MANUFACTURING AND ANALYSIS OF THERMAL AND
CHEMICAL PROPERTIES OF POTATO STARCH-GELATIN BIOFILMS

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MANUFACTURING AND ANALYSIS OF THERMAL AND CHEMICAL PROPERTIES OF POTATO STARCH-GELATIN BIOFILMS

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ABSTRACT

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CHEMICAL PROPERTIES OF POTATO STARCH-GELATIN BIOFILMS

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Today bioplastics, which are defined as environmentally friendly new
generation plastics, take place on the market as bags, foam fillers and food
tableware.

In this study, it was aimed to obtain biodegradable films using starch and
gelatin. Thermal, mechanical, permeability and optical properties of films
based on potato starch and bovine gelatin plasticized with glycerol and citric
acid monohydrate were investigated. Film formulations of different ratios of
potato starch to gelatin (1:0, 1:1, 2:1, 1:2, and 0:1) were prepared and casting
technique was used as film processing method. In the DSC analysis, one glass
transition temperature varying from 69.6 to 91.8ºC for different film
formulations was observed, which showed that potato starch and bovine gelatin
are compatible. It was determined that blending ratio had an important effect
on the mechanical properties. Tensile strength, elongation at break and Young's

modulus was found to be between 2.21-15.60 MPa, 93-130% and 14.20-151.50 MPa, respectively. The findings of this study showed that gelatin addition significantly increased the mechanical durability. The water vapor permeability of films ranged from 0.022×10^{-10} to 0.061×10^{-10} $g.m^{-1}.s^{-1}.Pa^{-1}$ and significantly depended on the blending ratio. It was determined that the films contained gelatin had lower water vapor permeability. It was observed that the blending level has an important effect on the opacity values of films with an opacity-decreasing effect of gelatin. It was also found that storage conditions affected the optical stability. Storage at 40ºC increased the opacity whereas storage at room temperature, +4ºC, and -20ºC had an opposite effect.

Keywords: Bioplastics, Biodegradable Films, Potato Starch, Gelatin

ÖZ

PATATES NİŞASTASI-JELATİN BİYOFİLMLERİNİN ÜRETİMİ, TERMAL VE KİMYASAL ÖZELLİKLERİNİN ANALİZİ

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Aralık 2012, 82 sayfa

Doğa dostu yeni nesil plastikler olarak tanımlanan biyoplastikler bugün; poşetler, köpük dolgu maddeleri ve gıda servis gereçleri şeklinde piyasada yer edinmeye başlamıştır.

Bu çalışmada, nişasta ve jelatin kullanılarak biyobozunur filmlerin elde edilmesi amaçlanmıştır. Patates nişastası ve sığır jelatini bazlı, gliserol ve sitrik asit monohidrat ile plastikleştirilmiş biyobozunur filmlerin termal, mekanik, geçirgenlik ve optik özellikleri incelenmiştir. Farklı patates nişastası-sığır jelatini (1:0, 1:1, 2:1, 1:2 ve 0:1) oranına sahip film formülasyonları hazırlanmış ve film işleme yöntemi olarak kalıba dökme tekniği kullanılmıştır. Diferansiyel taramalı kalorimetri analizlerinde, farklı film formülasyonları için 69.6ºC ila 91.8ºC arasında değişen bir adet camsı geçiş sıcaklığı izlenmiştir. Bu durum, patates nişastası ile sığır jelatininin uyumlu olduğunu göstermiştir. Harmanlama oranının, filmlerin mekanik özellikleri üzerinde önemli bir etkiye

sahip olduğu belirlenmiştir. Gerilme direnci, kopmadaki uzama ve Young modülü değerleri; sırasıyla 2.21-15.60 MPa, %93-130 ve 14.20-151.50 MPa olarak ölçülmüştür. Bu çalışmanın bulguları; jelatin ilavesinin, mekanik dayanıklılığı önemli ölçüde arttırdığını göstermiştir. Filmlerin su buharı geçirgenliklerinin 0.022×10^{-10} g.m^{-1}.s^{-1}.Pa^{-1} ile 0.061×10^{-10}g.m^{-1}.s^{-1}.Pa^{-1} arasında değiştiği ve önemli ölçüde harmanlama oranına bağlı olduğu bulunmuştur. Jelatin içeren filmlerin su buharı geçirgenliğinin daha düşük olduğu saptanmıştır. Jelatinin optik geçirgenliği düşürücü etkisi ile birlikte, harmanlama oranının optik geçirgenlik değerleri üzerinde önemli bir etkisi olduğu gözlenmiştir. Ayrıca depolama koşullarının da optik stabiliteyi etkilediği belirlenmiştir. 40ºC' de depolama, filmlerin opaklığını arttırırken; oda sıcaklığında, +4ºC' de ve -20ºC' de depolama ters yönde bir etki göstermiştir.

Anahtar Kelimeler: Biyoplastikler, Biyobozunur Film, Patates Nişastası, Jelatin

To my family

ACKNOWLEDGEMENTS

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NOMENCLATURE

A	Area, m^2
A*nm	Absorbance x wavelength
C	Concentration, $g\ m^{-3}$
D	Diffusion coefficient, $m^2\ s^{-1}$
e	Elongation, m
E	Young's modulus, MPa
F	Force, MPa
J	Mass flux, $g\ m^{-2}\ s^{-1}$
l	Final length of material, m
l_0	Initial length of material, m
p	Pressure, Pa
Q	Amount of permeant, g
RH	Relative humidity, %
S	Solubility of gas in solid, $g\ m^{-3}\ Pa^{-1}$
t	Time, s
Tg	Glass transition temperature, ºC
Tm	Melting temperature, ºC
x	Film thickness, m
w	Weight, g

Greek Letters

ε	Strain
σ	Stress

Subscripts

C	Carbon

Abbreviations

DMA	Dynamic mechanical analysis
DSC	Differential scanning calorimetry
EAB	Elongation at break
GLU	Gluteraldehyde
GLY	Glycerol
HDPE	High density polyethylene
3HB	3-hydroxybutyrate
3HV	3-hydroxyvalerate
5HV	5-hydroxyvalerate
IUPAC	International Union of Pure and Applied Chemistry
LDPE	Low density polyethylene
NMR	Nuclear magnetic resonance
Op	Opacity
PC	Polycarbonate
PCL	Polycaprolactone
PEG	Polyethylene glycol
PET	Poly(ethylene terephthalate)
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHBV	Poly-3-hydroxybutyrate-*co*-hydroxyvalerate
PHV	Polyhydroxyvalerate
PLA	Polylactic acid
PP	Polypropylene
PS	Potato starch
PVC	Poly(vinyl chloride)
WVP	Water vapor permeability
WVTR	Water vapor transmission rate

CHAPTER 1

INTRODUCTION

The current global consumption of conventional plastics which are manufactured through the treatment of petroleum has gone beyond 300 million tones (Siracusa et al., 2008). Since the conventional plastics are cost-effective and have good mechanical performance they have been used increasing day by day. However, in the course of time it was recognized that these synthetic polymers have adverse effects on the environment because of their accumulation in the nature. Moreover, their dependence on limited natural resources was also come into question as a problem. In recent years, sustainable development issue has gained importance and the awareness of international society about the protection of nature has increased. Thus, restriction of usage of petroleum-based plastics has been started to discuss in many countries. Especially in the last two decades, it has been concentrated on that the production of environmentally friendly biodegradable polymers as alternatives of petroleum-based plastics and as solution of environmental problems, and the studies about this issue has accelerated.

Bioplastics have been defined as polymers obtained from renewable resources, which reduce the environmental impact, perform as conventional plastics in use and completely degrade in the environment by the action of living organisms (Bastioli, 2001). Polysaccharides and proteins have been the most attractive materials for bioplastic production (Arvanitoyannis et al., 1998).

Potato starch is obtained from potato tubers and used industrially as a raw and an intermediate material throughout the world. The cheapness and abundance of starch and also its biodegradability property allow it to be commonly used in different industrial fields such as food, chemistry, pharmaceutical and

medicine. Furthermore, the wide knowledge about gelatinization and polymerization processes and processing technologies of is another factor that increases the industrial use of starch. However, in film and bioplastic applications of starch some problems have been faced such as poor mechanical and permeability properties. Because of these kinds of inadequacies native starch is used in bioplastic production blending with some natural based materials.

Gelatin is a soluble protein obtained by acid or alkaline treatment from collagen which is also a protein provides strength to tissues and organs of many vertebrates and invertebrates. Food coating and film applications of gelatin which has been commonly used as gelling agent have drawn attention mostly in recent years. Besides food industry, gelatin has found the possibility of usage in pharmaceutical, medical, photography, cosmetics, detergents, and paper processing industries. In the literature, gelatin isolated from various organisms such as pig, bovine and fish was used in bioplastic applications in combination with polysaccharides, lipids, proteins in order to strengthen the structure and obtain products that have superior characteristics (Arvanitoyannis et al., 1998a; Arvanitoyannis et al., 1998b; Lee et al., 2004; Bertan et al., 2005; Cao et al., 2007b; Denavi et al., 2009; Pérez-Mateos et al., 2009; Wang et al., 2009; Limpisophon et al., 2010).

Aid materials were needed in order to produce more flexible and processable films based on polysaccharides and proteins and at this point, plasticizers were utilized. Glycerol had been the most common plasticizer among various plasticizing agents including chloroform, low and high molecular weight glycols and sorbitol. On the other hand, use of citric acid as plasticizer has been an interesting improvement in recent years.

The objective of this study was to manufacture potato starch and gelatin-based biodegradable films and to determine the characteristics of these films which

can be alternatives for synthetic plastics. At the end of the study production and characterization of cost-effective bioplastics which are obtained from agricultural renewable resources, have determined thermal and physical properties, and help to protect the environment owing to their biodegradable property were carried out. In the current study, it was demonstrated that potato starch has alternative usage areas than food. From this point of view it is considered that this research can establish a basis to increase potato production in our country.

CHAPTER 2

LITERATURE SURVEY

2.1. Biodegradable Plastics

The terms compostable, biodegradable and bio-based plastics are generally used as they have the same meaning. However, they are distinct concepts and must be described separately.

The term biodegradable indicates the ability of polymers to be degraded in nature as a result of metabolic actions of live organisms. These metabolic actions convert the polymer into carbon dioxide, methane, water and biomass. It is important to note that biodegradable plastics are not always obtained from renewable resources; petroleum-based plastics can also be biodegradable. The term compostable, on the other hand, used for polymers that can be broken down into carbon dioxide, water and biomass as a result of biological processes occurred during composting in a specific compost site under specific composting conditions such as humidity, temperature, alkaline level. Compostable products can be used in agricultural lands as fertilizers and thus they participate in biological recycling in nature. They do not show toxic effect on nature including water, soil, plants and living organisms. Bio-based plastics refer to plastics obtained from renewable resources which can be polysaccharides, proteins, tree fibers. Bio-based plastics are not necessarily biodegradable (Packaging Recovery Organisation Europe, 2009). Many kinds of materials exist in the market launched in the name of bio-based or biodegradable. These kinds of materials create confusion among consumers. Especially oxo-degradable materials cause misunderstanding because they used in place of biodegradable materials. Oxo-degradable materials are derived from traditional plastics. These materials are made degradable with additives which

initiate degradation in the presence of ultraviolet light and oxygen. Oxo-degradable materials are not degradable in the soil and have adverse effects on nature particularly on marine life.

Another term frequently used in film applications is edible. The term edible means materials which can be consumed as food in human nutrition in the forms of coatings of foods or films alone. Edible films and coatings are used with the purposes of prevention of moisture loss, creation of a bright appearance on foods and enhancement of food stability.

Bioplastics can be produced synthetically as a result of the fermentation by certain microorganisms or from mixing of some renewable sources. Bioplastics that are synthesized by under different conditions or a part of microorganisms are also known as polyesters and are synthesized by microorganisms intra- or extracellularly. Some examples of bioplastics produced by microorganisms are polyhydroxyalkanoate (PHA), polyhydroxybutyrate (PHB), polyhydroxyvalerate (PHV), polylactic acid (PLA) and polycaprolactone (PCL). Chemical structure and usage areas of these biodegradable polymers are given in Table 2.1.

Depending on the raw material, bio-based polymers are divided into three groups: polyesters, starch-based polymers and others. (Siracusa et al., 2008) Polyesters include:

- polymers directly extracted from biomass such as proteins, lipids, or polysaccharides,

- aliphatic-aromatic copolymers, aliphatic polyesters, or polylactide aliphatic copolymers that are obtained by classical polymerization from renewable resources like polylactic acid (PLA) and polycaprolactones

- polymers that are produced by microorganisms such as polyhydroxyalkanoates (PHA) and their polyesters.
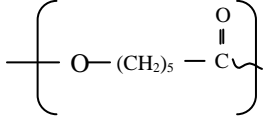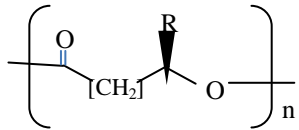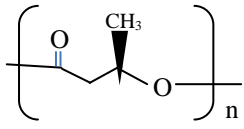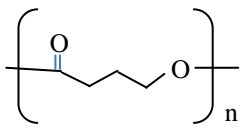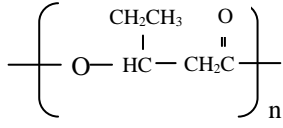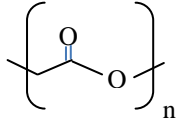
Among these progresses microbial polyesters draw a special attention because they are natural products that are synthesized and catabolized by different organisms and that have found broad biotechnological applications. They can be assimilated by many species (biodegradable) and do not cause toxic effects in the host organism (biocompatible) (Steinbüchel and Füchtenbusch, 1998; Angelova et al. 1999; Zinn et al. 2001; Williams and Martin, 2002; Luengo et al., 2003).

3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) were produced by Doi et al. (1987). The authors stated that these polyesters have attracted industrial attention as a possible candidate for large-scale biotechnological production, because they are environmentally degradable thermoplastics. They produced polyester from propionic acid in *Alcaligenes eutrophus.* In this study they defined the biosynthesis of 3HB, 3HV and 5-hydroxyvalerate (5HV) in *A. eutrophus* from different organic acids (Doi et al., 1987).

Holmes explained the physical properties of PHB and copolymers, their commercial importance, and their applications in detail (1988). The first patents for PHB were registered in the United States by J. N. Baptist in 1962 and the first industrial production of PHB and PHA was put into practice in 1982. Although PHB is the most abundant polyester polyhydroxyalkanoates (PHAs) were investigated as bioplastics by many researchers in various microorganisms.

Yu et al. (1998) produced PHAs and PHB by *Alcaligenes eutrophus* using various carbohydrates in the growth media, including sucrose, lactic acid, butyric acid, valeric acid as the carbon sources. In this study the authors investigated the usage of malt refuse as the C sources for the production of bioplastics. They reported that different polyhydroxyalkanoate copolymers with different properties could be produced by using different types of food wastes as C source.

Table 2.1. Chemical structure and usage areas of bioplastics produced by microorganisms

Polymer	Chemical Structure	Usage area	References
PCL		Drug delivery, packaging	Kumari et al., 2010
PHA		Production of cardiovascular products, tissue engineering, drug delivery	Zinn et al., 2001; Williams and Martin, 2002; Luengo et al., 2003
P3HB		Packaging, agriculture, tissue engineering, microencapsulation, drug delivery	Wiliams and Martin, 2002; Luengo et al., 2003
P4HB		Intravenous anesthetic agent, drug delivery	Sudesh, 2000; Williams and Martin, 2002
PHV		Pharmaceutical applications	Steinbüchel and Füchtenbusch, 1998
PLA		Drug delivery	Kumari et al., 2010

Besides bacteria yeasts also used for bioplastic production. Breuer et al. produced PHB in genetically engineered *Saccharomyces cerevisiae* transferring the PHB genes in *Ralstonia eutropha* and *Methylobacterium extorquens* to *S. cerevisiae*. They stated that the yeast could be used as a "cell factory" for the production of bioplastics (Breuer et al., 2002).

The main limitation for production of microbial bioplastics in large amounts is high production costs. For this reason alternatives have been sought which would be cost-effective and easy for processing by equipment used for conventional polymers. Herein some renewable materials such as polysaccharides, proteins, cellulosic and lignocellulosic materials have come into question in the last decade. Starch from various sources, egg white proteins or whey proteins and other renewable materials draw attention as biodegradable resources.

Alongside individual use of these renewable materials, they also used in combination with microbial bioplastics to reduce the costs. Godbole et al. was mixed PHB with starch to improve the properties of biodegradable polymers and for cost reduction (Godbole et al., 2003). In another study poly-3-hydroxybutyrate-*co*-hydroxyvalerate (PHBV) was used in combination with natural bamboo fiber and renewable resource based green biocomposites were prepared (Singh et al., 2008). Singh et al. (2010) also used PHBV with wood fiber and talc. PHBV was also blended with tapioca flour to increase the value of tapioca flour (Kaewkannetra et al., 2010). In a more recent study, as an interesting example, poultry feather fiber was used together with polylactic acid in order to obtain bioplastics (Ahn et al., 2011).

The study of Bradbury and Martin (1952) has been the leading research that enables the investigations of polysaccharides and proteins as biopolymers in production of packaging, coating materials and films. In the mentioned study the availability of gelatin for packaging and coating material was explained.

After this study many research has been conducted which examine the potential of polysaccharides and proteins in this area. Different biodegradable polymers produced by different processing methods are given in Table 2.2.

Among these resources starch seems to be more appropriate for biodegradable polymer production. Since starch is able to be fully degraded in nature, it has a potential for production of biodegradable products. Besides these properties its abundance and relatively low cost makes it a promising material for biodegradable film production.

2.2. Properties of Potato Starch and Starch-Based Films

Starch is a natural product that can be obtained from a number of crops such as wheat, corn, rice, potato and tapioca. It is totally biodegradable in different environments and enables the development of degradable products (Bastioli, 2001). Also, it has been used in industry for a long time. In 1939 it was reported that the industry knew how to produce a large number of commercial products from starch (Newkirk, 1939).

Potato starch consists of two major components, amylose and amylopectin, like other starches. These polysaccharides is formed by linking of α-D-glucose units by α-1, 4 linkages. Amylose is principally linear whereas amylopectin is highly branched. The structure of amylose and amylopectin is shown in Figure 2.1 (Parker and Ring, 2001). It is known that the linear structure of amylose molecule give the starch its film formation characteristics (Liu et al., 2009).

Potato starch is mostly composed of amylopectin which is in general 70-80% by weight independent of the size of the granules. Amylose is the minor component of potato starch (Hoover, 2001). Less than 0.5% of granules are proteins and there are almost no lipids in potato starch. It also contains phosphorus in the form of phosphate and potassium linked to phosphate groups (Bertoft and Blennow, 2009). The dominance of amylopectin is not unique for potato starch; similar amounts are found in corn, wheat and rice.

Table 2.2. Examples of biodegradable material production from various renewable resources

Resource	Processing method	Reference
Pectin and maize starch	Casting	Fishman et al., 1996
Chitosan and gelatin	Casting	Arvanitoyannis et al., 1998
Soy protein and starch	Extrusion and injection molding	Huang at el., 1999
Native corn starch or hydroxypropylated starch and coniferous tree fiber	Casting	Kim et al., 2003
Wheat or maize starch and montmorillonite organoclay	Extrusion	McGlashan and Halley, 2003
Potato starch and clay nanocomposites	Melt intercalation	Park et al., 2003
Chitosan and corn starch	Casting/solvent evoporation	Cervera et al., 2004
Chitosan and maize starch	Molding	Cervera et al., 2004
High-amylose rice starch or pea starch	Casting	Mehyar and Han, 2004

Table 2.2. Examples of biodegradable material production from various renewable resources *(continued)*

Resource	Processing method	Reference
Potato starch	Casting	Jansson and Järnström, 2005
Soy flour, polyester amide and pineapple leaf fiber	Extrusion and injection molding	Liu et al., 2005
Wheat gluten	Casting	Jerez et al., 2005
Yam starch	Casting	Mali et al., 2005
Potato starch	Casting	Cyras et al., 2006
Corn starch	Extrusion	Shujun et al., 2006
Wheat gluten and egg white proteins	Combined compression molding/thermosetting	Jerez et al., 2007
Potato starch	Casting	Talja, 2007a
Corn starch and nano silicon dioxide (nano-SiO_2)	Coating	Xiong et al., 2008
Wheat gluten (glutenin-rich fraction)	Molding	Song and Zheng, 2008
Wheat gluten	Compression-molding	Gómez-Martinez et al., 2009

Table 2.2. Examples of biodegradable material production from various renewable resources *(continued)*

Resource	Processing method	Reference
Egg white protein (albumen) and corn/potato starch	Mixing-Molding	Gonzalez-Gutierrez et al., 2010
Dialdehyde starch, sodium montmorillonitrile, and bovine gelatin	Heat compression	Martucci and Ruseckaite, 2010
Sago starch and fish gelatin	Casting	Al-Hassan and M. H. Norziah, 2012

Starch constitutes 15-20% of potatoes and hence it is considered as the major factor affecting then functionality of potato in terms of its applications. The granular and molecular structure of potato starch makes it a good source of biodegradable polymer technology (Bertoft and Blennow, 2009). Potato starch is more advantageous than other starches in terms of some aspects such as:

- The granules of potato starch are very large (nearly 10-100μm in diameter) and smooth.

- It contains high amount of covalently linked phosphate, long amylopectin chains and high molecular-weight amylose.

- The well-ordered and dense structure of potato starch makes it very resistant to enzymatic degradation by hydrolytic enzymes (Sun et al., 2006).

For these advantages, in the literature potato starch was investigated as a source of biodegradable/edible films. The effects of different plasticizers and various compounds have examined in order to improve the structure of potato starch films (Cyras et al., 2006; Talja, 2007a; Talja et al., 2007b; Wu et al., 2009; Zhou et al., 2009; Gonzalez-Gutierrez et al., 2010; Gupta et al., 2010).
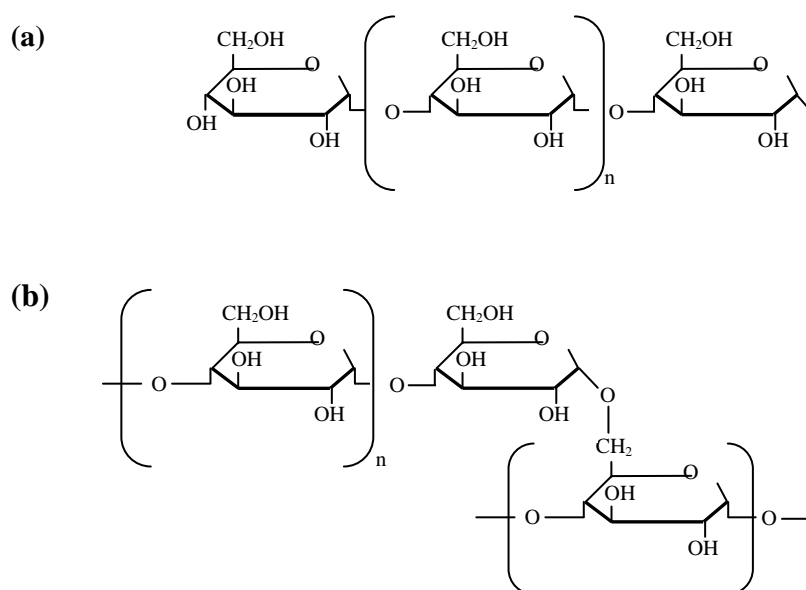
Figure 2.1. Structure of amylose (a) and amylopectin (b).

Starch-based film production is based on complete disruption of the granular structure of starch and formation of thermoplastic starch. Native starch can be transformed into thermoplastic starch through destruction of its crystalline structure by chemical, thermal and mechanical methods. The materials that are obtained by complexation of thermoplastic starch with other polymers exhibit good plastic properties. These properties explain the leading position of starch-based polymers in the biodegradable polymers market (Bastioli, 2005).

Besides potato-starch based films, in the literature there are a great number of studies about biodegradable films derived from other starches. In these studies mechanical, physicochemical, thermal and permeability characteristics of films were investigated (Parra et al., 2004; Mali et al., 2005; Romero-Bastida et al., 2005; Bertuzzi et al., 2006; Chang et al., 2006; Fama et al., 2006; Flores et al., 2007; Rodriguez et al., 2006; Zhang and Han, 2006; Talja et al., 2007a; Galdeano et al., 2009). However there are some limitations for developing starch-based polymers due to poor mechanical properties and moisture sensitivity (Wu et al., 2009). To overcome this problem three different procedures are recommended. The first recommendation is usage of starch with high amylose content (Ryu et al., 2002), the second one is chemical modification such as crosslinking (Parra et al., 2004). The last and most common method is blending with different materials. This method is the easiest and the most cost-effective procedure. In different studies cellulose, hemicellulose and zein (Gáspár et al., 2005), short pulp fiber (Kim et al., 2003), modified montmorillonite organoclay (McGlashan and Halley, 2003), agar (Wu et al., 2009), nano silicon dioxide (Xiong et al., 2008), nanoclay (Almasi et al., 2010), chitosan (Xu et al., 2005; Bourtoom and Chinnan, 2008; Mathew and Abraham, 2008) and pullulan (Kristo and Biliaderis, 2007) were added to starch in certain amounts. In these studies the properties of starch-based films were improved in a certain extent.

2.3. Properties of Gelatin

Gelatin is a protein that is obtained by controlled hydrolysis of collagen. Collagen is widely found in nature and it forms the major parts of skin, bones and connective tissue. Collagen is composed of unique sequence of amino acids which are mainly glycine, proline and hydroxyproline (Yıldız, 2010; Park et al., 2008). The proline and hydroxyproline content has particular importance for the gelling effect of gelatin (Gómez-Guillén et al., 2011). The chemical structure of gelatin is shown in Figure 2.2.

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In the presence of water above 37ºC, gelatin dissolve as colloidal sol; at lower temperatures it forms gel that behaves as soft thermoplastic polymers. The stiffness of gelatin gels is measured by the Bloom index which is determined via a test consisting a well-defined procedure which is performing at a certain gelatin concentration (6.67%), temperature (10ºC) and a maturation time which is 17 h (Usta et al., 2003; Gómez-Guillén et al., 2011). The structure of the network and the physical properties of the gelatin gels are designated by the source and the extraction conditions (Bigi et al., 2004). Gelatin is generally extracted from bovine or porcine tissues and as the extraction temperature lowers, the gel stiffs and the Bloom index increases (Usta et al., 2003).
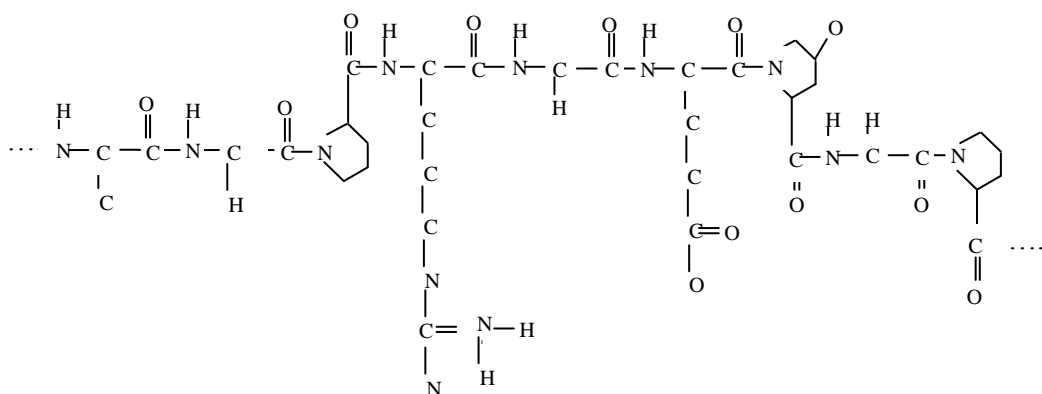
Figure 2.2. Chemical structure of gelatin (Kumari et al., 2010).

Gelatin is a low cost material and is obtained from bones and skins generated as waste during animal slaughtering and processing (Nur Hanani et al., 2012). The use of gelatin in film applications was well-established until sixties and this resulted in many patents, mainly in pharmaceutical area (Sobral et al., 2001; Park et al., 2008; Nur Hanani et al., 2012). Today the remarkable interest

in gelatin is a result of its biodegradability. It was reported by different scientists that gelatin films have good properties such as stability, strength and flexibility which make them suitable for packaging applications and other purposes (Sobral et al., 2001; Bigi et al., 2004; Park et al., 2008).

Gelatin has two groups of functional properties: i) properties related to the gelling behavior of gelatin, i.e. gel formation, texturizing, thickening and water binding capacity, and ii) properties associated with its surface behavior, that includes emulsion and foam formation and stabilization, protective colloid function, adhesion and cohesion and film-forming capacity (Schrieber and Gareis, 2007).

Gel formation of gelatin is mainly associated with structure, molecular size and temperature of the system. Since gelatin is composed of polymer chains of different lengths it does not form real solutions, instead colloidal solutions (sols) are formed. When gelatin solution is cooled the mobile molecules aggregate to small structures, these continuously grow and finally these sols convert to gels. This process is thermo-reversible; gelatin gels melt by raising the temperature (Schrieber and Gareis, 2007; Gómez-Guillén et al., 2011).

Gelatin also exhibits good film formation properties so it is considered as a useful protecting material for food to prevent drying and exposure to light and oxygen. Recent efforts focused on designing gelatin-based biodegradable materials with improved properties through blending gelatin with different polymers. In the literature, lipids (Bertan et al., 2005; Pérez-Mateos et al., 2009; Wang et al., 2009; Limpisophon et al., 2010), chitosan (Arvanitoyannis et al., 1998a; Kołodziejska and Piotrowska, 2007), gellan (Lee et al., 2004), konjac glucomannan (Li et al., 2006), soy and whey protein isolate (Cao et al., 2007b; Denavi et al., 2009; Wang et al., 2010), pectin (Liu et al., 2007) and starch (Arvanitoyannis et al., 1998b; Al-Hassan and Norziah, 2012) were combined with gelatin.

Furthermore, many researchers investigated the effects of various plasticizers such as citrate derivatives and soy lecithin (Andreucetti et al., 2009; Andreucetti et al., 2010), sucrose and organic acids (Cao et al., 2009) and modifications like cross-linking with different materials such as glutaraldehyde (Bigi et al., 2001), ferulic and tannic acid (Cao et al., 2007a), transglutaminase (Chambi and Grosso, 2006; Yi et al., 2006; Sztuka and Kołodziejska, 2009), carboxymethyl cellulose (Mu et al., 2012) on film formation of gelatin. In all these studies the characteristics of gelatin-based polymers could be partially improved.

Gelatin has been extensively used in many areas such as food, pharmaceutical, medical, cosmetic and photographic industries. It has founded its main application in the food industry. Gelatin is used in confectioneries, desserts, fruit gummies, and mallows, bar products, dairy products, ice cream, meat products and salad dressings. Gelatin has been also used in pharmaceutical and medicine industries successfully in the forms of capsules or coatings (Schrieber and Gareis, 2007).

On the other hand, gelatin applications in the scientific literature have shown an increase in the last 10-15 years. The contributing factors for this situation were the desire of economical valorization of industrial by-products, the management of wastes in an environmentally friendly way and the interest in innovative production practices. Gelatin has also an alternative usage area as the carrier of bioactive components (Gómez-Guillén et al., 2011).

2.4. Additives in Plastics

In the production of plastics, some additives are required in order to improve properties of final products. By the use of additives commercially available plastics can be more suitable to process and to use. Main groups of additives used in commercial plastics such as LDPE, HDPE, PP and PET are fillers,

flame retardants, colorants, stabilizers, lubricants, foaming agents, antistats and plasticizers (Harper, 2006).

2.4.1. Properties of Plasticizers

The Council of the International Union of Pure and Applied Chemistry (IUPAC) defined a plasticizer as ''a substance or material incorporated in a material (usually plastic or elastomer) to increase its flexibility, workability, or distensibility''. Plasticizers are low molecular weight non-volatile compounds and they have wide usage in polymer industries (Sejidov et al., 2005).

The use of plasticizers is not a new practice; their application to manipulate polymer characteristics goes back to 1800s. At the beginning, manufacturers of celluloid (the material used to make photographic film) used natural camphor and castor oil for plasticization purposes. However those were dissatisfying for many end uses. In 1912 triphenyl phosphate was tested to replace camphor oil, representing the beginning of the ester plasticizers era. Phthalic acid esters have used for the first time in 1920 and continue to be the largest class of plasticizers in the 21st century (Rahman and Brazel 2004). Today many different plasticizers that have been used for different materials exist. During the last decade, the worldwide production of plasticizers was around 60 polymers and more than 30 groups of products (Białecka-Florjańczyk E. and Florjańczyk Z., 2007). The most commonly used plasticizers are phthalic acid esters; glycerol is generally used for plasticization of biodegradable polymers (Rahman and Brazel, 2004).

Plasticizers are low molecular weight resins or liquids which improve the flexibility and processability of polymers forming secondary bonds to polymer chains and spread them apart. They allow macromolecules to be softer and flexible by reducing secondary bonding in polymer-polymer chains (Rahman and Brazel, 2004). They are also used to decrease brittleness and to avoid shrinking during storage (Vieira et al., 2011). Water is the main solvent in

biopolymer technology. Water molecules reduce the glass transition temperature and increase the free volume of biopolymers. Because of its features water is considered as the most powerful natural plasticizer (Cheng et al., 2006).

Apart from water, many natural substances such as glycerol, sorbitol, and organic acids, high and low molecular weight glycols and sucrose were used as plasticizers. Some plasticizers being used in edible and biodegradable films obtained from different raw materials are given in Table 2.3. In the production of edible or biodegradable films, glycerol and polyethylene glycols have been mostly used as plasticizers. Ethylene glycols have molecular weight above 200 are classified as polyethylene glycols. The numbers such as 200, 300 or 400 comes after the name of polyethylene glycol (PEG) indicates the molecular weight of the compound. Among these studies, it is clear that glycerol is the most common and thus, the most convenient plasticizer for biodegradable material production. Glycerol (1,2,3-propanetriol) is a polyhydric alcohol with a molecular weight of 92.1. Glycerol, which is also known as glycerin, has many uses in cosmetic, paint, automotive, food, tobacco, pharmaceutical and textile industries (Wang at al., 2001a).

Citric acid is an organic acid which is produced at the end of the Krebs (or citric acid) cycle. Citric acid is a nontoxic product of metabolism with its relatively new usage as a plasticizer.

In comparison with glycerol; the carboxyl groups of citric acid have a capability of forming stronger hydrogen bonds, especially with the hydroxyl groups of starch (Shi et al., 2007). It has been investigated in terms of plasticizing and also cross-linking effect in different materials (Jiugao et al., 2005; Ning et al., 2007; Shi et al., 2007; Holser, 2008). Molecular structure of glycerol and citric acid is given in Figure 2.3.

Table 2.3. Examples of plasticizers used in edible/biodegradable films

Plasticizer	Raw material of film	Reference
Ethylene glycol, diethylene glycol, triethylene glycol and glycerol	Pea proteins	Gueguen et al., 1998
Glycerol and PEG 400	Deacylated gellan	Yang and Paulson, 2000
Propylene glycol, PEG 200, PEG 400, glycerol, sorbitol and sucrose	β-lactoglobulin	Sothornvit and Krochta, 2001
Sorbitol	Chitosan, corn starch and pullulan	Lazaridou and Biliaderis, 2002
Sorbitol and glycerol	Maize starch	Krogars et al., 2003
Glycerol and i-erythriol	Chitosan and corn starch	Cervera et al., 2004
Glycerol	Rice and pea starch	Mehyar and Han, 2004
Monohydroxyl alcohols, low and high molecular weight glycols and sorbitol	Corn starch	Da Róz et al., 2006

Table 2.3. Examples of plasticizers used in edible/biodegradable films *(continued)*

Plasticizer	Raw material of film	Reference
Glycerol and sorbitol, and glucose, mannose and fructose	Pea starch	Zhang and Han, 2006
Glycerol, xylitol and sorbitol	Potato starch	Talja et al., 2007b
Tributyl citrate, acetyl tributyl citrate, triethyl citrate and acetyl triethyl citrate	Gelatin	Andreuccetti et al., 2009
Sucrose, organic acids and polyethylene glycols	Gelatin	Cao et al., 2009
Urea, glycerol and sorbitol	Oat starch	Galdeano et al., 2009
Sorbitol and glycerol	Sago starch	Abdorreza et al., 2011
Glycerol and sorbitol	Sago starch and fish gelatin	Al-Hassan and Norziah, 2012

(a)

(b)
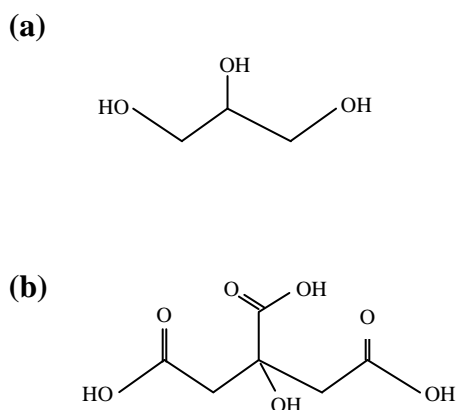
Figure 2.3. Molecular structures of glycerol (a) and citric acid (b)

2.5. Thermal Transitions in Polymers

Two different physical states are found in solid polymers: amorphous and crystalline. Amorphous polymers are characterized with glass transition (T_g); while crystalline polymers are characterized with melting temperature (T_m).

At sufficiently high temperature a thermoplastic polymer is liquid consisting of amorphous molecular chains. As the polymer is cooled thermal disorganization decreases and crystallization can occur at the T_m. In this state, all of the crystalline molecules are aligned regularly and form a compact structure. However, since the molecular chains are highly tortuous this state occurs rarely. As a result, many polymers crystallize slowly at supercooling. These supercooled polymers remain viscous until a lower temperature at which the polymer become glassy. The temperature at which the polymer vitrifies and becomes relatively more stiff and brittle is called glass transition temperature (T_g). T_g is the main transition temperature that is observed in amorphous polymers (Robertson, 2006).

Glass transition is generally explained in terms of the motion of chain atoms. Below T_g thermal energy does not allow chains to move as a whole. The motions of atoms are restricted to small movements. As T_g is approached thermal energy starts to allow larger motions. Glass transition temperature is also known as the temperature at which chain motions start (Billmeyer, 1962). Chain ends and low molecular weight plasticizers decrease the T_g of a polymer; a sufficient number of cross-links, on the other hand, increase the T_g. Above T_g, a few of carbon atoms can move in a relatively more free manner. However, below T_g, nearly all of the carbon atoms are fixed and only side groups and short chains can move (Robertson, 2006).

Semi-crystalline polymers do not separate from each other unless an external effect is applied to their crystalline regions. Due to these properties they show thermoplastic characteristics between glass transition and melting temperatures (Billmeyer, 1962).

Polymer chains can also form crystal regions aligning regularly in the polymer structure. The temperature at which these crystallized chains start to melt is defined as melting temperature (T_m). It has been known that an association exists between T_m and T_g (Billmeyer, 1962). This association is approximately expressed as:

$$Tg \approx \frac{2}{3} Tm \qquad \text{(unsymmetrical chains)} \qquad (2.1)$$

$$Tg \approx \frac{1}{2} Tm \qquad \text{(symmetrical chains)} \qquad (2.2)$$

The physical properties of a thermoplastic polymer are determined by T_m and T_g values. If T_m and T_g are below room temperature, the polymer is a liquid. If room temperature is between T_m and T_g, the polymer is a highly viscous liquid or a crystalline solid. If T_m and T_g are above room temperature, an amorphous polymer is glassy and brittle. These transition temperatures are among the most important properties which designate the usage areas of polymers. T_g and T_m

values of some of the common polymers are given in Table 2.4. At this table it is observed that flexible and linear polymers have lower T_g values whereas stiffer polymers have higher T_g values (Robertson, 2006). The melting and glass transition temperatures determine the temperature interval that a polymer can be used and processed. The temperature interval between T_g and T_m is the interval in which the polymer could be processed. Since the polymer is not able to move as a whole which means it becomes brittle below T_g, it could not be processed into any product. Similarly, polymers could not be processed above T_m because at this temperature melting of crystallized chains starts and this behavior renders polymer in a disorganized structure.

Table 2.4. Tg and Tm values of some common polymers (Robertson, 2006)

Polymer	T_g (ºC)	T_m (ºC)
High density polyethylene	-125	137
Low density polyethylene	-25	98
Polypropylene	-18	176
Poly(ethylene terephthalate) (PET)	69	267
Polycarbonate	150	220

2.5.1. Factors Affecting Glass Transition and Melting Temperature

Glass transition and melting temperatures change depending on the physical and chemical characteristics of the polymer. Chain flexibility, side chains,

geometric factors, molecular weight, branching, cross-linking, cristallinity, intermolecular forces and plasticizers change T_g and T_m (Ebewele, 2000; Robertson, 2006).

Bulky side chains decrease chain mobility and as a result increase T_g. Because side chains such as chloride and hydroxyl increase the strength of intermolecular bonds, they tend to decrease T_m and T_g. Plasticizers can decrease T_g up to 100ºC (Robertson, 2006).

Chain flexibility is a measure of the ability of the polymer chain to rotate around chemical bonds and is the most affecting factor on T_g. Long-chain ether and ester groups increase chain flexibility and decrease T_g while cyclic groups stiffen the chain and increase T_g. Bulky side chains decrease chain flexibility and increase T_g. The effectiveness of side groups to increase chain flexibility depends on the flexibility of the group. Highly flexible side groups lead to separation of polymer chains which increases free volume and consequently decreases T_g.

T_g is affected by backbone symmetry and the existence of double bonds. T_g of symmetric polymers is lower in comparison with asymmetric ones. *cis-trans* configuration is also one of the factors affecting T_g. Double bonds with *cis* configuration increase the chain flexibility and decrease T_g. As sum, all structural features tend to increase the distance between polymer chains decrease the glass transition temperature.

Molecular weight is another factor affecting glass transition temperature. As molecular weight increases, firstly T_g increases and then slows down and reach to a constant value.

Cross-linking is the process of formation of connections between molecules via chemical bonds. This process refers to reduction of the chain mobility and hence the increase of T_g. Moreover, as in long and flexible side groups,

branching increase separation between chains, which means the increase of free volume and decrease of Tg (Ebewele, 2000).

The glass transition temperature can be decreased by addition of plasticizers. The most known example of this case is poly(vinyl chloride) (PVC). PVC which is rigid can become a flexible polymer by addition of approximately 2-5% plasticizer (Ebewele, 2000; Robertson, 2006).

Intermolecular bonding, structural properties and chain flexibility also affect the melting temperature. A reduction in the density of intermolecular bonding, which refers to an increase in the space between polar groups, decreases T_m. Effects of structural properties on melting points are generally the same as that for the glass transition temperature. It has been found that rigid polymers have higher melting temperatures than flexible chains (Ebewele, 2000).

As yet, many methods have been developed regarding measurement of phase transitions in polymers. Among different techniques such as X-ray diffraction, nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA), DSC has become the most common method. Since Stevens and Elton first used DSC to measure the gelatinization in starch, it was accepted the most convenient method in determination of thermal transitions.

In the literature, Aguilar-Méndez et al. (2010) found that mixtures of starch and gelatin demonstrated higher T_m values than that of starch or gelatin alone. They also reported that Tg values of starch-gelatin films decreased as glycerol concentration increased. They observed that values of T_g and T_m changed from 80ºC to 67 ºC and from 192ºC to195ºC respectively, for starch concentrations of 0.2-0.4 and glycerol concentrations of 0.2-0.92 (%w/w). In a more recent study it was found that unplasticized sago starch-fish gelatin films had T_g values of 52.82-55.32 and T_m values of 144.91-163.76 depending on the ratio of starch to gelatin (Al-Hassan et al., 2012). They reported that addition of

glycerol to films increased T_g to the interval of 60.08-62.28 while decreasing T_m to the interval of 133.15-157.61. On the other hand they found that sorbitol, another plasticizer, significantly diminished T_g and T_m values. Sobral et al. (2001) produced edible films from bovine hide and pigskin gelatins and they used sorbitol as plasticizer. They reported that glass transition of gelatin films became broader as sorbitol concentration increased.

2.6. Mechanical Properties

The characteristics of polymers are of importance in terms of its applications. In addition to thermal and optical properties, polymers have some performance characteristics related with impact, pressure and fatigue. One of these characteristics is mechanical properties.

A common defined mechanical characteristic in polymers is tensile strength. The change in the geometrical shape of the material is called elongation and the response inside the material arisen to balance the external forces is called tensile. The tensile strength on a polymer, which is a significant measure of the ability of the polymer to be stretched, is defined as the force per unit area. When a simple elongation is considered, tensile strength can be defined as:

$$\sigma = \frac{F}{A} \qquad (2.3)$$

where F is the force (MPa) applied on the material and A is the cross-sectional area (m^2) of it.

The elongation, e, is given by the equation 2.4:

$$e = l - l_0 \qquad (2.4)$$

where l is the final length (m) and l_0 is the initial length (m) of the material.

Strain is the measure of the change in length of the material and expressed as:

$$\varepsilon = \frac{\Delta l}{l_0} \qquad\qquad (2.5)$$

The mechanical properties of polymers are affected by many external and internal factors. External factors create clear effects on the mechanical properties but they are not directly related with the structure and composition of the polymer. The major external factors are strain rate, temperature, and pressure. Internal factors produce direct changes in the chemical and physical nature of the polymer. Some of the important internal factors are chemical structure and composition, degree of crystallinity, molecular weight, polarity and presence of plasticizers.

In the literature, Parra et al. (2004) found that the tensile strength for cassava starch films plasticized with 1 g glycerol was approximately 0.357 MPa and elongation was 11.00 %. In another study, the tensile strength of rice starch films plasticized with 20, 25, 30 and 35% of glycerol was reported as 3.2, 2.2, 1.8 and 1.0 MPa respectively (Laohakunjit and Noomhorm, 2004). In a recent study Talja et al. (2007) reported that as glycerol increased, Young' modulus of potato starch films decreased and elongation at break of films increased. They found that increased glycerol and water content decreased the tensile strength.

The tensile strength and Young's modulus of 170 bloom-gelatin films was found as 1.9 MPa and 5.2 MPa respectively (Bigi et al., 2004). Researchers produced gelatin films with different Bloom indexes and they stated that Bloom index causes differences in mechanical properties of films.

Al-Hassan and Norziah (2012) reported tensile strength of sago starch-fish gelatin films in the ratios of 1:0, 2:1, 3:1, 4:1 and 5:1 plasticized with glycerol as 9.87, 1.28, 1.57, 1.70 and 1.67 MPa respectively. They found that as gelatin content in film formulations increased, % EAB increased and Young's modulus significantly decreased.

Mechanical properties of some packaging materials are given in Table 2.5. As shown in this table, mechanical properties of synthetic materials are better than that of biodegradable or edible materials. However, when biodegradable/edible materials are compared between each other, it is seen that polysaccharides have higher tensile strength values than proteins. On the other hand, it is observable that elongation of gelatin films is significantly higher than that of polysaccharide-based films.

2.7. Water Vapor Permeability

Packages produced from thermoplastic polymers are permeable to small molecules such as gases, water vapor and organic vapors (Robertson, 2006). Among these molecules water vapor is one of the most important factors affecting the shelf life of the product in the package because physical or chemical deterioration of this product is associated with the moisture content (Siracusa et al., 2008).

Water vapor permeability is defined as the amount of water vapor passes through the unit area in a given time per unit of vapor pressure under test conditions which are maintained at a constant temperature and relative humidity difference. It is generally expressed as grams per one second per meter per vapor pressure difference ($g\ m^{-1}\ s^{-1}\ Pa^{-1}$).

The main method using in determination of water vapor permeability of films includes the seal of film sample on a glass/Plexiglas/aluminum cup which contains a substance at relative humidity of 0%. The cup is then placed in a desiccator at constant relative humidity and weight increase in the film is measured as a function of time. Since pressure change (ΔP) is constant during the test, when the change in weight vs. time is plotted the points construct a straight line.

Table 2.5. Mechanical properties of some packaging materials

Material	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)
LDPE[1]	11,000	190	210
HDPE[1]	20.3	380	911
PP[1]	36.8	120	1,900
PET[1]	55	130	2,700
Cassava starch/Water[2]	0.069±0.050	11.10±0.45	-
Cassava starch/GLY (1/1)[2]	0.357±0.018	11.00±0.47	-
Cassava starch/GLY/GLU (1/1/1)[2]	0.421±0.020	12.1±1.35	-
Cassava starch /GLY/GLU/PEG (1/1/0.2/0.2)[2]	0.399±0.018	11.16±0.64	-
Pea starch/GLY (5/2)[3]	5.8±0.59	37.6±4.47	97.5±19.9
Pea starch/GLY (5/3)[3]	5.8±0.60	50.6±6.49	82.6±19.49
Pea starch/GLY (5/4)[3]	2.2±0.22	39.3±8.25	22.8±3.17
Pea starch/GLY (5/5)[3]	1.4±0.08	46.4±6.35	7.8±0.36

Table 2.5. Mechanical properties of some packaging materials *(continued)*

Material	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)
Chitosan/Water[4]	26.8±2.0	4.6±1.9	-
Chitosan/GLY(1/0.2)[4]	27.2±1.9	5.4±0.1	-
Chitosan/Erythritol (1/0.2)[4]	44.2±2.0	5.9±1.6	-
Gellan/GLY (2/1.2)[5]	37	29	31
Gellan/GLY (2/1.33)[5]	22.5	32	15
Gellan/GLY (2/1.5)[5]	16	37	9
Gellan/GLY (2/1.6)[5]	9	36	5
Gelatin-170 Bloom/GLU (5/1)[6]	1.9±0.6	227±70	5.2±0.6
Casein/GLY (5.7/1)[7]	29.1	4.1	-
Casein/GLY (2.3/1)[7]	13.9	30.8	-
Casein/Sorbitol (2.3/1)[7]	14.0	5.0	-
Casein/GLY (1.5/1)[7]	18.2	8.7	-

http://www.matweb.com[1]; Parra et al., 2004[2]; Zhang and Han, 2006[3]; Cervera et al., 2004[4]; Yang and Paulson, 2000[5]; Bigi et al., 2004[6]; McHugh and Krotcha, 2000[7].

Water vapor transmission rate (WVTR) is expressed as:

$$WVTR = \frac{Q}{A*t} \qquad (2.6)$$

$$= \frac{slope}{area} \qquad (2.7)$$

$$= \frac{g\ water}{m^2*second} \qquad (2.8)$$

In order to convert WVTR into permeability it should be divided by ΔP which is the driving force.

In the literature, water vapor permeability values of different edible/biodegradable films and some common packaging materials were investigated by researchers thus far. Various WVPs reported in the literature are given in Table 2.6. Because the water vapor permeability of packaging materials differs depending on environmental factors the relative humidity interval and temperature at which the measurement has done are given in the Table 2.6.

2.8. Optical Properties

Light initiates or accelerates many of the reactions that cause deterioration in foods. The catalytic effects of light are based on the rays come from lower wavelengths of visible spectrum or UV region. The intensity of light and the duration of light exposure are interpreted as the most important factors in color and flavor deteriorations. When light come to the surface of packaging material a part of it is reflected, a part is enters in the polymer and is absorbed converting to heat energy and the rest is transmitted through the material. The photochemical reactions in foods are proportional to transmittance percent.

Table 2.6. Water vapor permeability values of some edible/biodegradable films and packaging materials

Material	WVPx10^{-10} (g m^{-1} s^{-1} Pa^{-1})	T (ºC)	RH %
LDPE[1]	0,0087	38	95-0
HDPE[2]	0,0022	38	97-0
Edible gellan films[3]	1.9-7.8	21	54-0
Bovine hide gelatin films[4]	0.47-1.05	22	100-0
Pigskin gelatin films[4]	0.50-0,89	22	100-0
Fish gelatin-chitosan film[5]	6.7	25	50-0
Potato starch-based film/20% glycerol[6]	0.11	23.5	54-0
Rice starch-chitosan films[7]	0.48-0.90	25	60-0
Gelatin film[8]	0.00374–0.00692	25	50-0
Potato starch-agar film[9]	4.5-6.5	25	75-0
Pig hide gelatin/saponin[10]	0.33-0.94	25	75-0
Sago starch-fish gelatin film[11]	0.5-1.03	30	100-0

Myers, 1961[1]; Guilbert, 1996[2]; Yang and Paulson, 2000[3]; Sobral et al., 2001[4]; KołodziejskaandPiotrowska, 2007[5]; Talja et al., 2007[6]; Bourtoom and Chinnan, 2008[7]; Cao et al., 2009[8]; Wu et al., 2009[9]; Andreuccetti et al., 2010[10]; Al-Hassan and Norziah, 2012[11];

Optical transmittance is defined as the ratio of light intensity transmitting through the material to incident intensity and is given by:

$$T = \frac{I}{I_0} \qquad (2.9)$$

where I is the intensity of light transmitted through the material and I_0 is incident intensity (Tilley, 2011). Transmittance is generally expressed as percent and given by:

$$\% \, T = \frac{I}{I_0} x \, 100 \qquad (2.10)$$

Opacity, on the other hand, generally calculated by integration of absorbance spectrums with wavelength and expressed as multiplying absorbance by nanometer (A*nm). In the current study, opacity was calculated per unit thickness of films and expressed as multiplying absorbance by nanometer per millimeter (A*nm/mm).

It is well known that light accelerates the formation of free radicals in lipids during oxidation reactions. This oxidation decreases the nutritional value of fats and oils, leads production of toxic compounds from lipids and causes rancidity and also breaks fat-soluble vitamins particularly vitamins A and E.

The effects of light which plays an important role in deterioration of foods can be decreased or removed by suitable packaging. Depending on the characteristics of light transmittance of the packaging material, it can provide direct protection for foods by absorbing or reflecting whole light or a part of it (Robertson, 2006).

In the literature, opacity of corn starch films was reported as 138.0±0.8 A*nm while the opacity of corn starch films with glycerol was 109.6±0.9 A*nm (García et al., 2009). In another study, protein-starch based bioplastics in different ratios were produced using different procedures namely compression-

molding, extrusion and combination of both techniques. Researchers found that the type of starch had effects on the optical properties of films produced. They observed that potato starch added to formulation in a ratio of 20% (w/w) resulted in more transparent films than that of corn starch and also, compression-molding method revealed more transparent films than extrusion method (González-Gutiérrez et al., 2011).

Gelatin has stated in the literature as quite transparent. Pérez-Mateos et al. (2009) added sunflower oil to cod gelatin in order to improve the hydrophobic characteristics of gelatin films. They stored the films and observed the optical properties of films. They observed that adding oil increased optical absorbance and decreased transparency of gelatin films which were very transparent in sole form. They reported that the transparency of gelatin films was 9.51±0.75 and 8.00±0.41 with 1% oil added while it was 0.30±0.11 and 0.22±0.02 with no added oil, before and after storage respectively. In a more recent study, it has been found that increase of gelatin concentration in a formulation of gelatin and starch did not significantly affect the transparency of films (Al-Hassan and Norziah, 2012). They found transparency values of glycerol plasticized-sago starch-gelatin films in the ratios of 1:0, 2:1, 3:1, 4:1 and 5:1 as 1.34±0.09, 2.00±0.18, 1.91±0.02, 2.12±0.09 and 1.85±0.17 respectively. Higher light absorbance was denoted by some researchers as an excellent barrier to prevent light-induced lipid oxidation.

CHAPTER 3

MATERIALS AND METHODS

3.1. Chemicals

All chemicals were analytical grade and obtained from Merck Ltd and Surechem Products Ltd.

3.2. Preparation of Solutions and Production of Films

Commercial potato starch (PS, Soyyiğit Gıda San. ve Tic. A.Ş.) and gelatin from bovine skin (Sel Sanayi Ürünleri Ticaret ve Pazarlama A.Ş., Turkey) were used for the preparation of film solutions. Glycerol (Merck Chemicals) and citric acid monohydrate (Merck Chemicals) are added to film solutions as plasticizers.

3.2.1. Preparation of Potato Starch-based Films

6.6 g potato starch was added to 100 ml distilled water at room temperature. Then mixture was heated in a water bath (Nüve BM 402) at 60ºC for 30 min and obtained solution was vortexed (Nüve NM 110) for 1 min in order to disperse aggregation. Solutions were divided into four groups and different film formulations were prepared. Glycerol and citric acid monohydrate were kept constant at the ratio of 1 g/100 ml individually. Solutions were hold in water bath at 60 ºC for 30 min. After incubation solutions were poured on plastic petri dishes (30 ml). Finally films were dried in ambient conditions for 3 days or they were incubated in an oven (Nüve EN 500) at 40 ºC for 24 h.

3.2.2. Preparation of Gelatin-Based Films

3.4 g gelatin was added to 100 ml distilled water at room temperature. Mixture was heated in a water bath (Nüve BM 402) at 60ºC for 30 min and gelatin was solubilized. Solutions were divided into four groups and different film

formulations were prepared. Glycerol and citric acid monohydrate were kept constant at the ratio of 1 g/100 ml individually. Then the methods that were used for the preparation of PS-based films were applied for gelatin-based films.

3.2.3. Preparation of Combined Films

The method that was used for preparation of films is given in Figure 3.1. In the preparation of combined films, different ratios of potato starch and gelatin solutions (1:0, 1:1, 2:1, 1:2 and 0:1) based on total weight basis of 10 g in 200 ml distilled water were prepared. 2 g glycerol and 2 g citric acid monohydrate per 200 ml solution was used in the film production.

Figure 3.1. Preparation of combined film solutions and production of films

Starch solutions were prepared as described in Section 3.2.1 and gelatin solutions were prepared as described in Section 3.2.2. After solubilization, solutions were mixed and films in different formulations were prepared. Mixtures were hold in water bath at 60 ºC for 30 min. Solutions were poured on plastic petri dishes (30 ml). Films were dried in ambient conditions for 3 days or the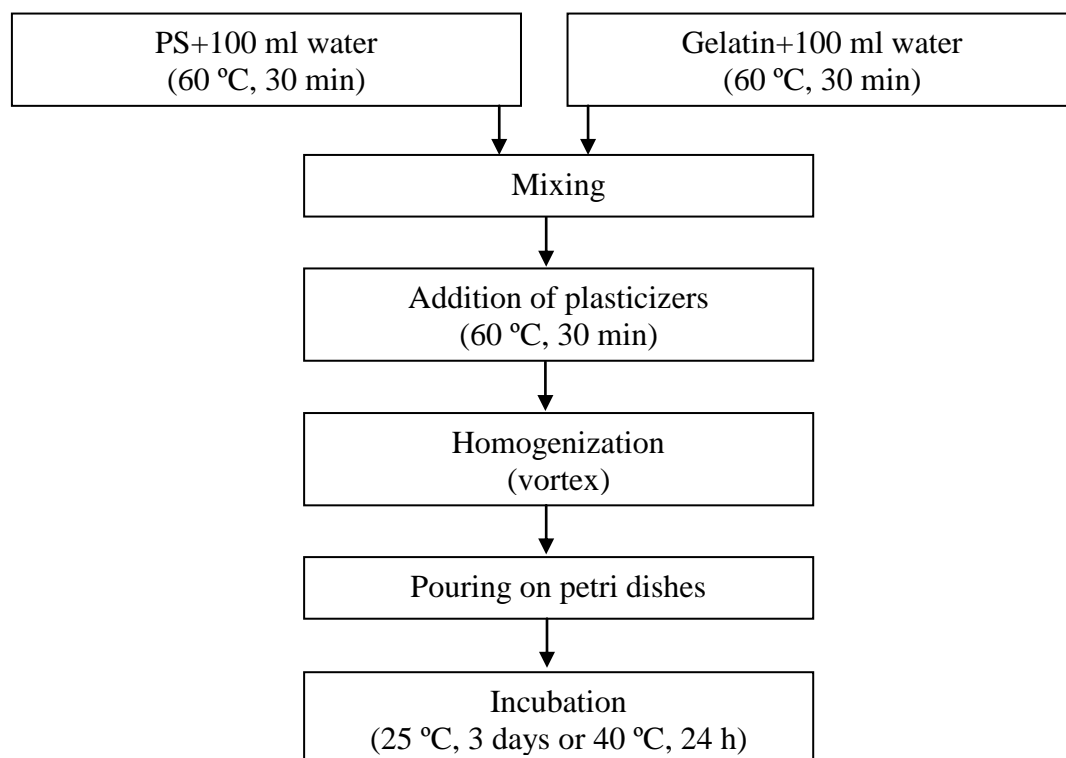y were incubated in an oven (Nüve EN 500) at 40 ºC for 24 h. Film formulations that were prepared for combined film production are given in Table 3.1.

Table 3.1. Description of film formulations

	1	**2**	**3**	**4**	**5**
Component	**Concentration, g/100 ml**				
Potato starch	5	2.5	3.3	1.7	--
Gelatin	--	2.5	1.7	3.3	5
Glycerol	1	1	1	1	1
Citric acid monohydrate	1	1	1	1	1

3.3. Analyses

Thicknesses of films were determined using a micrometer (BTS Digital Caliper) with an accuracy of 0.01 mm at 5 random positions on each film sample and average of those measurements was used. Film samples were stored in desiccator containing silica gel until analysis.

The analyses carried out on film samples were as follows: determination of thermal properties, mechanical analysis, determination of water vapor permeability and determination of opacity. Mechanical analyses, and determination of water vapor permeability and opacity were performed in triplicates and duplicates respectively.

3.3.1. Determination of Thermal Properties

Thermal analyses were conducted by METU Central Laboratory. Differential scanning calorimeter (Perkin Elmer 800) was used for determination of thermal properties of films. DSC data were recorded in the temperature range of (-90)-(100) ºC, under a nitrogen flow of 30 ml/min. 2-4 mg samples were encapsulated in aluminum pans and heated at a rate of 10 ºC/min. Thermograms were evaluated using Pyris program. T_m was taken from the DSC endotherm as the peak temperature of melting endotherm. T_g was determined as the midpoint of the gradual increase of the specific heat associated with the transition.

3.3.2. Determination of Mechanical Properties

Mechanical properties were determined on a universal testing machine (Zwick/Roell Z250) by METU Central Lab. After conditioning at 23±2 ºC, in RH = 50±5% for 88 h, three film specimens were cut from each film sample using dumbbell shaper (Zwick/Roell). The thicknesses of films were measured with a digital micrometer and specimens were mounted between the grips of the machine. Tests were performed at initial grip separation of 20 mm and cross-head speed of 5 mm/min. A loadcell at 100 N was applied until the break and the elongation was recorded.

3.3.3. Determination of Water Vapor Permeability

Water vapor permeability (WVP) was determined according to a gravimetric method at 25 ºC based on ASTM E96-80 test (ASTM, 1989). Films were sealed onto the opening of cells containing anhydrous $CaCl_2$ (RH: 0.0%) and

then, these cells were placed in desiccators containing saturated KCl solution (RH: 84%). The cells were weighed (± 0.01 g) in an analytical balance (EJ-610, A&D Co. Ltd.) hourly for 48 hours. The water vapor permeability of films was determined through the relation between weight and time using the equation (3.1) obtained from the calibration curve given in Figure 4.9:

$$Slope = \frac{P * A * \Delta p}{x} \tag{3.1}$$

where P is the water vapor permeability (g/m.s.Pa), x is the thickness of the film (m), A is the permeation area (m^2), and ΔP is the difference of partial vapor pressure of the atmosphere with $CaCl_2$ and KCl solution.

3.3.4. Determination of Optical Properties

The opacity of films was determined according to the method described by Cao et al. (2007b). Film samples were cut into a rectangle and placed on the internal part of a spectrophotometer cell. The area under the absorption curve in the visible region at 500 nm) was recorded using a UV-Vis spectrophotometer (Specord 50, Analytik Jena AG). The opacity of films was calculated using the equation (3.2).

$$Opacity = \frac{A_{500} * 500}{x} \tag{3.2}$$

where A_{500} is the absorption at 500 nm, and x is the thickness (mm). The measurements were repeated three times for each film sample and an average was taken as the result.

The opacity of film samples was determined immediately after the production as well as it was measured after storage of films at temperatures of -20º, +4º, +40º and ambient conditions for 10 days in order to investigate the effects of temperature conditions on the opacity of film samples.

3.3.5. Statistical Analysis

A one way analysis of variance (ANOVA) was used for statistical evaluation of the data obtained from this study using SPSS Package 17.0. Differences between formulations were detected by Duncan test ($p < 0.05$).

CHAPTER 4

RESULTS AND DISCUSSIONS

In this study, it was aimed to produce biodegradable potato starch-bovine gelatin films and investigate the properties of these films. In the first part of the study, films of different ratios of potato starch to bovine gelatin (1:0, 1:1, 1:2, 2:1, and 0:1) plasticized with glycerol and citric acid monohydrate were prepared. In the second part, in order to characterize the films, physical and mechanical properties including water vapor permeability, opacity, thermal transition temperatures, tensile strength and elongation-at-break were measured.

4.1. Visual Properties and Thicknesses of Films

Depending on the formulation, film samples had various visual properties that can be defined in a scale from opaque to transparent. It was observed that the films which were plasticized with both glycerol and citric acid monohydrate and dried at ambient conditions were separated from petri dishes easily and used in analyses due to their structural integrity. However, the films which were prepared by addition of glycerol alone did not exhibit film formation properties. Because these films were very brittle and extremely thin, they could not be analyzed. Moreover, it was observed that the films which were incubated in the oven at 40ºC gained a leathery structure so these films also could not be analyzed. It is known that the strength of starch decreased under stress especially under high temperature. Because glycoside bonds in starch start cracking at high temperature, cracking could be occurred in the films dried in oven. Within the scope of the thesis, it was studied on 5 different film formulation and the pictures of these samples are shown in Figure 4.1.
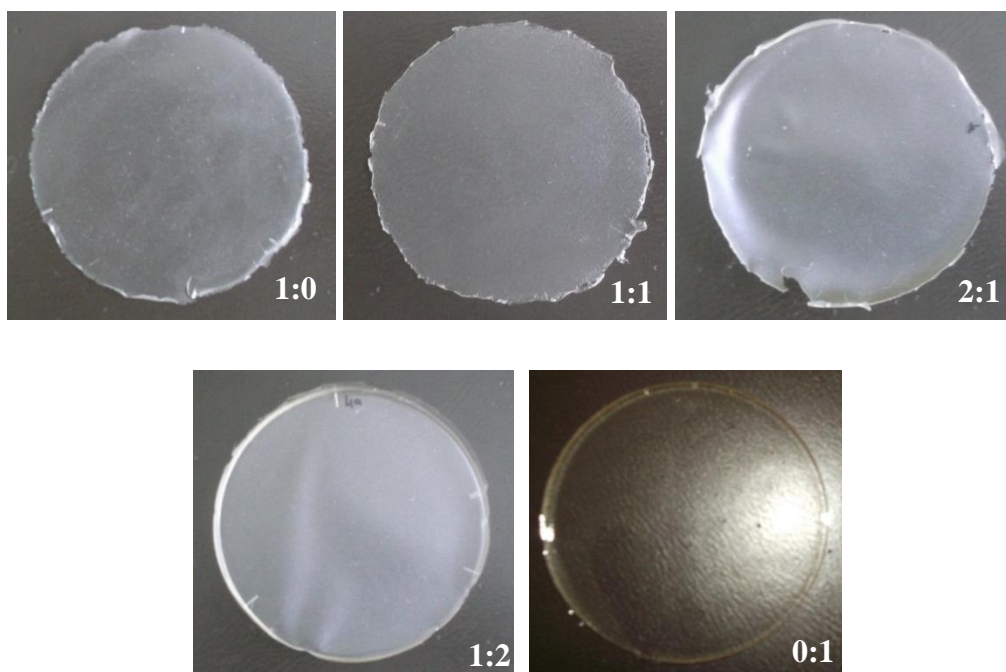
Figure 4.1. Visual properties of potato starch-gelatin films

The thickness values of films produced in this study are given in Table 4.1. Thicknesses of film samples were not affected by the film formulation. Statistically thickness values of all films fell within the same group.

Table 4.1. Thickness values of potato starch-gelatin films

Films (starch:gelatin)	Film thickness (mm)
1:0	0.19 ± 0.014^{a}
1:1	0.17 ± 0.007^{a}
2:1	0.16 ± 0.014^{a}
1:2	0.19 ± 0.028^{a}
0:1	0.15 ± 0.035^{a}

4.2. Differential Scanning Calorimetry (DSC) Analysis

Since it is known that plasticizers added to biodegradable/edible films in order to increase flexibility and processability affects the thermal properties of films, thermal properties of glycerol and citric acid monohydrate were firstly investigated with DSC. DSC thermograms of glycerol and citric acid monohydrate are given in Figure 4.2 and 4.3.

From the thermogram of glycerol its glass transition temperature was determined as -81.36ºC (Figure 4.2). Similar results were reported by other researchers. The glass transition temperature of pure glycerol was reported as -78ºC and -86ºC by Averous et al. (2000) and Murthy (1996), respectively.

Glass transition temperature of citric acid monohydrate was determined as 57.20ºC (Figure 4.3). In the literature melting temperature of citric acid was reported as between 153ºC and 160ºC (Shi et al., 2007; Holser, 2008). In the current study, since the temperature interval was (-90)-(100)ºC melting temperature of citric acid monohydrate was not determined. However, it is expected around 150-155ºC, depending on the literature.
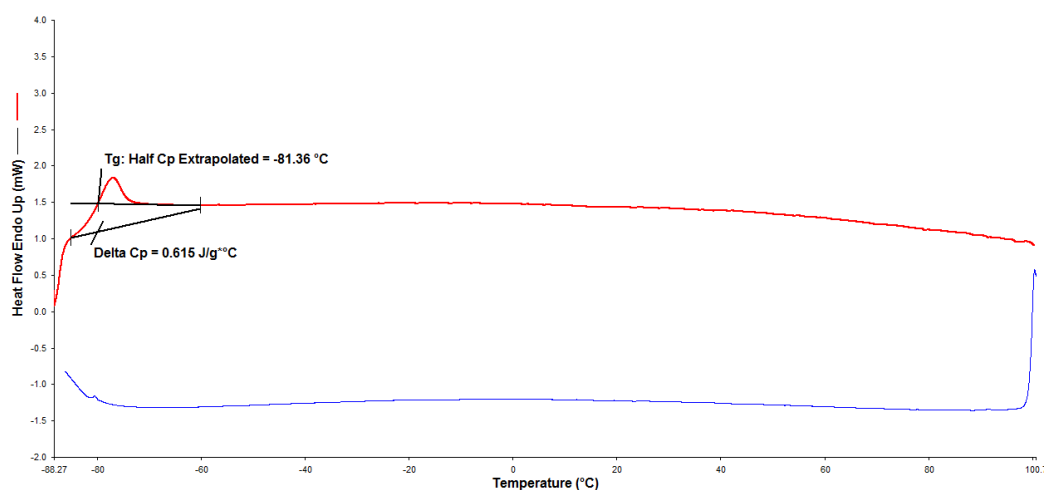
Figure 4.2. DSC thermogram of glycerol
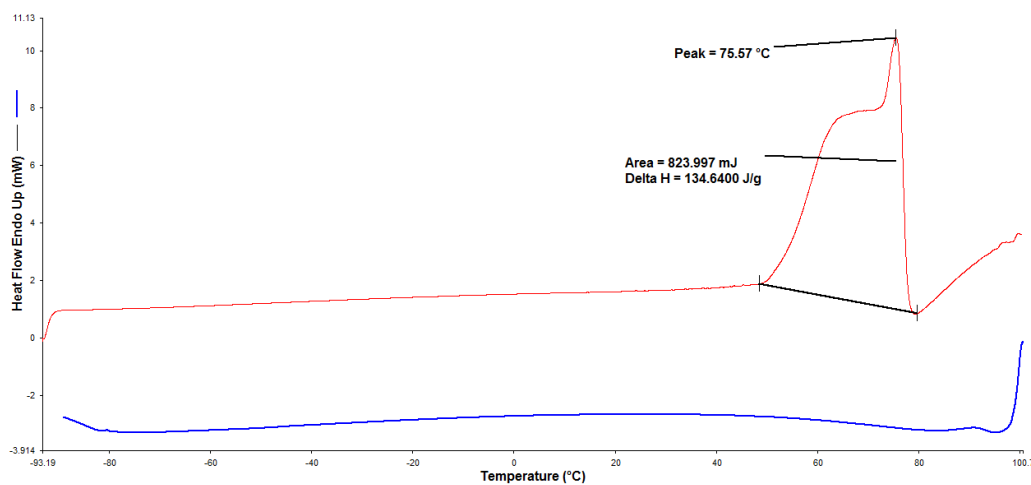
Figure 4.3. DSC thermogram of citric acid monohydrate

Measurement of glass transition temperature depends upon determination of the gradual effect in DSC curve created by the heat capacity change. DSC thermograms of film samples are given in Figure 4.4 – 4.8. Glass transition temperature is evaluated as an indication of the compatibility of polymers (Al-Hassan and Norziah, 2012). The findings of this study showed that potato starch-gelatin films had Tg values between 69.8ºC and 91.8ºC (Figure 4.5 – 4.7).

Films studied in this study showed only one glass transition temperature. This thermal behavior of films indicated that no phase separation occurred between plasticizers and potato starch or gelatin. The presence of only one Tg in multiple polymeric systems has been attributed to the polymer matrix of the system as a whole. In the literature, this behavior was also observed by Al-Hassan and Norziah (2012) in sago starch-gelatin blends. Arvanitoyannis et al. (1998) reported that chitosan and gelatin based films had shown no phase separation and they indicated the hydrophilic nature of gelatin as the reason of

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that observation. They stated that the hydrophilic structure of gelatin resulted in formation of hydrogen bonds between gelatin, water and glycerol. In all these studies the phenomena with only one Tg was related to entire polymeric matrix. In this study, the presence of only one Tg can also indicate that potato starch and gelatin are compatible. Furthermore, since no phase separation was observed, these findings confirmed that glycerol is an effective plasticizer.
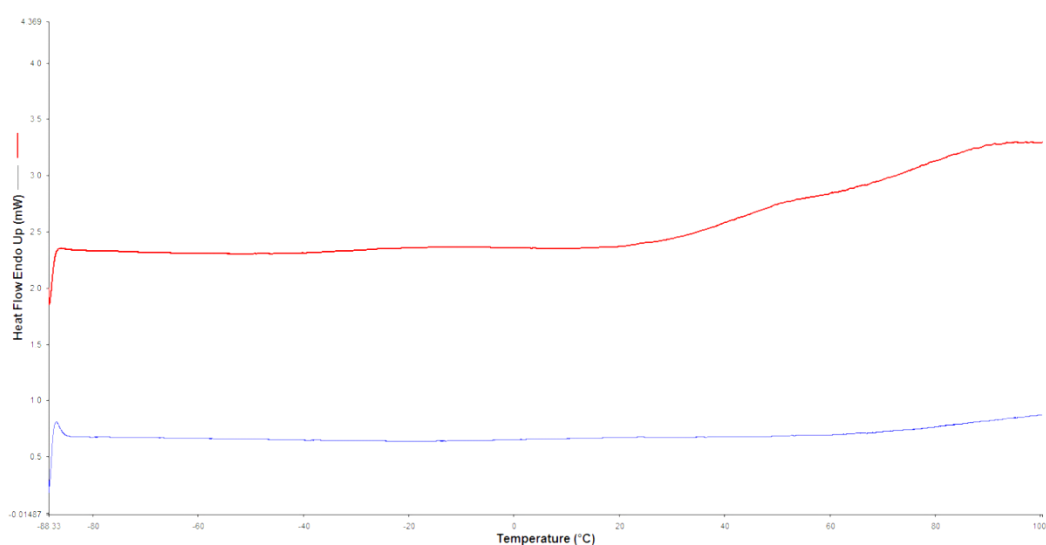
Figure 4.4. DSC thermogram of the film sample in the potato starch:gelatin ratio of 1:0

Glass transition temperature of film samples are given in Table 4.2. Tg values of films varied from 69.6 to 91.8°C depending on the formulation. The result of DSC analysis showed that Tg values of combined films lower than that of starch or gelatin alone except for the potato starch-gelatin ratio of 1:1.
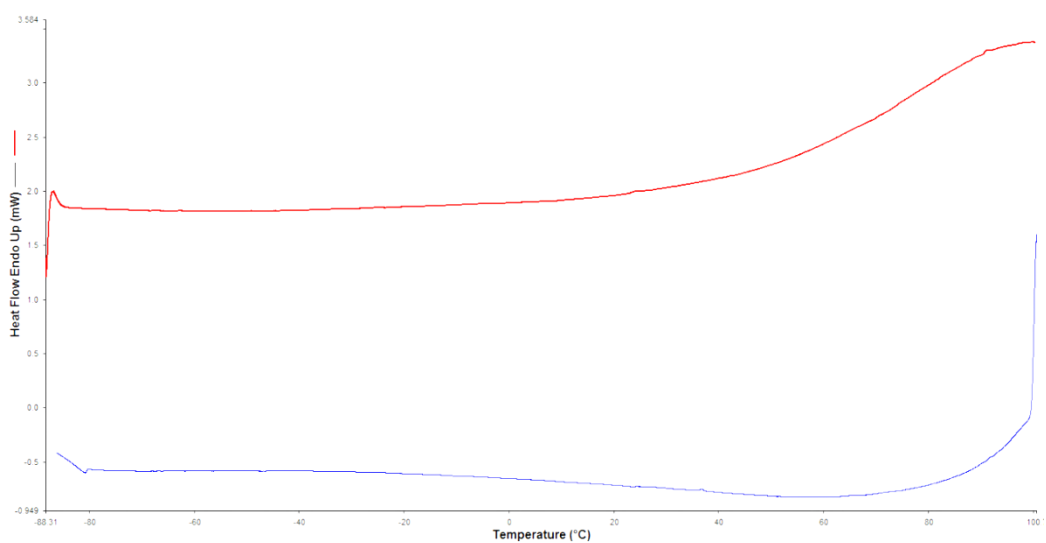
Figure 4.5. DSC thermogram of the film sample in the potato starch:gelatin ratio of 1:1
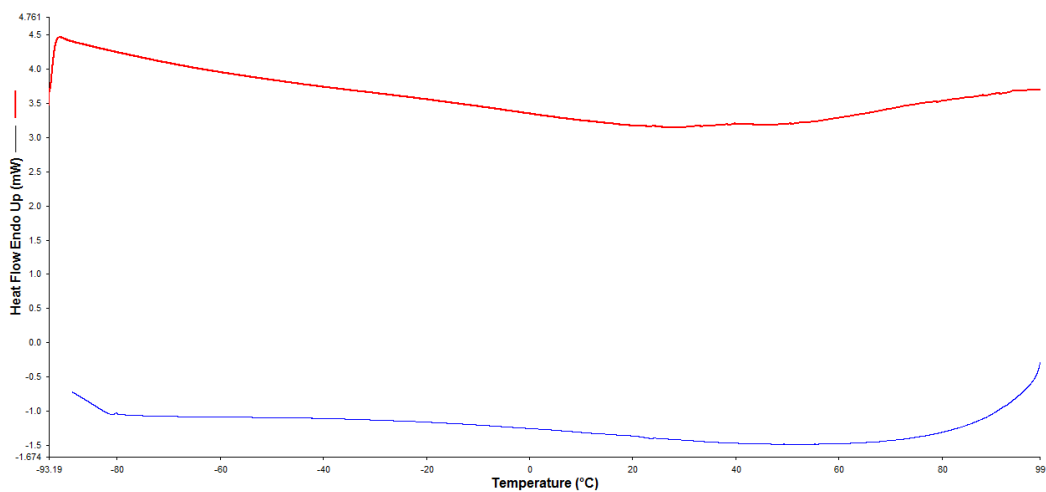
Figure 4.6. DSC thermogram of the film sample in the potato starch:gelatin ratio of 2:1
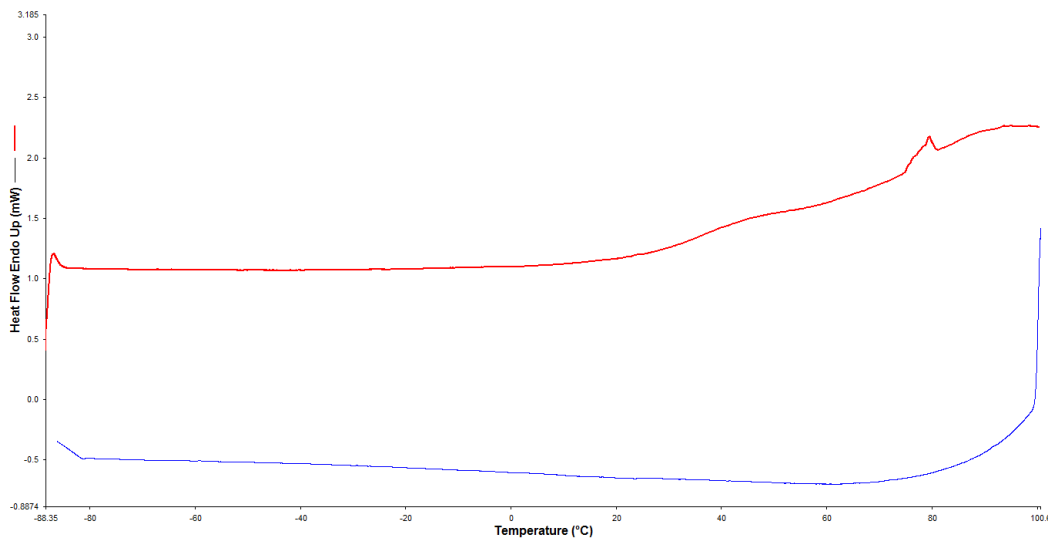
Figure 4.7. DSC thermogram of the film sample in the potato starch:gelatin ratio of 1:2
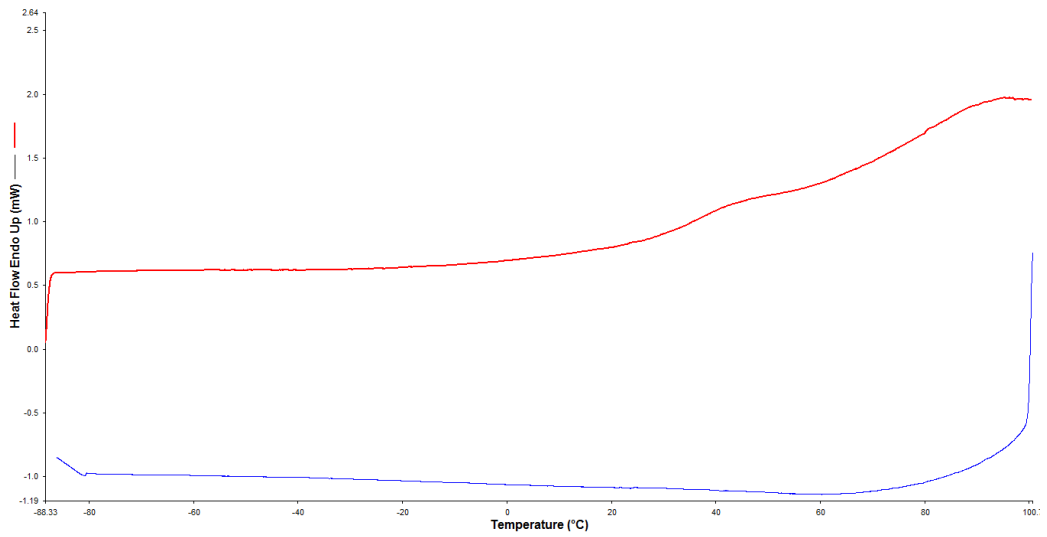
Figure 4.8. DSC thermogram of the film sample in the potato starch:gelatin ratio of 0:1

The lowest and highest Tg values were determined in the combined film samples in potato starch to gelatin ratio of 2:1 and 1:1, respectively. It was clearly observed that gelatin introduction into film formulation increased Tg. From this finding it can be concluded that gelatin diminished chain flexibility and hence decreased free volume, consequently Tg increased.

Table 4.2. Glass transition temperature of film samples

Films (starch:gelatin)	Tg (°C)
1:0	83.4
1:1	91.8
2:1	69.6
1:2	77.2
0:1	80.6

Findings of the current study are different from the results of studies on starch films. Tg value of potato starch film determined in this study is higher than that of potato and sago starch films which were reported by Talja et al. (2007) and Al-Hassan and Norziah (2012). However, Tg values of starch film demonstrated similarity with the results of Aguilar-Méndez et al. (2010) who reported glass transition temperature of starch film as 80°C.

Glass transition temperature of the gelatin film (0:1) is in parallel with the findings of Vanin et al. (2005) who reported the Tg value of gelatin film as 82.5°C. Tg values of the combined films are close to that values of sago starch-fish gelatin films (Al-Hassan and Norziah, 2012).

On the other hand, the films demonstrated higher Tg values than chitosan-gelatin, yam starch and pea starch films (Arvanitoyannis et al., 1998; Mali et al., 2005; Zhang and Han, 2006).

When the glass transition temperatures of the biodegradable films produced in the current study were compared to common synthetic polymers it was seen that Tg values of the films were significantly higher than that values of HDPE, LDPE and PP. However, it is also observed that Tg values of potato starch-gelatin films produced in this study were close to TG value of poly(ethylene terephthalate) which is 69ºC and are lower than Tg values of polycarbonate (Table 2.4).

Tm values of the films were not determined because temperature interval during DSC analysis was not sufficiently high to rise the temperature that the melting point could be observed. However, melting temperature of film samples are expected to be around 120ºC.

4.3. Mechanical Properties

The mechanical properties including tensile strength, percentage of elongation-at-break and Young's modulus values of the films produced in this study are given in Table 4.3. The mechanical properties were affected by the formulation.

The results demonstrated that the tensile strength of biodegradable potato starch-gelatin films increased with the addition of gelatin and the maximum tensile strength was observed in the ratio of 0:1. The tensile strength values of films varied from 2.21 to 15.60 MPa (Table 4.3). Tensile strength indicates the maximum load that sustained by the films. That is, it is an indication of mechanical durability. Higher tensile strength means stronger films so high tensile strength is generally required in materials used in packaging. In this study, films higher in gelatin content demonstrated higher tensile strength values than the starch films. Thus, it can be concluded that gelatin-based films are more durable than starch-based films.

Table 4.3. Mechanical properties of potato starch-gelatin films

Films (starch:gelatin)	Tensile strength (σ_M) (MPa)	Elongation at break (EAB) (%)	Young's modulus (E_t) (MPa)
1:0	2.21 ± 0.92^d	93 ± 32.2^b	14.20 ± 5.8^c
1:1	3.56 ± 0.31^c	123 ± 5.8^{ab}	80.07 ± 12.3^b
2:1	2.95 ± 0.38^{cd}	130 ± 17.3^a	86.57 ± 29.4^b
1:2	6.31 ± 0.28^b	130 ± 8.2^a	93.43 ± 11.6^b
0:1	15.60 ± 1.24^a	110 ± 14.1^{ab}	151.50 ± 25.1^a

Elongation at break (EAB) is a measure of the flexibility and extensibility of films. EAB is determined at the point when the films break under tensile testing (Bourtoom and Chinnan, 2008). In the current study, EAB varied between 93% and 130%, depending on the ratio of potato starch to gelatin (Table 4.3). In general EAB increased with gelatin and starch addition so the combined films demonstrated better flexibility than that of starch and gelatin alone. There is an explanation for EAB-increasing effect of gelatin which was reported by Al-Hassan and Norziah (2012). This explanation indicates that gelatin seems to act as a plasticizer with increased flexibility and reduced brittleness. With the results obtained in this study, the increased EAB can again be attributable to this effect of gelatin and also starch itself.

Young's modulus of films varied between 14.20 and 151.50 MPa and significantly increased with addition of gelatin in films. The gelatin film (0:1) showed the maximum Young's modulus value (Table 4.3). Since Young's

modulus is defined as the measure of the rigidity or stiffness, it can be concluded that films with gelatin are stiffer than starch films.

When the results were statistically evaluated, it was seen that the tensile strengths of the films took place in different statistical groups except for the films in the ratio of 1:1 and 2:1. Their tensile strength values were not statistically different. Tensile strengths of the starch film (1:0) and the film had the ratio of 2:1 also fell within the same group. For EAB values, it was determined that the values were statistically close to each other. There were two different statistical groups for EAB values and the values of the films in the potato starch-gelatin ratio of 1:1, 2:1, 1:2 and 0:1 fell in the same statistical group (a). The films had the ratio of 1:0, 1:1 and 0:1 also took place in the same statistical group (b). It can be concluded from these results that potato starch-gelatin ratio did not statistically affect EAB. As for Young's modulus values it was designated that there were three different statistical groups. The films in the ratio of 1:1, 2:1and 1:2 took place in the same group. Therefore it can be concluded that Young's modulus values of those films were not statistically different. However, the starch (1:0) and gelatin (0:1) films were in different groups from the combined films. It can be concluded that combination of potato starch and gelatin revealed stronger films than the films composed of potato starch or gelatin alone (Table 4.3).

In general, mechanical analysis revealed that the films higher in gelatin content were more durable, more rigid and more flexible than the films high in potato starch. Thus, it can be concluded that gelatin improved and starch weakened the structure of the biodegradable films. There are some explanations for these effects of gelatin and starch. One of them is that starch granules create heterogeneities in the protein matrix that acted as stress points, induce cracking and result in lower values of strength and elongation (Wang et al., 2001b). Another explanation is that networks between starch and gelatin in anionic domains of the starch and cationic domains of the gelatin could be formed

(Fonkwe et al., 2003). As a result, this formation makes the films stronger. Moreover, the possibility of the formation of these networks is significantly increased by the introduction of small molecules such as water and glycerol to the blend (Tolstoguzov, 1994).

When the mechanical properties of the biodegradable films produced in the current study were compared to common synthetic polymers it was seen that tensile strength and Young's modulus values of the films were lower than that of those common polymers. However, elongation-at-breaks of the biodegradable films were comparable to those values of PP and PET. Although the films demonstrated lower tensile strength than chitosan, gellan and casein films, their elongation-at-break and Young's modulus values are significantly higher than that of films composed of cassava starch, pea starch, chitosan, whey protein (casein) and gellan (Table 2.5).

4.4. Water Vapor Permeability

Water vapor permeability was determined according to the ASTM method (1989) at $25\pm2^{\circ}$C and $0\text{-}84\pm2$ RH. The weight gained was plotted versus time and the slope of this plot was used to determine the WVP. The slope yielded a coefficient of determination of (R^2) of 0.996-0.999. The data of a WVP determination experiment is given in Figure 4.9.

Although water vapor permeability is considered as a constant value, in hydrophilic materials such as protein and polysaccharide films it can deviate from the ideal behavior due to the interaction of water permeated with polar groups in the film (Hagenmaier and Shaw, 1990). It is generally accepted that water vapor permeability of food packaging materials should be as low as possible. The WVPs of potato starch-gelatin films produced in this study are given in Table 4.4. The WVP values of films varied between 0.022×10^{-10} and 0.061×10^{-10} $g.s^{-1}.m^{-1}.Pa^{-1}$ depending on the formulation.

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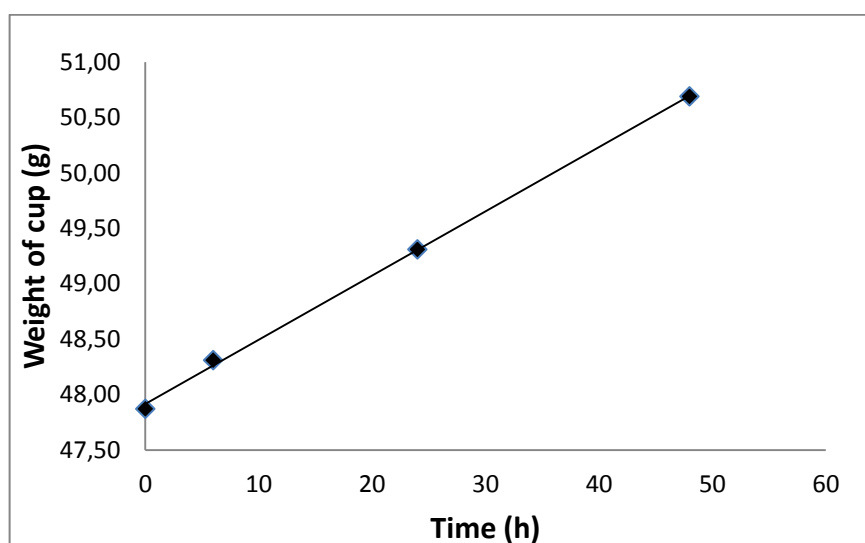
Figure 4.9. Change in weight of the cup containing $CaCl_2$ with time for a potato starch film (Slope $= 2 \times 10^{-5}$ $g.s^{-1}$; WVP $= 0.064 \times 10^{-10}$ $g.s^{-1}.m^{-1}.Pa^{-1}$).

The lowest permeability value occurred at the potato starch-gelatin ratio of 0:1 (gelatin film). The results demonstrated that WVP decreased with the presence of gelatin in the formulation.

Table 4.4. Water vapor permeability values of potato starch-gelatin films

Films (starch:gelatin)	WVP $(g.s^{-1}.m^{-1}.Pa^{-1})$ x 10^{-10}
1:0	0.061 ± 0.004^a
1:1	0.026 ± 0.001^b
2:1	0.031 ± 0.005^b
1:2	0.025 ± 0.002^b
0:1	0.022 ± 0.004^b

Statistically, the starch film (1:0) fell in a different group from other films. The films had the potato starch-gelatin ratio of 1:1, 2:1, 1:2 and 0:1 took place in the same statistical group. From the statistical analysis it was concluded that water vapor permeability difference between combined films (1:1, 2:1, 1:2) and the gelatin film (0:1) was not statistically significant whereas the WVP difference of the starch film (1:0) was significant. However, it is apparent that the ratio of 1:2 had the best permeability property among combined films (Table 4.4).

These results are in contrast with results obtained by Al-Hassan and Norziah (2012) who reported that higher WVP was found with films containing more protein. Though gelatin is more hydrophilic than potato starch, the lowest WVP value that it possessed and the reduction in the WVP of films produced with higher gelatin content demonstrated that the intramolecular polymer-polymer interactions in gelatin are stronger than that of potato starch. Tensile strength values determined by mechanical analysis supported this situation. The tensile strength which is an indication of the interaction between polymer chains was significantly high in gelatin film. In mechanical analysis it was observed that the tensile strength increased as the gelatin content increased. In can be concluded that gelatin increased the tortuosity factor of the way that water molecules diffused and clouded the diffusion. The decrease in WVP with the increase of gelatin content can be attributable to a formation of hydrogen bonding between amino groups of gelatin which were protonated to NH_3^+ due to the presence of citric acid monohydrate and OH^- of partially gelatinized and destroyed starch molecules.

Water vapor permeability values of some polymers are summarized in Table 2.6. WVP of LDPE and HDPE is 0.087×10^{-10} and 0.0022×10^{-10} $g.s^{-1}.m^{-1}.Pa^{-1}$. The WVPs of the films produced in the current study are 2-10 folds higher than these values. However, the films studied in this study demonstrated better

permeability properties compared to other films based on gellan, chitosan or other protein and polysaccharide sources (Table 2.6).

4.5. Optical Properties

The opacity of films before and after storage at room temperature, 40ºC, +4ºC and -20ºC was measured as an indication of the shelf life of films. Opacity determination based on the measurement of the light absorbed by the material and it was measured in the visible region. Opacity values of films (Op) before and after storage are given in Table 4.5. Obviously, opacity values of films were affected by formulation and storage.

Opacity measurement indicated that the introduction of gelatin significantly reduced the opacity of films. Opacity values of films before storage varied between 208.7 and 1194.4 A.nm/mm (Table 4.5). These results also observed with naked eye. The films containing higher gelatin were less opaque than the films high-in-starch (Figure 4.1). It was determined from visual analysis that with increasing gelatin content there were less starch particles in films. It can also be concluded that as the content of potato starch increased the light transmittance decreased. These findings can be related to the effect of gelatin to decrease the surface roughness of films and the amount of starch particles in the film structure (Figure 4.1). Similarly, González-Gutiérrez et al. (2010) reported for albumen/starch-based bioplastics that the transmittance decreased as starch content increased. In another study, it was reported that the introduction of a polysaccharide (chitosan) into gelatin films increased the opacity (Rivero et al., 2009).

Nevertheless increased opacity is an undesirable property in many applications, in some food packaging applications this is required to provide protection against deterioration reactions triggered by light (Andreucetti et al., 2009).

Table 4.5. Opacity (O$_p$) of potato starch-gelatin films before and after storage

Films (starch: gelatin)	Op before storage (A.nm/mm)	Op after storage at room temperature (A.nm/mm)	Op after storage at 40°C (A.nm/mm)	Op afterstorage at +4°C (A.nm/mm)	Op after storage at -20°C (A.nm/mm)
1:0	1194.4±17.96$^{a/yz}$	1407.3±58.97$^{a/x}$	1923.8±42.14$^{a/w}$	1109.4±8.84$^{a/z}$	1268.8±16.19$^{a/y}$
1:1	709.4±18.17$^{c/x}$	685.2±13.08$^{c/x}$	1997.2±8.63$^{a/w}$	667.6±9.19$^{c/x}$	532.7±24.47$^{b/y}$
2:1	1070.2±3.61$^{b/x}$	1020.6±112.29$^{b/x}$	1561.0±227.62$^{b/w}$	1070.1±17.40$^{b/x}$	1122.6±194.10$^{a/x}$
1:2	548.7±11.60$^{d/x}$	445.7±2.97$^{d/z}$	608.2±11.81$^{c/w}$	524.1±14.99$^{d/xy}$	494.4±13.44$^{b/z}$
0:1	208.7±6.01$^{e/wx}$	235.3±8.34$^{e/w}$	229.2±20.65$^{d/w}$	198.6±2.05$^{e/xy}$	179.4±8.34$^{c/y}$

$^{a-e}$ Different letters in the same columns indicate significant differences between the averages obtained in the Duncan test (p<0.05).

$^{x-z}$ Different letters in the same rows indicate significant differences between the averages obtained in the Duncan test (p<0.05).

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It can be concluded in this regard that increase of starch content in the films produced in the current study, which resulted in an increase in the film opacity, can be suitable for the packaging applications that will aim to protect the food, particularly oils, fats and some oily food products, from the effect of light.

On the other hand storage at different temperatures affected the opacity, which is an indication of the shelf life of packaging materials. Storage at 40ºC had the maximum effect on the films. In general, opacity of films significantly increased after storage at 40ºC whereas it reduced after storage of combined films at room temperature, +4ºC and -20ºC. This could mean that, in the case of the usage of these films in food packaging, they could lose their optical stability in refrigerator or freezer while they could be suitable packaging materials for foods which are kept at temperatures above room temperature.

Among all film samples, starch film (1:0) demonstrated the greatest change towards storage at different temperatures. It could not sustain its stability during storage. However, the introduction of gelatin to the film offered it the stability that protected the optical properties. Alongside the starch film (1:0), gelatin film (0:1) also exhibited greater changes than the combined films with storage. With the addition of gelatin the fluctuations in the opacity values during storage reduced and the most stable structure was observed in the potato starch-gelatin ratio of 2:1.

Storage at room temperature for 10 days increased the opacity of starch (1:0) and gelatin (0:1) films whereas reduced these values of the combined films. Among the combined films the highest and the lowest percent reduction was determined in the ratios of 2:1 and 1:2 respectively. The percent increase in the opacity of starch and gelatin films after storage at room temperature was 17.9% and 12.8%, respectively. On the other hand the percent decrease in the opacity of combined films had the ratio of 1:1, 2:1 and 1:2 was 3.4%, 4.6% and 1.9%,

respectively. According to these results, it can be concluded that the most stable film after storage at room temperature for 10 days is the film in the ratio of 1:2.

Storage at 40ºC for 10 days significantly increased the opacity of all film samples in comparison with the films before storage. The highest increase was determined in the film sample with the ratio of 1:1 with 181.5% increase. The most stable film after storage at 40ºC was the gelatin film with 9.8% increase. The film sample which was composed of higher gelatin amount (1:2) was more stable than the other combined films. It can be concluded that gelatin provided stability to the films during storage.

Storage at 4ºC for 10 days decreased the opacity of all film samples except for the film in the ratio of 2:1 which did not demonstrated change in opacity value. This film sample was the most stable one after storage at refrigerator. On the other hand, the highest reduction was observed in the starch film (1:0).

Storage at -20ºC generally decreased the opacity of films as storage at 4ºC. However, the opacity of the films high in starch content demonstrated an increase after storage at -20ºC. The highest decrease was observed in the second film sample (1:1).

Obviously higher storage temperature increased the opacity whereas lower storage temperature reduced it. When the optical properties evaluated, it was observed that the most stable film is the third film (2:1) and the least stables are the first (1:0) and the fourth (1:2) films. Statistical analysis supported these findings (Table 4.5). The optical values of the starch film (1:0) fell within different statistical groups. In the second film (1:1), opacity change by storage at room temperature, 40ºC and -20 ºC was not statistically important; these values took place in the same statistical group. From these finding, it can be concluded that storage of this film at these temperatures will not significantly affect its stability. On the other hand, storage of the second film at 40ºC was in a different statistical group and this indicated that storage of this film at 40ºC

will affect its optical stability. However, the opacity values of the third film (2:1) before and after storage statistically fell within the same group except for the value of storage after 40ºC. This difference can be related to the strong effect of high temperature on the opacity. Therefore, it can be interpreted that the third film could be an appropriate packaging material for food which should be stored at room temperature, in refrigerator or in freezer. Statistically, opacities of the fourth film fell into different groups except for values after storage at room temperature and -20ºC. The mentioned opacity values fell into the same group. Finally, opacity values of gelatin film (0:1) before and after storage showed significant statistical differences in comparison to the third film (2:1). However, opacity values before and after storage at room temperature, 40ºC and 4ºC were in the same statistical group. Inside the values of gelatin film it can be concluded that storage at these temperatures will not significantly affect the optical stability, so the shelf life of the material.

The opacity differences occurred with different potato starch to gelatin ratios found in the current study are different from the study of Al-Hassan and Norziah (2012) who reported that the increase of fish gelatin concentration did not significantly affect the light absorbance of the films. The opacity values before storage found in the current study are lower than the films composed of soy protein isolate and gelatin and plasticized with glycerin which were investigated by Cao et al. (2007b) and were found to have opacity values between 1184 and 5676 (A.nm/mm). However, opacity values obtained in this study are significantly higher than unplasticized or glycerol-plasticized gelatin-chitosan films which had opacity values around 280-470 A.nm/mm (Rivero et al., 2009).

CHAPTER 5

CONCLUSION

In this study, it was aimed to produce potato starch and gelatin based bioplastics and to investigate the characteristics of these materials. In order to investigate the effects of potato starch to gelatin ratio on the properties of films, film samples with different potato starch to gelatin ratios were manufactured by casting method. In order to determine the characteristics of these biodegradable films thermal, mechanical, two physical (permeability and optical) properties were examined.

From the results of DSC analysis, it was concluded that glycerol was an effective plasticizer. In DSC thermograms only one T_g was observed for all film samples. According to this result it was concluded that potato starch and bovine gelatin were compatible. It was decided that the effectiveness of glycerol as plasticizer was maximum in the third film sample (2:1).

It was determined that the mechanical properties affected by potato starch to gelatin ratio. The tensile strength increased with the increased content of gelatin. Elongation at break increased as both starch and gelatin content in the films increased. Young's modulus also increased as both starch and gelatin content in the films increased. It was determined that combined films demonstrated higher mechanical durability than the films composed of potato starch or gelatin alone. Therefore, it was concluded that mechanical properties of starch-based or gelatin-based films could be improved by the addition of gelatin.

According to the results of water vapor permeability measurement, it was determined that WVP decreased as the content of gelatin increased in the

formulation. The lowest water vapor permeability was observed in the gelatin film which was in the potato starch to gelatin ratio of 0:1. Reduction effect of gelatin on permeability values despite its higher hydrophilic characteristics over potato starch was depended on that polymer-polymer interactions were stronger between gelatin chains that that of potato starch. It was determined that the findings obtained in this study were well-adjusted to the tensile strength values. Higher tensile strength values of combined films then potato starch film is another indication of stronger polymer-polymer interactions between chains. It was stated as a reason for the reduction in WVP with the introduction of gelatin that the formation of hydrogen bonds between OH^- groups of partially gelatinized potato starch and NH_3^+ groups of gelatin.

Opacity of the films was determined before storage as a measure of their transparency and after storage at different temperatures for 10 days as an indication of the shelf life of the materials. It was observed that the opacity of films was significantly affected by the ratio of potato starch to gelatin. It was determined that the increase in the starch content resulted in an increase of opacity whereas the increase in the gelatin content made the films more transparent. This effect of gelatin which has very high transparency was interpreted as an expected result. Opacity change was determined after storage at room temperature, 40ºC, +4ºC and -20ºC for 10 days. It was determined that storage at room temperature decreased the opacity values of combined films. On the contrary, optical transmittance of starch and gelatin films increased after 10-day storage at room temperature. It was determined that opacity values of films after storage at 40ºC similarly affected. Storage at a relatively high temperature prominently increased the opacities of films. The greatest raise was observed in the film sample which had the potato starch to gelatin ratio of 1:1 and the minimum change was determined in the gelatin film. Opacity of the film sample in the potato starch to gelatin ratio of 2:1 was not affected by storage at +4ºC while opacity values of the other films decreased. It was concluded that the least stable film was the starch film. It was determined that

the majority of films demonstrated reduction in their opacity values. On the contrary, it was observed that opacity of the films high in potato starch increased after storage at -20ºC.

When the results entirely evaluated, the optimum formulation among combined films determined as the potato starch gelatin ratio of 1:2. This formulation displayed the best properties, specifically in terms of mechanical behavior and permeability value. The glass transition temperature of this film sample was the second-lowest value among all films. It had the highest tensile strength, elongation at break, Young's modulus and water vapor permeability values among combined films. It was also superior to the other films in terms of optical stability after storage. It was determined that optimum storage condition for this film formulation was storage at +4ºC, whereby it can be concluded that this film could be suitable for foods which should be stored at refrigerator.

As a conclusion, the production of potato starch/gelatin-based films which were characterized in physical properties and associated with each other was carried out. It was revealed that thermal, mechanical and physical properties (water vapor permeability) of starch-based films could be significantly improved by the addition of gelatin. Although the films obtained in this study had higher glass transition temperature than that value of many biodegradable/edible films in the literature, demonstrated thermal properties comparable with PET and PC. Also, the films had higher mechanical durability than many bioplastics in the literature composed of cassava starch, pea starch, casein, chitosan and gellan. Additionally, elongation at break values of the films was comparable with synthetic polymers PP and PET. Furthermore, the films had significantly lower water vapor permeability degrees than other polysaccharide-based films such as chitosan and gellan but higher values than common synthetic polymers. It is thought that the films obtained in this study will be suitable packaging materials with some improvements, depending on both permeability and light transmittance properties the films.

This study holds the qualification that to be a basis for studies about biodegradable plastics would be done in the future. The high cost of raw materials of biodegradable plastics is supposed as the main disadvantage of their applications. It is considered that the investigation of the recovery of these raw materials from industrial wastes can make the production of biodegradable plastics more economic. It is also evaluated that this kind of applications will attain a place in the packaging market which is dominated by commercial petroleum-based products, as user-friendly and environmentally friendly options and will reduce the dependence on petroleum.

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