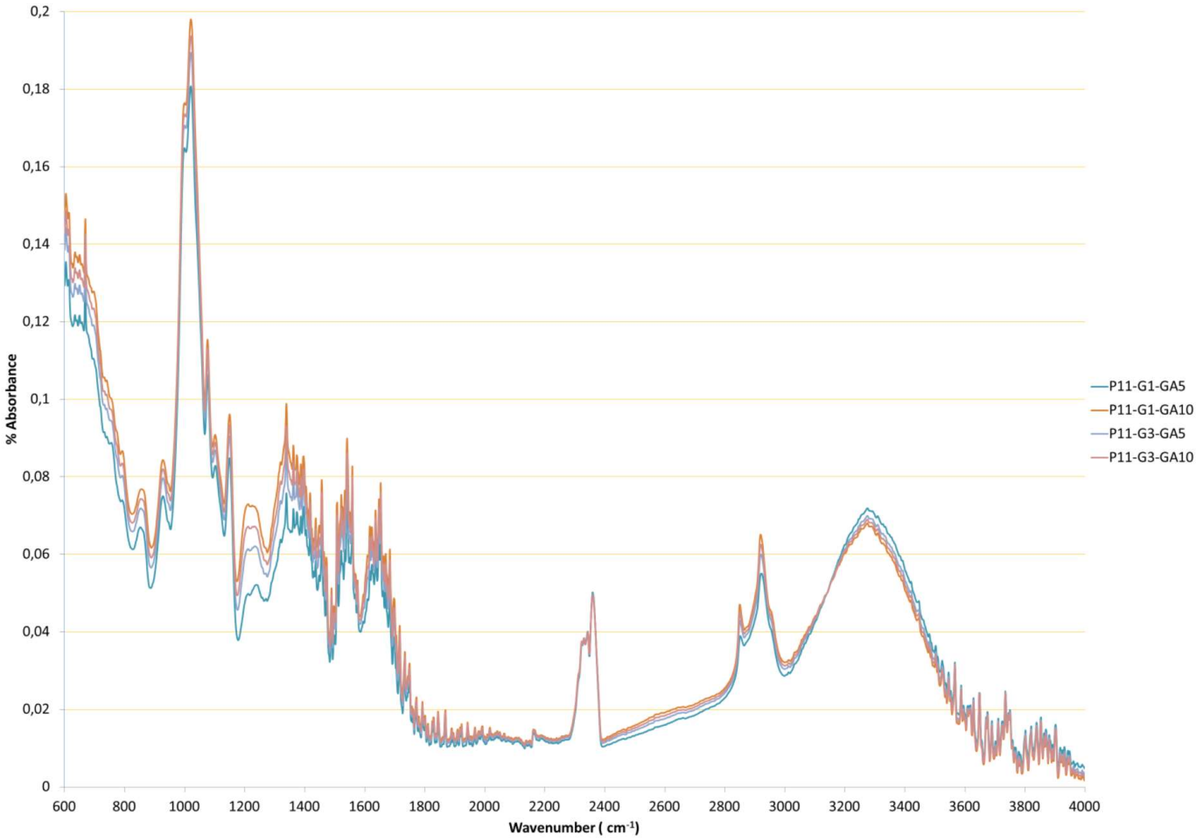

Figure 3.11. FTIR spectra of chickpea flour based films containing gallic acid at pH 11

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

Incorporation of gallic acid into chickpea based films provided great antioxidant activity and total phenolic content to films. Chickpea flour based film at pH 11 with glycerol concentration of 10% showed lower WVP than most of the flour based films. Moreover, higher gallic acid concentration gave the films compact structure, flexibility and opacity. Films with lower concentration of glycerol increased antioxidant activity, mechanical strength and decreased WVP which resulted stronger barrier properties. At pH 11, films were found to be stronger with denser microstructure supported by SEM images, darker and also had higher antioxidant activity. Thus, chickpea flour based films containing gallic acid can be a good alternative of composite based active films in terms of low WVP, higher antioxidant activity and good mechanical properties. Chickpea based films containing gallic acid could be used for wrapping of highly oxidative food product such as nuts, fish, meat as a second protective material.

For the future studies, film solution may be prepared with another solvent different from water such as ethanol. Chickpea flour may be used at different concentrations. Different type of plasticizer may be investigated in order to obtain the strongest chickpea flour based films. Instead of gallic acid, different antioxidant substances may be added to films and they may be characterized. Other substances like cross-linking agents may be added to the film solution to strengthen the films. The effect of drying conditions and methods may be investigated.

REFERENCES

Abbasi, S., Daneshfar, A., Hamdghadareh, S., & Farmany, A. (2011). Quantification of sub-nanomolar levels of gallic acid by adsorptive stripping voltammetry. *International Journal of Electrochemical Science, 6,* 4843–4852.

Alkan, D., Aydemir, L. Y., Arcan, I., Yavuzdurmaz, H., Atabay, H. I., Ceylan, C., & Yemenicioğlu, A. (2011). Development of flexible antimicrobial packaging materials against Campylobacter jejuni by incorporation of gallic acid into zein-based films. *Journal of Agricultural and Food Chemistry*, *59*(20), 11003–11010. https://doi.org/10.1021/jf202584b

Amorati, R., Pedulli, G. F., Cabrini, L., Zambonin, L., & Landi, L. (2006). Solvent and ph effects on the antioxidant activity of caffeic and other phenolic acids. *Journal of Agricultural and Food Chemistry*, *54*, 2932–2937.

Andrade-Mahecha, M. M., Tapia-Blácido, D. R., & Menegalli, F. C. (2012). Development and optimization of biodegradable films based on achira flour. *Carbohydrate Polymers*, *88*(2), 449–458. https://doi.org/10.1016/j.carbpol.2011.12.024

Aydogdu, A., Kirtil, E., Sumnu, G., Oztop, M. H., & Aydogdu, Y. (2018). Utilization of lentil flour as a biopolymer source for the development of edible films. *Journal of Applied Polymer Science*, *135*(23), 1–10. https://doi.org/10.1002/app.46356

Bamdad, F., Goli, A. H., & Kadivar, M. (2006). Preparation and characterization of proteinous film from lentil (Lens culinaris): Edible film from lentil (Lens culinaris). *Food Research International*, *39*(1), 106–111. https://doi.org/10.1016/j.foodres.2005.06.006

Bergo, P., Moraes, I. C. F., & Sobral, P. J. A. (2013). Effects of plasticizer concentration and type on moisture content in gelatin films. *Food Hydrocolloids*, *32*(2), 412–415. https://doi.org/10.1016/j.foodhyd.2013.01.015

Bitencourt, C. M., Fávaro-Trindade, C. S., Sobral, P. J. A., & Carvalho, R. A. (2014). Gelatin-based films additivated with curcuma ethanol extract : Antioxidant activity and physical properties of films. *Food Hydrocolloids*, *40*, 145–152. https://doi.org/10.1016/j.foodhyd.2014.02.014

Bonilla, J., Atarés, L., Vargas, M., & Chiralt, A. (2012). Edible films and coatings to prevent the detrimental effect of oxygen on food quality: Possibilities and limitations. *Journal of Food Engineering*, *110*(2), 208–213. https://doi.org/10.1016/j.jfoodeng.2011.05.034

Bourtoom, T. (2008a). Factors affecting the properties of edible film prepared from mung bean proteins. *International Food Research Journal*, *15*(2), 167–180. https://doi.org/10.1037//1099-9809.8.2.157

Bourtoom, T. (2008b). Review article edible films and coatings : characteristics and properties. *International Food Research Journal*, *15*(3), 237–248.

Cao, N., Yang, X., & Fu, Y. (2009). Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films. *Food Hydrocolloids*, *23*(3), 729–735. https://doi.org/10.1016/j.foodhyd.2008.07.017

Cazón, P., Velazquez, G., Ramírez, J. A., & Vázquez, M. (2017). Polysaccharide-based films and coatings for food packaging: A review. *Food Hydrocolloids*, *68*, 136–148. https://doi.org/10.1016/j.foodhyd.2016.09.009

Cheng, S., Wang, B., & Weng, Y. (2015). LWT - Antioxidant and antimicrobial edible zein / chitosan composite films fabricated by incorporation of phenolic compounds and dicarboxylic acids. *LWT - Food Science and Technology*, *63*(1), 115–121. https://doi.org/10.1016/j.lwt.2015.03.030

Cilek, B., Luca, A., Hasirci, V., Sahin, S., & Sumnu, G. (2012). Microencapsulation of phenolic compounds extracted from sour cherry pomace: Effect of formulation, ultrasonication time and core to coating ratio. *European Food Research and Technology*, *235*(4), 587–596. https://doi.org/10.1007/s00217-

012-1786-8

Daneshfar, A., Ghaziaskar, H. S., & Homayoun, N. (2008). Solubility of gallic acid in methanol, ethanol, water, and ethyl acetate. *Journal of Chemical & Engineering Data*, *53*(3), 776–778. https://doi.org/10.1021/je700633w

Dias, A. B., Müller, C. M. O., Larotonda, F. D. S., & Laurindo, J. B. (2010). Biodegradable films based on rice starch and rice flour. *Journal of Cereal Science*, *51*(2), 213–219. https://doi.org/10.1016/j.jcs.2009.11.014

Du, S., Jiang, H., Yu, X., & Jane, J. lin. (2014). Physicochemical and functional properties of whole legume flour. *LWT - Food Science and Technology*, *55*(1), 308–313. https://doi.org/10.1016/j.lwt.2013.06.001

Elsabee, M. Z., & Abdou, E. S. (2013). Chitosan based edible films and coatings: A review. *Materials Science and Engineering C*, *33*(4), 1819–1841. https://doi.org/10.1016/j.msec.2013.01.010

Emami, S., & Tabil, L. G. (2008). Friction and compression characteristics of chickpea flour and components. *Powder Technology*, *182*(1), 119–126. https://doi.org/10.1016/j.powtec.2007.07.012

Ghanbarzadeh, B., & Almasi, H. (2011). Physical properties of edible emulsified films based on carboxymethyl cellulose and oleic acid. *International Journal of Biological Macromolecules*, *48*(1), 44–49. https://doi.org/10.1016/j.ijbiomac.2010.09.014

Gómez-Estaca, J., López-de-Dicastillo, C., Hernández-Muñoz, P., Catalá, R., & Gavara, R. (2014). Advances in antioxidant active food packaging. *Trends in Food Science and Technology*, *35*(1), 42–51. https://doi.org/10.1016/j.tifs.2013.10.008

Guo, X., Lu, Y., Cui, H., Jia, X., Bai, H., & Ma, Y. (2012). Factors affecting the physical properties of edible composite film prepared from zein and wheat gluten. *Molecules*, *17*(4), 3794–3804.

https://doi.org/10.3390/molecules17043794

Gutiérrez, T. J., Suniaga, J., Monsalve, A., & García, N. L. (2016). Influence of beet flour on the relationship surface-properties of edible and intelligent films made from native and modified plantain flour. *Food Hydrocolloids*, *54*, 234–244. https://doi.org/10.1016/j.foodhyd.2015.10.012

Hager, A. S., Vallons, K. J. R., & Arendt, E. K. (2012). Influence of gallic acid and tannic acid on the mechanical and barrier properties of wheat gluten films. *Journal of Agricultural and Food Chemistry*, *60*(24), 6157–6163. https://doi.org/10.1021/jf300983m

Hamaguchi, P. Y., WuYin, W., & Tanaka, M. (2007). Effect of pH on the formation of edible films made from the muscle proteins of Blue marlin (Makaira mazara). *Food Chemistry*, *100*(3), 914–920. https://doi.org/10.1016/j.foodchem.2005.10.045

Hasebe, T., Sasaki, S., Imoto, S., & Ochiai, A. (2004). Histological Characteristics of Tumor in Vessels and Lymph Nodes Are Significant Predictors of Progression of Invasive Ductal Carcinoma of the Breast: A Prospective Study. *Human Pathology*, *35*(3), 298–308. https://doi.org/10.1016/j.rser.2013.06.017

Hoque, M. S., Benjakul, S., & Prodpran, T. (2010). Effect of heat treatment of film-forming solution on the properties of film from cuttlefish (Sepia pharaonis) skin gelatin. *Journal of Food Engineering*, *96*(1), 66–73. https://doi.org/10.1016/j.jfoodeng.2009.06.046

Jafarzadeh, S., Alias, A. K., Ariffin, F., & Mahmud, S. (2018). Physico-mechanical and microstructural properties of semolina flour films as influenced by different sorbitol/glycerol concentrations. *International Journal of Food Properties*, *21*(1), 983–995. https://doi.org/10.1080/10942912.2018.1474056

Janjarasskul, T., & Krochta, J. M. (2010). Edible Packaging Materials. *Annual Review of Food Science and Technology*, *1*(1), 415–448.

https://doi.org/10.1146/annurev.food.080708.100836

Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2010). Effect of lipid self-association on the microstructure and physical properties of hydroxypropyl-methylcellulose edible films containing fatty acids. *Carbohydrate Polymers*, *82*(3), 585–593. https://doi.org/10.1016/j.carbpol.2010.05.014

Jomlapeeratikul, P., Poomsa-Ad, N., & Wiset, L. (2016). Effect of Drying Temperatures and Plasticizers on the Properies of Konjac Flour Film. *Journal of Food Process Engineering*, *40*(3). https://doi.org/10.1111/jfpe.12443

Jouki, M., Khazaei, N., Ghasemlou, M., & Hadinezhad, M. (2013a). Effect of glycerol concentration on edible film production from cress seed carbohydrate gum. *Carbohydrate Polymers*, *96*(1), 39–46. https://doi.org/10.1016/j.carbpol.2013.03.077

Jouki, M., Khazaei, N., Ghasemlou, M., & Hadinezhad, M. (2013b). Effect of glycerol concentration on edible film production from cress seed carbohydrate gum. *Carbohydrate Polymers*, *96*(1), 39–46. https://doi.org/10.1016/j.carbpol.2013.03.077

Jouki, M., Mortazavi, S. A., Yazdi, F. T., & Koocheki, A. (2014). Characterization of antioxidant – antibacterial quince seed mucilage films containing thyme essential oil. *Carbohydrate Polymers, 99*, 537–546.

Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (Cicer arietinum L.): a review. *British Journal of Nutrition*, *108*(S1), S11–S26. https://doi.org/10.1017/S0007114512000797

Kavoosi, G., Rahmatollahi, A., Dadfar, S. M. M., & Purfard, A. M. (2014). Effects of essential oil on the water binding capacity, physico-mechanical properties, antioxidant and antibacterial activity of gelatin films. *LWT-Food Science and Technology*, *57*(2), 556–561. https://doi.org/DOI 10.1016/j.lwt.2014.02.008

Kokoszka, S., Debeaufort, F., Lenart, A., & Voilley, A. (2010). Water vapour permeability, thermal and wetting properties of whey protein isolate based edible films. *International Dairy Journal*, *20*(1), 53–60. https://doi.org/10.1016/j.idairyj.2009.07.008

Kowalczyk, D., & Baraniak, B. (2011). Effects of plasticizers, pH and heating of film-forming solution on the properties of pea protein isolate films. *Journal of Food Engineering*, *105*(2), 295–305. https://doi.org/10.1016/j.jfoodeng.2011.02.037

Li, Y., Jiang, B., Zhang, T., Mu, W., & Liu, J. (2008). Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH). *Food Chemistry*, *106*(2), 444–450. https://doi.org/10.1016/j.foodchem.2007.04.067

Limpisophon, K., & Schleining, G. (2017). Use of gallic acid to enhance the antioxidant and mechanical properties of active fish gelatin film. *Journal of Food Science*, *82*(1), 80–89. https://doi.org/10.1111/1750-3841.13578

Limpisophon, K., Tanaka, M., & Osako, K. (2010). Characterisation of gelatin-fatty acid emulsion films based on blue shark (Prionace glauca) skin gelatin. *Food Chemistry*, *122*(4), 1095–1101. https://doi.org/10.1016/j.foodchem.2010.03.090

Liu, C., Tellez-garay, A. M., & Castell-perez, M. E. (2004). Physical and mechanical properties of peanut protein films. *LWT-Food Science and Technology, 37(7)*, 731–738. https://doi.org/10.1016/j.lwt.2004.02.012

Lu, L. L., & Lu, X. Y. (2007). Solubilities of gallic acid and its esters in water. *Journal of Chemical and Engineering Data*, *52*(1), 37–39. https://doi.org/10.1021/je0601661

Mauri, A. N., & Añón, M. C. (2008). Mechanical and physical properties of soy protein films with pH-modified microstructures. *Food Science and Technology International*, *14*(2), 119–125. https://doi.org/10.1177/1082013208092130

Mendis, E., Rajapakse, N., Byun, H. G., & Kim, S. K. (2005). Investigation of jumbo squid (Dosidicus gigas) skin gelatin peptides for their in vitro antioxidant effects.

Life Sciences, *77*(17), 2166–2178. https://doi.org/10.1016/j.lfs.2005.03.016

Mohammad, S., Razavi, A., Amini, A. M., & Zahedi, Y. (2015). Characterisation of a new biodegradable edible film based on sage seed gum : Influence of plasticiser type and concentration. *Food Hydrocolloids*, *43*, 290–298. https://doi.org/10.1016/j.foodhyd.2014.05.028

Motedayen, A. A., Khodaiyan, F., & Salehi, E. A. (2013). Development and characterisation of composite films made of kefiran and starch. *Food Chemistry*, *136*(3–4), 1231–1238. https://doi.org/10.1016/j.foodchem.2012.08.073

Muehlbauer, F. J., & Sarker, A. (2017). The Chickpea Genome, 5–13. https://doi.org/10.1007/978-3-319-66117-9

Nadaud, P., Pérez-Gago, M. B., & Krochta, J. M. (2006). Water vapor permeability, solubility , and tensile properties of heat-denatured versus native whey proteins. *Journal of Food Science, 64*(6), 1034–1037.

Nemet, N. T., Šošo, V. M., & Lazić, V. L. (2010). Effect of glycerol content and pH value of film-forming solution on the functional properties of protein-based edible films. *Acta Periodica Technologica*, *41*, 57–67. https://doi.org/10.2298/APT1041057N

Noronha, C. M., Matos de Carvalho, S., Lino, R. C., & Manique Barreto, P. L. (2014). Characterization of antioxidant methylcellulose film incorporated with α-tocopherol nanocapsules. *Food Chemistry*, *159*, 529–535. https://doi.org/10.1016/j.foodchem.2014.02.159

Pagliaro, M., & Rossi, M. (2008). Glycerol : properties and production. In *The Future of Glycerol: New Uses of a Versatile Raw Material*, 1–17

Pérez-Gago, M. B., & Krochta, J. M. (2001). Lipid particle size effect on water vapor permeability and mechanical properties of whey protein/beeswax emulsion films. *Journal of Agricultural and Food Chemistry*, *49*(2), 996–1002. https://doi.org/10.1021/jf000615f

Piñeros-Hernandez, D., Medina-Jaramillo, C., López-Córdoba, A., & Goyanes, S. (2017). Edible cassava starch films carrying rosemary antioxidant extracts for potential use as active food packaging. *Food Hydrocolloids*, *63*, 488–495. https://doi.org/10.1016/j.foodhyd.2016.09.034

Rachtanapun, P., & Tongdeesoontorn, W. (2009). Effect of antioxidants on properties of rice flour/ cassava starch film blends. *Kasetsart Journal - Natural Science*, *43*(5), 252–258. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-77749345989&partnerID=40&md5=ac787faf0b3619b433733097c49fc33c

Riveros, C. G., Martin, M. P., Aguirre, A., & Grosso, N. R. (2017). Film preparation with high protein defatted peanut flour: characterisation and potential use as food packaging. *International Journal of Food Science & Technology*, 969–975. https://doi.org/10.1111/ijfs.13670

Rui, L., Xie, M., Hu, B., Zhou, L., Yin, D., & Zeng, X. (2017). A comparative study on chitosan/gelatin composite films with conjugated or incorporated gallic acid. *Carbohydrate Polymers*, *173*, 473–481. https://doi.org/10.1016/j.carbpol.2017.05.072

Ruiz-Navajas, Y., Viuda-Martos, M., Sendra, E., Perez-Alvarez, J. A., & Fernández-López, J. (2013). In vitro antibacterial and antioxidant properties of chitosan edible films incorporated with Thymus moroderi or Thymus piperella essential oils. *Food Control*, *30*(2), 386–392. https://doi.org/10.1016/j.foodcont.2012.07.052

Salehan, N. A., Sulaiman, A. Z., & Ajit, A. (2016). Effect of temperature and sonication on the extraction of gallic acid from labisia pumila (Kacip Fatimah). *ARPN Journal of Engineering and Applied Sciences, 11*(4), 2193–2198.

Sanyang, M. L., Sapuan, S. M., Jawaid, M., Ishak, M. R., & Sahari, J. (2015). Effect of plasticizer type and concentration on tensile, thermal and barrier properties of biodegradable films based on sugar palm (arenga pinnata) starch. *Polymers*, *7*, 1106–1124. https://doi.org/10.3390/polym7061106

Saremnezhad, S., Azizi, M. H., Barzegar, M., Abbasi, S., & Ahmadi, E. (2011). Properties of a new edible film made of faba bean protein isolate. *Journal of Agricultural Science and Technology*, *13*, 181–192.

Schmidt, V. C. R., Porto, L. M., Laurindo, J. B., & Menegalli, F. C. (2013). Water vapor barrier and mechanical properties of starch films containing stearic acid. *Industrial Crops and Products*, *41*(1), 227–234. https://doi.org/10.1016/j.indcrop.2012.04.038

Setyaningsih, W., Saputro, I. E., Palma, M., & Barroso, C. G. (2016). Stability of 40 phenolic compounds during ultrasound-assisted extractions (UAE). *AIP Conference Proceedings*, *1755*. https://doi.org/10.1063/1.4958517

Shiku, Y., Hamaguchi, P. Y., & Tanaka, M. (2003). Effect of pH on the preparation of edible films based on fish myofibrillar proteins. *Fisheries Science*, *69*(5), 1026–1032. https://doi.org/10.1046/j.1444-2906.2003.00722.x

Shit, S. C., & Shah, P. M. (2014). Review article: Edible polymers: Challenges and Opportunities, Journal of Polymers, *2014*.

Siripatrawan, U., & Harte, B. R. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids*, *24*, 770–775. https://doi.org/10.1016/j.foodhyd.2010.04.003

Skurtys, O., Acevedo, C., Pedreschi, F., Enrione, J., Osorio, F., & Aguilera, J. (2010). Food hydrocolloid edible films and coatings. *Food Hydrocolloids; Characteristics, Properties and Structures*, 41–80. https://doi.org/10.1016/j.freeradbiomed.2014.10.731

Sothornvit, R., & Krochta, M. (2005). Innovations in food packaging: 23-Plasticizers in edible films and coatings, *Food Science and Technology*, 403–433.

Sreerama, Y. N., Sashikala, V. B., & Pratape, V. M. (2012). Phenolic compounds in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their antioxidant and enzyme inhibitory properties associated with hyperglycemia and hypertension. *Food Chemistry*, *133*, 156–162. https://doi.org/10.1016/j.foodchem.2012.01.011

Sun, C., Xiong, L., & Sun, Q. (2015). Mechanical, barrier and morphological properties of pea starch and peanut protein isolate blend films. *Carbohydrate Polymers*, *121*, 155–162. https://doi.org/10.1016/j.carbpol.2014.12.040

Sun, X., Wang, Z., Kadouh, H., & Zhou, K. (2014). The antimicrobial , mechanical , physical and structural properties of chitosan- gallic acid films. *Food Science and Technology*, *57*, 83–89.

Tapia-Blácido, D., Mauri, A. N., Menegalli, F. C., Sobral, P. J. A., & Añón, M. C. (2007). Contribution of the starch, protein, and lipid fractions to the physical, thermal, and structural properties of amaranth (Amaranthus caudatus) flour films. *Journal of Food Science*, *72*(5), 293–300. https://doi.org/10.1111/j.1750-3841.2007.00359.x

Tongnuanchan, P., Benjakul, S., & Prodpran, T. (2011). Roles of lipid oxidation and pH on properties and yellow discolouration during storage of film from red tilapia (Oreochromis niloticus) muscle protein. *Food Hydrocolloids, 25, 426–433.*

Valderrama Solano, A. C., & Rojas de Gante, C. (2014). Development of biodegradable films based on blue corn flour with potential applications in food packaging. Effects of plasticizers on mechanical, thermal, and microstructural properties of flour films. *Journal of Cereal Science*, *60*(1), 60–66. https://doi.org/10.1016/j.jcs.2014.01.015

Vanin, F. M., Sobral, P. J. A., Menegalli, F. C., Carvalho, R. A., & Habitante, A. M. Q. B. (2005). Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. *Food Hydrocolloids*, *19*(5), 899–

907. https://doi.org/10.1016/j.foodhyd.2004.12.003

Vargas, C. G., Costa, T. M. H., Rios, A. de O., & Flôres, S. H. (2017). Comparative study on the properties of films based on red rice (Oryza glaberrima) flour and starch. *Food Hydrocolloids*, *65*, 96–106. https://doi.org/10.1016/j.foodhyd.2016.11.006

Vieira, M. G. A., Da Silva, M. A., Dos Santos, L. O., & Beppu, M. M. (2011). Natural-based plasticizers and biopolymer films: A review. *European Polymer Journal*, *47*(3), 254–263. https://doi.org/10.1016/j.eurpolymj.2010.12.011

Wahyuni, E. S., & Arifan, F. (2018). Optimization of Chitosan Drying Temperature on The Quality and Quantity of Edible Film. In *E3S Web of Conferences 31* (Vol. 03012, pp. 1–5).

Wani, S. A., & Kumar, P. (2014). Comparative study of chickpea and green pea flour based on chemical composition , functional and pasting properties. *Journal of Food Research and Technology*, *2*(3), 124–129. https://doi.org/10.13140/2.1.3470.4964

Wittaya, T. (2012). Protein-Based Edible Films: Characteristics and Improvement of Properties. *Structure and Function of Food Engineering*. https://doi.org/10.5772/48167

Won-Seok, C., & Jung, H. H. (2001). Physical and Mechanical Properties of Pea-Protein-based Edible Films. *Journal of Food Science*, *66*(2), 319–322.

Yildirim, A., & Öner, M. D. (2015). Electrical conductivity, water absorption, leaching, and color change of chickpea (Cicer arietinum L.) during soaking with ultrasound treatment. *International Journal of Food Properties*, *18*(6), 1359–1372. https://doi.org/10.1080/10942912.2014.917660

APPENDICES

A. Calibration Curves

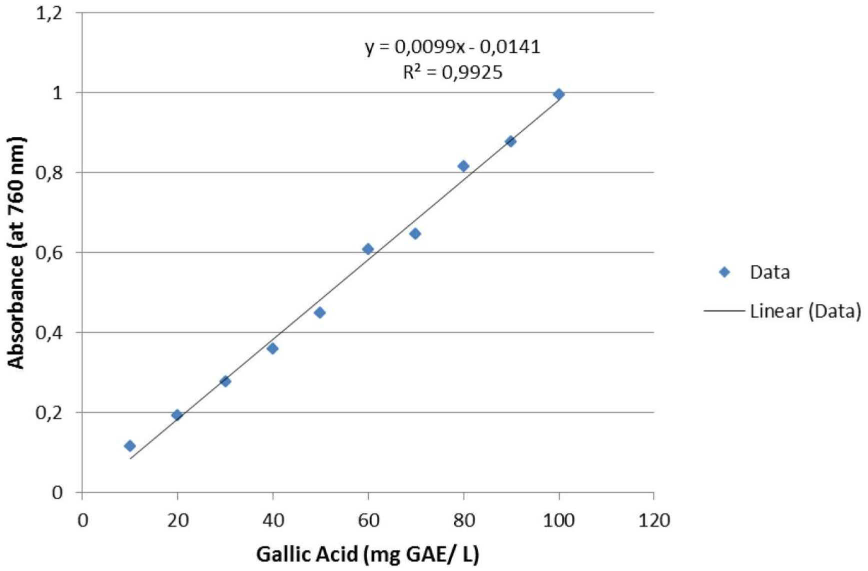

Figure A.1. Calibration curve prepared by gallic acid in ethanol:acetic acid:water mixture (50:8:42 v/v) for determination of total phenolic contents

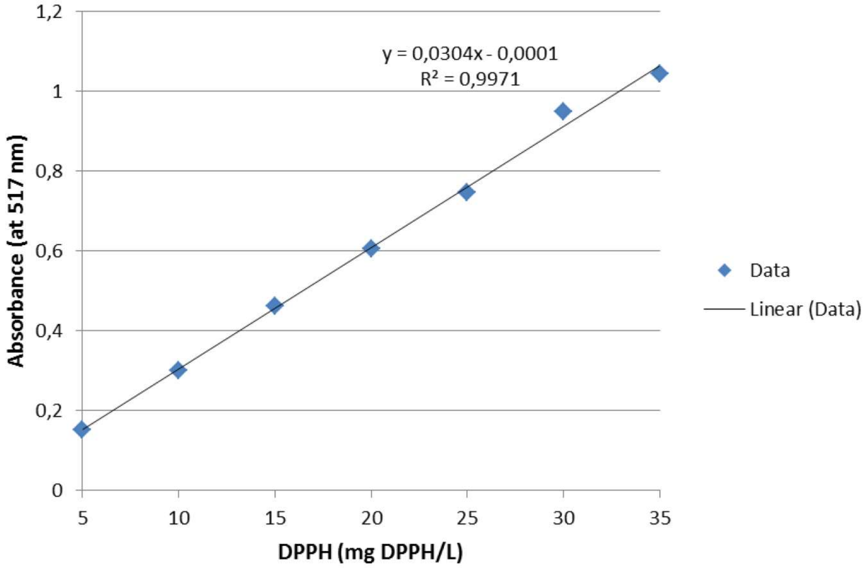

Figure A.2. Calibration curve prepared by DPPH˙ radical in methanol for determination of antioxidant activity

B. Water Vapor Transmission Rates

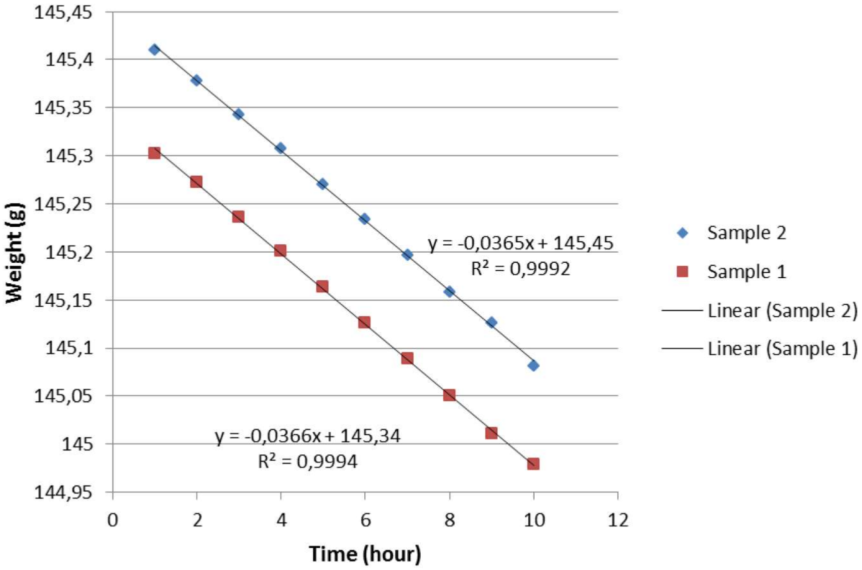

Figure B.1. Water Vapor Transmission Rate of P9-G1-GA5

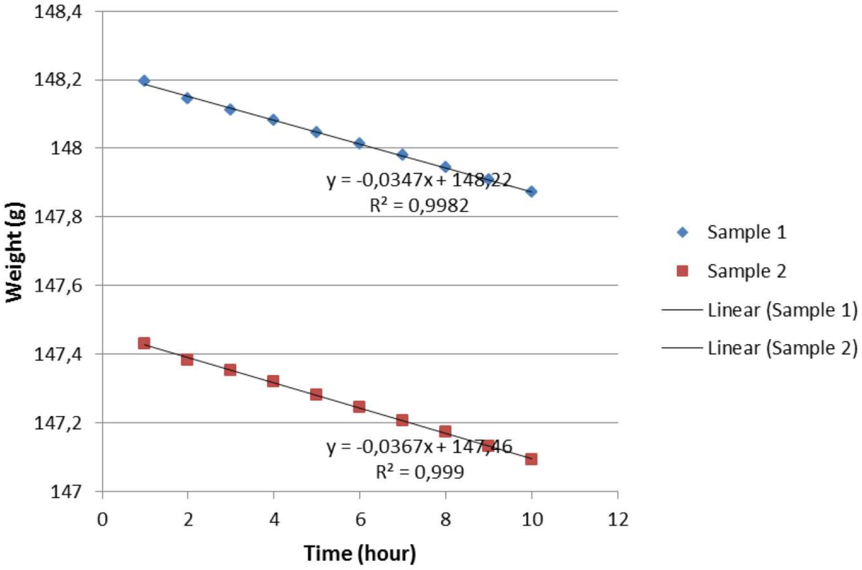

Figure B.2. Water Vapor Transmission Rate of P9-G1-GA10

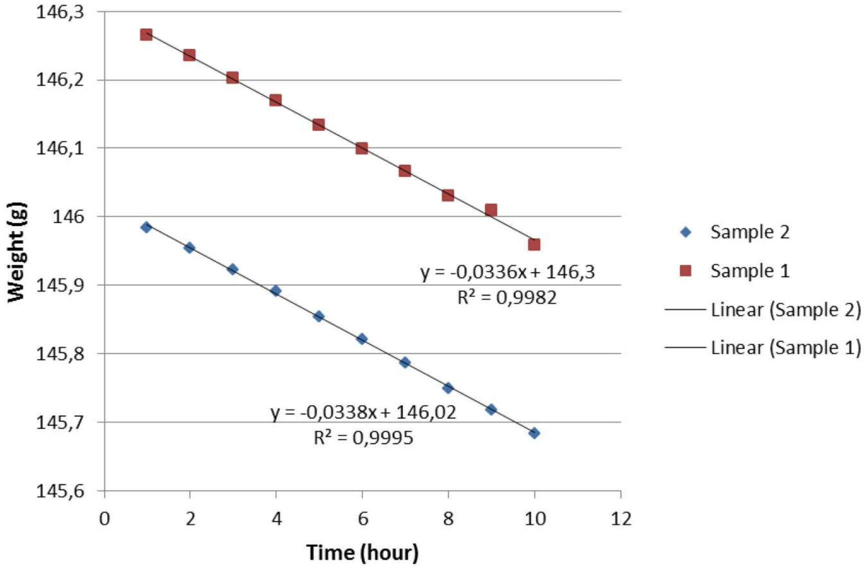

Figure B.3. Water Vapor Transmission Rate of P9-G3-GA5

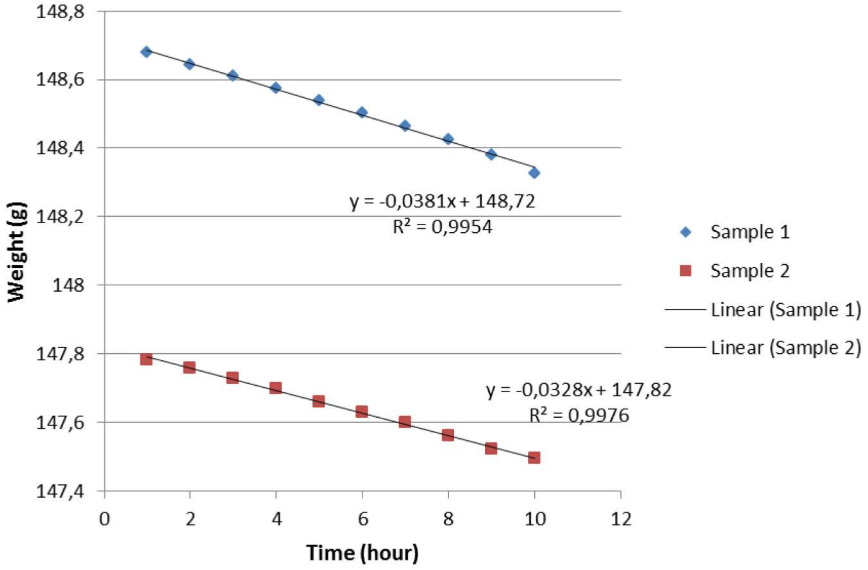

Figure B.4. Water Vapor Transmission Rate of P9-G3-GA10

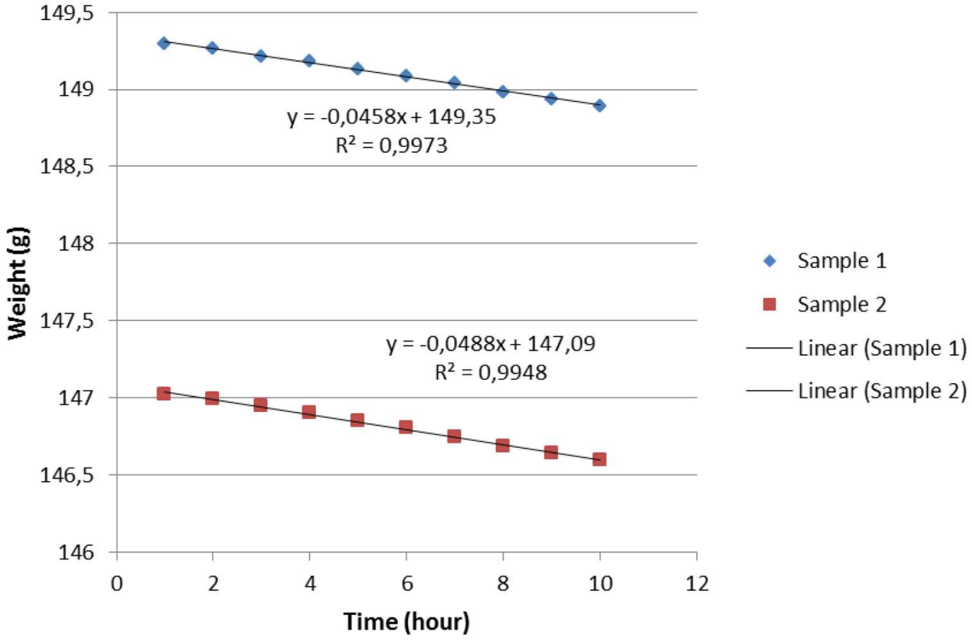

Figure B.5. Water Vapor Transmission Rate of P11-G1-GA5

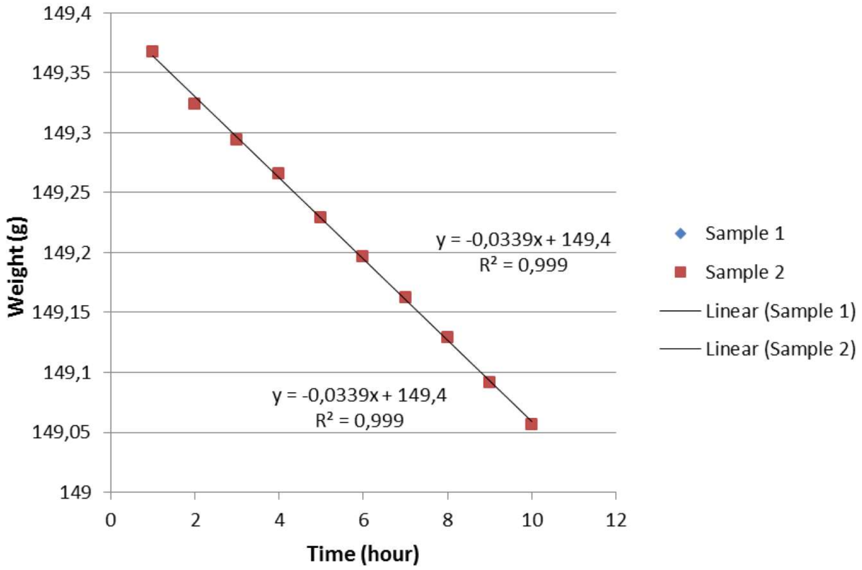

Figure B.6. Water Vapor Transmission Rate of P11-G1-GA10

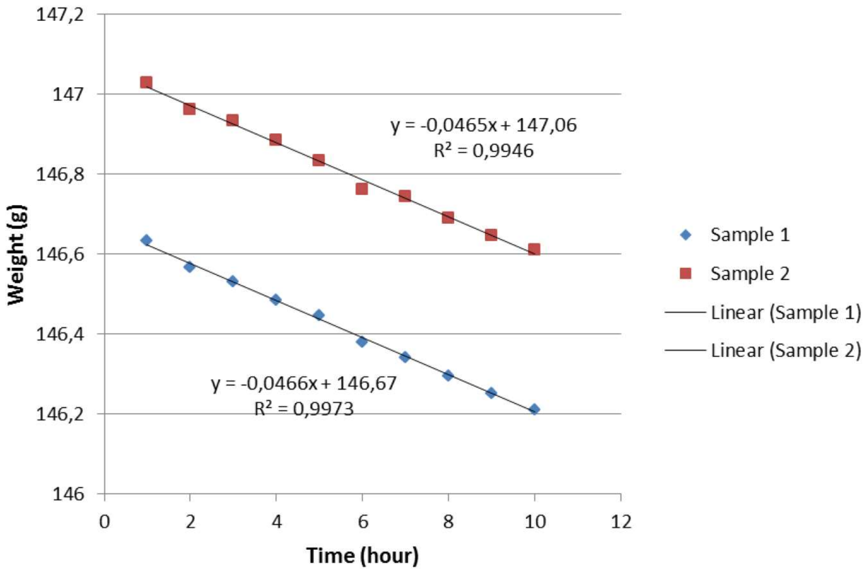

Figure B.7. Water Vapor Transmission Rate of P11-G3-GA5

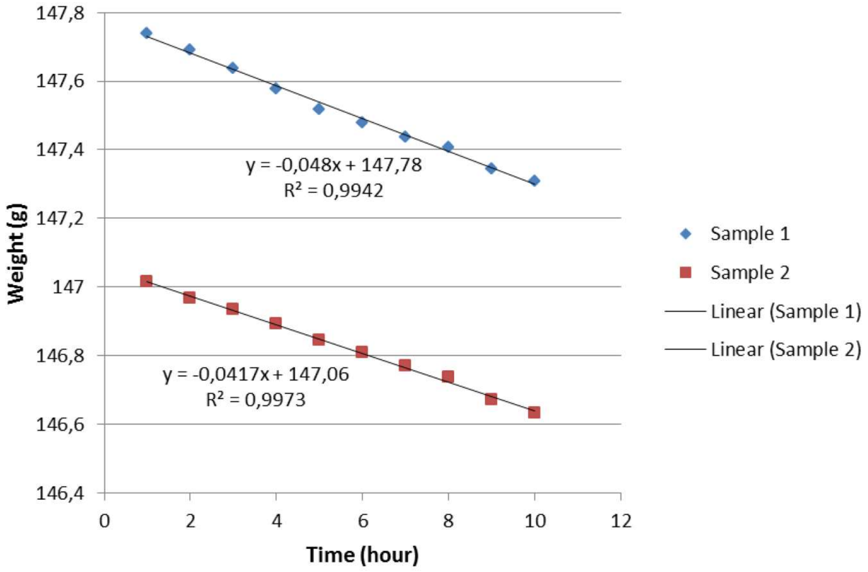

Figure B.8. Water Vapor Transmission Rate of P11-G3-GA10

C. Statistical Analysis

Table C.1. *Lightness of Films*

General Linear Model: L versus pH; Glycerol; Gallic Acid

Factor	Type	Levels	Values
pH	Fixed	2	9; 11
Glycerol	Fixed	2	1; 3
Gallic Acid	Fixed	2	5; 10

Analysis of Variance:

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	33,820	33,820	19,31	0,000
Glycerol	1	2,426	2,426	1,39	0,242
Gallic Acid	1	17,289	17,289	9,87	0,002
Error	92	161,105	1,751		
Lack-of-Fit	4	25,370	6,342	4,11	0,004
Pure Error	88	135,735	1,542		
Total	95	214,639			

Model Summary:

S	R-sq	R-sq(adj)	R-sq(pred)
1,32330	24,94%	22,49%	18,27%

Coefficients:

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	95,582	0,135	707,70	0,000	
pH 9	0,594	0,135	4,39	0,000	1,00
Glycerol 1	-0,159	0,135	-1,18	0,242	1,00
Gallic Acid 5	-0,424	0,135	-3,14	0,002	1,00

Regression Equation:

L=95,582+ 0,594 pH_9- 0,594 pH_11- 0,159 Glycerol_1+ 0,159 Glycerol_3- 0,424 Gallic Acid_5 + 0,424 Gallic Acid_10

Fits and Diagnostics for Unusual Observations:

Obs	L	Fit	Resid	Std Resid
26	89,540	95,910	-6,370	-4,92 R

Comparisons for L

Tukey Pairwise Comparisons: Response = L, Term = pH

Grouping Information Using the Tukey Method and 95% Confidence

pH	N	Mean	Grouping
9	48	96,1754	A
11	48	94,9883	B

Tukey Pairwise Comparisons: Response = L, Term = Glycerol

Glycerol	N	Mean	Grouping
3	48	95,7408	A
1	48	95,4229	A

Tukey Pairwise Comparisons: Response = L, Term = Gallic Acid

Gallic Acid	N	Mean	Grouping
10	48	96,0063	A
5	48	95,1575	B

Means that do not share a letter are significantly different.

Table C.2. a^* *value of Films*

General Linear Model: a versus pH; Glycerol; Gallic Acid

Factor	Type	Levels	Values
pH	Fixed	2	9; 11
Glycerol	Fixed	2	1; 3
Gallic Acid	Fixed	2	5; 10

Analysis of Variance:

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	0,56427	0,564267	60,27	0,000
Glycerol	1	0,00007	0,000067	0,01	0,933
Gallic Acid	1	0,00540	0,005400	0,58	0,450
Error	92	0,86132	0,009362		
Lack-of-Fit	4	0,33305	0,083262	13,87	0,000
Pure Error	88	0,52827	0,006003		
Total	95	1,43105			

Model Summary:

S	R-sq	R-sq(adj)	R-sq(pred)
0,0967581	39,81%	37,85%	34,46%

Coefficients:

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,02625	0,00988	2,66	0,009	
pH 9	-0,07667	0,00988	-7,76	0,000	1,00
Glycerol 1	0,00083	0,00988	0,08	0,933	1,00
Gallic Acid 5	-0,00750	0,00988	-0,76	0,450	1,00

Regression Equation:

a=0,02625- 0,07667 pH_9+ 0,07667 pH_11+ 0,00083 Glycerol_1- 0,00083 Glycerol _3- 0,00750 Gallic Acid_5 + 0,00750 Gallic Acid_10

Fits and Diagnostics for Unusual Observations:

Obs	a	Fit	Resid	Std Resid	
3	-0,2500	-0,0571	-0,1929	-2,04	R
7	-0,2900	-0,0571	-0,2329	-2,46	R
11	-0,2600	-0,0571	-0,2029	-2,14	R
50	0,3200	0,0962	0,2238	2,36	R

R: Large residual

Comparisons for a

Tukey Pairwise Comparisons: Response = a, Term = pH

Grouping Information Using the Tukey Method and 95% Confidence

pH	N	Mean	Grouping
11	48	0,102917	A
9	48	-0,050417	B

Tukey Pairwise Comparisons: Response = a, Term = Glycerol

Glycerol	N	Mean	Grouping
1	48	0,0270833	A
3	48	0,0254167	A

Tukey Pairwise Comparisons: Response = a, Term = Gallic Acid

Gallic Acid	N	Mean	Grouping
10	48	0,03375	A
5	48	0,01875	A

Means that do not share a letter are significantly different.

Table C.3. b^* *value of Films*

General Linear Model: b versus pH; Glycerol; Gallic Acid

Factor	Type	Levels	Values
pH	Fixed	2	9; 11
Glycerol	Fixed	2	1; 3
Gallic Acid	Fixed	2	5; 10

Analysis of Variance:

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	29,515	29,5149	20,09	0,000
Glycerol	1	0,436	0,4361	0,30	0,587
Gallic Acid	1	0,055	0,0546	0,04	0,848
Error	92	135,159	1,4691		
Lack-of-Fit	4	64,492	16,1231	20,08	0,000
Pure Error	88	70,666	0,8030		
Total	95	165,164			

```
Model Summary:
    S          R-sq      R-sq(adj)    R-sq(pred)
1,21207     18,17%     15,50%      10,90%
Coefficients:
Term               Coef    SE Coef  T-Value  P-Value   VIF
Constant           6,418      0,124      51,88       0,000
pH 9              -0,554      0,124      -4,48       0,000     1,00
Glycerol 1         0,067      0,124       0,54       0,587     1,00
Gallic Acid 5     0,024     0,124       0,19       0,848     1,00
Regression Equation:
b  =  6,418   - 0,554 pH_9   + 0,554 pH_11   + 0,067 Glycerol_1   - 0,067 Glycerol_3
+ 0,024 Gallic Acid_5 - 0,024 Gallic Acid_10
Fits and Diagnostics for Unusual Observations:
Obs      b           Fit      Std Resid     Resid
 50     10,310    7,064     3,246       2,74  R
 56     10,140    7,064     3,076       2,59  R
 58       9,460    7,064     2,396       2,02  R
R:  Large residual
Comparisons for b
Tukey Pairwise Comparisons: Response = b, Term = pH
Grouping Information Using the Tukey Method and 95% Confidence
pH          N    Mean     Grouping
11          48   6,97292    A
9           48   5,86396        B
Tukey Pairwise Comparisons: Response = b, Term = Glycerol
Glycerol       N       Mean        Grouping
1              48       6,48583     A
3              48       6,35104     A
Tukey Pairwise Comparisons: Response = b, Term = Gallic Acid
```

Gallic Acid	N	Mean	Grouping
5	48	6,44229	A
10	48	6,39458	A

Means that do not share a letter are significantly different.

Table C. 4. *ΔE^* value of Films*

General Linear Model: ΔE versus pH; Glycerol; Gallic Acid

Factor	Type	Levels	Values
pH	Fixed	2	9; 11
Glycerol	Fixed	2	1; 3
Gallic Acid	Fixed	2	5; 10

Analysis of Variance:

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	58,190	58,190	22,93	0,000
Glycerol	1	1,384	1,384	0,55	0,462
Gallic Acid	1	6,453	6,453	2,54	0,114
Error	92	233,438	2,537		
Lack-of-Fit	4	76,455	19,114	10,71	0,000
Pure Error	88	156,983	1,784		
Total	95	299,465			

Model Summary:

S	R-sq	R-sq(adj)	R-sq(pred)
1,59291	22,05%	19,51%	15,12%

Coefficients:

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7,847	0,163	48,26	0,000	
pH 9	-0,779	0,163	-4,79	0,000	1,00
Glycerol 1	0,120	0,163	0,74	0,462	1,00
Gallic Acid 5	0,259	0,163	1,59	0,114	1,00

Regression Equation:

ΔE=7,847- 0,779 pH_9+ 0,779 pH_11+ 0,120 Glycerol_1- 0,120 Glycerol_3+ 0,259 Gallic Acid_5 - 0,259 Gallic Acid_10

Fits and Diagnostics for Unusual Observations:

Obs	ΔE	Fit	Std Resid	Resid	
26	12,294	7,207	5,087	3,26	R
50	12,842	9,004	3,838	2,46	R
56	12,268	9,004	3,263	2,09	R

R: Large residual

Comparisons for ΔE

Tukey Pairwise Comparisons: Response = ΔE, Term = pH

Grouping Information Using the Tukey Method and 95% Confidence

pH	N	Mean	Grouping	
11	48	8,62512	A	
9	48	7,06801		B

Tukey Pairwise Comparisons: Response = ΔE, Term = Glycerol

Glycerol	N	Mean	Grouping
1	48	7,96664	A
3	48	7,72649	A

Tukey Pairwise Comparisons: Response = ΔE, Term = Gallic Acid

Gallic Acid	N	Mean	Grouping
5	48	8,10583	A
10	48	7,58730	A

Means that do not share a letter are significantly different.

Table C.5. *Opacity of Films*

General Linear Model: Opacity versus pH; Glycerol; Gallic Acid

Factor	Type	Levels	Values
pH	Fixed	2	9; 11
Glycerol	Fixed	2	1; 3
Gallic Acid	Fixed	2	5; 10

Analysis of Variance:

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	0,0210	0,0210	0,02	0,889
Glycerol	1	1,3115	1,3115	1,24	0,273
Gallic Acid	1	23,1105	23,1105	21,88	0,000
Error	36	38,0292	1,0564		
Lack-of-Fit	4	23,7209	5,9302	13,26	0,000
Pure Error	32	14,3083	0,4471		
Total	39	62,4721			

Model Summary:

S	R-sq	R-sq(adj)	R-sq(pred)
1,02780	39,13%	34,05%	24,85%

Coefficients:

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7,107	0,163	43,73	0,000	
pH 9	0,023	0,163	0,14	0,889	1,00
Glycerol 1	-0,181	0,163	-1,11	0,273	1,00
Gallic Acid 5	-0,760	0,163	-4,68	0,000	1,00

Regression Equation:

Opacity = 7,107 + 0,023 pH_9 - 0,023 pH_11 - 0,181 Glycerol_1+ 0,181 Glycerol_3 - 0,760 Gallic Acid_5 + 0,760 Gallic Acid_10

Fits and Diagnostics for Unusual Observations:

Obs	Opacity	Fit	Std Resid	Resid	
6	9,661	7,709	1,952	2,00	R
7	9,712	7,709	2,003	2,05	R
31	8,612	6,505	2,107	2,16	R

R Large residual

Comparisons for Opacity

Tukey Pairwise Comparisons: Response = Opacity, Term = pH

Grouping Information Using the Tukey Method and 95% Confidence

pH	N	Mean	Grouping
9	20	7,12982	A
11	20	7,08404	A

Tukey Pairwise Comparisons: Response = Opacity, Term = Glycerol

Glycerol	N	Mean	Grouping
3	20	7,28801	A
1	20	6,92586	A

Tukey Pairwise Comparisons: Response = Opacity, Term = Gallic Acid

Gallic Acid	N	Mean	Grouping
10	20	7,86704	A
5	20	6,34683	B

Means that do not share a letter are significantly different.

Table C.6. *Total Phenolic Content of Films*

General Linear Model: mg gallic acid/ g film versus pH; Glycerol; Gallic Acid

Factor	Type	Levels	Values
pH	Fixed	2	9; 11
Glycerol	Fixed	2	1; 3
Gallic Acid	Fixed	2	5; 10

Analysis of Variance:

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	0,2	0,21	0,00	0,957
Glycerol	1	254,0	253,98	3,66	0,066
Gallic Acid	1	7855,2	7855,17	113,10	0,000
Error	28	1944,7	69,45		
Lack-of-Fit	4	1350,0	337,51	13,62	0,000
Pure Error	24	594,6	24,78		
Total	31	10054,0			

Model Summary:

```
    S         R-sq     R-sq(adj)    R-sq(pred)
8,33383   80,66%     78,59%      74,74%
Coefficients:
Term                 Coef   SE Coef   T-Value   P-Value   VIF
Constant             50,99      1,47     34,61      0,000
pH 9                  0,08      1,47      0,05      0,957     1,00
Glycerol 1            2,82      1,47      1,91      0,066     1,00
Gallic Acid 5       -15,67      1,47    -10,63      0,000     1,00
Regression Equation:
mg gallic acid/ g film = 50,99 + 0,08 pH_9 - 0,08 pH_11 + 2,82 Glycerol_1
- 2,82 Glycerol_3- 15,67 Gallic Acid_5 + 15,67 Gallic Acid_10
Fits and Diagnostics for Unusual Observations:
Obs    mg gallic acid/g film   Fit      Resid    Std Resid
 23          49,26             69,39   -20,13     -2,58  R
R:  Large residual
Comparisons for mg gallic acid/ g film
Tukey Pairwise Comparisons: Response = mg gallic acid/ g film, Term = pH
Grouping Information Using the Tukey Method and 95% Confidence
pH      N      Mean       Grouping
9       16     51,0663      A
11      16     50,9053      A
Tukey Pairwise Comparisons: Response = mg gallic acid/ g film, Term = Glycerol
Glycerol       N     Mean        Grouping
1              16    53,8030      A
3              16    48,1686      A
Tukey Pairwise Comparisons: Response = mg gallic acid/ g film, Term = Gallic Acid
Gallic Acid       N       Mean        Grouping
10                16      66,6534      A
5                 16      35,3182         B
Means that do not share a letter are significantly different.
```

Table C.7. *Antioxidant Activity of Films*

General Linear Model: mg DPPH/g film versus pH; Glycerol; Gallic Acid

Factor	Type	Levels	Values
pH	Fixed	2	9; 11
Glycerol	Fixed	2	1; 3
Gallic Acid	Fixed	2	5; 10

Analysis of Variance:

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	22,96	22,96	4,37	0,046
Glycerol	1	293,87	293,87	55,97	0,000
Gallic Acid	1	1889,91	1889,91	359,98	0,000
Error	28	147,00	5,25		
Lack-of-Fit	4	106,46	26,62	15,76	0,000
Pure Error	24	40,54	1,69		
Total	31	2353,74			

Model Summary:

S	R-sq	R-sq(adj)	R-sq(pred)
2,29130	93,75%	93,09%	91,84%

Coefficients:

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	19,738	0,405	48,73	0,000	
pH 9	-0,847	0,405	-2,09	0,046	1,00
Glycerol 1	3,030	0,405	7,48	0,000	1,00
Gallic Acid 5	-7,685	0,405	-18,97	0,000	1,00

Regression Equation:

mg DPPH/g film = 19,738 - 0,847 pH_9 + 0,847 pH_11 + 3,030 Glycerol_1 - 3,030 Glycerol_3 - 7,685 Gallic Acid_5 + 7,685 Gallic Acid_10

Comparisons for mg DPPH/g film

Tukey Pairwise Comparisons: Response = mg DPPH/g film, Term = pH

Grouping Information Using the Tukey Method and 95% Confidence

pH	N	Mean	Grouping
11	16	20,5851	A
9	16	18,8910	B

Tukey Pairwise Comparisons: Response = mg DPPH/g film, Term = Glycerol

Glycerol	N	Mean	Grouping
1	16	22,7685	A
3	16	16,7076	B

Tukey Pairwise Comparisons: Response = mg DPPH/g film, Term = Gallic Acid

Gallic Acid	N	Mean	Grouping
10	16	27,4231	A
5	16	12,0530	B

Means that do not share a letter are significantly different.

Table C.8. *Tensile Strength of Films*

General Linear Model: Tensile Strength versus pH; Glycerol; Gallic Acid

Factor	Type	Levels	Values
pH	Fixed	2	9; 11
Glycerol	Fixed	2	1; 3
Gallic Acid	Fixed	2	5; 10

Analysis of Variance:

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	2,28766	2,28766	15,75	0,002
Glycerol	1	1,04960	1,04960	7,23	0,020
Gallic Acid	1	0,00087	0,00087	0,01	0,940
Error	12	1,74271	0,14523		
Lack-of-Fit	4	1,16105	0,29026	3,99	0,045
Pure Error	8	0,58167	0,07271		
Total	15	5,08084			

Model Summary:

S	R-sq	R-sq(adj)	R-sq(pred)
0,381085	65,70%	57,13%	39,02%

Coefficients:

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,8490	0,0953	8,91	0,000	
pH 9	-0,3781	0,0953	-3,97	0,002	1,00
Glycerol 1	0,2561	0,0953	2,69	0,020	1,00
Gallic Acid 5	-0,0074	0,0953	-0,08	0,940	1,00

Regression Equation:

Tensile Strength = 0,8490 - 0,3781 pH_9 + 0,3781 pH_11 + 0,2561 Glycerol_1 - 0,2561 Glycerol_3 - 0,0074 Gallic Acid_5 + 0,0074 Gallic Acid_10

Fits and Diagnostics for Unusual Observations:

Obs	Tensile Strength	Fit	Std Resid	Resid
4	2,200	1,491	0,709	2,15 R

R Large residual

Comparisons for Tensile Strength

Tukey Pairwise Comparisons: Response = Tensile Strength, Term = pH

Grouping Information Using the Tukey Method and 95% Confidence

pH	N	Mean	Grouping
11	8	1,22713	A
9	8	0,47087	B

Tukey Pairwise Comparisons: Response = Tensile Strength, Term = Glycerol

Glycerol	N	Mean	Grouping
1	8	1,10513	A
3	8	0,59287	B

Tukey Pairwise Comparisons: Response = Tensile Strength, Term = Gallic Acid

Gallic Acid	N	Mean	Grouping
10	8	0,856375	A
5	8	0,841625	A

Means that do not share a letter are significantly different.

Table C.9. *Young's Modulus of Films*

General Linear Model: Modulus versus pH; Glycerol; Gallic Acid

Factor	Type	Levels	Values
pH	Fixed	2	9; 11
Glycerol	Fixed	2	1; 3
Gallic Acid	Fixed	2	5; 10

Analysis of Variance:

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	1827,6	1827,56	12,80	0,004
Glycerol	1	1794,4	1794,37	12,57	0,004
Gallic Acid	1	177,3	177,29	1,24	0,287
Error	12	1712,8	142,73		
Lack-of-Fit	4	1243,7	310,91	5,30	0,022
Pure Error	8	469,1	58,64		
Total	15	5512,0			

Model Summary:

S	R-sq	R-sq(adj)	R-sq(pred)
11,9469	68,93%	61,16%	44,76%

Coefficients:

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	19,84	2,99	6,64	0,000	
pH 9	-10,69	2,99	-3,58	0,004	1,00
Glycerol 1	10,59	2,99	3,55	0,004	1,00
Gallic Acid 5	3,33	2,99	1,11	0,287	1,00

Regression Equation:

Modulus=19,84- 10,69 pH_9+ 10,69 pH_11+ 10,59 Glycerol_1- 10,59 Glycerol_3+ 3,33 Gallic Acid_5 - 3,33 Gallic Acid_10

Comparisons for Modulus

Tukey Pairwise Comparisons: Response = Modulus, Term = pH

Grouping Information Using the Tukey Method and 95% Confidence

pH	N	Mean	Grouping
11	8	30,5275	A
9	8	9,1525	B

Tukey Pairwise Comparisons: Response = Modulus, Term = Glycerol

Glycerol	N	Mean	Grouping
1	8	30,43	A
3	8	9,25	B

Tukey Pairwise Comparisons: Response = Modulus, Term = Gallic Acid

Gallic Acid	N	Mean	Grouping
5	8	23,1688	A
10	8	16,5112	A

Means that do not share a letter are significantly different.

Table C.10. *Elongation of Films*

General Linear Model: Elongation versus pH; Glycerol; Gallic Acid

Factor	Type	Levels	Values
pH	Fixed	2	9; 11
Glycerol	Fixed	2	1; 3
Gallic Acid	Fixed	2	5; 10

Analysis of Variance:

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	9,000	9,000	1,48	0,247
Glycerol	1	151,290	151,290	24,87	0,000
Gallic Acid	1	91,202	91,202	14,99	0,002
Error	12	73,007	6,084		
Lack-of-Fit	4	64,077	16,019	14,35	0,001
Pure Error	8	8,930	1,116		
Total	15	324,500			

Model Summary:

S	R-sq	R-sq(adj)	R-sq(pred)
2,46657	77,50%	71,88%	60,00%

Coefficients:

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	11,800	0,617	19,14	0,000	
pH 9	-0,750	0,617	-1,22	0,247	1,00
Glycerol 1	-3,075	0,617	-4,99	0,000	1,00
Gallic Acid 5	-2,388	0,617	-3,87	0,002	1,00

Regression Equation:

Elongation=11,800- 0,750 pH_9+ 0,750 pH_11- 3,075 Glycerol_1+ 3,075 Glycerol_3- 2,388 Gallic Acid_5 + 2,388 Gallic Acid_10

Comparisons for Elongation

Tukey Pairwise Comparisons: Response = Elongation, Term = pH

Grouping Information Using the Tukey Method and 95% Confidence

pH	N	Mean	Grouping
11	8	12,55	A
9	8	11,05	A

Tukey Pairwise Comparisons: Response = Elongation, Term = Glycerol

Glycerol	N	Mean	Grouping
3	8	14,875	A
1	8	8,725	B

Tukey Pairwise Comparisons: Response = Elongation, Term = Gallic Acid

Gallic Acid	N	Mean	Grouping
10	8	14,1875	A
5	8	9,4125	B

Means that do not share a letter are significantly different.

Table C.11. *WVP of Films*

General Linear Model: WVP versus pH; Glycerol; Gallic Acid

Factor	Type	Levels	Values
pH	Fixed	2	9; 11
Glycerol	Fixed	2	1; 3
Gallic Acid	Fixed	2	5; 10

Analysis of Variance:

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	0,000673	0,000673	1,89	0,194
Glycerol	1	0,008118	0,008118	22,77	0,000
Gallic Acid	1	0,008400	0,008400	23,56	0,000
Error	12	0,004278	0,000357		
Lack-of-Fit	4	0,002919	0,000730	4,29	0,038
Pure Error	8	0,001360	0,000170		
Total	15	0,021469			

Model Summary:

S	R-sq	R-sq(adj)	R-sq(pred)
0,0188816	80,07%	75,09%	64,57%

Coefficients:

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,26371	0,00472	55,87	0,000	
pH 9	0,00649	0,00472	1,37	0,194	1,00
Glycerol 1	-0,02252	0,00472	-4,77	0,000	1,00
Gallic Acid 5	0,02291	0,00472	4,85	0,000	1,00

```
Regression Equation:

WVP   =   0,26371   + 0,00649 pH_9   - 0,00649 pH_11   - 0,02252 Glycerol_1
+ 0,02252 Glycerol_3+ 0,02291 Gallic Acid_5 - 0,02291 Gallic Acid_10

Fits and Diagnostics for Unusual Observations:

Obs     WVP     Fit         Resid     Std Resid

 7    0,18990   0,22476   -0,03486    -2,13  R

R:  Large residual

Comparisons for WVP

Tukey Pairwise Comparisons: Response = WVP, Term = pH

Grouping Information Using the Tukey Method and 95% Confidence

pH   N     Mean    Grouping

9    8   0,270200    A

11   8   0,257225    A

Tukey Pairwise Comparisons: Response = WVP, Term = Glycerol

Glycerol   N     Mean     Grouping

3          8   0,286238   A

1          8   0,241188      B

Tukey Pairwise Comparisons: Response = WVP, Term = Gallic Acid

Gallic Acid   N      Mean    Grouping

5             8    0,286625   A

10            8    0,240800      B

Means that do not share a letter are significantly different.
```