PREPARATION AND CHARACTERIZATION OF CORN ZEIN COATED POLYPROPYLENE (PP) FILMS FOR FOOD PACKAGING APPLICATIONS

A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in Chemical Engineering

by
İsa Doğan ATİK

July 2007
İZMİR

We approve the thesis of **İsa Doğan ATİK**

                                                    Date of Signature

……………………………..   **19 July 2007**

Associate Professor Funda TIHMINLIOĞLU

Supervisor

Department of Chemical Engineering

İzmir Institute of Technology

……………………………..   **19 July 2007**

Assistant Professor Banu ÖZEN

Co-Supervisor

Department of Food Engineering

İzmir Institute of Technology

……………………………..   **19 July 2007**

Associate Professor Sacide Alsoy ALTINKAYA

Department of Chemical Engineering

İzmir Institute of Technology

……………………………..   **19 July 2007**

Assistant Professor Figen KOREL

Department of Food Engineering

İzmir Institute of Technology

……………………………..   **19 July 2007**

Prof. Dr. Devrim BALKÖSE

Head of Department

İzmir Institute of Technology

....

Prof. Dr. M. Barış ÖZERDEM

Head of the Graduate School

ACKNOWLEDGEMENTS

ABSTRACT

PREPARATION AND CHARACTERIZATION OF CORN-ZEIN COATED POLYPROPYLENE (PP) FILMS FOR FOOD PACKAGING APPLICATIONS

The plasticized corn-zein coatings on polypropylene (PP) films as an alternative to multilayer packaging films consisting of non-degradable polymers were prepared to evaluate barrier, mechanical, thermal, surface and optical properties of the resulting coated film, as affected by coating formulation (solvent, corn-zein, plasticizer concentration, and plasticizer type). PP films coated with corn zein were obtained through a simple solvent casting method. Corn-zein with different amounts (5% and 15%) was dissolved in 70% and 95% aqueous ethanol solution at $50^{o}C$, respectively. Solutions of corn-zein plasticized by polyethylene glycol (PEG) and glycerol (GLY) with various levels (20% and 50%) were applied on corona-discharged-treated PP film.

The resulting corn-zein coated PP films showed good appearance, flexibility and adhesion between the coating and the base film. The coated PP films showed a significant ($P<0.05$) increase in barrier (water and oxygen) properties and improvement in mechanical properties when coating formulation consisted of higher corn-zein content and lower amount of GLY as plasticizer. Furthermore, zein coating increased the service temperature range of the PP films, and promised good printability on the surface due to contact angle results. The statistical analysis defined that the key parameters of coating formulation that had major effect on the final properties of coated PP films as corn-zein concentration, plasticizer concentration, and plasticizer type while ethanol concentration was found to be as less effective parameter compared to others.

In conclusion, corn zein coatings with appropriate formulation on PP films could have potential as an alternative to conventional synthetic coatings for food packaging applications.

ÖZET

GIDA AMBALAJI UYGULAMALARI İÇİN MISIR PROTEİNİ KAPLI POLİPROPİLEN (PP) FİLMLERİN HAZIRLANMASI VE KARAKTERİZASYONU

Günümüzde biyobozunur özellikteki polimerlerin petrol türevli sentetik polimerlere kıyasla doğal ve geri dönüşebilir olmaları nedeniyle, yüksek miktarda tüketilen gıda ambalajlarında kullanılmaları hedeflenmektedir. Bu çalışmada, mısırın yapısındaki en önemli protein olan ve film oluşturma özelliğiyle dikkat çeken zein'in, polipropilen (PP) ambalaj filmleri üzerine kaplanmasıyla elde edilen yeni bir tür kaplı filmlerin gıda ambalajlarında kullanılmasına yönelik uygunluğunun incelenmesi amaçlanmıştır. Uygulanan kaplamanın, yüzeyden kimyasal olarak kolayca ayrılabilmesi ve bu sayede PP'nin yüksek oranda geriye dönüşmesine olanak sağlaması nedeniyle, pahalı ve sentetik kaplama amaçlı polimer filmlere alternatif olabilecektir. En uygun ambalaj ölçütlerini elde etmek açısından kaplama içeriğinde yer alan çözücü, mısır proteini, plastikleştirici yüzdesi ve tipinin kaplı PP filmlerinin su buharı ve oksijen geçirgenliği, mekanik, ısıl, optik ve yüzey özelliklerine etkileri detaylıca saptanmıştır. Mısır proteini (zein) sırasıyla %70'lik ve %95'lik etil alkol ile 50°C'de farklı oranlarda (%5 ve 15%) çözülmüş, ve kırılganlığının azaltılmasına yönelik iki tür plastikleştirici (polietilenglikol ve gliserol) düşük ve yüksek oranlarda (%20, %50) ilave edilerek, PP'nin önceden korona ile modifiye edilmiş yüzeyine çözüğü döküm tekniğiyle kaplanmıştır. Mısır proteininin kaplanmasıyla elde edilen PP filmler iyi dış görünümleri ve esneklikleriyle göze çarpmışlardır. Elde edilen sonuçların istatistiksel çözümlemesi, kaplama içeriğinde yer alan mısır proteini ve plastikleştirici oranlarının, ayrıca plastikleştirici türünün kaplı PP film özelliklerine en etkili parametreler olduğunu göstermiştir. Yüksek oranlarda mısır proteininin düşük miktarlardaki gliserol ile plastikleştirilmesinden sonra PP filmlerine kaplanması ile , filmlerin bariyer ve mekanik özelliklerinde belirgin ($P<0.05$) artış sağlanmıştır. Ayrıca, PP filmlerinin kaplamayla birlikte erime sıcaklığı azalırken, bozulma sıcaklığında artış göze çarpmış, bu da kaplı PP filmlerin process çalışma aralığını genişletmiştir. Sonuç olarak, uygun içerikle kaplanan PP filmlerin, günümüzde gıda ambalajlamada kullanılan sentetik kaplamalara alternatif bir malzeme olabileceği bulunmuştur.

Dedicated to my mother; Sabiha Atik
for her love, support, and encouragement,
and who has brought patience, and sacrificed many things to give me a start…

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

The plastics, polypropylene (PP), polyethylene (PE) and polystyrene (PS) used for food packaging today consist of petroleum-derived (non-degradable) polymers because of their low cost, durability, and structures that resulted in wide ranges of strengths and shapes (Herdmand 1993, Reddy et al. 2003). The purpose of food packaging is to preserve the quality and safety of the food it contains from the time of its manufacture to the time it is used by the consumer (Cutter 2006). The polymers used for food packaging should have combination of moisture and gas barrier, and also mechanical properties. Most of the food packaging materials consist of multilayer films made usually from thermoplastic polymers (Weber 2000). With regard to barrier properties of packaging materials, the critical compounds that can penetrate the packaging materials and degrade the food quality are the water vapor and oxygen of the surrounding atmosphere. They can be absorbed and diffused by and through the packaging materials (Hong and Krochta 2004). The protection of packaged food against water and oxygen is one of the most important requirements, and they can be blocked by the use of coatings on the packaging materials. Conventional barrier coatings on packaging materials typically consist of expensive and synthetic polymers such as ethylene vinyl alcohol (EVOH), polyvinylidene chloride (PVDC), and polyesters (Hong and Krochta 2006).

Despite the availability of a variety of excellent synthetic coatings, the disadvantage is the difficulty entailed in their recycling. The coating films containing layers of different plastic materials may not be recycled, because typically only recycling of single component plastics is feasible. Furthermore, the growing reliance on these coated packaging films has raised a number of environmental concerns. Most of the plastic materials, for instance, are not biodegradable and are derived from non-degradable resources, and also these polymers are noxious or toxic materials. Furthermore, the increased consumption of these laminated packaging films has resulted in increased waste and the effects of this waste on the environment can no longer be neglected (Haugaard et al. 2001, Reddy et al. 2003, Tharanathan 2003).

Since the multilayer food packaging materials are being demanded that to be natural, disposable, potentially biodegradable, as well as recyclable, the replacement of non-degradable polymers by degradable plastics is a major interest of plastic packaging industry. Therefore, degradable polymers is now being considered for packaging materials because of their film-forming abilities and also their favorable environmental advantages in terms of recyclability and reutilization compared to conventional synthetic polymeric films (Chandra and Rustgi 1998).

However, biopolymer films have a susceptibility to drying, brittleness and lower mechanical properties. In an attempt to overcome this problem, a novel approach can be explored for the use of biopolymer coatings on conventional synthetic plastic films (e.g.polyethylene, and polypropylene) to produce a multilayer film structure. One advantage of these multilayer films is an easy separation of the films in solvent, which provides a higher possibility for recycling. Another advantage is to improve the barrier and mechanical properties of synthetic monolayer films (Lai and Padua 1997).

To date, many studies based on polysaccharide coatings have been studied. Hong et al. studied on the properties of polypropylene (PP) coated with polysaccharides (Hong et al. 2005). They observed high glossy surfaces, when chitosan and k-carrageenan, were used as coating. PP films coated with chitosan, and k-carrageenan had greater transparency, tensile strength and elongation than the other coated films. Whey protein, one of the animal based proteins, is another important research area and new studies have been focusing on this topic (Hong and Krochta 2004). Hong and co-workers studied the feasibility of whey protein isolate (WPI) coating as an alternative oxygen barrier for food packaging. They observed a decrease in oxygen permeability of WPI-coated LDPE films. They found that oxygen barrier properties of coated films were inversely proportional with plasticizer amount. As a result, it was suggested that WPI coating could work successfully as an oxygen barrier coating (Hong and Krochta 2004). To our knowledge, however, there is no study about corn zein coated synthetic monolayer polymeric films. Existing studies are limited only with freestanding films of corn zein (Lai and Padua 1997, Lawton 2004, Andrianaivo et al. 2003).

In this work, the surface of the polypropylene film was coated with a biodegradable corn-zein polymer for food packaging applications. These films were prepared as an alternative to multilayer packaging films which consist of non-degradable polymers. The effectiveness of these films for food packaging applications was evaluated in terms of barrier (water vapor and oxygen), mechanical, thermal,

optical and surface properties of the resulting coated film which are affected by coating formulation (ethanol as solvent, corn-zein and plasticizer concentration, and also plasticizer type).

This thesis consists of six chapters which altogether aim at covering preparation and characterization of corn-zein coated polypropylene (PP) films for food packaging applications. Chapter 1 gives a general introduction to the background of the thesis as well as to the interest in biodegradable polymers and their importance. Potential food packaging and requirements for excellent packaging are summarized in Chapter 2. Biodegradable-based packaging materials and especially details of corn-zein are presented in Chapter 3 together with an introduction to their properties. The literature survey based on opportunities of biodegradable packaging materials, coated packaging materials used today, their applications and biobased coatings on synthetic films are summarized in this chapter. In Chapter 4, materials and preparation of coating formulation are defined, and then characterization methods of corn zein coated PP films and statistical planning of experiments are presented. In Chapter 5, the criteria for packaging applications of corn-zein coated PP films such as water vapor and oxygen permeability, mechanical, thermal and optical properties are presented and discussed to make clear the effect of coating formulations on these properties by using statistical analysis. Finally in Chapter 6, a joined conclusion of the potential of corn zein coated PP film for the food packaging industry is outlined.

CHAPTER 2

THE REQUIREMENTS AND PROPERTIES OF FOOD PACKAGING MATERIALS

During the second half of the last century, plastics played an increasing role as materials for packaging all types of foods. Packaging materials have become an integral part of life because of their many advantages. Packaging of a food is aimed to prevent most of possible kinds of degradation that may render it a lower quality or make it unsuitable for consumption. The efficient packaging materials ensure the proper properties of food products from processing and manufacturing through handling and storage and ultimately to consumer (Tice 2003, Weber et al. 2002).

Since foods may undergo biological, chemical and physical deterioration during storage and distribution, an equally important function of packaging is to protect the product from these damages. Several interactions, including oxygen and moisture, exposure to light, which may catalyze certain reactions, can be accounted for the majority of deterioration sources. Foods, either in their processed form or in the raw material stage, depending upon storage conditions are highly perishable, therefore they need a careful technological intervention to preserve them longer (Tharanathan 2003). The most well-known packaging materials that meet these criteria are petrochemical based plastics such as polypropylene (PP), and polyethylene (PE) which have been in use by the food industry for over 50 years because of their availability in large quantities at low cost and favourable functionality characteristics. These materials are not only safe, inexpensive, versatile, but also flexible (Hong and Krochta 2006).

Global productions of these plastic packaging materials are estimated at more than 180 million tons per year, with growth and demand increasing annually and it is known that food packaging is the largest growing sector in the plastic packaging market. It is estimated that of the $100 billion packaging market in the world, 70% is attributed to food production. While many plastic consumer products are designed for a relatively long lifetime, almost all of the plastics materials for food packaging is meant to be discarded (Comstock et al. 2004). In fact, during the 1990s less than 10% of all plastic packaging materials were recycled by consumers. In addition, the presence of these types of packaging materials in landfills can be problematic on many fronts

(Cutter 2006). If plastic is not recycled, these items end up in landfills, where they can last forever and never degrade. Therefore, increased consumption of food packaging materials creates a large waste problem (Haugaard et al. 2001).

The multilayer structures and coated synthetic films ensure that the quality and safety of the packaged foodstuffs are maintained effectively. The gas barrier properties (water vapor, oxygen, carbon dioxide) of packaging films are improved with coatings or multilayer structures, but recyclability is decreased due to created difficulty in separation of multilayers from each other. Packaging films might have good barrier properties against loss of food aromas, but the coatings and the multilayer structures with barrier resins provide improvements where necessary (Tice 2003). However, since serious ecological problems has caused due to their total non-degradability, jointly packaging and the food industries have tried to reduce the amount of these wastes. Thus, over the last decades, there has been growing needs to find alternatives to petroleum-based products because of environmental concerns. Due to creation of these environmental problems, a series of discoveries and inventions led to today's multitude of primarily synthetic polymer packaging materials (Martin et al. 2001). Thus, eco-friendly atmosphere has led to a paradigm shift on the use of biodegradable packaging materials for packaging, derived from renewable resources such as agriculture feedstock and marine food processing industry wastes. As society has increased its understanding of the environmental implications of its industrial practices, greater attention has been given to concept of sustainable eco-friendly systems that rely on renewable resources. The use of biologically derived polymers for food packaging applications can emerge as an important component of this new paradigm of environmental development (Tharanathan 2003).

Unfortunately, it is important to realize that no single biodegradable material will satisfy all potential markets or applications. Therefore, a growing interest is seen in developing packaging concepts consisting of multilayer biologically based materials, the conventional approach to produce multilayer and coated conventional synthetic films (Weber et al. 2002). Their complete replacement for synthetic packaging may be just impossible to achieve packaging criteria. Properties of biodegradable packaging films depend on the raw material that they are based on, the additives used and on the (chemical) modifications during production (Olabarrieta 2005).

The combination of synthetic and biodegradable packagings is proposed to increase the efficiency of food quality preservation by the packaging and recyclability

in terms of easy separation of laminated layers from each other. In other respects, as mentioned before, biodegradable packagings are non-pollutant products because they consist of natural and biodegradable substances from agriculture. Thus, they contribute to the protection of the environment and also the amount of synthetic packaging is reduced and recyclability is increased. Therefore, to meet the growing demand of recyclable packaging material and consumer demands for safer and better quality foods, new and novel food-grade packaging materials or technologies have been and continue to be developed (Debeaufort et al. 1998).

In addition, designing of food packaging materials is a multistep process and involves careful and numerous considerations to researcher where the final package should satisfy successfully with all the required properties. In fact, the packaging requirements of foods are complex. In addition to preserving food products, good packaging can contribute significantly to profit. The requirements for a package include efficient packaging materials, suitable structure and form, convenience, and especially consideration of disposal of the package at the end of its useful life (Weber et al., 2002). Furthermore, these properties to be considered in relation to food distribution may include gas and water vapour permeability, mechanical properties, thermoforming properties, resistance (towards water, acid, UV light, etc.), transparency, printability, availability, and cost. In addition to this development, researchers are employing various types of packaging materials to be active, intelligent, or interactive (Chandra and Rustgi 1998).

Regardless of whether it is synthetic or biodegradable packaging material, food industry is concerned with the preservation and protection of all types of foods particularly from oxidative conditions. Foods are often dynamic systems with limited shelf-life and very specific packaging requirements. The characteristics required in packaging depend on what item will be packaged as well as the environment in which the package will be stored. Physical characteristics of the packaging films are greatly influenced by the chemical structure, molecular weight, crystallinity and the processing conditions of the polymers used (Tharanathan 2003).

In addition, since foods are consumed to maintain life, the safety aspect is a critical packaging parameter. Packaging materials produced must meet the criteria which is related to transport properties (water vapor, gases, light and aroma), optical and mechanical properties, marking and printing properties, chemical and temperature properties. A good packaging film should allow for a selective barrier to gases and a

slow but controlled respiration, maintain mechanical handling, and prevent microbial spoilage during storage. In addition to these, product shelf life is controlled by three factors; product characteristics, properties and storage and distribution conditions (Haugaard et al. 2001).

2.1. Mass Transport in Packaging

Mass transport properties of packaging materials are often described by three common coefficients which are diffusion, solubility and permeability coefficients. Mass transport through the packaging is influenced by composition and structure of packaging material. Since mass transport properties of a packaging material is responsible for product quality deterioration and shelf-life reduction, a detailed understanding of mass transport characteristics is a great practical and commercial importance. Therefore, design and processing conditions that affect the composition and structure of polymer films should be also considered (Crank 2003, Miller and Krochta 1997).

For many packaging materials, the relation between three common coefficients can be described by the following expression:

$$P_m = D \times S \tag{2.1}$$

where P is the permeability coefficient, D is the diffusion coefficient, and S is solubility coefficient.

2.1.1. Diffusion through the Packaging Films

Diffusion is the process by which permeant molecule moves through the polymer matrix as a result of random molecular motions. Thus, the diffusion coefficient, D represents a kinetic property of the polymer-permeant system. Figure 2.1 shows the activated diffusion process through the packaging polymer films (Crank 2003).

Activated diffusion is described as the opening of a void space among a series of segments of polymer chain due to oscillations of the segments. Then, this active state is followed by translational motion of the permeant within the void space before the segments return to their normal state. Both active and normal states are long-lived, as compared with the translational of the permeant. Factors affecting the structure of a

polymer have a direct effect on segmental mobility, and therefore, influence its mass transport properties (Miller and Krochta 1997).
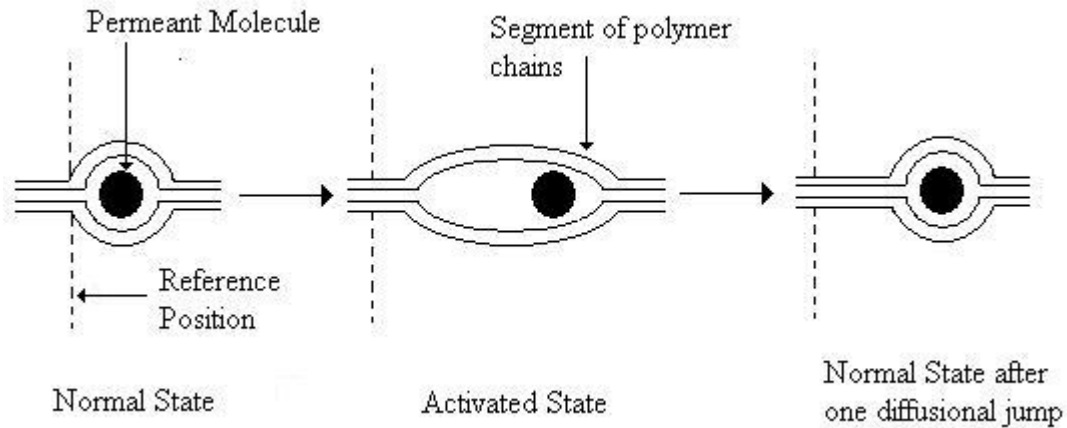
Figure 2.1. The Activation Process for Diffusion (Crank 2003)

The mathematical theory of diffusion called Fick's first law is based on the hypothesis that the rate of transfer of diffusing substance through unit area of a section is proportional to the concentration difference;

$$J = -D\frac{\partial c}{\partial x}$$ (2.2)

where J is the diffusive mass transfer rate of permeant per unit area, c is the concentration of permeant, x is the length and D is the diffusion coefficient (Crank 2003).

2.1.2. Solubility of Permeants in Packaging Films

Solubility is the partitioning behavior of a permeant molecule between the surface of the polymer and the surroundings headspace. It is a measure of the mass of permeant molecules sorbed by a unit of polymer mass per unit of partial pressure. The solubility coefficient, S describes the dissolution of a permeant in a polymer, and thus represents a thermodynamic property of polymer-permeant system. The solubility coefficient may be defined by an adaptation of the Nerst distribution function as:

$$S = \frac{c}{p}$$ (2.3)

8

where p is the vapor pressure of the permeant, c is the concentration of permeant and S is the solubility coefficient. The solubility coefficient is a function of temperature and may be a function of the vapor pressure (Crank 2003, Barr et al. 2000).

When S is independent of the sorbed permeant concentration and vapor pressure (i.e. for low permeant concentrations), then the relationship between c and p becomes linear and S is referred as the Henry's law solubility coefficient. This relation is often used to calculate the solubility coefficient from sorption isotherms, which are plots of the permeant concentration in the headspace above a polymer versus the concentration of the permeant within the polymer (Crank 2003).

2.1.3. Permeability of Packaging Films

Permeability is the steady-state rate of transport of a permeant molecule through a polymer of unit area per unit thickness as a result of the combined effects of diffusion and solubility. The permeability coefficient, P includes both kinetic and thermodynamic properties of the polymer-permeant system, and thus provides a gross mass transport property. The permeability coefficient is most commonly related to diffusion and solubility as given Equation 2.1 when both D and S are independent of concentration (Barr et al. 2000).

Several packaging material properties influence permeability, such as chemical structure, method of preparation, and processing, conditions, free volume, crystallinity, polarity, cross linking, presence of additives, and use of other polymer blends, moisture sensitivity and temperature, thus packaging film properties should be compared at as near identical testing conditions as possible as the conditions at which the test or analysis is carried out (Miller and Krochta 1997).

The barrier properties of packaging materials to gases and vapors correlate with permeability properties of material used. A barrier polymer inhibits permeant progress. Packaging materials do allow the transport of gases and vapors to some extent. Since quality and shelf life are reduced when food, through interaction with the outside environment, gains or loses moisture or aroma, takes up oxygen, there is a continuing interest in optimizing property sets of barrier packaging materials to provide an efficient and also economical method for packaging (Olabarrieta 2005).

Barrier properties are determined by the steady-state rate of mass transport through the films with constant D and S, and permeability coefficient can be defined by integrating Equation 2.1. with combining Equations 2.2 and 2.3 to obtain:

$$P = \frac{(dM / dt)_{steady-state} \times L}{A \times \Delta p}$$ (2.4)

where M is the quantity of permeant (which can be expressed as either mass or volume), t is time, L is the polymer film thickness, A is the cross-sectional area of the polymer, Δp is the partial pressure difference across the polymer. The term (dM/dt) is the slope of the transmission curve and is required to be at steady-state (Miller and Krochta 1997). In packaging industry, water vapor and oxygen permeability are two of the most important parameters that affect the food quality.

i) *Water Vapor Permeability*

Water vapor permeability (WVP) can be useful to understand possible mass transport mechanisms and solute and polymer interactions in all packaging materials. Water chemical potential difference is the driving force of the water vapor transfer through the packaging film. This driving force is proportional to water vapor concentration difference between two surfaces of films at constant temperature and pressure (Bertuzzi et al. 2007).

According to this driving force, water vapor transport through the packaging films follows typical pathway: absorption of water vapor on the packaging material surface, solution of water vapor into polymer matrix and diffusion through the polymer, and finally, desorption of water vapor from the other surface of the packaging material. It shows major differences between the hydrophobic and hydrophilic films, i.e. water vapor permeability of hydrophobic films, such as polyethylene, is independent of water vapor pressure. However, hydrophilic films due to interactions of permeating water molecules with polar groups, exhibit water vapor pressure-dependent permeability (Flores et al. 2007).

The determination of WVP is generally performed by sealing a specimen to the open mouth of a test cup containing either desiccant or water and placing the assembly into a controlled atmosphere (ASTM E-96). This allows the conditions with low

humidity on one side of the packaging film and high humidity on the other. The test cup is weighed periodically and the weight is plotted as a function of time. Water vapor transmission rate is taken as the slope of the curve (in the linear region) divided by the area of the dish opening as described above;

$$WVTR = Slope / A \tag{2.5}$$

The Equation 2.5 is multiplied by the thickness of the film and divided by the pressure difference to obtain the WVP;

$$WVP = WVTR \times L / \Delta p \tag{2.6}$$

where L is the film thickness (mm) and Δp is the partial pressure differences of water vapor at underside of the film and at the film surface. In addition to this calculation, WVP can be also determined from weight differences of test cups at the end of test duration by using data obtained from this standard method.

$$WVP = \frac{\Delta W}{A \times \Delta t} \times \frac{L}{\Delta p} \tag{2.7}$$

where ΔW is the weight gain by silica gel (g), A is the transfer area of the exposed film surface (m^2), Δt is test time (h).

The another alternative method to calculate WVP includes the assumption of one dimensional transport in the film that is taken place only according to Fickian Diffusion given as Equation 2.3. When the steady-state condition is quickly achieved in the film even though the concentrations of the water vapor at both sides of the film may change with time, the Equation 2.3 can be integrated from $x=0$ to L to find the following expression of the flux for constant diffusion coefficient:

$$J = \frac{D}{L} \times (C_{x=0} - C_{x=L}) \tag{2.8}$$

where C_1 and C_2 are the concentration of water vapor at both sides of the film. If a linear equilibrium relationship between the concentrations of the permeant in vapor and

the film is assumed where the concentrations become as $C_{x=0} = p_{x=0} S$ and $C_{x=L} = p_{x=L} S$, then the flux of the permeant is given as follows:

$$J = \frac{D \times S}{L} \times (p_{x=0} - p_{x=L})$$ (2.9)

Furthermore, the molecules permeating through the film cause an increase in partial pressure of the water vapor, then this increase can be expressed as follows if vapor phase is assumed to be ideal:

$$\frac{V}{RT} \frac{dp_{x=0}}{dt} = JA$$ (2.10)

If all these Equations (2.1), (2.9) and (2.10) are combined;

$$\frac{dp_{x=0}}{dt} = \frac{RT}{L} \times \frac{A}{V} \times P \times (p_{x=0} - p_{x=L})$$ (2.11)

and if partial pressure of the water vapor in the higher humidity is maintained constant by controlled atmosphere, then Equation 2.11 can be integrated between limits;

$$t = 0 \quad p_{x=0} = p_i$$
$$t = t \quad p_{x=0} = p_t$$

to give the following expression;

$$\ln \frac{p_{x=0} - p_i}{p_{x=0} - p_t} = P_m \times \frac{ART}{VL} t$$ (2.12)

Finally, WVP can be calculated from slope of $\ln(p_{x=0} - p_i)/(p_{x=0} - p_t)$ versus time. These test methods used in the permeability measurement should be used at most nearly food conditions (Hu et al. 2000). Table 2.1 shows water vapor permeability values of some conventional synthetic and biodegradable packaging materials.

Table 2.1. Water Vapor Permeability of Common Packaging Materials (Sources: Miller and Krochta 1997, Olabarrieta 2005, Tice 2003, Cuq et al. 1998).

Packaging Film	T (^{o}C)	RH (%)	WVP$\times 10^{12}$ (mol.m/Pa.sec.m^2)
High Density Polyethylene (HDPE)	38	90	0.0289
Polyvinylidene Chloride (PVDC)	27	100	0.0289
Low Density Polyethylene (LDPE)	38	90	0.0482
Ethylene Vinyl Alcohol (EVOH)	38	90	0.0964
Polypropylene (PP)	38	90	0.1238
Polyethyleneterephthalate (PET)	25	100	0.289
Polyvinylchloride (PVC)	28	100	0.868
Chitosan	25	11	1.928
Wheat Gluten (WG)	30	75	5
Corn-Zein (CZ)	20	75	6.45
Methyl Cellulose (MC)	30	11	8.676
Soy Protein Isolate (SPI)	28	80	77
Starch-Based	38	75	142

Normally, biodegradable films have quite high water vapor permeability compared to other packaging materials which are often used as moisture barrier coatings. The poor resistance of films to water vapor is due to the hydrophilic nature of biodegradable packaging materials and to the substantial amount of hydrophilic plasticizer added to increase flexibility.

ii) Oxygen Permeability

Oxygen permeability is another important as transport property of the packaging films. The most common quality loss of packaged foods resulted from the oxidation of its ingredients. Oxygen is strongly and irreversibly reacted with food ingredients. The oxidation of fats, oils and other food components produces off-flavors, and nutrient loss. Therefore, the protection of food against oxygen becomes one of the most important requirements for packaging. Good packaging barrier properties are critical for maintaining the initial quality of foods. The oxygen permeability of common synthetic packaging films and also biodegradable packaging films are given in Table 2.2. which

shows impressive oxygen barrier properties for biodegradable based films (Cuq et al. 1998)

Table 2.2. Oxygen Permeability of Common Packaging Materials (Cuq et al. 1998, Hong and Krochta 2004, Hong and Krochta 2006, Miller and Krochta, 1997, Olabarrieta 2005).

Packaging Film	T (^{o}C)	RH (%)	Oxygen Perm.$\times 10^{18}$ (mol.m/Pa.sec.m^2)
Chitosan	25	0	0.251
Polyvinylidene Chloride (PVDC)	25	90	0.40
Soy Protein Isolate (SPI)	25	90	2
Ethylene Vinyl Alcohol (EVOH)	25	90	6
Corn-Zein (CZ)	25	90	30
Polyvinylchloride (PVC)	23	50	35
Polyethyleneterephthalate (PET)	23	75	40
Whey Protein Isolate (WPI)	23	50	45
High Density Polyethylene (HDPE)	23	50	224
Polypropylene (PP)	25	50	450
Methyl Cellulose (MC)	30	50	522
Low Density Polyethylene (LDPE)	23	50	1003
Starch-Based	25	90	1085
Wheat Gluten (WG)	25	90	1290

For instance, O_2 permeability of chitosan films is 4000× lower than low density polyethylene (LDPE) and 150× lower than polyvinylchloride (PVC). Furthermore, O_2 permeability of corn-zein is 15× lower than polypropylene (PP) and also 30× lower than LDPE. Due to the large amount of hydrogen bonds, biodegradable films are hydrophilic, which makes them excellent barriers to non-polar substances such as oxygen. This hydrophilicity makes their oxygen barrier properties very much dependent on the humidity conditions for the measurements. That is why the gas permeability of these materials may increase manifold when humidity increases (Olabarrieta 2005).

In general, permeability of multilayer structures (water vapor and oxygen) can be approximately determined by following equation;

$$\frac{L}{P} = \sum_{i=1}^{n} \frac{L_i}{P_i} \qquad\qquad (2.13)$$

where n is the number of layers, L and P are the thickness and permeability of the multilayer film, L_i and P_i is the thickness and permeability of each layer, respectively. With the knowledge of intrinsic thickness and permeabilities, the permeability of multilayer structures can be calculated from Equation 2.13.

2.2. Mechanical Properties

Mechanical properties are important for packaging materials as barrier properties. Having sufficient mechanical strength and being free of minor defects ensure the integrity of a packaging film. Among the many mechanical properties of plastic materials, tensile properties are the most frequently considered, evaluated, and used throughout the industry. Tensile testing of films provides these useful data: yield strength, tensile strength, modulus of elasticity (Young's modulus), and elongation at yield and break (Olabarrieta 2005, Nielsen and Landel, 1994).

The maximum yield strength is the maximum tensile stress that a film can sustain. It is a very important property of the packaging films that gives information on the maximum allowable load before plastic deformation occurs. It is a measure of the strength of a polymeric material at the yield point. Tensile strength is the ultimate strength of the packaging film. Polymers are used under loads approaching their ultimate tensile strength in many packaging applications, for that reason tensile strength is also an important parameter. The yield strength and tensile strength can be increased or decreased by the addition of fillers to film structure. The increase in yield strength with increase in filler content is due to the ability of filler to carrying higher loads than the matrix. Young's modulus is the ratio of stress to strain below the elastic limit, gives information about stiffness of the packaging material (Nielsen and Landel 1994).

Interfacial adhesion between the fillers and the polymer matrix is also important factor affecting the tensile testing results of the packaging materials. For that reason, theoretical tensile yield strength and tensile strength of the packaging films are formulated corresponding to the cases of adhesion and no adhesion between the filler particles and the polymer matrix. In the case of no adhesion between the matrix and the

filler, the interfacial layer can not transfer stress. Quantitative information on the mechanical parameters of packaging films is also essential for the packaging design process. The test conditions are also important since the mechanical properties of hydrophilic films are also affected by the interactions with the surrounding environment. The stress-strain behavior of these polymers is strongly dependent on temperature and relative humidity. As temperature increases, Young's modulus and the tensile yield strength as well as maximum tensile strength generally decrease. However, the elongation at yield and break tend to increase. Additives and plasticizers also affect the mechanical properties. As the plasticizer content increases, tensile strength decreases and elongation increases (Olabarrieta 2005).

2.3. Optical and Surface Properties

Transparent packaging films are available as competitive products for food. Transparency and appearance of packaging films allow looking at packaged food without having to touch it, cutting down on bruising and other damages.

Appearance is affecting the seal ability of coated packaging film. Gloss is an appearance attributes highly demanded by packaging applications. From a physical point of view, gloss is related to amount of light scattered around specular direction and usually is associated with surface roughness in many cases. It is used extensively to quantify the visual appearance and functional properties of packaging materials. Gloss depends not only on the material properties but also on process variables (Hong et al. 2003, Alexander Katz and Barrera 1997).

Another aspect of appearance of packaging materials is haze, a term that refers to the tendency of light-transmitting packaging film to scatter light, producing a cloudy appearance as a result. The haze is largely aesthetic, although in extreme cases haziness may interfere with one's ability to distinguish details of contents in packages that are important to consumers (Hong et al. 2003).

The surface energy or surface tension is a controlling factor in the processes involving wetting and coating of packaging films. The surface energy of packaging films is found to be generally anisotropic, and depends on the spatial variations in surface and composition, surface roughness, and orientation of crystal-growth faces. The surface energy of packaging thin films can not be measured directly. The most

useful method is to measure the contact angles that several probe liquids make with the surface. The contact angles provide a simple and inexpensive technique for examining the surface energy of films. The surface energy of film is then related to the surface tensions of the liquids and contact angles. This method involves various assumptions and requires extrapolation of probe liquid contact angle results (Hong et al. 2003, Extrand and Kumagai 1997).

A greater understanding of these properties of packaging materials will allow packaging technologists to optimize the visual and printing characteristics of the coated films.

CHAPTER 3

BIODEGRADABLE BASED FOOD PACKAGING MATERIALS

Biodegradable packaging materials are materials derived from renewable resources. Today, to a large extent, packaging materials have been based on non-renewable materials. Biodegradable packaging materials are not made directly from petroleum resources, but from rapidly renewable agricultural feedstock. Since these biodegradable materials can be used for food packaging applications, the trend in packaging industry over 21st century has been towards the development of biodegradable plastics to replace existing synthetic products. Current research and studies in the area cover modified biodegradable polymers, coatings and also blends. In addition to the development of packaging materials from these polymers, researchers are employing various types to be active, intelligent and interactive. But these approaches still require further research and development before they become competitive to commonly used synthetic polymers (Kaplan 1998, Petersen et al. 1999, Cutter 2006).

3.1. Biodegradable Polymers

Biodegradable polymers are generated from renewable natural resources, are often biodegradable, and not toxic to produce. They can also be produced by biological systems. Over the past several decades, several biopolymers have received increased attention for their applications in chemical, biomedical and especially food industries. They have no adverse impact on human or environmental health (Weber 2000).

In general, biodegradable polymers are classified into three main categories according to their origin and method of production.

- Polymers produced from microorganisms or genetically modified bacteria. Polyhydroxyalkonoates (PHAs) are the important members of this group of biopolymers.

- Polymers produced by chemical synthesis using renewable bio-derived monomers, for example synthesis of polylactic acid (PLA) from lactic acid monomers.

- Polymers such as polysaccharides and proteins directly extracted from biomass.

These three categories are further outlined in Figure 3.1 (Weber 2000, Kolybaba et al. 2003).
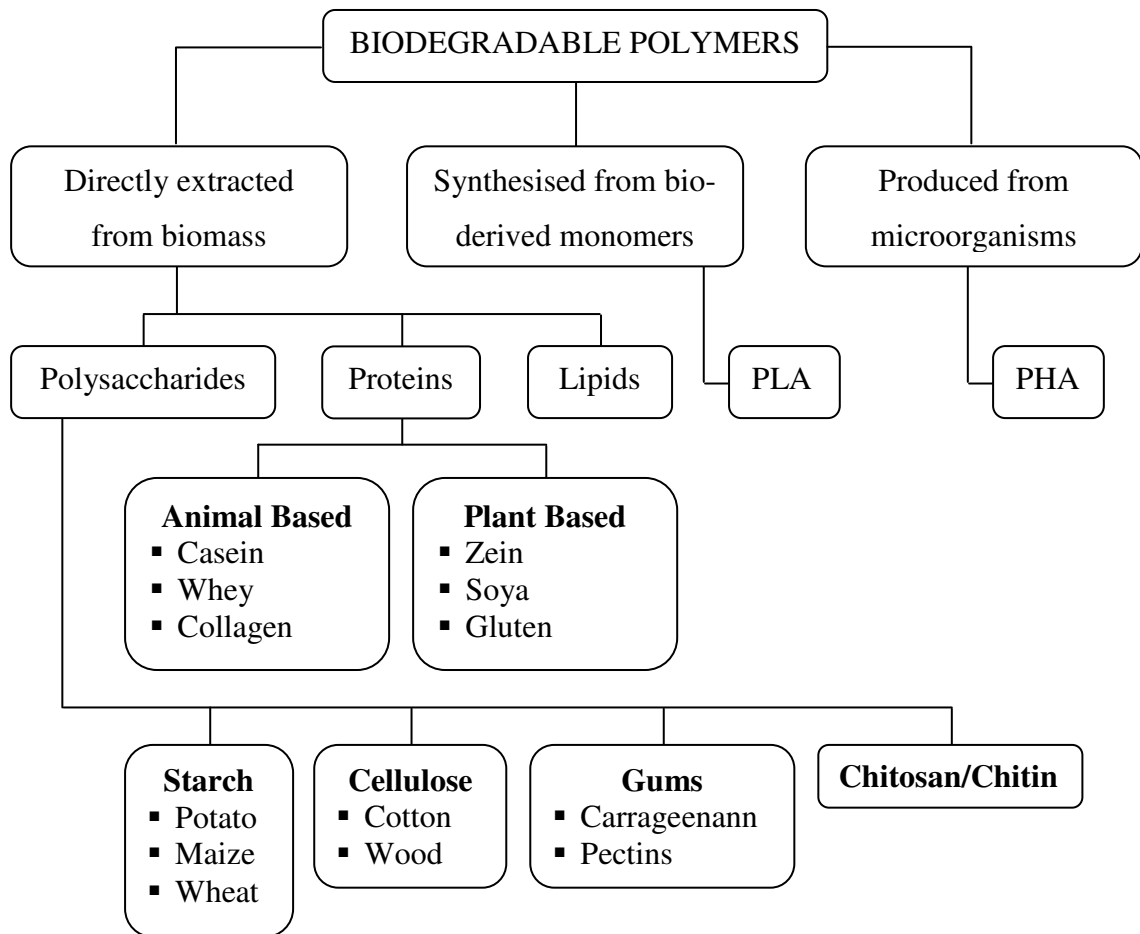
Figure 3.1. Schematic Presentation of types of biodegradable polymers

(Source: Weber 2000, Kolybaba et al. 2003)

3.1.1. Biopolymers Produced from Microorganisms

These biopolymers are synthesized by many gram-positive and gram-negative bacteria. They are accumulated intracellularly to levels as high as 90% of the cell dry weight under conditions of nutrient stress and act as a carbon and energy reserve. These

polymers are susceptible to microbial degradation; the microorganisms produce enzymes that cleave polymers into smaller segments amenable to metabolization by microbial flora (Nayak 1999).

Polyhydroxyalkonoates (PHAs) are important member of this family with renewable, biologically degradable and biocompatible properties. They are thermoplastic polyesters prepared by a bacterial fermentation process using a variety of feedstocks including especially glucose and acetic acid. Since the properties of PHAs are dependent on their monomer composition, a large variety of PHAs can be synthesized. The monomer compositions of PHAs are controlled by choosing the nature of the carbon source and microorganisms used (Reddy et al. 2003).

Polyhydroxybutyrate (PHB), a class of PHA's has been studied more extensively, and its presence has often been used as a taxonomic characteristic (Savenkova et al. 2000, Godbole et al. 2003). PHB is a typical highly crystalline thermoplastic. PHB has high melting point (175-180°C) similar to polypropylene (PP) and better oxygen barrier properties than polyethylene terephthalate (PET) and PP. Mechanical properties of PHB resembles to those of polystyrene (PS) and PP. It has a water vapor transmission rate about threefold lower than that of PP. All these properties are notable for food packaging applications (Weber 2000).

Applications that have been developed from PHB and similar based materials can be found in very different areas and cover especially packaging industry. Technically, the prospects for PHAs are very promising. When the price of these materials can be further reduced, application of biopolyesters will also become economically attractive (Holmes 1985, Westhuis et al. 2000).

3.1.2. Biopolymers Produced from Chemical Synthesis

The classical chemical synthesis using renewable biobased monomers for the production of biopolymers gives a wide spectrum of possible "bio-polyesters". The monomers themselves may be produced via fermentation of carbohydrate feedstock such as agricultural resources, e.g. corn or wheat, or alternatively agricultural waste products, such as whey or green juice (Weber et al. 2002).

Polylactic acid (PLA) synthesized by the condensation polymerization of lactides is most important member of this category with the highest potential for a

commercial major scale production of renewable packaging materials. PLA with high molecular weight PLA is a material with very high melting point, high crystallinity, and high tensile strength. These properties make PLA useful in packaging applications (Noyak 1999, Viljanmaa et al. 2002). In theory, all conventional packaging materials derived from mineral oil can be produced from renewable monomers gained by e.g. fermentation in the future. Today, this approach is not economically feasible due to the cost of the production of the monomers. Considerable research and development activities are dedicated to preparation and characterization of PLA based films (Petersen et al. 2001, Södergard and Stolt 2002, Chen et al. 2003).

3.1.3. Biopolymers Extracted from Biomass

This category includes the most commonly available biopolymers extracted from marine sources from animal and plants. Examples are polysaccharides such as cellulose, starch, and chitosan, and proteins such as casein, whey and zein, and also lipids. These polymers represent truly renewable resources since they are biodegradable. All are considered environmentally acceptable degradable polymers (Chandra and Rustgi 1998, Weber et al. 2002).

3.1.3.1. Lipids

The coating of foods with fats has been performed to reduce shrinkage of food product, as well as to provide oxygen or moisture barriers. Waxes and other types of fat-based oils also have been added to protein- or polysaccharide-based films to impart flexibility and to improve coating characteristics. There are numbers of advantages for coating foods with lipids. Lipids not only impart hydrophobicity, and flexibility, but they also provide excellent moisture barrier due to the tightly packed crystalline structure of lipids (Weber 2000, Cutter 2006). Many studies have aimed to investigate the factors affecting the water vapor permeability of films for the lipid presence (Garcia et al. 2000, Morillon et al. 2002). Despite these advantages, lipid-based films at higher storage temperatures may show lower permeability to gases such as oxygen, carbon dioxide leading to potentially anaerobic conditions which may cause food safety issues. Another problem is poor adherence of lipid films to hydrophilic surfaces.

3.1.3.2. Polysaccharides

The principal polysaccharides (especially starch, cellulose, and chitosan) of interest for packaging material production have been especially cellulose, starch, gums, and chitosan. These polymers generally contain more than one type of carbohydrate unit, and in many cases these polymers have regularly arranged branched structures (Weber et al. 2002).

The packaging films made from polysaccharides showed excellent gas permeability properties resulting in desirable packaging material that improve the shelf-life of the food product. Additionally, polysaccharide coatings on synthetic materials were observed as functional to extend the shelf-life of packaged food by preventing dehydration, oxidative rancidity, and surface browning. However, because of the structure of the polymer chains, polysaccharide films showed high oxygen permeability; but their hydrophilic nature makes them poor barriers for water vapor (Immirzi et al. 2003).

Starch and Derivatives: Starch, the storage polysaccharide of cereal grains and potatoes, is composed of amylose and amylopectin, and also it is a renewable and widely available raw material suitable for a variety of packaging applications. Starch alone does not form packaging films with sufficient mechanical properties unless it is first treated by either plasticization or blending with other materials. Starch is economically competitive with petroleum and has been used in several methods for preparing compostable plastics. It provides a good barrier to oxygen and carbon dioxide transmission. However, one of the most important limitations of the use of starch for packaging films or coatings is the hydrophilicity of this material which makes them poor barrier to water vapor (Chandra and Rustgi 1998, Weber 2000, Cutter 2006).

Literature showed that starch-based films exhibit physical characteristics similar to synthetic films in where they can be odorless, tasteless, colorless, non-toxic, semi-permeable to carbon dioxide and resistant to oxygen. The incorporation of plasticizers into starch resulted in more flexible and higher water vapor permeability with lower tensile strength, higher elongation. The films containing high starch content are more flexible (Rodriguez et al. 2006, Bertuzzi et al. 2007). Starch blend systems were also studied by several researches to design a novel packaging structure (Myllymaki et al.

1998, Wang et al. 2003, Baldev et al. 2004). The water vapor permeability of these films was increased by an addition of starch into the biodegradable polyesters. For polypropylene (PP)-starch blend films, it was observed that there is a gradual decrease in tensile strength and elongation of the films with an increase in starch content, while increase in water vapor and oxygen permeability were also observed (Wang et al., 2003; Baldev et al. 2004). Furthermore, Myllymaki et al. investigate that the addition of polycaprolactone (PCL) had a significant effect on the mechanical and water vapor permeability properties of the starch films and improved the processability. The extrusion blending of PCL into starch improved the mechanical properties and water vapour barrier properties relative to those of pure starch films (Myllymaki et al. 1998).

Cellulose and Derivatives: Cellulose is the most abundantly occurring natural polymer and is an almost linear polymer of anhydroglucose, a non-digestible component of plant cell walls. It is a fully biodegradable, water-insoluble renewable resource and a cheap raw material, but is difficult to use because of its hydrophilic nature, insolubility and crystalline structure. Cellulose based packaging materials have mechanical properties comparable to those of polystyrene (PS) and they can be melt processed using the same techniques adopted for commodity thermoplastic polymers by addition of a suitable plasticizer (Nayak, 1999). The most commonly used cellulose derivatives produced are methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, and cellulose acetate. Cellulose acetate among cellulose derivative polymers is most used polymer in food packaging applications. Although many cellulose derivatives have excellent film-forming properties, they are simply too expensive (Weber 2000). Studies demonstrated that cellulose based films applied to food products can reduce moisture loss resulting in improvement of shelf-life. The films or coatings made from cellulose derivatives were transparent and readily peel able and prevent desiccation. Furthermore, they had good mechanical, oxygen barrier properties for food packaged with appropriate formulation (Park et al. 1993, Park et al., 1995, Erdohan and Turhan, 2005).

Chitosan/Chitin: Chitosan is the second most abundant polymer available in nature which is an edible and biodegradable polymer derived from chitin found in the major structural component of the exoskeleton of invertebrates (crab shrimps, krill, etc.) and of arthropods (insects, crustaceans, and some fungi). Some desirable properties of chitosan are that it has good film forming property without the addition of any additives,

shows good oxygen and carbon dioxide permeability, as well as excellent mechanical properties and it has been widely used as an edible coating. Furthermore, chitosan may also be used as coatings on other packaging films to improve gas barrier properties (Tharanathan 2001, Srinivasa and Tharanathan 2007). However, one disadvantage of chitosan is its high sensitivity to moisture. Its film properties are also affected by plasticizer and solvent used. Caner et al. (1998) studied the effects of acid concentrations, plasticizer concentration and storage time on mechanical and permeability properties of chitosan films. They observed the significant decrease in mechanical, water and oxygen barrier properties with increasing plasticizer content and neither property was affected by storage time (Caner et al., 1998).

In addition, since the extraction of chitosan is so expensive, the production volumes are relatively much lower than cellulose. Thus its production cost is more expensive compared to plastic packaging films even though other biopolymers are much cheaper (Olabarrieta 2005). So far, studies indicated that usage of chitosan by itself has been limited. Therefore, many different studies have been focused on the combination of chitosan with other biopolymers to make a desirable food packaging materials (Park et al. 2001, Suyatma et al. 2004). Suyatma et al. (2004) studied mechanical and water vapor barrier properties of chitosan with PLA. The results showed that the incorporation of PLA to chitosan improved water vapor barrier properties; however tensile strength and elastic modulus decreased with the addition of PLA.

3.1.3.3. Proteins

A protein can be considered to be a random copolymer of amino acids and the side chains that are highly suitable for chemical modification to adapt the required packaging properties. Proteins can be divided into proteins from animal origin (e.g. whey protein) and proteins from plant origin (e.g. corn-zein). Protein-based packaging films adhere well to hydrophilic surfaces, provide barriers for oxygen and carbon dioxide, but do not resist water vapor diffusion. Due to their excellent gas barrier properties, packaging materials based on proteins are highly suitable of packaging applications (Gennadios 2002). The major disadvantage of all protein-based film is their sensitivity towards relative humidity. Therefore, blending and especially coating on other packaging films may overcome this challenge with lower sensitivity towards

humidity (Cutter 2006). Among biopolymers, the extremely low oxygen permeability of protein films, in addition to good gloss and mechanical properties, makes them potentially useful as a transparent coating material for improving the oxygen-barrier property of food packages (Cuq et al. 1998).

Whey Protein: Whey proteins are by-products from the cheese production and are separated at a range of different levels of purity. They have a relatively high nutritional content. Since whey proteins are available in large amounts world-wide, extensive studies were performed to their use as edible coatings and films (Olabarrieta 2005). Today, most research has focused on film formation and film properties of whey proteins. However, more research is still needed to better understand the effect of film structure on film properties. Whey protein has been shown to make transparent films with good oxygen and aroma barrier properties. Whey protein films produce transparent, blend, flexible water based edible films with excellent oxygen and aroma barrier properties at low relative humidities. They are readily processable and have some potential as exterior films, if suitable modification strategies can be developed to reduce moisture sensitivity. These films are also characterized by their water insolubility, which can be beneficial in maintaining film and food integrity. However, their hydrophilic nature causes them to be less effective moisture barriers (Miller and Krochta 1997, Sothornvit and Krochta 2000, Kim and Ustunol 2001, Fang et al. 2002).

Corn-Zein Protein: Corn-zein proteins are by-products from the corn wet-milling industry and comprise a group of alcohol soluble proteins (prolamines) found in corn endosperm. It is a thermoplastic protein with a hydrophobic nature, which is related to its high content of non-polar amino acids. Corn zein protein has been used as a good renewable and biodegradable material for package film forming, coatings, and plastics applications (Shukla and Cheryan, 2001). It has excellent film forming properties and can be used for fabrication of biodegradable films. Therefore, the most promising application of zein appears to be for biodegradable films and coatings for packaging applications. These films are formed by drying of alcoholic aqueous dispersions. They have been shown to have higher strength and lower gas permeability which are required for packaging applications than other biopolymer films. All these studies are based on free standing corn-zein films (Lai and Padua 1997, Miller and Krochta 1997, Gioia et al. 2000). However, because of high gas barrier properties of corn-zein films, the

combination of it with other polymers should be put into practice. Therefore, further information about the characteristics and production of zein films are considered in the next section of this chapter.

3.2. Corn-Zein as Packaging Material

Zein (prolamin of corn) is located in small round particles (1-2 µm in diameter) called protein bodies in maize endosperm. It constitutes 47% of the total protein in corn. Zein is produced commercially from corn gluten meal, which is a coproduct obtained during starch production from corn by ethanolic extraction and has low price. Zein has been examined as a possible raw material for polymeric applications since the early part of the 20th century. It offers several potential applications for packaging films, coatings and plastic applications. The ability of zein and its resins to form tough, glossy, flexible and hydrophobic coatings have been of commercial interest (Lawton 2001).

3.2.1. Characteristics of zein

Zein is to be found in mixture of peptides varying in molecular size, solubility and charge depending on the corn variety and separation method used. It is distributed uniformly throughout the cytoplasm of corn endosperm cells between starch granules. Zein's defining characteristic is its insolubility in water except in the presence of alcohol due to its amino acid composition. Zein is particularly rich in glutamic acid (21-26%), and non-polar groups of leucine (20%), proline (10%), and alanine (10%) (Shukla and Cheryan 2001). The hydrophobic properties of zein are primarily due to the larger amounts of these peptides. Furthermore, this high proportion of non-polar amino acid residue is responsible for the solubility behavior of zein (Swallen 2002).

Guo and coworkers studied on nanostructure properties of zein by atomic force microscopy (AFM) to observe and investigate protein bodies in maize endosperm. They observed a series of zein globules narrowly distributed between 1-5 µm in diameter with heights ~20 nm (Guo et al 2005).

According to molecular size and solubility of proteins present in zein, zein can be classified into different categories such as α and β-zein. α-Zein is by far the most abundant, accounting for 70% of total prolamine present in corn. β-Zein is soluble in

60% ethanol and insoluble in 95% ethanol. This zein is relatively unstable, precipitating and coagulating frequently. It is not a constituent of commercial zein preparations. α-Zein can be extracted using only aqueous alcohol, whereas the other β-zein needs a reducing agent in the solvent to be extracted. Commercial zein is made up of α-zeins. (Lawton 2004, Momany et al. 2006).

3.2.2. Solvents for Zein Films

Zein is known for its solubility in binary solvents containing a lower aliphatic alcohol and water, such as aqueous ethanol (50-95%), but not in anhydrous alcohol solutions (except methanol) and for its insolubility in water alone. Therefore, aqueous alcohols are the most common solvents for zein today. Also, several researches based on zein isolation by using aqueous alcohols because alcohols are easily recovered from the zein. Dickey and coworkers studied the ethanolic extraction of zein. Zein was found to be dispersible in high concentrations of alcohol and in absolute alcohol when temperatures are above the normal boiling point of the solvent. Similarly, they dissolved zein in 40% ethanol at high temperatures. For lower concentrations of ethanol, zein had a tendency to denature before reaching the temperatures required for it to disperse (Dickey et al 2001, Swallen 2002).

Table 3.1 lists the solvents for zein generally used in studies and their critical cloud point temperatures (peptization) at which the film solution turns cloudy upon cooling. For a single substance to be a good solvent for zein, the molecule needs to have the proper balance between polar and non-polar groups. Otherwise, non-homogenous film forming solutions are obtained in which the final film properties are affected. For example, propylene glycol is a good solvent for zein, but absolute propanol is not. Adding additional hydroxyl groups seems to lower the solvent power. Propylene glycol can dissolve zein at room temperature, whereas glycerol needs to be heated to 150°C. Polypropylene glycols with a molecular weight >3,000 do not dissolve zein at all (Shukla and Cheryan, 2001, Lawton 2002).

Researchers noted that aqueous ethanol solution in which zein is freely soluble is the best solvent to form zein films. It is known that temperature and the ratio of the aqueous ethanol in film forming solution have a significant affect on homogeneity of film forming solutions and of course on final properties of zein films.

Table 3.1. Solvents for zein film forming solutions.

(Source: Lawton, 2002)

Solvent	Temperature, $^{o}C^{*}$	Solvent	Temperature, ^{o}C
Acetic Acid	14	Glycerol	139
Acetone (aqueous)	70	Isobutanol (aqueous)	≤40
Chloroform	20	Isopropanol (aqueous)	≤40
Diethanolamine	30	Lactic acid	≤40
Diethylene glycol	≤40	Methanol	63
Diethylenetriamine	≤40	Phenol	40
Ethanol (aqueous)	≤50	Propanol (aqueous)	≤40
Ethylene glycol	18	Propylene Glycol	≤40
Ethylenediamine	11	Trietyhlene Glycol	≤40
Formic acid	7	Toluene	≤40

*Temperature at which the 10% (w/v) zein solution became cloudy.

The solubility behavior of zein is shown in the form of for a ternary phase diagram in Figure 3.2 (Shukla and Cheryan 2001). At constant temperature, the solubility of zein varies between 2 and 60% (w/v), depending on the ethanol concentration. At lower (<50%) and higher (>95%) concentrations of ethanol, two liquid phases appear. This corresponds to a transition state between complete solubilization and precipitation of zein. Higher temperatures increase the solubility curve and temperatures below $50^{o}C$ give non-homogenous solutions, thus this phenomenon has been used to form zein films. The zein solutions prepared above $50^{o}C$ temperatures evaporate the ethanol and give an increasingly concentrated solution without discontinuity. The formation of precipitates at low ethanol concentrations can be suppressed by low temperatures (Dickey et al. 2001, Shukla et al. 2000).
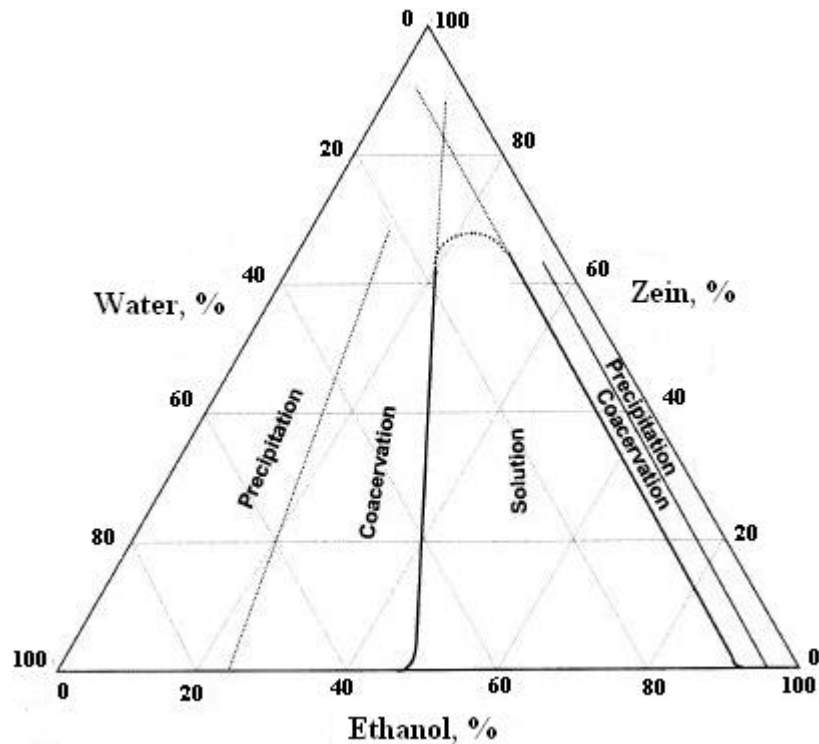
Figure 3.2. Ternary Phase Diagram for the Solubility of Zein in Aqueous Ethanol at T=50°C (Source: Shukla and Cheryan 2001).

Furthermore, the study by Yoshino and his workers compared final properties of films in which aqueous ethanol and acetone used as solvents. Tensile strengths of films made from ethanol solution were higher than films from acetone. There was no large difference in water vapor permeabilities between the zein acetone films and the zein ethanol films. However, zein films obtained by ethanol solution showed good oxygen barrier property (Yoshino et al. 2002). Therefore, studies showed that appropriate solvent for zein can be ethanol and film forming temperature should be at least 50°C.

3.2.3. Plasticizers for Zein Films

The packaging films formed by zein are brittle because of strong intermolecular forces between the protein chains. Thus plasticizers are needed to improve their flexibility. Plasticizers are low molecular weight organic compound. Plasticizers added to zein solutions to reduce these attractive intermolecular forces by modification of the three-dimensional organization. They also increase the mobility of the protein chains

and free volume, resulting in flexibility and extensibility (Lawton 2002). As with solvents for zein, plasticizers for zein should satisfy a proper balance between polar and non-polar groups. Therefore, they must be compatible with the polymer. Researches showed that plasticizers tabulated as in Table 3.2. can be added to zein film forming solutions at different concentrations ranging from 10 to 60% of dry weight, depending on the rigidity of the matrix polymer. In addition, according to these studies, glycerol, oleic acid, polyethylene glycol, sugars and stearic acid were suggested as some of the more useful plasticizers for zein (Lai and Pauda 1997, Gioia and Guilbert 1999, Paramawati et al. 2001, Lawton 2004).

Table 3.2. The most common plasticizers for zein film forming solutions.
(Source: Lawton, 2002)

Plasticizer Type	Plasticizer Type
Glycerol	Polypropylene Glycol
Dibutyl Tartrate	Propylene Glycol
Lauric Acid	Sugars (glucose, fructose)
Oleic Acid	Stearic Acid
Polyethylene Glycol	Water

Although water is generally not used as plasticizer in corn protein film forming solutions, numerous studies have reported that of water has great effect on glass transition temperature (T_g) of zein (Madeka and Kokini 1996, Gioia and Guilbert 1999). Madeka and Kokini investigated zein-water interactions by differential scanning calorimety (DSC). They reported that even a few percentages of water resulted in a dramatic decrease in T_g of zein (Madeka and Kokini 1996). In addition to this study, Santosa and Padua reported decrease in T_g of zein plasticized with oleic acid (Santosa and Padua 2000).

Many studies have been also reported on the effects of plasticizers on mechanical and barrier properties of zein films (Lai et el. 1997, Parris and Coffin 1997, Lai and Padua 1998, Tillekeratne and Easteal 2000, Paramawati et al. 2001, Wu et al. 2003, Lawton 2004, Ghanbarzadeh et al. 2006, Ghanbarzadeh et al. 2007).

Lai and coworkers observed the mechanical and water absorption properties of zein films plasticized with stearic acid. Tensile strength increased with addition of low

levels of plasticizer. However, beyond a critical point, tensile strength decreased with further addition of plasticizer. They also observed decrease in water absorption with increasing plasticizer content. They concluded that zein-stearic acid interaction is responsible for these improvements in zein films (Lai et al. 1997).

In another study of Lai and coworkers the effects of oleic acid (OA) plasticization and environmental conditions on water and oxygen barrier and mechanical properties of zein films were investigated. The films containing higher OA at high relative humidity conditions resulted higher water and oxygen permeability values. Toughness, elongation and tensile strength of zein films were considerably improved with OA when relative humidity increased from 50 to 85%. They obtained zein films with good mechanical properties and could be used as potential food packaging films through humidity levels from 50 to 98% depending on food applications (Lai and Padua 1998).

Parris and Coffin examined the composition factors (solvent and plasticizer type) affecting the water vapor permeability (WVP) and mechanical properties of corn-zein films. Zein films containing no plasticizers found to be have the lowest WVP values. The films prepared in acetone had lower WVP values than those prepared in ethanol. Incorporation of plasticizers, polyethylene glycol (PEG) and glycerol (GLY) into zein films significantly reduced water vapor barrier properties. However, films prepared with PEG were more flexible than GLY plasticized films resulting in an almost doubling in tensile strength values. As a result, elongation and strength of the films increased approximately a 2-3 fold without significantly compromising water vapor barrier properties (Parris and Coffin 1999).

Tillekeratne and Easteal improved the water resistance of zein films by using PEG as a plasticizer (Tillekeratne and Easteal 2000). Wu et al. studied on dibutyl tartrate (DBT) plasticization to improve toughness and water resistance properties of zein based films. The toughness was improved significantly by a small amount of DBT due to the existence of microphase separation structure in the sheets. In particular, zein films exhibited better mechanical properties when modified by 20–50 wt% DBT, and when the corresponding sheets were immersed in water for 24 h then when stored in 75% relative humidity. This implies an excellent water resistance (Wu et al. 2003).

Paramawati et al. used lauric acid (LA) as plasticizer to evaluate characteristics changes in tensile and barrier properties of zein films. They noted that incorporation of LA decreased the elastic modulus value by 45%, while an increase in film flexibility

was obtained at higher values of elongation of break. Water vapor permeability through the zein film decreased with LA addition into zein (Paramawati et al. 2001).

The final two studies based on characterization of zein films plasticized with sugars were done by Ghanbarzadeh and his coworkers (galactose, glucose, and fructose). They found that zein films containing galactose have better tensile properties than other films plasticized with sugar. The addition of sugars decreased T_g of all samples considerably and T_g values were lower than the value observed in zein films plasticized with oleic acid prepared. However, there was not any significant difference between T_g of the three sugar plasticizers due to the similar molecular weights. Furthermore, they observed high WVP for pure zein film and adding sugars caused a decrease of WVP. The significant decrease in WVP was obtained for the films plasticized with galactose (Ghanbarzadeh et al. 2006, Ghanbarzadeh et al. 2007).

As a summary, researches on plasticizers used for zein film making showed that plasticizers should be absolutely used to overcome film brittleness for corn-zein. Furthermore, the proper amount of these plasticizers can improve characteristic properties of zein films. According to previous study results, especially polyethylene glycol (PEG) and glycerol (GLY), and sugars found to be the most suitable and common plasticizers for film forming solutions of zein.

3.2.4. Packaging Applications of Zein Films

One of the most promising applications of corn-zein appears to be for biodegradable films and plastics for packaging because of its film forming ability. Recent interest in the development of edible and biodegradable films has driven research on the formation of free-standing zein films for food and packaging applications. Zein can form tough, glossy, hydrophobic films with good flexibility and compressibility. Films have good mechanical strength and barrier properties especially for oxygen (Cuq et al. 1998). Even though solvent type, plasticizer content, type of substrate used for casting, and drying conditions vary among studies, the general film-forming procedure is always the same. To prepare films, zein is dissolved in an appropriate solvent and plasticizers are added to this solution. Then, the solution is cast onto a non-stick substrate and the solvent is allowed to evaporate. Finally, the free-standing film is peeled off from the nonstick substrate (Lawton 2002).

Although solution casting method for zein film preparation is the most common method in literature. Some researchers have also investigated preparation of zein films by molding process and blown film extrusion (Lai and Padua 1997, Wang and Padua 2003, Wang and Padua 2004, Wang et al. 2005). Lai and coworker investigated the effect of film preparation methods on the zein film properties. They compared a method involving plasticization of zein with oleic acid to form an intermediate moldable resin and conventional film casting method. It was observed that cast films resulted in stiff and brittle material property, whereas resin films showed more flexible and tougher films. Differences in mechanical properties were also obtained and attributed to the microstructure development and orientation (Lai and Padua 1997).

In addition to solvent casting and resin stretching methods, Wang and Padua have studied and reported the feasibility of extrusion forming of zein films through slit die and blow head. The extruded zein sheets showed higher elongation at break, lower tensile strength and lower modulus than non-extruded samples. Since blown film extrusion was affected by feed moisture content and barrel temperatures, these parameters were also optimized. Similar tensile properties were observed for film samples, blown after extrusion and slit die extruded samples. However, water vapor permeability of films produced by blown extrusion was lower compared to films by solvent casting. (Wang and Padua 2003, Wang and Padua 2004). Further study by Wang and coworkers was performed to analyze and compare the microstructure of the films prepared by solvent casting, and resin stretching. The cast film cross-section showed a large amount of voids of several micrometers in size, which were believed to be caused by evaporated solvent droplets. However, the voids were not observed on cross-section of stretched films and also no apparent characteristics were detected on the surface of these films. The extruded sheets had a great number of irregular voids caused by entrapped air and water droplets. During extrusion blowing, the most of the voids were eliminated to result in more uniform morphology with fewer and smaller voids in where films became more transparent (Wang et al. 2005). Therefore, these results explain the earlier findings of Wang and Padua related to the permeability decrease in blown extruded films.

There have been several reports in literature related to the possible use of zein blends with biodegradable polymers such as starch (Corradini et al. 2004), and synthetic polymers (Herald et al. 2002) or chemical modification of zein with polycaprolactone (PCL) (Wu et al. 2003). Wu and his coworkers established improvement of mechanical

properties of zein by modification with PCL in this study. Corradini et al. studied the mechanical and morphological properties of blends of starch and zein plasticized with glycerol. They reported that the Young's modulus and tensile strength increased with increasing zein content. On the other hand, increasing glycerol content caused a decrease in mechanical property of blends. It was found that starch-zein blends are immiscible in the range of composition studied (Wu et al. 2003, Corradini et al. 2004).

Herald et al. studied the tensile properties of extruded corn zein with low density polyethylene (LDPE). The tensile strength of LDPE decreased with zein addition. With increasing the level of zein in the blended films, the tensile properties decreased (Herald et al. 2002).

For the last years, several different studies based on usage of zein as a food coating were presented as its further food based applications (Park et al. 1994, Wong et al. 1996, Herald et al. 1996, Bai et al. 2003). Park and coworkers coated tomatoes with zein to increase their shelf life. Shelf life of the coated tomatoes was extended by six days. Zein coatings had been tried as protective coating for eggs by Wong and his workers. Eggs coated with zein also exhibited low moisture loss and maintained their freshness for 21 days of storage at room temperature (Park et al. 1994, Wong et al. 1996). Herald compared the properties of corn-zein with PVDC polymer and observed no significant differences in elongation, tensile strength, or Young Modulus values of corn zein and polyvinylidine chloride (PVDC) films (Herald et al. 1996). Bai and coworkers have studied the possibility of zein coating on apples to improve their shelf life. They observed an increase in gloss with higher amounts of zein in coating formulations. Whitening was observed on the coated apple surfaces upon wetting. This was reduced by decreasing zein content. It was found that permeability to oxygen and water vapor through zein films was strongly dependent on the zein content in the coating (Bai et al. 2003).

Studies summarized above have been about the applications of free-stand alone zein films and only some of them were about zein coatings on foods directly. These studies showed that coatings based on zein films appear to be one of its most promising applications due to its low gas permeability properties.

Recently, it has been shown that biopolymer coating on synthetic polymers could be developed as a novel approach to obtain better film properties (mechanical and barrier properties). In this work, this approach has been explored for the use of corn protein (zein) coatings with an oxygen barrier function on a conventional synthetic

packaging film, polypropylene (PP) to improve the properties of synthetic monolayer PP films.

3.3. Biopolymer Coated Food Packaging Materials

The coated packaging films comprise a unique category of packaging materials varying from biodegradable polymers to conventional polymers. Coated packaging materials differ in their function, mode of formation and also application to foods. They are formed by casting and drying film-forming solutions on a substrate film. The biopolymer coated food packaging materials provide barriers towards moisture, oxygen (O_2), carbon dioxide (CO_2), aromas, and/or improve the mechanical integrity or handling of the food product. They may be used to reduce the amount of synthetic packaging materials and allow reprocess of multilayer, multi-component packaging materials (Weber 2000, Cutter, 2006).

When it comes to improvements in conventional packaging films, most research has addressed formulations on coated films using various combinations of biopolymeric materials (Shin et al. 2002, Hong and Krochta 2004, Hong et al. 2004, Hong and Lee 2004, Hong and Krochta 2005, Mohareb and Mittal 2006). The additional study about coating of biopolymers onto synthetic plastic films to improve their properties was carried out by Shin and coworkers. Chitosan and corn-zein were used to coat low density polyethylene (LDPE). The plasma source ion implantation technique was used to improve the adhesion between LDPE and biopolymer coating because of the non-polar surface of LDPE film. After modification, chitosan or corn zein was used for coating on LDPE. Bilayer films coated with chitosan or corn zein showed 10 times lower oxygen permeability. Tensile strength of multilayer films was decreased a little compared with that of LDPE. It was concluded that the plastic and biopolymer multilayer films have potential for food packaging application because of their O_2 gas barrier properties and easy recyclability of the multilayer film (Shin et al. 2002).

Hong and his coworkers have studied the coatings of biopolymer on several conventional plastic films such as PP, polyethylene (PE) and polyvinylchloride (PVC). Their first work was aimed to examine the feasibility of glycerol plasticized whey protein isolate (WPI) coating on low density polyethylene (LDPE) films as an alternative oxygen barrier in the composite structure for food packaging. WPI-coated

LDPE films showed significant decrease in oxygen permeability, therefore they concluded that WPI coating could work successfully as an oxygen barrier (Hong and Krochta 2004). Hong et al have also studied optical and surface properties of whey protein coatings on PP and PVC films as influenced by substrate, protein concentration and plasticizer type. Regardless of the base films, WPI-coated films possessed excellent gloss and no color, as well as good adhesion between the coating and the base film when an appropriate plasticizer was added to the coating formulations. The WPI concentration did not significantly affect gloss of WPI-coated plastic films. Among the five plasticizers (Glycerol, polyethylene glycol, polypropylene glycol, sorbitol and sucrose) applied, sucrose conferred the most highly reflective and homogenous surfaces to the coated films. Therefore, it was concluded that WPI coatings formulated with a proper plasticizer can improve the visual characteristics of the polymeric substrate (Hong et al. 2004).

Then, the further study by Hong and Krochta was carried out to understand the effect of temperature, relative humidity (RH) and base films (polypropylene, PP and polyethylene, PE) on oxygen barrier performance properties of WPI-coated plastic films. The resulting whey-protein-coated films showed increase in oxygen permeability (OP) as temperature increased. An exponential effect of RH on OP with excellent oxygen-barrier properties was observed at low to intermediate RH. Furthermore, overall OP values of the WPI coated PP were found to be significantly lower than those of the coated PE. Consequently, the good oxygen barrier was obtained under low moisture conditions (Hong and Krochta 2005).

Last study of Hong and coworkers was based on preparation of plasticized polysaccharide coatings on PP film to characterize optical and tensile properties of the resulting coated films as affected by biopolymer (carrageenan, chitosan, dextrin, hydroxy methylcellulose, methylcellulose) and plasticizer type (glycerol, polyethylene glycol, polypropylene glycol, sorbitol and sucrose). Results indicated that chitosan could be used as an excellent coating on PP films with proper plasticizers, and resulting films have better mechanical and optical properties than the other coated films Hong and Krochta 2005).

Mohareb and Mittal investigated the effects of the biopolymer (soy protein isolate, gellan gums, and carrageenan) and plasticizer types (glycerol, mannitol, and sorbitol) and their concentrations on mechanical properties of the coated polystyrene (PS) films. The results showed that when soy protein isolate plasticized with glycerol at

low levels was coated on PS, higher tensile strength was obtained. Gellan gums coated films provided more uniform color while carrageenan coating exhibited browning. They concluded that glycerol was the most suitable plasticizing agent, as the coatings did not fail completely when it was used (Mohareb and Mittal 2006).

Studies showed that biopolymer coatings on conventional plastic films have potential as an alternative and to replace existing expensive coatings in multilayer films for packaging applications. Especially, whey protein films are the most used one and formed as a transparent coating material for improving the oxygen-barrier property of packaging materials. It can be expected that a multilayer film could be obtain with a high barrier, mechanical and surface properties by coating common plastic films such as LDPE and PP with corn-zein. In addition, the corn-zein coatings can be separated chemically or enzymatically from the substrates, enhancing recyclability and reutilization of the plastic layer.

CHAPTER 4

EXPERIMENTAL

4.1. Materials

Corn zein (regular grade) used in this work was obtained from Sigma-Aldrich. Ethyl alcohol (99,5%) used as a solvent was supplied from Panreac and it was diluted with distilled water to prepare two different solvent concentrations which are 70 v % and 95 v %. Polyethyleneglycol (PEG) (M_w=400 g/mol), and Glycerol (GLY) (M_w=92 g/mol) used as plasticizer to overcome film brittleness was obtained from Merck and Sigma, respectively. Commercial corona discharge-treated polypropylene (PP) cast film of 40 µm thickness (C11/40 µm) obtained from Polinas Company (Manisa, Turkey) was used as a substrate for corn-zein coatings.

4.2. Preparation of Corn-zein coated PP films

Corn-zein film solutions were prepared by dissolving zein with two different concentrations (5% and 15% (w/v)) in 70 v % and 95 v % aqueous ethanol solution, respectively. The solution was stirred on a magnetic stirrer for 2 hours at 50°C. Zein solutions were plasticized by both polyethylene glycol (PEG) and glycerol (GLY) with various concentration levels which were 20% and 50% based on corn-zein weight. Following the addition of plasticizer, stirring was continued for a further period of 2 hours. Then, film solutions were cast by using automatic film applicator (Sheen 1133N, England) on corona discharge-treated polypropylene (PP) film with wet coating thickness of 30 μm. Corn-zein coated PP films were dried at 50°C for 2 hours under vacuum of 200 mbar. Then, the temperature of the vacuum oven was increased to 120°C to allow further evaporation of solvent.

4.3. Film Thickness

Thicknesses of the films were measured with an electronic digital micrometer (Mitutoyo, Japan) which has a sensitivity of 0.001 mm and measurements were taken at least from five random positions on each film. Then, the mean value was used for the calculations in water vapor permeability and mechanical properties.

4.4. Water Vapor Permeability

Water vapor permeability (WVP) of coated films was determined with two different methods. The first method was the standard method of ASTM E96, where WVP is determined gravimetrically. The method was performed by sealing a film to the open mouth of a test cup containing silica gel (20 g) and placing the assembly into the controlled environmental chamber at 60% relative humidity (RH) and 25°C. This allows the conditions with low humidity on one side of the film and high humidity on the other side. The water vapor transferred through the film and absorbed by the silica gel was detected by weighing the cups periodically until a stationary state was reached. Initially, weights of the cups were recorded at 30 min. intervals for a period of 8 h. The final measurement was done after 24 h. Then, WVP was determined from weight differences of test cups at the end of test duration by using equation 2.7.

In the second method, the water vapor permeability apparatus which consist of two chambers separated with a test film sample was used to determine WVP as shown in Figure 4.1. The upper chamber includes a humidity probe connected to a Datalogger SK-L 200 TH to monitor relative humidity (RH) and temperature versus time. The lower chamber contains a small bath filled with deionized water to maintain high relative humidity (95%). During the permeation experiments, air was dried in a fixed bed column. Upper chamber was exposed to dry air until the relative humidity was reduced to nearly 5 %, and then valves were closed at the inlet and outlet of permeation cell, water vapor at lower chamber allowed to pass through the film and the program was started and data was recorded by computer. Water vapor permeabilities were estimated from Equation 2.12 using experimentally collected relative humidity versus time data. For each film, results were reported as an average of three measurements.
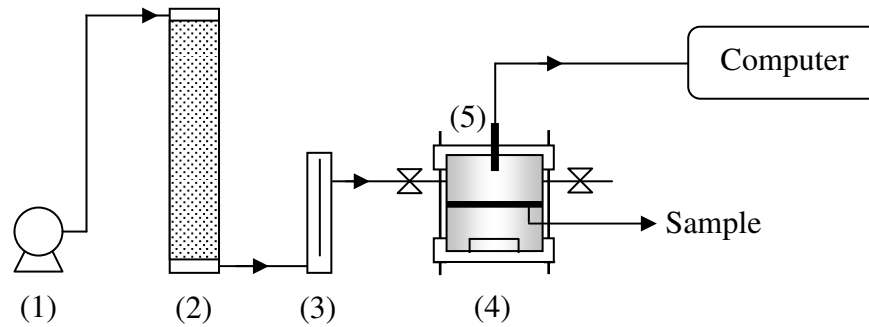
Figure 4.1. Schematic presentation of permeation set-up (1) Pump; (2) fixed bed column; (3) flowmeter; (4) permeation cell; (5) humidity probe

4.5. Oxygen Permeability

The oxygen permeability (OP) of coated PP films was measured in Polinas Company (Manisa, Turkey) by using Macon II Instrument (England) according to standard method of ASTM E96. Oxygen permeabilities of coated films were determined at constant temperature ($23^{o}C$) and relative humidity (0%RH) conditions. The coated film was placed between two sides of test chamber. One side was exposed to carrier gas containing 98% N_2 and 2%H_2 while the other side was exposed to test gas of 5% O_2 and 95% N_2. The sensor monitoring the exit port of the carrier gas side measured the amount of oxygen present. The measurement was completed when the concentration of oxygen in the exit of carrier gas was constant and then OP was calculated by dividing exit concentration with transfer film area and time required to reach for stationary state.

4.7. Mechanical Properties

The universal testing machine Shidmadzu AG-I 5 kN (Japan) was used to perform mechanical behavior of coated PP films according to ASTM D638 method. Specimens were prepared 10 mm in width, and preconditioned at 23 °C and 50% RH for 24 h prior to testing. Initial gauge length and cross-head speed were set at 50 mm and 10 mm/min, respectively. Tensile strength was calculated by dividing the maximum load for breaking the film by cross-sectional area, and percentage of elongation at break by dividing film elongation at the moment of rupture to initial gauge length. Percent elongation is the ratio of extension to the length of the sample. The modulus of

elasticity is the ratio of stress to strain at the linear portion of the curve. A total of 5 samples were tested for each film sample.

4.8. Thermal Analysis

Differential scanning calorimetry measurements were carried out in Shidmadzu DSC-50 (Japan) equipment. Film samples (3 mg) were scanned at a rate of 10$^\text{o}$C/min between temperature ranges of 20 and 500$^\text{o}$C. Glass transition temperatures of the plasticized corn-zein films were determined from resulting thermograms as the midpoint between onset and end temperatures of step changes in heat flow observed during heating. The melting and degradation temperatures of the coated PP samples were also determined by using DSC.

4.9. Color Measurements

The color of corn-zein coated PP films was assessed using a colorimeter (Avantes). PP film having no biopolymer coating (L = 90,56, a = 0,35, b = 0,55) was used as a background for color measurements of the coated films. In this system (Hunter system), color is represented as a position in a three-dimensional sphere, where the vertical axis L indicates the lightness (ranging from black to white), and the horizontal axes, indicated by a and b, are the chromatic coordinates (ranging from a: greenness to redness and b: blueness to yellowness). Hunter L, a, and b values were averaged from three readings across for each coating replicate. The total color difference (ΔE) can be calculated by the following equation;

$$\Delta E = \sqrt{\left(\Delta L\right)^2 + \left(\Delta a\right)^2 + \left(\Delta b\right)^2} \tag{4.1}$$

The results were expressed as ΔE values, with the substrate PP having no biopolymer coatings as reference. For each film, at least 3 measurements on different positions of film surface were made.

4.10. Optical Properties

The ultraviolet (UV) and visible light barrier properties of the films were measured at selected wavelengths from 200 to 700 nm using a UV spectrophotometer (UV-1201, Shimadzu, Japan) according to ASTM1003 method. Light transmission through non-coated PP films was also measured for comparison. For each film, at least 5 measurements were made and the average was taken.

4.11. Contact Angle Analysis

The surface hydrophobicity or wettability of the films was evaluated by contact angle measurements carried out with water using a goniometer (Kruss G10, Germany). To perform the measurements, a syringe was filled with 5 ml of water, and a drop was placed on top of the film surface that was glued on a well-leveled plastic platform. The angle between the baseline of the drop and the tangent at the drop boundary was measured. For each film type, at least 5 measurements on different positions of film surface were made and the average was taken.

4.12. Statistical Analysis

Statistical analysis based on full factorial design was performed to examine the influence of the three variables on the final properties of corn-zein coated PP films. The model included factors of two replications at center point, for corn zein concentration (5 wt% and 15 wt%), ethanol concentration (70 wt% and 95 wt%) and plasticizer concentration (20% and 50%) for each plasticizer containing films; GLY and PEG. These variables were examined at three levels: upper, midpoint and lower limits. Testing of all three factors (ethanol, corn-zein and plasticizer concentrations) simultaneously would involve factorial design with eleven experiments for a single plasticizer containing coating solution (Table 4.1) determined by statistical analysis system, MODDE version 7.0 (Umetrics, Sweden).

Table 4.1. The Experiments Performed According To The Experimental Design For A Certain Plasticizer Type

Coated Film no	Ethanol Concentration, % (v/v)	Corn-Zein Concentration, % (w/v)	Plasticizer Concentration, % (w/w)
1	70	5	20
2	95	5	20
3	70	15	20
4	95	15	20
5	70	5	50
6	95	5	50
7	70	15	50
8	95	15	50
9	82.5	10	35
10	82.5	10	35
11	82.5	10	35

CHAPTER 5

RESULTS AND DISCUSSION

In this study, the plasticized corn-zein coatings with polyethylene glycol (PEG) and glycerol (GLY) on PP films were prepared to evaluate water and oxygen barrier, mechanical, optical and surface properties of resulting coated films as affected by ethanol, corn-zein and plasticizer concentrations and also plasticizer type in order to develop a novel film structure of corn-zein coating on common packaging film (PP) for food packaging applications. Several factors were hypothesized to affect properties of corn-zein coated PP films and a complete factorial design was set to examine the relationship among these different factors.

The plasticized corn-zein coatings films showed good adhesion with PP surface. The total thicknesses of coated films are tabulated in Table 5.1. It was found that the thickness of the corn-zein coated PP films is affected by the coating formulation. GLY plasticized coating solution with low zein content gave relatively thicker films.

Table 5.1. The Thickness of the Corn-Zein Coated PP Films

PEG Plasticization		GLY Plasticization	
Coated Film no	Thickness (μm)	Coated Film no	Thickness (μm)
1	42.8 ± 0.72	12	42.9 ± 0.84
2	42.6 ± 0.45	13	43.1 ± 0.82
3	43.3 ± 0.19	14	43.2 ± 0.59
4	43.5 ± 0.98	15	43.5 ± 0.64
5	43.5 ± 0.49	16	42.8 ± 0.73
6	43.8 ± 0.36	17	43.4 ± 0.96
7	43.8 ± 0.26	18	43.2 ± 0.38
8	43.9 ± 0.58	19	43.4 ± 0.15
9	43.8 ± 0.84	20	43.8 ± 0.17
10	43.3 ± 0.88	21	43.2 ± 0.14
11	43.3 ± 0.75	22	43.1 ± 0.16

5.1. Water Vapor Permeability

It is well known that a proper barrier to water vapor would have a significant effect on the shelf-life of food product. Water vapor transport properties of coated packaging materials are often influenced by coating composition. Since the water transport properties of packaging material is responsible for product quality deterioration and shelf-life reduction, the detailed understanding of water vapor permeability characteristics of coated films has a great practical and commercial importance.

The water vapor transferred through the film was determined according to ASTM E96 . The absorbed vapor by the silica gel was reported by measuring the weight gain after 24 h (Table 5.2). Then, WVP was calculated by using Equation 2.7.

Table 5.2. The Weight Gain (ΔW) of Test Cups used in WVP Calculations According to ASTM E96 Method

PEG Plasticization		GLY Plasticization	
Coated Film no	The weight gain (ΔW) $\times 10^2$ (g)	Coated Film no	The weight gain (ΔW) $\times 10^2$ (g)
1	1.44 ± 0.18	12	0.42 ± 0.14
2	0.88 ± 0.08	13	0.88 ± 0.07
3	1.54 ± 0.19	14	0.22 ± 0.06
4	4.33 ± 0.40	15	0.23 ± 0.02
5	6.28 ± 0.98	16	2.05 ± 0.93
6	3.88 ± 1.58	17	3.70 ± 0.98
7	1.42 ± 0.32	18	2.21 ± 0.28
8	3.34 ± 0.45	19	0.11 ± 0.05
9	2.89 ± 0.72	20	0.29 ± 0.04
10	2.57 ± 1.56	21	0.28 ± 0.04
11	0.57 ± 0.05	22	0.29 ± 0.02

The weights of the cups were recorded periodically at 30 min. intervals, until a stationary state was reached for each film nearly after 10 hours. The weight gain vs. time plot of a sample test cup was shown in Figure 5.1.
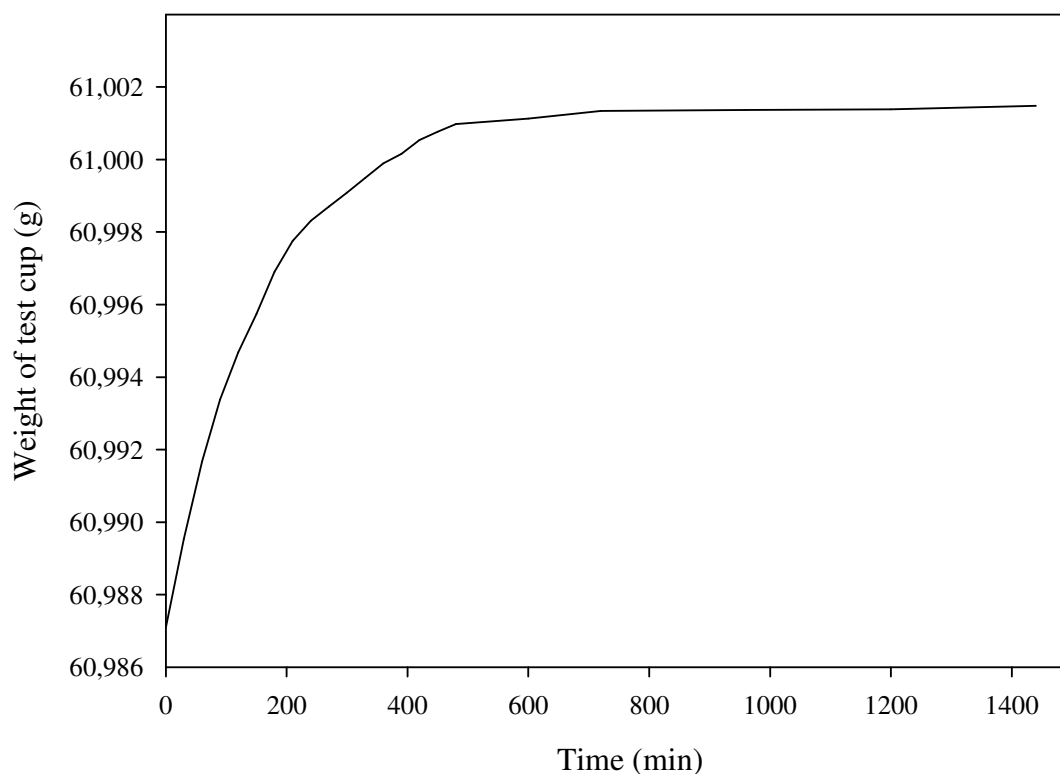
Figure 5.1. The Change in Weight of a Sample Test Cup for 24 hours.

Figure 5.2 illustrates the effect of coating formulation on the WVP values of the different corn-zein coated PP films obtained by standard method of ASTM E96. The corn-zein, ethanol and plasticizer concentration and plasticizer type in coating solution produced statistically significant differences ($p < 0.05$) in water barrier properties of coated PP films. The considerable effect of amount of zein and plasticizer, ethanol concentration, and also plasticizer type (PEG and GLY) on WVP of coated PP films can be easily observed from Figure 5.2.
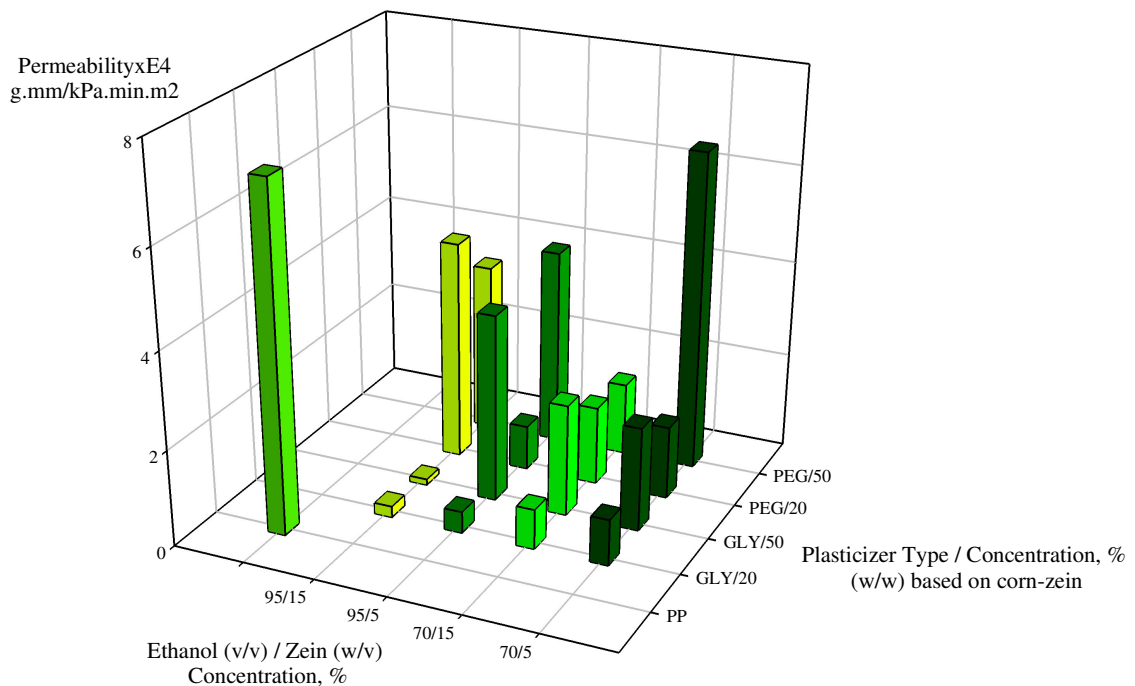
Figure 5.2. The Effects of Coating Composition on WVP of Corn-zein Coated PP Films

In fact, it can be stated that WVP is directly related with the hydrophobic and hydrophilic character of the film and with the presence of voids in its structure. Since films having hydrophobic character provide good water barrier performances, hydrophobicity is resulted by non-polar nature of polymer used. Thus, corn-zein coatings used in this study was expected to improve water vapor barrier of PP due to hydrophobic character of zein due to its high non-polar amino acid content. As seen from Figure 5.2., significant improvement in water vapor barrier of PP base films was obtained, and these results were also tabulated in Table 5.3. The improvement in WVP was observed in all formulations compared to uncoated PP film having WVP value of 7.140×10^{-4} g.mm/kPa.min.m^2

Table 5.3. The WVP of Corn-zein Coated PP Films Prepared According to Experimental Design (ASTM E96 Method)

PEG Plasticization		GLY Plasticization	
Coated Film no	Water Vapor Permeability$\times 10^4$ (g.mm/kPa.min.m^2)	Coated Film no	Water Vapor Permeability$\times 10^4$ (g.mm/kPa.min.m^2)
1	1.51 ± 0.41	12	0.95 ± 0.14
2	0.92 ± 0.05	13	0.44 ± 0.02
3	1.62 ± 0.39	14	0.93 ± 0.11
4	4.55 ± 048	15	0.23 ± 0.03
5	6.60 ± 3.20	16	2.15 ± 0.53
6	4.08 ± 1.39	17	3.89 ± 1.06
7	1.50 ± 0.36	18	2.32 ± 0.38
8	3.51 ± 0.67	19	0.12 ± 0.05
9	3.04 ± 0.82	20	0.31 ± 0.04
10	2.70 ± 1.38	21	0.29 ± 0.04
11	0.60 ± 0.15	22	0.31 ± 0.06

The coating solution plasticized with smaller amount of plasticizer showed increase in WVP barrier. Generally plasticizers increase permeability of the films depending on the concentration of plasticizer used. Plasticizer was used to increase the flexibility of corn-zein films. They work as spacers between protein chains resulting a less dense structure where chain ends became more mobile and increase permeability. The effect of different plasticizers, PEG and GLY, and also their concentrations used in zein coating solution on WVP of resulted films are tabulated in Table 5.4. The hypothesis testing showed statistically differences in WVP for two different plasticization of coating solution.

In comparison, among the films coated by zein solutions containing two different plasticizers, the coating solution plasticized by glycerol (GLY) had the highest water vapor barrier properties. The both plasticizers, PEG and GLY, have polar and hydrophilic character. However, it was seen that PEG has more the ability to attract water molecules through either absorption or adsorption and also strong affinity for moisture, thus PEG plasticized zein coating on PP films resulted in relatively large amounts of water sorption and higher permeability values.

Table 5.4. The Effects of Plasticizer Type and Concentration on the WVP of Coated PP Films[a] (ASTM E96 Method)

Plasticizer Type	Plasticizer Concentration, % (w/w) (based on corn-zein content)	Water Vapor Permeability×10^4 (g.mm/kPa.min.m^2)
PEG	20	0.92 ± 0.05
PEG	50	4.08 ± 0.39
GLY	20	0.44 ± 0.02
GLY	50	3.89 ± 0.06

[a]where ethanol and corn-zein concentrations are constant at 95% and 5%, respectively.

Furthermore, the higher WVP of corn-zein plasticized by PEG coated PP films as compared to GLY plasticized films may be related to the hydration of the plasticizers in which water molecules associated by PEG were more than GLY for the same concentration. Thus, the concentration of hydrophilicity becomes the key factor for the barrier efficiency, so, the more hydrophilic nature of PEG than GLY increased the WVP.

Several authors studied the influence of the plasticizer content on barrier properties of zein films, and they showed that the increase in WVP of films caused by the increase of plasticizer content (Lai et el. 1997, Parris and Coffin 1997, Lai and Lawton 2004, Ghanbarzadeh et al. 2007). The results from these studies were similar to those reported in this study.

Parris and Coffin studied the effect of some of plasticizers on the WVP of the free-standing zein films. Water vapor barrier properties were the best for unplasticized zein films, and incorporation of plasticizers which were PEG and GLY into the zein films resulted in an almost doubled WVP values. The increase in WVP was more pronounced for films plasticized with PEG which is similar with our results obtained from this study (Parris and Coffin 1997). Lawton studied on the relationship between plasticizer type and water sorption of zein films and they reported that films increasingly absorbed water depending on the plasticizers used in the film in the order of GLY>PEG and they found that plasticizing efficiency increased with the molecular weight of the plasticizer used. Thus, PEG plasticized films had higher plasticizing efficiency than GLY plasticized films. So, their results confirm our permeability results that the films plasticized with PEG have higher values than GLY plasticized films.

Furthermore, most of the published works in literature deals with water vapor transfer properties of other biodegradable based films. The results showed that plasticizers generally increase permeability when high ratio of plasticizer was used in polymer (Caner et al. 1998, Sothornvit and Krochta 2000, Srinivasa et al. 2007)

As seen from Figure 5.2, ethanol and zein concentrations in coating formulation were important and affect the WVP coated PP films. Corn-zein coated films were expected to have variable behavior against water vapor depend on zein concentration because of major hydrophobic character of zein. In addition, the ethanol concentration affects the solubility of corn zein that resulted in different permeability properties of coated films. The effects of ethanol and corn-zein concentration on WVP values of coated PP films are summarized in Table 5.5.

Table 5.5. The Effects of Ethanol and Corn-zein Concentration on the WVP of Coated PP Films[b]

Ethanol Concentration, %	Corn-zein Concentration (g/100 ml ethanol)	Water Vapor Permeability$\times 10^4$ (g.mm/kPa.min.m^2)
70	5	0.95 ± 0.14
70	15	0.93 ± 0.11
95	5	0.44 ± 0.02
95	15	0.23 ± 0.03

[b]where plasticizer is glycerol, and its concentration is constant as 20% (w/w) based on corn-zein content.

The corn-zein films having zein concentration of 5 and 15 g/100 ml ethanol were prepared to coat PP films at the same ethanol concentration, and then the effect of ethanol concentration of 70 v % and 95 v % used in preparation of film-forming solution was also investigated. As the corn-zein concentration in the coating formulations increased, the water vapor barrier of coated PP films was also increased.

Corn-zein and ethanol concentrations in coating film-forming solutions strongly affected film structure, and high water vapor permeability properties of coated PP films were obtained at low concentrations of ethanol. Since the amount of corn-zein present in the coating formulations affects the hydrophobicity of coated PP films which was the

key factor for the barrier efficiency, the WVP of films was influenced by corn-zein concentration. The films coated with solutions included higher amount of zein created the more hydrophobic nature than the coated PP films containing low corn-zein in the coating solutions, thus the improvement of water barrier was observed for high concentrations of zein. In addition, results showed that WVP decreased significantly when the ethanol concentration increased. Although an increase in concentration of zein decreased WVP, for film solutions plasticized with 20% (w/w) of PEG resulted in an increase in WVP at both low and high ethanol concentrations while it was expected to cause a decrease in WVP of coated films. This can be explained by non-homogeneous distribution of corn-zein in the coating solution, therefore, the effect of corn-zein may not be observed for these films.

The WVP of coated PP films were also determined by using the permeability apparatus consisting of two chambers separated with a test film as described in section 4.4. The relative humidity change in upper chamber for a sample film was shown in Figure 5.3.
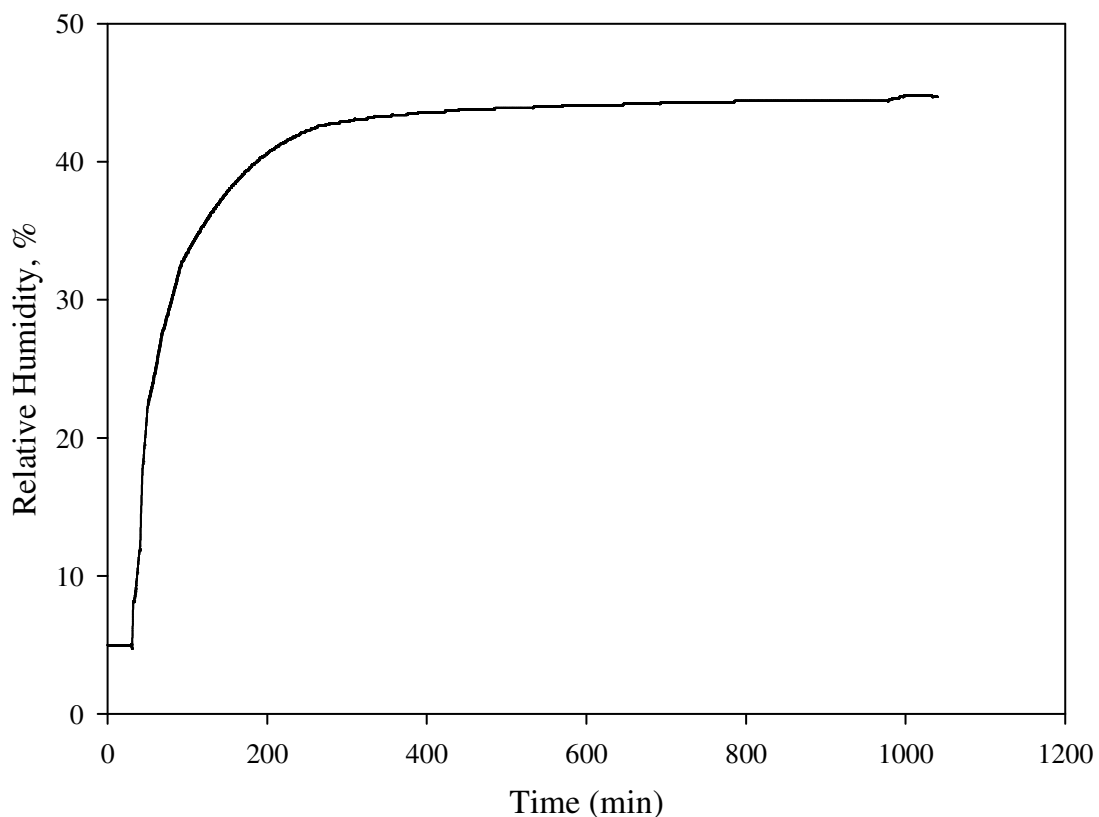
Figure 5.3. The Relative Humidity Change in the Upper Chamber of Permeability Apparatus

The slope of $\ln(p_{x=0} - p_i)/(p_{x=0} - p_t)$ versus time plot derived from relative humidity data was used by using equation 2.12 to calculate WVP of films. The slope was determined from linear portion of the $\ln(p_{x=0} - p_i)/(p_{x=0} - p_t)$ vs time as shown in Figure 5.4 for a sample film, and the slopes of the all films studied in this study were reported in Table 5.6 .
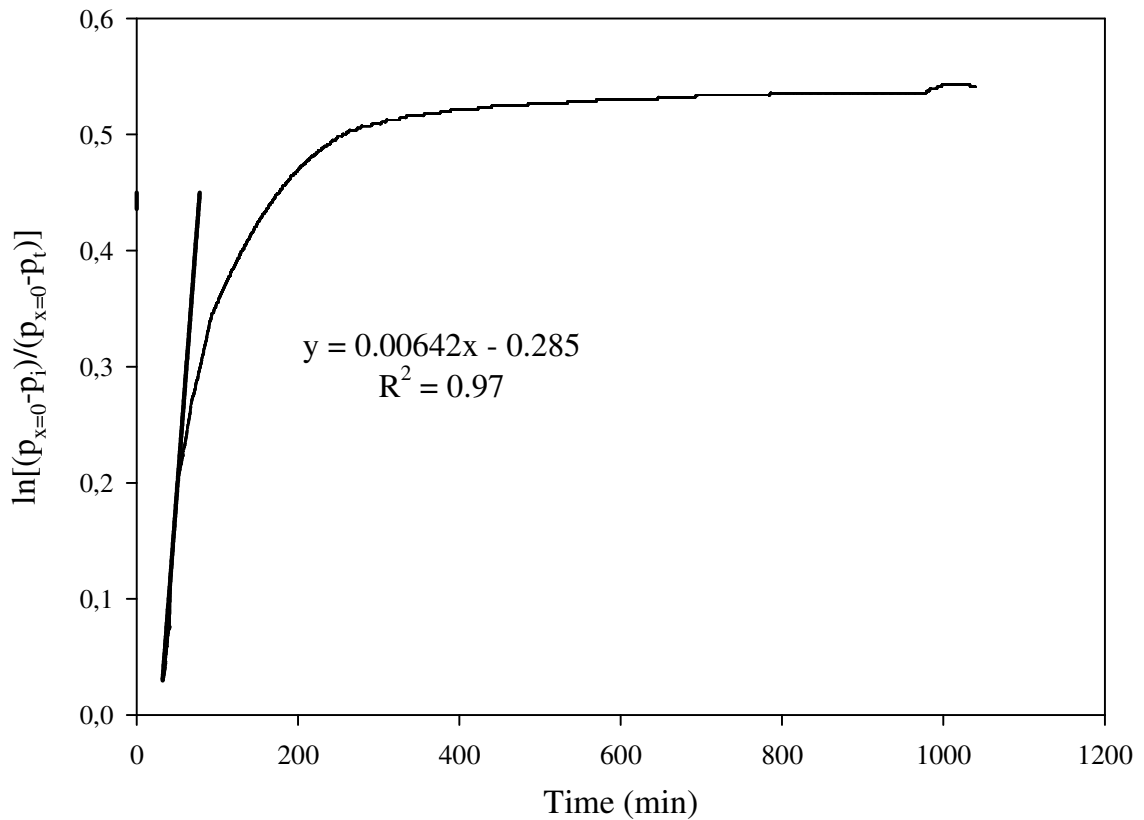
Figure 5.4. The Slope Calculation for $\ln(p_{x=0} - p_i)/(p_{x=0} - p_t)$ vs Time Plot of Sample Film

By using the slope value, the WVP was estimated from Equation 2.12 and the WVP data for all corn zein coated PP films according to experimental design by using water vapor transport apparatus method were listed in Table 5.7.

Table 5.6. The Slopes of $\ln(p_{x=0} - p_i)/(p_{x=0} - p_t)$ vs Time Plots Used in WVP Calculations According to Water Vapor Transport Apparatus Method.

PEG Plasticization		GLY Plasticization	
Coated Film no	Slope×10^2 (min^{-1})	Coated Film no	Slope×10^2 (min^{-1})
1	0.64 ± 0.13	12	0.50 ± 0.21
2	0.52 ± 0.06	13	0.22 ± 0.07
3	0.80 ± 0.12	14	0.22 ± 0.04
4	2.05 ± 0.44	15	0.10 ± 0.02
5	0.13 ± 0.09	16	1.09 ± 0.39
6	2.65 ± 0.43	17	1.35 ± 0.34
7	2.59 ± 0.71	18	1.40 ± 0.22
8	0.61 ± 0.05	19	0.84 ± 0.03
9	0.05 ± 0.02	20	0.13 ± 0.04
10	1.42 ± 0.52	21	0.01 ± 0.01
11	0.09 ± 0.02	22	0.11 ± 0.03

Table 5.7. The WVP of Corn-Zein Coated PP Films Prepared According to Experimental Design (Water Vapor Transport Apparatus Method).

PEG Plasticization		GLY Plasticization	
Coated Film no	Water Vapor Permeability×10^4 (g.mm/kPa.min.m^2)	Coated Film no	Water Vapor Permeability×10^4 (g.mm/kPa.min.m^2)
1	1.33 ± 0.34	12	1.26 ± 0.89
2	1.08 ± 0.51	13	2.26 ± 1.89
3	1.65 ± 0.48	14	2.79 ± 0.37
4	4.24 ± 2.01	15	2.89 ± 0.14
5	1.04 ± 0.27	16	1.74 ± 0.33
6	0.44 ± 0.02	17	0.10 ± 0.14
7	0.46 ± 0.34	18	2.94 ± 0.55
8	0.20 ± 0.02	19	0.18 ± 0.02
9	0.27 ± 0.05	20	0.27 ± 0.03
10	5.49 ± 0.98	21	0.02 ± 0.04
11	5.37 ± 0.97	22	0.23 ± 0.06

The WVP values of coated PP films obtained from water vapor permeability apparatus were comparable with those calculated from standard method of ASTM E96. Hypothesis testing showed that no statistically significant differences for WVP values calculated from two different methods were observed. The experimental results of the coated films suggested that corn-zein could be used as an alternative coating material to improve water barrier properties of polypropylene (PP) films within the optimum film forming conditions. Corn zein coated PP film is a laminated structure consisting of a base PP film layer and corn zein coating layer. For using two layer-model, the WVP of corn zein coating can be calculated from Equation 2.13. The estimated WVP values of corn zein coating plasticized using PEG and GLY according to experimental design were listed in Table 5.8. In Table 5.9, the comparison of WVP values corn zein coating determined in this study were compared with the common coating materials used in the industry. From these results, the corn-zein coatings could have potential as an alternative to water vapor barrier packaging materials.

Table 5.8. The WVP of Corn-Zein Coating Plasticized using PEG and GLY According to Experimental Design (by Two Layer-Model)

PEG Plasticization		GLY Plasticization	
Coated Film no	Water Vapor Permeability$\times 10^4$ (g.mm/kPa.min.m^2)	Coated Film no	Water Vapor Permeability$\times 10^4$ (g.mm/kPa.min.m^2)
1	0.205 ± 0.036	12	0.087 ± 0.016
2	0.073 ± 0.005	13	0.033 ± 0.002
3	0.163 ± 0.015	14	0.074 ± 0.010
4	0.800 ± 0.224	15	0.017 ± 0.002
5	6.027 ± 0.380	16	0.213 ± 0.075
6	0.699 ± 0.055	17	0.595 ± 0.304
7	0.200 ± 0.049	18	0.234 ± 0.056
8	0.467 ± 0.175	19	0.009 ± 0.004
9	0.365 ± 0.152	20	0.023 ± 0.003
10	0.332 ± 0.280	21	0.021 ± 0.003
11	0.046 ± 0.013	22	0.023 ± 0.005

Table 5.9. The Comparison of the Conventional Coating Materials with Corn-Zein Films

Film type	Water Vapor Permeability$\times 10^4$ (g.mm/kPa.min.m^2)	References
Corn-zein	0.009-6.027	This study
Polyvinylidene Chloride (PVDC)	6.860	Cuq et al.,1998
Ethylene Vinyl Alcohol (EVOH)	23.34	Cuq et al., 1998
Uncoated PP	7.140	This study
PVDC coated PP	1.938	Eq. (2.13)
EVOH coated PP	4.442	Eq. (2.13)

The analysis of variance (ANOVA) table for WVP values of films is given in Table A.1 and A.2 in Appendix. The general linear models procedure was used for ANOVA, then results showed that model is significant for WVP results using both experimental methods. The statistical analysis also defined the key parameters that had dominant effect on WVP of coated PP films as corn-zein, plasticizer concentration and plasticizer types. The coefficient table related to these results is given in Appendix (Table A.3 and A.4).

Finally, the corn-zein coatings could work successfully as a water vapor barrier and have a potential to be used as an alternative to existing packaging materials under optimum levels of these coating parameters mentioned above.

5.2. Oxygen Permeability

The packaged foods may undergo biological, chemical and physical deterioration by interaction with oxygen and water. The some of the quality loss of packaged foods is caused by oxygen, thus the oxygen barrier properties of the coated PP films is the one of the most important requirements to maintain initial high quality of the packaged foods. The good oxygen-barrier properties of corn-zein coated PP films are critical for achieving a long shelf life for the packaged food products as water barrier properties. The oxygen permeabilities of some of the corn-zein coated PP films

measured at 23°C and 0%RH were given in Table 5.10 as a function of plasticizer type and its concentration.

Table 5.10. The Effects of Plasticizer Type and Concentration on the OP of Coated PP Films[a]

Plasticizer Type	Plasticizer Concentration, % (w/w) (based on corn-zein content)	Oxygen Permeability ($cc/m^2 \times day$)
Uncoated PP films		2000
PEG	20	811
PEG	50	858
GLY	20	609
GLY	50	488

[a]where ethanol and corn-zein concentrations are constant at 95% and 5%, respectively.

The O_2 permeability of PP films was significantly decreased upon with corn-zein coating. The application of plasticized corn-zein coating on PP films showed nearly more than two or three order of reduction in O_2 permeability of PP films when compared to without coating (in where O_2 permeability of PP film was determined as 2000 $cc/m^2 \times day$).

The effect of plasticizer concentration on O_2 permeability was also examined. Oxygen permeability value increased with the plasticizer content. The increase in O_2 permeability with plasticizer content is associated with the hydrophilic nature of plasticizers used. The further breaking down of hydrogen bonds created additional sites for the dissolution of oxygen and increase mobility of the O_2 molecules within the coating layer. The higher mobility of the corn-zein chains resulted in easy O_2 diffusion through the film. Furthermore, type of plasticizer used also influenced O_2 permeability of the corn-zein coating on PP films. Corn-zein coatings plasticized with PEG on PP films had higher O_2 permeability values compared to GLY plasticized films. The larger size and relatively high ability of PEG to affect hydrogen bonding between chains caused to increase molecular diffusion through the coated film. Due to its straight chain structure and greater availability of oxygen atoms to interact with corn-zein proteins resulted in negative effect on O_2 barrier properties of PP films.

The increase in corn-zein concentration of coating solution from 5 to 15% (w/v) resulted in higher O_2 barrier property of coated PP films. The O_2 permeability of PP coated films with solution containing 15% (w/v) corn-zein plasticized with 20% PEG was measured to investigate the effect of corn-zein concentration on O_2 permeability. The O_2 permeability of this coated PP film was determined as 466 cc/m^2×day which was lower than PP film coated with solution containing 5% (w/v) corn-zein plasticized with 20% PEG shown in Table 5.10 as 811 cc/m^2×day. This improvement was caused by the excellent oxygen barrier properties of freestanding zein films, thus higher amount of zein present in coating formulation resulted in lower O_2 permeability of coated PP film. The high oxygen barrier property of freestanding zein films is well known from several reviews published in literature (Cuq et al. 1998, Rakotonirainy and Padua 2001, Hsu et al. 2005). They reported that oxygen permeability values of zein films are lower than those of common synthetic plastic films such as low density polyethylene (LDPE), high-density polyethylene (HDPE), PP, polystyrene (PS), and poly (vinyl chloride) (PVC). Furthermore, Shin and coworkers obtained excellent oxygen barrier of corn-zein coated LDPE films in agreement with our results. They showed that LDPE films coated with zein reduced oxygen permeability compared to uncoated LDPE. Bilayer films including corn zein coating showed nearly 10 times lower oxygen permeability (Shin et al., 2002).

For using two layer-model, the O_2 permeability (OP) of corn zein coating can be calculated from Equation 2.13. The estimated OP values of corn zein coating plasticized using PEG and GLY according to experimental design were listed in Table 5.11.

Table 5.11. The Oxygen Permeability of Corn-zein Coatings

Plasticizer Type / Concentration	Ethanol % (v/v) / Corn Zein (w/v) Concentration	Oxygen Permeability (cc/m^2×day)
PEG/20	95/5	90.9
PEG/50	95/5	99.6
GLY/20	95/5	59.3
GLY/50	95/5	44.0
PEG/20	95/15	41.5

[a]where ethanol and corn-zein concentrations are constant at 95% and 5%, respectively.

In Table 5.12, the comparison of OP values corn zein coating determined in this study were compared with the common coating materials used in the industry. From the results, it can be said that the corn-zein coatings show potential to use as a oxygen barrier packaging material. Overall, according to reported studies of freestanding zein films and our obtained results for zein coated PP films, it can be suggested that proper and optimum formulation of corn-zein coatings on PP films could be used as an alternative oxygen barrier layer in multicomponent synthetic packaging films.

Table 5.12. The Comparison of Oxygen Permeability of the Conventional Coating Materials with Corn-zein Films

Film type	Oxygen Permeability (cc/m$^2\times$day)	References
Corn-zein	41.5-99.6	from this study
Polyvinylidene Chloride (PVDC)	35.8	Cuq et al. 1998
Ethylene Vinyl Alcohol (EVOH)	7.89	Cuq et al. 1998
Uncoated PP	2000	from this study
PVDC coated PP	553	from Eq. (2.13)
EVOH coated PP	153	form Eq. (2.13)

5.3. Mechanical Properties

Mechanical properties of corn-zein coated PP films that are important for packaging applications as barrier properties were also determined in this study to investigate the effect of coating formulation on resulted PP films. The quantitative information on the mechanical parameters of coated PP films is necessary for the packaging design process. Also, the mechanical properties of coated or laminated films in multilayer structure generally depend strongly on the substrate rather than the coating. For corn-zein coated PP films, however tensile properties can be influenced by coating material having different molecular characteristics than PP. Interactions between zein protein and plasticizers dispersed in the space of the polymer matrix, and the coating solvent concentration could contribute to the mechanical behavior of final coated PP films.

Tensile testing of coated PP films was performed to obtain tensile strength, elongation at break, and also modulus of elasticity (Young's modulus) which give enough information about mechanical behavior of packaging films. Tensile strength was calculated by dividing the maximum load necessary for breaking the film by cross-sectional area, and percent elongation is the ratio of extension to the length of the sample. The elastic modulus is the fundamental measure of film stiffness. The mechanical behavior of the coated films generally depends on coating formulation. The results indicated that these properties were influenced by the nature of coating composition. The Figure 5.5 shows tensile strength of coated PP films, obtained from tensile testing measurements.
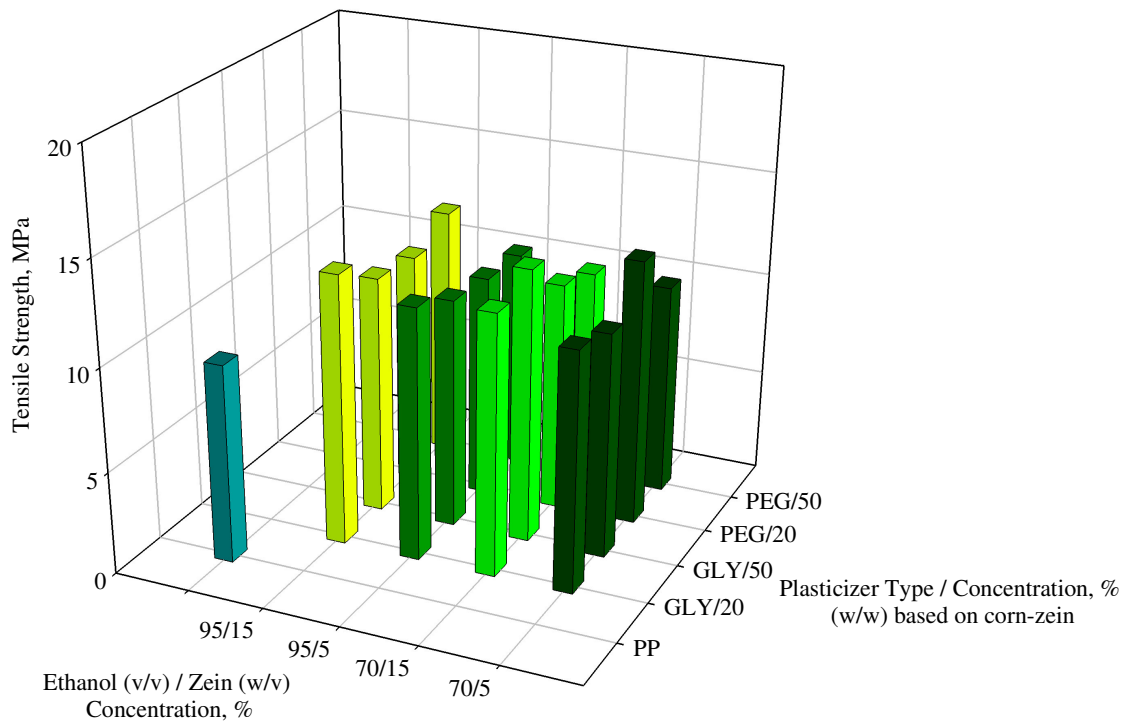
Figure 5.5. The Presentation of the Tensile Strength of Coated PP Films Affected by Coating Formulation

The tensile strength of the commercial corona discharge-treated polypropylene (PP) film used in this study was found as 9.65 MPa without corn-zein coating. Therefore, it was clearly observed that from experimental results (Table 5.13) the maximum amount of tensile stress of PP films subjected to before failure was improved

with corn-zein coating. Tensile strength values of all coated films were in the range of 10.31 and 14.66 MPa.

The plasticizing of corn-zein coating solutions with glycerol (GLY) and polyethylene glycol (PEG) caused differences in mechanical properties of coated PP films. The increasing plasticizer concentration in corn zein coating solutions decreased the tensile properties of resulted PP films. GLY and PEG could form numerous hydrogen bonds with the zein polypeptide chain because of the carbonyl of protein and hydroxyl groups of these plasticizers. Thus, these plasticizers introduced between polypeptide chains affected the polymer-polymer interaction causing decrease in tensile strength of coated films. Therefore, as the plasticizer content increased, tensile strength had tendency to decrease due to weak polymer-polymer interaction. It was observed that the plasticizing efficiencies of each PEG and GLY strongly depend on their concentration.

Table 5.13. The Tensile Strengths of Corn-Zein Coated PP Films Prepared According to Experimental Design

PEG Plasticization		GLY Plasticization	
Coated Film no	Tensile Strength (MPa)	Coated Film no	Tensile Strength (MPa)
1	12.94 ± 0.45	12	11.65 ± 0.39
2	10.86 ± 0.68	13	12.24 ± 0.60
3	11.17 ± 0.28	14	12.65 ± 0.58
4	11.27 ± 0.95	15	13.13 ± 1.03
5	10.31 ± 1.00	16	10.93 ± 0.17
6	10.69 ± 1.95	17	11.17 ± 0.39
7	10.38 ± 1.68	18	13.27 ± 0.85
8	12.20 ± 0.95	19	11.58 ± 0.26
9	10.42 ± 1.21	20	13.37 ± 1.35
10	10.59 ± 1.28	21	14.66 ± 0.48
11	10.36 ± 0.59	22	14.09 ± 1.71

PP films coated with corn-zein containing high amounts of GLY and PEG in coating formulation showed the lowest values for tensile stress. Therefore, low level of

plasticizer was enough to increase association within zein polymer chains in coating, otherwise further increase in plasticizer content negatively affected mechanical properties. The effect of plasticizers PEG and GLY, and also their concentrations used in zein coating solution on tensile strength of resulted films are tabulated in Table 5.14.

Table 5.14. The Effects of Plasticizer Type and Concentration on the Tensile Strength of Coated PP Films[a]

Plasticizer Type	Plasticizer Concentration, % (w/w) (based on corn-zein content)	Tensile Strength (MPa)
PEG	20	10.86 ± 0.68
PEG	50	10.69 ± 1.95
GLY	20	13.13 ± 1.03
GLY	50	11.17 ± 0.39

[a]where ethanol and corn-zein concentrations are constant at 95% and 5%, respectively.

Similar results were obtained for freestanding zein films by several authors (Lai et al. 1997, Di Gioa et al. 2000, Wang and Padua 2006). Lai and his coworkers reported that 25% stearic acid used as plasticizer for zein films increased tensile strength of zein sheets substantially, but above this concentration the tensile strength decreased (Lai et al. 1997). In the work of Di Gioa et al., glycerol was used as plasticizer for zein-based films. An increase in the glycerol content resulted in a decrease of the tensile strength which was also consistent with our results (Di Gioa et al. 2000).

In comparison, the coating solution plasticized with PEG caused to relatively lower tensile strength of resulted PP films than films prepared by coating containing GLY. This could be attributed to more hygroscopic nature of zein coating solution including PEG (the ability to attract water molecules) than those containing GLY. This showed that PEG has more hydrophilic plasticizer than GLY which is in agreement with results obtained from water vapor permeability of coated PP films in this study.

Furthermore, the results showed that high corn-zein concentrations at coating formulations induced the formation of coated PP films with higher mechanical strength due to the stiffness of corn-zein. Also the increase in percentage of aqueous ethanol solution in coating resulted in slightly increase in tensile properties as shown in Table 5.15.

Table 5.15. The Effects of Ethanol and Corn-Zein Concentration on the Tensile Strength of Coated PP Films[b]

Ethanol Concentration, %	Corn-zein Concentration (g/100 ml ethanol)	Tensile Strength (MPa)
70	5	11.65 ± 0.39
70	15	12.65 ± 0.58
95	5	12.24 ± 0.60
95	15	13.13 ± 1.03

[b]where plasticizer is glycerol, and its concentration is constant as 20% (w/w) based on corn-zein content.

ANOVA table for tensile strength of coated films for both plasticizers is given in Tables A.3 and A.4 in Appendix part. The results showed that model is significant for both plasticizers. Tensile strengths of coated films were found to be different from each other and uncoated PP's tensile strength according to Hypothesis testing. The key parameters that had dominant effect on tensile strength of coated PP films were estimated as corn-zein concentration and plasticizer concentration. The coefficient table related to these results is given in Appendix for two different plasticizers (Tables A.5 and A.6). The importances of interactions were also observed from coefficient tables.

An increase in film flexibility was indicated with a higher value in elongation to break that is the maximum elongation of sample (before failure occurred) divided by its original length and usually indicated as percentage. The plasticizers both PEG and GLY are used to make coating solution more flexible. They work as spacers between zein protein chains decreasing intermolecular forces and this resulted in improvement in flexibility of corn-zein coating and finally coated PP films. The elongations at break (%) of coated PP film were presented in Figure 5.6 as a function of coating parameters and also their numerical values were given in Table 5.16.
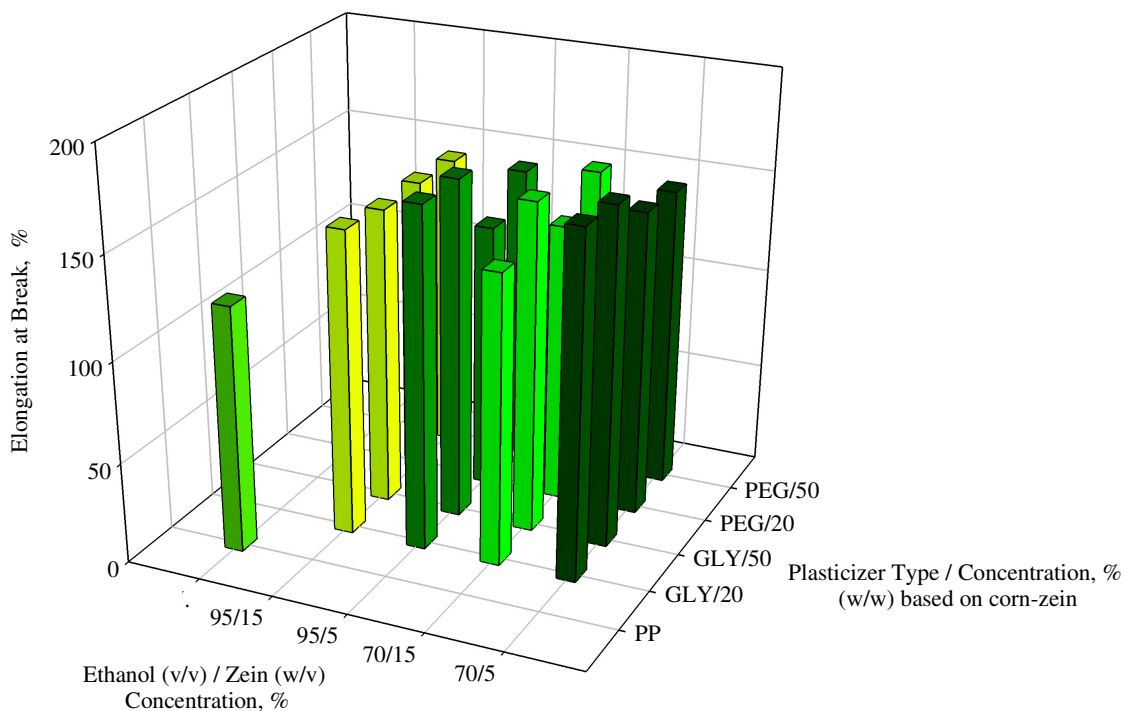
Figure 5.6. The Elongation at Break Values of Corn-Zein Coated PP Films as Affected by Coating Formulation

The elongation at break of base PP film was found to be 121.69 %. When the base PP film was coated with corn zein solution, the improvements in elongation at break of the corn zein coated PP films were obtained. As seen in Figure 5.6 and Table 5.16., the significant increase in elongation at break of all corn zein coated PP films was obtained. These mechanical behaviors of coated films were related to the structural modifications of zein network with changing coating formulation parameters. The separation of zein protein chains by breaking the bonds that hold the polymer chains together, and then covering the centers of forces resulted in the plasticizer-zein interaction due to plasticization effect.

Table 5.16. The Elongation at Break Values of Corn-Zein Coated PP Films Prepared According to Experimental Design

PEG Plasticization		GLY Plasticization	
Coated Film no	Elongation at Break (%)	Coated Film no	Elongation at Break (%)
1	150.98 ± 12.9	12	169.48 ± 13.6
2	131.74 ± 9.22	13	167.90 ± 17.7
3	138.11 ± 6.87	14	142.88 ± 7.41
4	148.23 ± 17.6	15	150.52 ± 16.7
5	148.52 ± 17.6	16	166.88 ± 11.1
6	165.53 ± 7.71	17	147.61 ± 8.58
7	152.77 ± 8.53	18	162.63 ± 9.20
8	147.43 ± 4.81	19	147.48 ± 6.76
9	143.91 ± 2.18	20	149.15 ± 5.08
10	147.48 ± 2.10	21	147.49 ± 3.02
11	148.36 ± 2.31	22	155.01 ± 12.3

The elongations at break of coated PP films increased with increasing plasticizer levels for all coating formulations as shown in Table 5.17. The coating solution plasticized with GLY contributed to relatively higher elongation of resulted PP films than films coated by zein containing PEG. It can be said that PEG based coated films are less flexible than GLY based coated PP film.

Table 5.17. The Effects of Plasticizer Type and Concentration on Elongation at Break of Coated PP Films[a]

Plasticizer Type	Plasticizer Concentration, % (w/w) (based on corn-zein content)	Elongation at Break (%)
PEG	20	131.74 ± 9.22
PEG	50	165.53 ± 7.71
GLY	20	147.61 ± 8.58
GLY	50	167.90 ± 17.6

[a]where ethanol and corn-zein concentrations are constant at 95% and 5%, respectively.

In addition, since GLY has low molecular mass compared to PEG, the number of moles of GLY incorporated in the coating solution was higher than the PEG ones, and this certainly is more important to the plasticizing effect. The coating of zein containing GLY on PP films produced more flexible film structures because of easily fit of GLY into zein protein chains and establish hydrogen bonds with reactive groups of proteins.

Since the increasing amount of corn-zein present in coating solution caused in more hydrophobic nature, and also due to the brittleness of corn-zein, the elongation of coated PP films decreased. The effects of corn-zein and ethanol concentration on elongations of the resulted films were shown in Table 5.18. The decrease in water content of coating matrix was characterized by decrease in elongation at break values of coated PP films. It is proven that water acts a good plasticizer for corn-zein films, therefore the changing the water content in the film changed the properties of the coated films.

Table 5.18. The Effects of Ethanol and Corn-Zein Concentration on Elongation At Break of Coated PP Films[b]

Ethanol Concentration, %	Corn-zein Concentration (g/100 ml ethanol)	Elongation at Break (%)
70	5	169.48 ± 13.60
70	15	142.88 ± 7.413
95	5	167.90 ± 17.70
95	15	150.52 ± 16.77

[b]where plasticizer is glycerol, and its concentration is constant as 20% (w/w) based on corn-zein content.

ANOVA table for elongation at break values of coated films was given in Tables A.7 and A.8 in Appendix for different plasticizers. The results showed that model is significant for both plasticizers. The key parameters that had dominant effect on elongation at break values of coated PP films were found as ethanol concentration and corn-zein concentration in coating solution. The coefficient tables are presented in Appendix (Tables A.9 and A.10). Elongation at break values of coated films was found

to be different from each other and uncoated PP's tensile strength according to Hypothesis testing.

Furthermore, elongation results of coated PP films were also in agreement with the study of Parris and Coffin where zein films were plasticized with PEG and GLY (Parris and Coffin 1997). The films plasticized with GLY was found to be more flexible. Furthermore, they observed significant improvement in zein film flexibility in where mixing of GLY and poly (propylene glycol), PPG was used as plasticizer. The films containing GLY:PPG ratio of 1:3 exhibited elongation at break values almost fifty times greater than GLY plasticized zein films. However, a similar increase in elongation was not observed when PEG replaced with PPG in the plasticizer mixture.

The Young's Modulus (modulus of elasticity) is the measure of the force that is required to deform the film by a given amount of tension. It is also a measure of the intrinsic stiffness of the film and related to the rigidity of the film, as higher Young's modulus, higher the stiffness of the material. The Young modulus was determined from the slope of the stress-strain curve at elastic limit. The modulus of zein coated PP films as affected by coating formulation were presented in Figure 5.7 and numerical values were tabulated in Table 5.19. The significant improvements in stiffness of coated PP films were achieved while it was 842.82 MPa for PP base film without zein coating. In this study, the coating of PP with zein led to significant increase in stiffness values as seen in Table 5.19. All modulus values of coated films were higher than PP base film without coating. Increasing plasticizer content of coating solution resulted in lower Young's modulus and higher flexibility of the coated films. The increase of flexibility of resulted PP films with increasing plasticizer content in coating solution was obtained previously in this study (Figure 5.6).
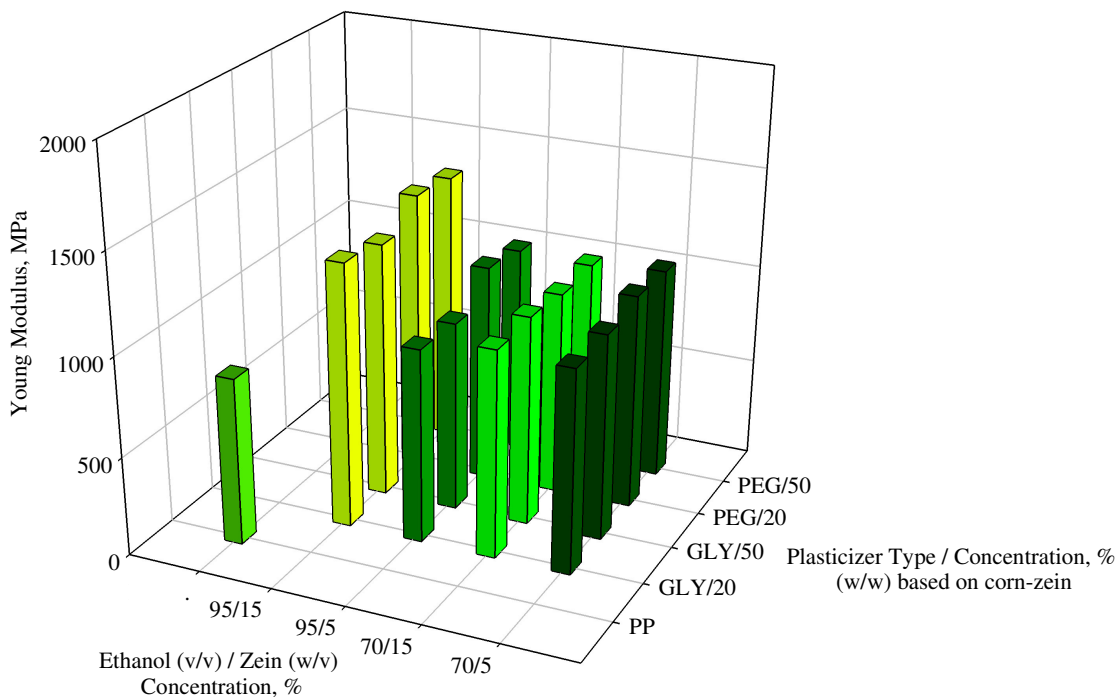
Figure 5.7. The Young Modulus Values of Corn-Zein Coated PP Films as Affected by
Coating Formulation

Table 5.19. The Young Modulus of Corn-Zein Coated PP Films Prepared According to
Experimental Design

PEG Plasticization		GLY Plasticization	
Coated Film no	Young Modulus (MPa)	Coated Film no	Young Modulus (MPa)
1	1086.01 ± 65.16	12	1028.28 ± 134.1
2	1100.67 ± 48.44	13	977.24 ± 64.301
3	1030.24 ± 173.7	14	1046.68 ± 100.3
4	1404.69 ± 112.9	15	1331.31 ± 80.49
5	1074.50 ± 122.4	16	1101.34 ± 31.77
6	1056.48 ± 21.94	17	958.62 ± 25.06
7	1043.44 ± 30.98	18	1159.26 ± 154.3
8	1372.32 ± 269.3	19	1287.07 ± 55.83
9	982.64 ± 6.325	20	1050.68 ± 82.07
10	991.38 ± 33.10	21	1057.56 ± 90.19
11	982.42 ± 40.84	22	1099.73 ± 75.47

The effects of plasticizer type and its concentration on modulus of coated film can be seen clearly from Table 5.20. The results showed that PP films coated with zein solution plasticized with PEG were much stiffer than the films coated with zein solution plasticized with GLY. This could be concluded that the incorporation of GLY resulting in increase in chain flexibility, thus the brittleness of zein coating solution overcame.

Table 5.20. The Effects of Plasticizer Type and Concentration on Young's Modulus of Coated PP Films[a]

Plasticizer Type	Plasticizer Concentration, % (w/w) (based on corn-zein content)	Young Modulus (MPa)
PEG	20	1100.67 ± 48.44
PEG	50	1056.48 ± 21.94
GLY	20	977.24 ± 64.301
GLY	50	958.62 ± 250.6

[a]where ethanol and corn-zein concentrations are constant at 95% and 5%, respectively.

Since zein is a brittle material because of strong intermolecular forces between the protein chains, its high presence favored stiffer behavior of the coated PP films. Therefore, Young's modulus increased with zein content. The observation of this effect of corn-zein amount on modulus was summarized in Table 5.21.

Table 5.21. The Effects of Ethanol and Corn-Zein Concentration on Young's Modulus of Coated PP Films[b]

Ethanol Concentration, %	Corn-zein Concentration (g/100 ml ethanol)	Young Modulus (MPa)
70	5	1028.28 ± 134.1
70	15	1046.68 ± 100.3
95	5	977.24 ± 64.30
95	15	1331.31 ± 80.49

[b]where plasticizer is glycerol, and its concentration is constant as 20% (w/w) based on corn-zein content.

The higher zein concentrations in coating formulation induced formation of coated PP films with higher resistance to deformation. Modulus of elasticity was enhanced with increasing zein content as seen in Table 5.21. Similar results obtained by Parris and Coffin in the literature were consistent with our results. They observed that the change in plasticizer composition resulted in decrease in initial modulus values from approximately 300 MPa to 100 MPa (Parris and Coffin 1997). The results based on mechanical properties of polysaccharide-coated PP film studied by Hong and his co-workers were also in agreement with our experimental study. They showed that depending on the type and amount of plasticizers, some polysaccharides, including especially chitosan could be used as an excellent coating material when a proper plasticizer is applied (Hong et al. 2005).

ANOVA table for Young Modulus of coated films for both plasticizers was given in Tables A.11 and A.12 in Appendix part. The results showed that model is significant for both plasticizers. The most effective parameters on Young Modulus of coated PP films were determined as corn zein-plasticizer and ethanol-plasticizer interaction. The coefficient tables related to these results were given in Appendix for two different plasticizers (Tables A.12 and A.13). The solvent-corn zein interaction was also observed as the most important parameter.

Finally, it was realized that developing of corn-zein coated PP films with desirable mechanical properties represented a rather challenging multidisciplinary problem. The results showed the mechanical behaviors of corn-zein PP films strongly depend on formulation of coating solution, which depends among other factors, on polymer-polymer and polymer-plasticizer interactions. Many studies showed that the films become weak in tensile strength and elongation at break increases at high plasticizer content. The incorporation of plasticizers into coating solution also induced the formation of protein-plasticizer interactions, consequently the loss of protein-protein interactions. These observations were also in agreement with our results obtained from this study. Elongation, tensile strength and modulus showed a stronger effect of plasticizer content. Also the smaller size of GLY present in coating solution influenced the mechanical property of the coated PP film, giving more elongation to the film compared to PEG. Similar changes in the mechanical properties of films as affected by different plasticizers were observed previously for various freestanding zein films (Lai et al. 1997, Di Gioa et al. 2000, Tillekeratne and Easteal 2000, Paramawati et al. 2001, Ghanbarzadeh et al. 2006)

Tillekeratne and Easteal studied the mechanical properties of corn zein films plasticized with PEG. Incorporation of PEG at low levels substantially enhances the tensile strength and elongation at break values of zein films (Tillekeratne and Easteal 2000). Paramawati and coworkers aimed to evaluate characteristics changes in tensile properties of zein-based film as affected by the composition of PEG. The flexibility of plasticized-zein films increased as the amount of PEG increased, as shown by the trend of decreasing elastic modulus and increasing elongation at break of zein films were observed (Paramawati et al. 2001). Ghanbarzadeh and his workers used sugars as plasticizers to determine mechanical properties of zein films. They reported that the tensile strength and elongation at break values of zein films increased with increasing plasticizer levels. In addition, Young modulus of zein films decreased continuously with increasing sugar plasticizer concentration (Ghanbarzadeh et al. 2006).

Similar changes in the mechanical properties of other protein-based and polysaccharide films were observed by several authors (Galietta et al. 1998, Turhan and Şahbaz 2004, Gao et al. 2006, Srinivasa et al. 2007). Galietta and coworkers studied the plasticizing effect of GLY on mechanical properties of whey protein-based films. They observed that increased plasticizer content decreased the mechanical resistance, especially Young modulus (Galietta et al. 1998). Turhan and Şahbaz studied the effect of plasticizer on mechanical properties of methylcellulose-based films. Consistent with our results, they found that PEG had the largest effect on tensile strength (Turhan and Şahbaz 2004). Thus, mechanical properties of methylcellulose-based edible films were compared as a function of plasticizer content by Gao et al. (Gao et al. 2006). They showed that the plasticizer had a strong effect on the protein-based films. Tensile strength, elongations at break and Young's modulus have shown strong dependence on amount of plasticizer used. Consistent with our results, tensile strength and modulus decreased with plasticizer level while elongation at break of films increased. Srinivasa and coworkers purposed to determine the mechanical properties of chitosan blend films with different plasticizers (GLY, sorbitol and PEG). The tensile strength of the blended films decreased with the addition of plasticizer, whereas the percent elongation was increased. Researches summarized above seemed to be in agreement with the results obtained from this study for zein-coated PP films (Srinivasa et al. 2007). Statistically differences were observed for all mechanical properties and uncoated PP film by hypothesis testing. Statistical analysis results showed that plasticizer and corn-zein

concentrations in coating formulation were the most effective parameters for final properties of resulted PP films.

5.3. Thermal Properties

The T_g of polymers depend on molecular weight, side branches, covalent or non-covalent bonding within the molecular structure, ratio of amorphous and crystalline regions, presence of a plasticizers, copolymers, and other physical conditions (Andrianaivo et al. 2003). Therefore, in this study, the thermal behavior of corn-zein coating films and coated PP films were investigated by DSC analysis to see the effects of plasticizer type and its concentrations on the glass transition temperature of freestanding corn-zein films, and also thermal properties (melting and degradation temperatures) of corn-zein coated PP films.

The glass transition temperature (T_g) of plasticized corn-zein coating solution was expected to show plasticizer compatibility with zein and effectiveness of corn-zein plasticization process. Thus, corn-zein film without plasticizer was initially analyzed to examine plasticizer effect on T_g of coating solution and T_g was found as approximately ~125°C for without plasticizer as shown in Figure 5.7. Since T_g is the function of polymer chain flexibility, the decrease in T_g of the zein coating was expected to decrease by increasing plasticizer content. The T_g values of corn-zein films plasticized with 20% PEG and GLY can be seen in Figure 5.7 and Table 5.22.
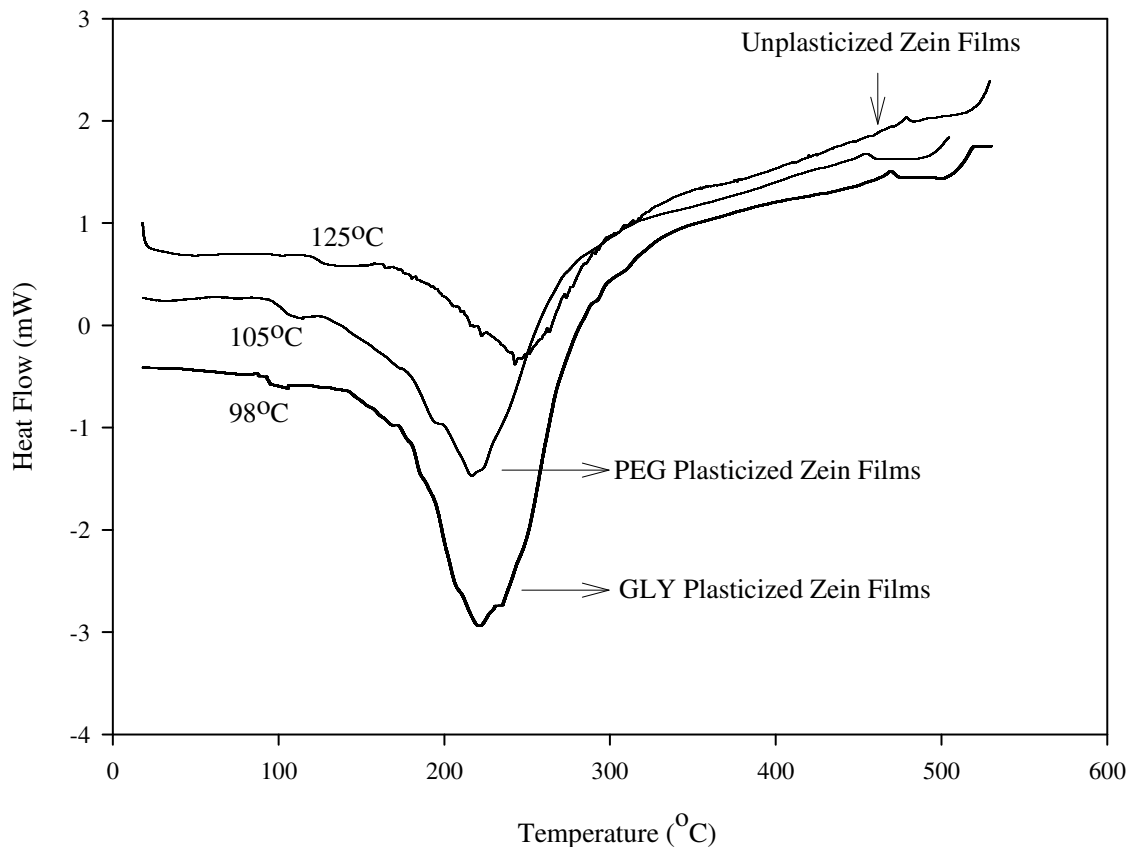
Figure 5.8. Differential Scanning Calorimetry (DSC) Thermograms of Corn-zein Films

Table 5.22. The Glass Transition Temperatures of Corn-zein Film as Affected by Plasticizer Content[a]

Plasticizer Type	Plasticizer Concentration, % (w/w) (based on corn-zein content)	T_g (^{o}C)
Control film	(without plasticizer)	125
PEG	20	105
GLY	20	98

[a] where ethanol and corn-zein concentrations are constant at 95% and 5%, respectively.

The incorporation of plasticizer into corn-zein solution resulted in a decrease in T_g value from 125 to 105°C and 98°C for PEG and GLY plasticized films, respectively. The slightly lower T_g value was obtained for GLY plasticizer corn-zein films. Since the T_g of GLY plasticized films was slightly lower than PEG, elongation at break values is higher,

but plasticizing effect of PEG on WVP is more pronounced than GLY due to hydrophilicity of PEG. Furthermore, these observations were similar to previous studies based on plasticizer effects on thermal behavior of zein films (Kokini et al. 1995, Madeka and Kokini 1996, Gioia and Guilbert 1999, Gioa et al. 2000, Ghanbarzadeh et al. 2006). Kokini and coworkers investigated the plasticizing effect of water on cereal proteins. They reported that the first few percentages of water resulted in a decrease in T_g of zein. Then, they measured T_g of zein at various moisture contents and reported its decrease from 139°C to 47°C when water content increased (Kokini et al. 1995). Gioa and Guilbert studied plasticization with various polar (water, glycerol) and amphiphilic (palmitic acids, dibutyl tartrate) plasticizers. They reported that the first amounts of added plasticizer (<10%) were the most effective at lowering T_g. However, at higher plasticizer content (between 10 and 30%) plasticization effectiveness slowed down due to difficulty in diffusion into polymeric matrix (Gioia and Guilbert 1999). Then, in another study of Gioa et al., the T_g values were reported and oleic acid plasticized zein films led to a decrease in T_g from 98°C to 48°C (Gioa et al. 2000). Finally, Ghanbarzadeh et al. observed T_g for zein resins plasticized containing various sugar levels and types in the range of 60-70°C range. However, they concluded that water content in zein resins has most important effect on T_g compared to sugars (Ghanbarzadeh et al. 2006).

Since T_g of freestanding corn-zein film reduced with the addition of plasticizer, corn-zein plasticized coating on PP would expected to have effects on the properties of PP coated films. For example, the change in mechanical properties as a function of water content could be related to the change in T_g of corn-zein coatings. The decrease in T_g of coating solution resulted in higher flexibility of coated PP films. Therefore, the corn-zein coating solution having lower T_g could result in more flexible of coated PP film structures. Therefore, coated PP films with corn-zein containing GLY was expected to have higher elongation at break values due to the lower temperature of the transition from glass to rubbery. This hypothesis was proved by elongation at break results as shown in Figure 5.6 and thermal behavior results of corn-zein developed into conformity with elongation values of coated PP films obtained in this study. Therefore, the mechanical properties of coated PP films were found to be directly correlated to the structure characteristics of the corn-zein, and particularly to the glass transition.

In addition, two major endothermic peaks were observed from DSC thermograms of plasticized zein coated PP films which were attributed to melting and degradation of bilayer films. The thermograms showed that the melting and degradation

peaks of the coated PP film were similar to uncoated PP film (Table 5.23), however shift in melting (T_m) and degradation temperatures (T_d) were observed. T_m and T_d of PP base film without coating were 170.01^{o}C and 457.79^{o}C, respectively. The peaks related to corn-zein were not seen in the thermogram due to PP base film thickness, and also small amount of plasticizers present in the whole film structure.

The coating of corn zein on PP films increased their degradation temperature while decreased melting temperature which can allow wide service temperature range of coated PP films without losing mechanical property such as strength, stiffness and toughness. The melting temperature of coated PP films decreased slightly due to increase in mobility of polymeric chains in coating by plasticization.

Table 5.23. The Melting (T_m) and Degradation Temperatures (T_d) of Corn-zein Coated PP Films Prepared According to Experimental Design

PEG Plasticization			GLY Plasticization		
Coated Film no	Melting Temp. (T_m)	Degradation Temp. (T_d)	Coated Film no	Melting Temp. (T_m)	Degradation Temp. (T_d)
1	164.91	459.76	12	167.32	461.09
2	166.56	460.07	13	169.07	460.21
3	168.23	462.86	14	169.76	462.53
4	169.08	463.42	15	169.30	463.40
5	168.55	461.57	16	168.74	463.03
6	165.52	463.96	17	168.97	463.44
7	169.28	462.66	18	167.85	461.29
8	168.85	462.32	19	169.22	464.70
9	167.25	460.70	20	165.12	459.22
10	166.98	461.23	21	165.88	458.94
11	166.56	460.59	22	166.90	459.65

5.4. Surface and Optical Properties

The appearance of films is the most important property for packaging applications requiring package visibility. Transparency is the property of allowing transmission of light through a film and color of the films are highly desirable

appearance attribute in packaging applications. Transparent film materials are highly desirable for a number of packaging applications. In addition, the wetting of film surface by liquids is important fundamental phenomenon in many applications such as printing, and water proofing. The contact angle of the film surface is the controlling factor for wetting. Therefore, the improved understanding of surface properties allows optimizing the visual and printing characteristics of coated PP films. In this study, the surface properties of corn-zein coated PP films were measured in terms of transparency, color, and contact angle related to degree of hydrophilic nature of surface. The property of corn-zein coating PP films that may be as important as barrier and mechanical properties is appearance they impart. The color of the corn-zein coated PP films differed with coating formulation. The total color difference (ΔE) values were calculated by using Hunter L, a, and b values measured with taking PP base film without coating as a reference were given in Table 5.24. This conversion of Hunter values to ΔE is necessary to obtain better differentiation of the color measurements.

Table 5.24. The Total Color Differences (ΔE) of Corn-zein Coated PP Films Prepared According to Experimental Design

PEG Plasticization		GLY Plasticization	
Coated Film no	Total Color Difference (ΔE)	Coated Film no	Total Color Difference (ΔE)
1	1.33 ± 0.84	12	1.69 ± 0.61
2	1.50 ± 1.14	13	1.38 ± 1.10
3	1.91 ± 0.72	14	5.11 ± 0.81
4	1.93 ± 0.51	15	1.56 ± 0.29
5	1.52 ± 0.98	16	1.00 ± 0.91
6	0.81 ± 0.66	17	0.48 ± 0.36
7	2.58 ± 0.21	18	1.43 ± 0.82
8	1.80 ± 0.70	19	1.88 ± 0.63
9	2.15 ± 0.53	20	1.17 ± 0.71
10	2.49 ± 0.26	21	1.11 ± 0.21
11	2.42 ± 0.32	22	1.60 ± 0.87

The color of corn-zein coated PP films with different coating formulations did not showed significant differences compared to PP base film without coating. Since it is generally known that ΔE values less than 3.0 can not be detected by naked human eye, it was very difficult to visually observe any color difference between coated PP and non-coated PP films. The higher transparency of coated PP films caused to approach ΔE to the zero in where it was assumed to be zero for PP base film without coating. The effect of plasticizer used and its concentration on color of coated films were tabulated in Table 5.25.

Table 5.25 The Effects of Plasticizer Type and Concentration on Color of Coated PP Films[a]

Plasticizer Type / Concentration	L	a	b	ΔE
PEG/20	90.68 ± 1.50	0.207 ± 0.003	1.016 ± 0.022	1.505 ± 1.145
PEG/50	91.24 ± 0.75	0.227 ± 0.003	0.899 ± 0.013	0.816 ± 0.667
GLY/20	90.78 ± 0.85	0.377 ± 0.006	0.557 ± 0.046	1.383 ± 1.105
GLY/50	90.98 ± 0.65	0.445 ± 0.006	0.699 ± 0.031	0.482 ± 0.363

[a]where ethanol and corn-zein concentrations are constant at 95% and 5%, respectively.

The color values of the corn-zein coated PP films were similar to those L = 90.56; a = 0.35 and b = 0.55 for the non-coated PP films which indicate lightness, redness, and yellowness, respectively. The considerable changes in transparency related to L values were not observed with corn-zein coating onto PP films. However, the increase in plasticizer content resulted to obtain more opaque film relating to higher L values. The application of con-zein coating plasticized with PEG reduced film redness as evidence by lower "a " values for coated films while redness was increased by using GLY plasticized coating. Furthermore, coating increased the yellowness of the PP films due to zein content in coating formulation. This result was significantly observed when the effect corn-zein concentration on yellowness of coated PP films was examined as shown in Table 5.26.

Table 5.26. The Effects of Ethanol and Corn-zein Concentrations on Color of Coated PP Films[a]

Ethanol / Zein Concentration	L	a	b	ΔE
70/5	89.53 ± 1.33	0.138 ± 0.002	1.581 ± 0.022	1.699 ± 0.612
70/15	90.65 ± 1.40	-1.06 ± 0.015	5.364 ± 0.075	5.118 ± 0.814
95/5	90.98 ± 0.85	0.377 ± 0.006	0.557 ± 0.046	1.383 ± 1.105
95/15	91.00 ± 0.75	-0.09 ± 0.001	1.897 ± 0.026	1.568 ± 0.290

[b]in where plasticizer is glycerol, and its concentration is constant as 20% (w/w) based on corn-zein content.

Since the commercial zein powder used in coating formulation has a yellow color, b (yellowness) values increased with corn-zein concentration. As concentration of zein present in coating increased, coated PP films became more yellowish. Thus, the greater b values of coated PP films were attributed to more intense yellowish coloration.

To date, some of the published works were based on color differences of freestanding zein films. Weller and coworkers investigated the color properties of bilayer films consist of wax layer as a coating layer and zein layer as a subtrate. They observed that the single-layer zein film had more intense yellowish coloration because of greater b (more yellow) and a (greener) values similar to our results. The application of wax layer on zein films significantly reduced film yellowness by lowering b values (Weller et al. 1998).

All these results were also related to transparency of coated PP films. The transparency of films affected by coating formulations was shown in Figure 5.9. Considering that the transparency value for PP base film was about 95, the PP films coated with corn-zein showed approximately similar and acceptable appearance properties for high ethanol concentrations (95%) in coating formulations.
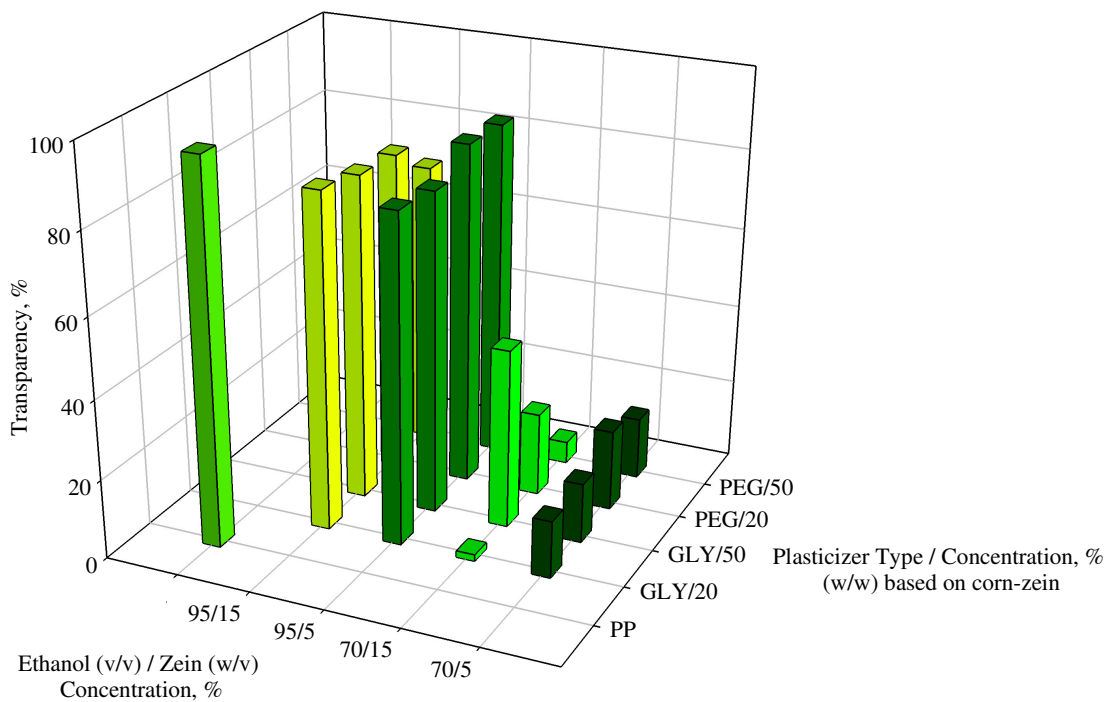
Figure 5.9. The Transparency of Corn-zein Coated PP Films as Affected by Coating Formulation

A little decrease in transparency can be caused by formation thin corn-zein coating layer onto PP. However, the reduction in transparency of coated films was significantly observed for lower ethanol concentrations of coating due to solubility behavior of zein in solvent. Since corn-zein comprises a group of alcohol soluble proteins, the concentration of ethanol affected the optical clarity. The coated films turned from transparent to opaque. Similar remarks could be considered for the effect of plasticizer to appearance. The transparency of the coated PP films had tendency to decrease less with the increasing plasticizer concentration used in coating formulation due to their molecular size. Moreover, the transparency values of coated PP films (Table 5.27) can be correlated with color differences in where the lower ΔE indicated that higher transparency.

Table 5.27. The Transmittance of Corn-zein Coated PP Films Prepared According to Experimental Design

PEG Plasticization		GLY Plasticization	
Coated Film no	Transmittance (%)	Coated Film no	Transmittance (%)
1	20.27 ± 6.39	12	14.45 ± 4.41
2	85.79 ± 1.58	13	82.15 ± 4.25
3	21.02 ± 3.99	14	2.300 ± 0.62
4	80.53 ± 1.25	15	83.97 ± 1.69
5	15.50 ± 7.96	16	17.77 ± 0.74
6	84.93 ± 3.47	17	86.53 ± 1.22
7	5.54 ± 0.74	18	45.11 ± 4.45
8	18.87 ± 4.82	19	85.57 ± 0.72
9	40.46 ± 6.46	20	88.56 ± 1.07
10	37.45 ± 3.15	21	89.49± 1.72
11	34.32 ± 4.71	22	90.29 ± 1.20

ANOVA tables for transparency values of coated films were given in Tables A.15 and A.16 in Appendix part. The general linear models procedure was used for ANOVA, and then results showed that model is significant for each plasticizer types. The statistical analysis defined the key parameters that had dominant effect on transparency of coated PP films as corn-zein concentration and ethanol concentration. The coefficient tables related to these results were also given in Appendix (Tables A.17 and A.18).

Lastly, surface characteristics of coated PP films were investigated. Water contact angles were determined to understand degree of hydrophobic nature of the coated film surface. The degree of hydrophobicity of surface is important for barrier and printable properties of coated films. The contact angle formed between the water droplets placed on the surface of the coated film and the kinetics of spreading is related to the hydrophobicity of the film. The schematic representation of contact angle of a liquid placed on the film surface was shown in Figure 5.10.

(a)   (b)   (c)

$\theta = 0^{o}$ $30^{o}<\theta<90^{o}$ $\theta>90^{o}$
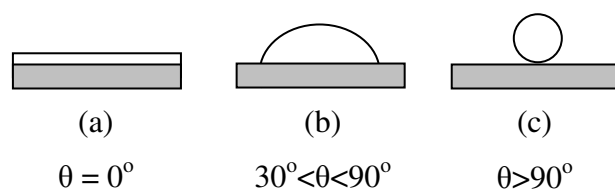
Figure 5.10. The Schematic Presentation of Contact Angles of Liquid Placed on a Film
Surface

The drop of water placed on the film surface spread either across the surface to form a thin, uniform film (Figure 5.9a) or to limited extent but remain as discrete drop on the surface (Figure 5.9b and Figure 5.9c). The final condition of water drop on the film surface was taken as the indication of wettability and hydrophobicity of the coated PP film surface. The wettability of the film was estimated by measuring of the contact angle, θ formed by the water droplet. The contact angle values of the corn-zein coated PP films measured were given in Table 5.28.

Table 5.28. The Contact Angles of Corn-zein Coated PP Films Prepared According to
Experimental Design

PEG Plasticization		GLY Plasticization	
Coated Film no	Contact Angle (deg)	Coated Film no	Contact Angle (deg)
1	33.00 ± 2.40	12	51.40 ± 3.37
2	42.40 ± 2.01	13	50.20 ± 3.24
3	27.80 ± 4.57	14	55.60 ± 2.01
4	50.20 ± 0.39	15	51.40 ± 2.01
5	30.20 ± 4.40	16	61.80 ± 2.35
6	51.40 ± 0.78	17	54.40 ± 2.67
7	29.00 ± 3.09	18	45.40 ± 2.11
8	19.80 ± 1.99	19	54.00 ± 1.63
9	30.80 ± 1.99	20	43.60 ± 1.92
10	40.20 ± 2.99	21	38.80 ± 2.43
11	43.40 ± 1.81	22	47.00 ± 0.87

It is well known that the water contact angle increases with increasing surface hydrophobicity. The surface hydrophobicity of zein coated films depends on the coating formulation included corn-zein concentration, plasticizer concentration, and plasticizer type. Therefore, the effects of these parameters examined on the surface hydrophobicity were given in Table 5.29.

Table 5.29. The Effects of Plasticizer Type and Concentration on Contact Angles of Coated PP Films[a]

Plasticizer Type	Plasticizer Concentration, % (w/w) (based on corn-zein content)	Contact Angle (deg)
PEG	20	51.40 ± 0.78
PEG	50	42.40 ± 2.01
GLY	20	54.40 ± 2.67
GLY	50	50.20 ± 3.24

[a]where ethanol and corn-zein concentrations are constant at 95% and 5%, respectively.

Since zein protein contains many non-polar amino acids, and can be considered a hydrophobic protein, it is expected to have high water contact angle reported as in the range of 60 and 70° in the literature (Ghanbarzadeh et al. 2006, Muthuselvi and Dhathathreyan 2006). In our study, it was found that the addition of plasticizer decreased water contact angle of corn zein coating. Therefore, the hydrophobicity of the coating layer decreased.

The coating formulated with GLY had higher water contact angle compared to PEG plasticized film. This result was caused by the strong hydrophilic interaction of PEG with water due to its higher molecular weight and hydrophilic nature than GLY. The increase in amount of plasticizer used in coating resulted in further decrease in water contact angle. The higher hydrophilic nature of coating formed by PEG plasticization was also confirmed by water vapor permeability (WVP) results of coated PP films. The higher WVP was obtained for PP films coated by PEG plasticized corn-zein compared to GLY plasticized films.

Since hydrophobic nature of coating would increase with corn-zein concentration, the increase in water contact angle of the coating surface was observed as

seen in Table 5.30. Hydrophobic coating on PP film decreased the surface energy of the coated films, along with decreasing water affinity by increasing the water contact angle.

Table 5.30. The Effects of Ethanol and Corn-zein Concentration on Contact Angle of Coated PP Films[b]

Ethanol Concentration, %	Corn-zein Concentration (g/100 ml ethanol)	Contact Angle (deg)
70	5	51.40 ± 3.37
70	15	55.60 ± 2.01
95	5	50.20 ± 3.24
95	15	51.40 ± 2.01

[b]where plasticizer is glycerol, and its concentration is constant as 20% (w/w) based on corn-zein content.

ANOVA tables for contact angles of coated films were given in Tables A.19 and A.20 in Appendix. The results showed that model is significant for each plasticizer types. Furthermore, the statistical analysis defined the key parameter that had dominant effect on contact angle results as only corn-zein concentration. The related coefficient table was given in Table A.21 in Appendix .

Similar results were obtained by several researchers (Ghanbarzadeh et al. 2006a, 2006b, Muthuselvi and Dhathathreyan 2006). Ghanbarzadeh and coworkers studied to determine the effects of plasticizers on hydrophobicity of zein films. The addition of sugar plasticizers to freestanding zein films increased the surface tension of zein films, and resulted in reduction of water contact angle of zein films. However, no significant differences within ethanol and ethylene glycol contact angle of zein films were observed. Similar to our results, the zein films containing GLY had the highest water contact angle compared to other plasticized films (Ghanbarzadeh et al. 2006). Muthuselvi and Dhathathreyan also found comparable results with this study. They observed significant decrease in contact angle of zein films compared to films of pure zein (Muthuselvi and Dhathathreyan 2006).

In addition to these studies based on corn-zein protein, polysaccharide based films showed consistent results with corn-zein film studies and also with our study. Hong and coworkers purposed to determine optical and surface properties of the whey

protein isolate (WPI) coated PP and polyvinyl chloride (PVC) films in terms color, contact angle as influenced by protein concentration and plasticizer type. WPI coatings were obtained as very transparent and coated films with various protein concentrations and plasticizer showed no remarkable changes in color compared to films without coating. Although ΔE of coated PP and PVC films increased with WPI content, those values were too small to differentiate the films from each other which were agreement with color results of corn-zein coated and non-coated PP films obtained in our study. WPI coatings on films represented lower contact angles than uncoated ones. Among the coated films, WPI concentration did not affect the contact angles. However, the plasticizer content in coating formulations decreased contact angles of coated films and surface energies with the order of PEG < sorbitol < GLY < polypropylene glycol (PG). According to their results PEG plasticized WPI coating has lower contact angle than other plasticized coatings due to its higher molecular weight again (Hong et al. 2004, Hong and Krochta 2004, Hong et al. 2005).

CHAPTER 6

CONCLUSION

In this study, the corn-zein coatings on polypropylene (PP) films were examined as an alternative to commercial coated films which consist of expensive and non-degradable polymers. The plasticized corn-zein coatings on PP films were prepared to investigate their barrier, mechanical, thermal, surface and optical properties as affected by corn-zein, ethanol, and plasticizer concentration and plasticizer types. Thus, the novel film structure for food packaging applications which provides better possibility in recycling processes because of easy separation of coating from the base plastic was proposed by optimizing coating formulation.

The final corn-zein coated PP films showed good appearance, flexibility and adhesion between the coating and the base film. The water vapor permeability (WVP) of PP films significantly decreased by corn-zein coating depending on coating formulation. The improvement of water vapor barrier properties of coated PP films was obtained for high concentrations of both corn-zein and ethanol in coating solutions. In addition, the WVP of coated PP films increased with increased concentrations of polyethylene glycol (PEG) and glycerol (GLY) used as plasticizers. The higher water barrier of corn-zein coated PP films was observed by plasticization of GLY as compared to PEG due to higher hydrophilicity of PEG. Corn-zein coating on PP film improved O_2 permeability of PP base film by showing nearly 4 times lower oxygen permeability than uncoated PP film. O_2 permeability of coated PP films increased with an increase in plasticizer concentration. The excellent oxygen barrier for coated PP films was obtained by applying coating solution which consists of higher amount corn-zein plasticized with GLY at low level.

The mechanical behavior of PP films (tensile strength, elongation at break, and Young's modulus) was significantly improved at different levels by all coating formulations of corn-zein. The effect of plasticization degree of coating solutions was considerably observed where elongations of coated PP films increased while elastic modulus and tensile strengths decreased by rising content of plasticizer used in coating formulations. GLY worked well as a plasticizer in corn-zein solution and the better improvements were obtained in mechanical properties of the corn zein coated PP films.

The statistical analysis of the results showed that corn zein, plasticizer concentrations and plasticizer type used in coating formulation were more effective parameters and had significant effect on barrier and mechanical behavior of coated PP films.

The coating of corn zein on PP films increased their degradation temperature which can allow higher service temperature without losing mechanical property such as strength, stiffness and toughness. Due to increase in mobility of polymeric chains in coating by plasticization, melting temperature of coated PP films decreased which allow high processability of films even at low temperatures. The color of corn-zein coated PP films with different formulations did not showed any significant differences (P<0.05) compared to PP base film without coating. The final corn-zein coated PP films showed good appearance and plasticizer used increased hydrophilicity of coated surface of PP which resulted in good printability by completely wetting the film surface.

In summary, results suggested that the corn-zein coated PP films showed tendency to provide packaging criteria with proper coating formulations especially high corn-zein content films plasticized with low level of GLY. Furthermore, the film coating formulation can be changed depending on property required. In conclusion, corn zein coatings on PP films could have potential as an alternative to synthetic coating materials with appropriate formulation.

REFERENCES

Alexander Katz, R. and Barrera, R. G. 1998. "Surface Correlation Effects on Gloss", *Journal of Polymer Science: Part B: Polymer Physics.* Vol. 36, pp. 1321-1334

Bai, J., Alleyne. V., Hagenmaier. R. D., James. P., Mattheis, J. P. and Baldwin E. A., 2003. "Formulation of Zein Coating for Apples", *Postharvest Biology and Technology.* Vol. 28, pp. 259-268.

Barr, C. D. Giacin, J. R. and Hernandez, R. J. 2000. "A Determination of Solubility Coefficient Values Determined by Gravimetric and Isostatic Permeability Techniques", *Packaging Technology and Science.* Vol. 13, pp. 157-167.

Bertuzzi, M. A., Vidaurre. C. E. F., Armada, M., Gottifredi, J.C., 2007. "Water vapor Permeability of Edible Starch Based Films", *Journal of Food Engineering.* Vol. 80 pp. 972-978.

Caner, C., Vergano, P. J., and Wiles, J. L., 1998. "Chitosan Film Mechanical and Permeation Properties as Affected by Acid, Plasticizer, and Storage", *Journal of Food Science.* Vol. 63, No. 6, pp. 1049-1053.

Chandra, R. and Rustgi, R. 1998. "Biodegradable Polymers", *Progressive Polymer Science.* Vol. 23, pp. 1273-1335.

Chen, C. C., Chueh, J. Y., Tseng, H., Huang, H. M. and Lee, S. Y., 2003. "Preparation and Characterization of Biodegradable PLA Polymeric Blends", *Biomaterials.* Vol. 24, pp. 1167-1173.

Comstock, K., Farrell, D., Christina Godwin, C. and Xi, Y., 2004. *From Hydrocarbons to Carbohydrates: Food Packaging of the Future*, (John Wiley, New York), pp. 1-25.

Corradini, E., Mattoso, L. H. C., Guedes, C. G. F. and Rosa, D. S. 2004. "Mechanical, Thermal and Morphological Properties of Poly(e-caprolactone)/Zein Blends", *Polymers for Advanced Technologies.* Vol. 15, pp. 340-345.

Crack, J., 2003. *The Mathematics of Diffusion*, (Oxford Science Publications), pp.25-35.

Cuq, B., Gontard, N. and Guilbert, S. 1998. "Proteins as Agricultural Polymers for Packaging Production", *The American Association of Cereal Chemists.* Vol. 75, No. 1, pp. 1-9

Cutter, C. N. 2006. "Opportunities for Bio-based Packaging Technologies to Improve the Quality and Safety of Fresh and Further Processed Muscle Foods", *Meat Science*. Vol. 74, pp. 131-142.

Debeaufort, F., Quezada-Gallo, J. A. and Voilley, A. 1998. "Edible Films and Coatings: Tomorrow's Packagings: A Review", *Critical Reviews in Food Science.* Vol. 38, No. 4, pp. 299-313.

Dickey, L.C., Parris N., Craig, J.C. and Kurantz, M.J., 2001. "Ethanolic Extraction of Zein from Maize", *Industrial Crops and Products.* Vol. 13, pp. 67-76.

Extrand, C. W. and Kumagai, Y., 1997. "An Experimental Study of Contact Angle Hysteresis", *Journal of Colloid and Interface Science.* Vol. 191, pp. 378-383.

Fang, Y., Tung, M. A., Britt, I.J., Yada, S., and Dalgleish, D.G. 2002. "Tensile and Barrier Properties of Edible Films Made from Whey Proteins", *Journal of Food Science.* Vol. 67, No. 1, pp.188-193.

Flores, S., Conte, A., Campos, C., Gerschenson, L. and Del Nobile, M. 2007. "Mass Transport Properties of Tapioca-based Active Edible Films", *Journal of Food Engineering.* Vol. 81, pp. 580-586.

Galietta, G., Gioia, L. D., Guilbert, S. and Cuq, B., 1997. "Mechanical and Thermomechanical Properties of Films Based on Whey Proteins as Affected by Plasticizer and Crosslinking Agents", *Journal of Dairy Science.* Vol. 81, pp. 3123-3130.

Gao, C., Stading, M., Wellner, N., Parker, M. L., Noel, T. R., Mills, E. N. C. and Belton, P. S., 2006. "Plasticization of a Protein-Based Film by Glycerol: A Spectroscopic, Mechanical, and Thermal Study", *Journal of Agricultural Food Chemistry.* Vol. 54, pp. 4611-4616.

García, M. A., Martino, M. N. and Zaritzky, N. E. 2000. "Lipid Addition to Improve Barrier Properties of Edible Starch-based Films and Coatings", *Journal of Food Science.* Vol. 65, No. 6, pp. 941-947.

Geankoplis, C. J., 1993. *Unit Operations and Transport Processes*, (Prentice Hall), pp.100-120

Gennadios, A., 2002. *Protein-Based Films and Coatings*, (CRC Press), pp.35-37

Ghanbarzadeh B., Musavi M., Oromiehie A.R., Jomeh Z. E.., Rad E. R. and Milani J., 2006. "Effect of Plasticizing Sugars on Water Vapor Permeability, Surface Energy and Microstructure Properties of Zein Films", *Food Research International.* Vol.39, pp. 882-890

Ghanbarzadeh, B., Musavi, M., Oromiehie, A.R., Rezayi, K., Razmi, E. and Milani, J. 2006. "Investigation of Water vapor Permeability, Hydrophobicity and Morphology of Zein Films Plasticized by Polyols", *Iranian Polymer Journal.* Vol.15, No.9, pp. 691-700

Gioia, L. D., Cuq, B. and Guilbert, S. 1998. "Effect of Hydrophilic Plasticizers on Thermomechanical Properties of Corn Gluten Meal", *Cereal Chemistry.* Vol. 75, No. 4, pp. 514-519.

Gioia, L. D. and Guilbert, S. 1999. "Corn Protein-Based Thermoplastic Resins: Effect of Some Polar and Amphiphilic Plasticizers", *Journal of Agricultural Food Chemistry.* Vol. 47, pp. 1254-1261.

Gioia, L. D., Cuq, B. and Guilbert, S. 2000. "Mechanical and Water Barrier Properties of Corn-protein Based Biodegradable Plastics", *Journal of Material Research.* Vol. 15, No. 12, pp. 2612-2619.

Godbol, S., Gote, S., Latkar, M. and Chakrabarti, T. 2000. "Preparation and characterization of biodegradable poly-3-hydroxybutyrate–starch blend films", *Bioresource Technology.* Vol. 86, pp. 33-37.

Guo, Y., Liu, Z., An, H., Li, M. and Hu, J. 2005. "Nano-structure and properties of maize zein studied by atomic force microscopy", *Journal of Cereal Science.* Vol. 41 pp. 277-281.

Haugaard, V. K., Udsen, A. M., Mortensen, G., Høegh, L., Petersen, K. and Monahan, F. 2001. "Potential Food Applications of Biobased Materials-An EU-Concerted Action Project", *Starch.* Vol. 53 pp. 189-200.

Herald, T. J., Obuz, E., Twombly, W. W. and Rausch, K. D. 2002. "Tensile Properties of Extruded Corn Protein Low-Density Polyethylene Films", *Cereal Chemistry.* Vol. 79, No. 2, pp. 261-264.

Herald, T.J., Hachmeister, K.A., Huang, S. and Bowers, J.R., 1996. "Corn-zein Packaging Materials for Cooked Turkey", *Journal of Food Science.* Vol.61 (2), pp.415-417

Hermand, R.C., 1993. *Biopolymers-Making Materials Natures' Way*, (John Wiley), pp. 25-48

Holmes, P. A. 1985. "Applications of PHB-Microbially Produced Biodegradable Thermoplastic", *Physical Technology.* Vol. 16, pp. 32-36

Hong, S. I. and Krochta, J. M. 2006. "Oxygen Barrier Performance of Whey-protein Coated Plastic Films as Affected by Temperature, Relative Humidity, Base Film and Protein Type", *Journal of Food Engineering.* Vol. 77, pp. 739-745.

Hong, S. I. and Krochta, J. M. 2004. "Whey Protein Isolate Coating on LDPE Film as a Novel Oxygen Barrier in the Composite Structure", *Packaging Technology and Science.* Vol.17, p. 13-21.

Hong, S. I., Han, J. H. and Krochta, J. M. 2004. "Optical and Surface Properties of Whey Protein Isolate Coatings on Plastic Films as Influenced by Substrate, Protein Concentration, and Plasticizer Type", *Journal of Applied Polymer Science.* Vol. 92, pp. 335-343.

Hong, S. I., Lee, J. W. and Son, S. M. 2005. "Properties of Polysaccharide-coated Polypropylene Films as Affected by Biopolymer and Plasticizer Types", *Packaging Technology and Science.* Vol.18, pp. 1-9

Hsu, B., Weng, Y., Liao, Y. and Chen, W. 2005. "Structural Investigation of Edible Zein Films/Coatings and Directly Determining Their Thickness by FT-Raman Spectroscopy", *Journal of Agricultural and Food Chemistry.* Vol 53, pp. 5089-5095

Hu, Y., Topolkaraev, V., Hiltner, A. and Baer, E., 2001. "Measurement of Water Vapor Transmission Rate in Highly Permeable Films", *Journal of Applied Polymer Science.* Vol. 81, pp. 1624-1633.

Immirzi, B., Malinconico, M., Romano, G., Russo, R. and Santagata, G. 2003. "Biodegradable Films of Natural Polysaccharides Blend", *Journal of Materials Science Letters.* Vol. 22, pp. 1389-1392.

Kaplan, D.L., 1998. *Biopolymers from Renewable Resources*, (Springer), pp.14-27

Kim, S.J. and Ustunol, Z. 2001. "Solubility and Moisture Sorption Isotherms of Whey-Protein-Based Edible Films as Influenced by Lipid and Plasticizer Incorporation", *Journal of Agricultural Food Chemistry.* Vol. 49, pp. 4388-4391.

Kolybaba, M., Tabil, L.G., Panigrahi, S., Crerar, W.J., Powell, T. and Wang, B. 2003. "Biodegradable Polymers: Past, Present, and Future", Proceedings on an ASAE Meeting, USA, (October 3-4), Elsevier, Vol.12, pp.15-24

Lai, H. M. and Padua, G.W. 1997. "Properties and Microstructure of Plasticized Zein Films", *Cereal Chemistry*, Vol. 74(6), pp. 771-775.

Lai, H. M. and Padua, G.W. 1998. "Water Vapor Barrier Properties of Zein Films Plasticized with Oleic Acid", *Cereal Chemistry.* Vol. 75, No.2, pp. 194-199.

Lai, H. M., Padua, G.W. and Wei, L. S. 1997. "Properties and Microstructure of Zein Sheets Plasticized with Palmitic and Stearic Acids", *Cereal Chemistry.* Vol. 74, No.1, pp. 83-90.

Lawton, J. W. 2004. "Plasticizers for Zein: Their Effect on Tensile Properties and Water Absorption of Zein Films", *Cereal Chemistry.* Vol. 81, No.1, pp. 1-5.

Lawton, J. W. 2002. "Zein: A History of Processing and Use", *Cereal Chemistry.* Vol. 79, No.1, pp. 1-18.

Madeka, H. and Kokini, J. L. 1996. "Effect of Glass Transition and Cross-Linking on Rheological Properties of Zein: Development of a Preliminary State Diagram", *Cereal Chemistry.* Vol. 73, No.4, pp. 433-438.

Martin, O., Schwach, E., Avérous, L. and Couturier Y. 2001. "Properties of Biodegradable Multilayer Films Based on Plasticized Wheat Starch" *Starch/Stärke.* Vol. 53, pp. 372-380.

Miller, K. S. and Krochta, J.M. 1997. "Oxygen and Aroma Barrier Properties of Edible Films: A Review", *Trends in Food Science & Technology.* Vol. 8, pp. 228-237.

Mohareb, E. and Mittal, G. S. 2006. "Formulation and Process Conditions for Biodegradable/Edible Soy-based Packaging Trays", *Packaging Technology and Science.* In press

Momany, F.A., Sessa, D., Lawton, J.W., Selling, G.W., Sharon, A., Hamaker, H. and Willett, J.L. 2006. "Structural Characterization of α-zein", *Journal of Agricultural and Food Chemistry*.Vol. 54, pp.543

Morillon, V., Debeaufort, F., Blond, G., Capelle, M. and Voilley, A. 2002. "Factors Affecting the Moisture Permeability of Lipid-Based Edible Films: A Review", *Critical Reviews in Food Science and Nutrition*. Vol. 42, No.1, pp. 67-89.

Muthuselvi, L. and Dhathathreyan, A. 2000. "Contact Angle Hysteresis of Liquid Drops as Means to Measure Adhesive Energy of Zein on Solid Substrates", *Pramana Journal of Physics*. Vol.66 (3), p.563

Nayak, P. L. 1999. "Biodegradable Polymers: Opportunities and Challenges", *Rev. Macromol. Chem. Phys.*. Vol. C39, No.3, pp. 481-505

Nielsen, L.E. and Landel, R.F. 1994. *Mechanical Properties of Polymers and Composites*, (John Wiley, New York), pp. 45-52

Olabarrieta, I. 2005. "Strategies to Improve the Aging, Barrier and Mechanical Properites of Chitosan, Whey and Wheat Gluten Protein Films", *KTH Fibre and Polymer Technolgy*. Vol. 42, pp.12-22

Paramawati, R., Yoshino, T. and Isobe, S. 2001. "Properties of Plasticized-Zein Film as Affected by Plasticizer Treatments", *Food Science Technology*. Vol. 7, No.3, pp. 191-194.

Park, H.J, Weller, C.L, Vergano, P.J. and Testin, R.F. 1993. "Permeability and Mechanical Properties of Cellulose-based Edible Films", *Journal of Food Science*. Vol. 58, No. 6, pp. 1361-1368.

Park, H. J., Chinnan, M. S. and Shewfelt, R. L. 1994. "Edible Coating Effects on Storage Life and Quality of Tomatoes", *Journal of Food Science*. Vol. 59, pp.568-572

Park, H.J and Chinnan, M. S. 1995. "Gas and Water Vapour Barrier Properties of Edible Films from Protein and Cellulosic Materials". *Journal of Food Engineering*. Vol 25, pp. 497-503.

Park, S. Y., Lee, B. I., Jung, S. T. and Park, H. J. 2001. "Biopolymer Composite Films Based on k-carrageenan and Chitosan", *Materials Research Bulletin*. Vol. 36, pp. 511-519.

Parris, N. and Coffin, D. R. 1997. "Composition Factors Affecting the Water Vapor Permeability and Tensile Properties of Hydrophilic Zein Films", *Journal of Agricultural Food Chemistry*. Vol. 45, pp. 1596-1599.

Peterson, K., Nielsen, P. V. and Olsen, M. B. 2001. "Physical and Mechanical Properties of Biobased Materials – Starch, Polylactate and Polyhydroxybutyrate", *Starch/Stärke*. Vol. 53, pp. 356-361.

Pouplin, M., Redl, A. and Gontard, N. 1999. "Glass Transition of Wheat Gluten Plasticized with Water, Glycerol, or Sorbitol", *Journal of Agricultural Food Chemistry.* Vol. 47, pp. 538-543.

Raj, B. and Sankar, K.U. 2004. "LDPE-Starch Blend Films for Food Packaging Applications", *Advances in Polymer Technology.* Vol 23, pp.32-45.

Rakotonirainy, A.M. and Padua, G.W. 2001. "Effects of Lamination and Coating with Drying Oils on Tensile and Barrier Properties of Zein Films", *Journal of Agricultural Food Chemistry.* Vol.43, pp. 2860-2863.

Reddy, C.S.K., Ghai, R., Rashmi, V.C. 2003. "Polyhydroxyalkanoates: An Overview", *Bioresource Technology.* Vol. 87, pp. 137-146.

Rodríguez, M., Osés, J., Ziani, K. and Mate, J. I. 2006. "Combined Effect of Plasticizers and Surfactants on the Physical Properties of Starch Based Edible Films", *Food Research International.* Vol. 39, pp. 840-846.

Santosa, F. X. B. and Padua, G. W. 2000. "Thermal Behavior of Zein Sheets Plasticized with Oleic Acid", *Cereal Chemistry.* Vol. 77, No.4, pp. 459-462.

Savenkova, L., Gercberga, Z., Nikolaeva, V., Dzene, A., Bibers, I. and Kalnin, M. 2000. "Mechanical properties and biodegradation characteristics of PHB-based films", *Process Biochemistry.* Vol. 35, pp. 573-579.

Shin, G.H., Lee, Y.H., Lee, J.S., Kim, Y.S., Choi W.S. and Park, H.J. 2002. "Preparation of Plastic and Biopolymer Multilayer Films by Plasma Source Ion Implantation", *Journal of Agricultural Food Chemistry.* Vol. 50, pp.4608-4615

Shukla, R. and Cheryan, M. 2001. "Zein: the Industrial Protein from Corn", *Industrial Crops and Products.* Vol. 13, pp. 171-192.

Shukla, R., Cheryan, M. and DeVor, R. E. 2000. "Solvent Extraction of Zein from Dry-Milled Corn", *Cereal Chemistry.*Vol. 77, No.6, pp. 724-730.

Sothornvit, R. and Krochta, J.M. 2000. "Water Vapor Permeability and Solubility of Films from Hydrolyzed Whey Protein", *Journal of Food Science.* Vol. 65, pp. 700-703.

Södergård, A. and Stolt, M. 2002. "Properties of Lactic Acid Based Polymers and Their Correlation With Composition", *Progress in Polymer Science.* Vol 27, pp. 1123-1163.

Srinivasaa, P.C., Rameshb, M.N. and Tharanathan, R.N. 2006. "Effect of Plasticizers and Fatty Acids on Mechanical and Permeability Characteristics of Chitosan Films", *Food Hydrocolloids.* In Press

Suyatma, N. E., Copinet, A., Tighzert, L. and Coma, V. 2004. "Mechanical and Barrier Properties of Biodegradable Films Made from Chitosan and Poly (Lactic Acid) Blends", *Journal of Polymers and the Environment.* Vol. 12, pp. 1-6.

Swalley, L. C. 2002. "ZEIN-A New Industrial Protein", *Corn Products Refining Company Journal.* Vol. 4, pp. 394

Tharanathan, R.N. 2003. "Biodegradable Films and Composite Coatings: Past, Present and Future", *Trends in Food Science & Technology.* Vol. 14, pp. 71-78.

Viljianmaa, M., Södergård, A. and Törmälä, P. 2002. "Lactic Acid Based Polymers as Hot Melt Adhesives for Packaging Applications", *International Journal of Adhesion & Adhesives.* Vol. 22, pp. 219-226.

Wang, Y. and Padua, G. W. 2003. "Tensile Properties of Extruded Zein Sheets and Extrusion Blown Films", *Macromolecular Materials and Engineering.* Vol. 288, pp. 886-893.

Wang, X.L., Yang, K.K. and Wang, Y.Z. 2003. "Properties of Starch Blends with Biodegradable Polymers", *Journal of Macromolecular Science.* Vol. C43, pp. 385-391.

Wang, Y., Rakotonirainy, M.A. and Padua, G. W. 2004. "Thermal Behavior of Zein-based Films", *Starch.* Vol.55, pp.25-32

Wang, Y. and Padua, G. W. 2004. "Water Sorption Properties of Extruded Zein Films", *Journal of Agricultural Food Chemistry.* Vol. 52, pp. 3100-3105.

Wang, Y., Filho, F. L., Geil P. and Padua, G. W. 2005. "Effects of Processing on the Structure of Zein/Oleic Acid Films Investigated by X-Ray Diffraction", *Macromolecular Bioscience.* Vol. 5, pp. 1200-1208.

Wang, Y. and Padua, G. W. 2006. "Water Barrier Properties of Zein-Oleic Acid Films", *Cereal Chemistry.* Vol. 83, No.4, pp. 331-334.

Weber, C.J., 2000. *Biobased Packaging Materials for the Food Industry: Status and Perspectives*, (KVL), pp.15-69.

Weller, C. L., Gennadios, A. and Saraiva, R.A. 1998. "Edible Bilayer Films from Zein and Grain Sorghum Wax or Carnauba Wax" , *LWT Journal.* Vol.31, pp.279-285.

Westhuis, R. A., Van der Walle, G. A.M. and Eggink, G. 2000. "Potential of PHA Based Packaging Materials for the Food Industry", Proceedings of the Food Biopack Conference, 27-9 August, Copenhagen, pp. 24-28

Wong, Y. C., Herald, T. J. and Hachmeister, K. A. 1996. "Evaluation of Mechanical and Barrier Properties of Protein Coatings on Shell Eggs" *Journal of Poultry Science.* Vol. 75, pp. 417-427

Wu, Q., Yoshino, T., Sakabe, H., Zhang, H. and Isobe, S. 2003. "Chemical Modification of Zein by Bifunctional Polycaprolactone (PCL)", *Polymer.* Vol. 44, pp. 3909-3019.

Wu, Q., Sakabe, H. and Isobe, S. 2003. "Studies on the Toughness and Water Resistance of Zein-based Polymers by Modification", *Polymer.* Vol. 44, pp. 3901-3908.

Yoshino, T., Isobe, S. and Maekawa, T. 2002. "Influence of Preparation Conditions on the Physical Properties of Zein Films", *Journal of the American Oil Chemists' Society.* Vol. 79, pp. 345-349.

APPENDIX A

Table A.1. ANOVA Table for Water Vapor Permeability Results of Corn-zein Coated
PP Films for PEG Plasticization

WVP	DF	SS	MS (variance)	F	p	SD
Total	30	2,465e-006	8,217e-008			
Constant	1	1,986e-006	1,986e-006			
Total Corrected	29	4,789e-007	1,651e-008			0,0001285
Regression	6	3,672e-007	6,121e-008	12,6121	**0,000**	0,0002474
Residual	23	1,116e-007	4,853e-009			6,96e-005
Lack of Fit (Model Error)	2	3,414e-008	1,707e-008	4,62644	**0,022**	0,0001306
Pure Error (Replicate Error)	21	7,749e-008	3,690e-009			6,07e-005
	N = 30	Q2 =	0,634	Cond. no.=	1,1180	
	DF = 23	R2 =	0,767	Y-miss =	0	
		R2 Adj. =	0,706	RSD =	0,0001	

Table A.2. ANOVA Table for Water Vapor Permeability Results of Corn-zein Coated
PP Films for GLY Plasticization

WVP	DF	SS	MS (variance)	F	p	SD
Total	33	3,396e-006	1,029e-007			
Constant	1	1,295e-006	1,295e-006			
Total Corrected	32	2,101e-006	6,566e-008			0,000256
Regression	6	1,729e-006	2,882e-007	20,156	**0,000**	0,0005368
Residual	26	3,717e-007	1,429e-008			0,000119
Lack of Fit (Model Error)	2	3,550e-007	1,775e-007	253,97	**0,000**	0,0004213
Pure Error (Replicate Error)	24	1,677e-008	6,989e-010			2,643e-005
	N = 33	Q2 =	0,762	Cond. no. =	1,1726	
	DF = 26	R2 =	0,823	Y-miss =	0	
		R2 Adj. =	0,782	RSD =	0,0001	

Table A.3. The Coefficient Table Water Vapor Permeability Results of Corn-zein Coated PP Films for PEG Plasticization

WVP	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	0,000257	1,271e-005	3,768e-016	2,631e-005
Solvent Concentration	5,502e-005	1,422e-005	7,781e-4	2,941e-005
Corn Concentration	8,753e-005	1,422e-005	**2,801e-006**	2,941e-005
Plasticizer Concentration	4,377e-005	1,422e-005	**0,005311**	2,941e-005
Solvent-Corn Interaction	1,114e-005	1,422e-005	**0,441355**	2,941e-005
Solvent-Plasticizer Interaction	-1,294e-005	1,422e-005	**0,372109**	2,941e-005
Corn- Plasticizer Interaction	-4,905e-005	1,422e-005	0,002180	2,941e-005
N = 30	Q2 = 0,634		Cond. no. = 1,1180	
DF = 23	R2 = 0,767		Y-miss = 0	
	R2 Adj. = 0,706		RSD = 0,0001	
			Conf. lev. = 0,95	

Table A.4. The Coefficient Table Water Vapor Permeability Results of Corn-zein Coated PP Films for GLY Plasticization

WVP	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	0,000198	2,081e-005	5,878e-010	4,278e-005
Solvent Concentration	-0,000121	2,440e-005	3,625e-005	5,017e-005
Corn Concentration	-0,000173	2,440e-005	**1,564e-007**	5,017e-005
Plasticizer Concentration	0,000126	2,440e-005	**2,161e-005**	5,017e-005
Solvent-Corn Interaction	5,134e-005	2,440e-005	0,04526	5,017e-005
Solvent-Plasticizer Interaction	-2,065e-005	2,440e-005	**0,40518**	5,017e-005
Corn- Plasticizer Interaction	-9,158e-005	2,440e-005	0,000889	5,017e-005
N = 33	Q2 = 0,762		Cond. no. = 1,1726	
DF = 26	R2 = 0,823		Y-miss = 0	
	R2 Adj. = 0,782		RSD = 0,0001	
			Conf. lev. = 0,95	

Table A.5. ANOVA Table for Tensile Strength Results of Corn-zein Coated PP Films
for PEG Plasticization

Tensile Strength	DF	SS	MS (variance)	F	p	SD
Total	40	5430,23	135,756			
Constant	1	5268,92	5268,92			
Total Corrected	39	161,313	4,13622			2,03377
Regression	6	23,7638	3,96064	0,950217	**0,473**	1,99014
Residual	33	137,549	4,16814			2,0416
Lack of Fit (Model Error)	1	6,03442	6,03442	1,46829	**0,000**	2,45651
Pure Error (Replicate Error)	32	131,514	4,10982			2,02727
	N = 40	Q2 =	0,008	Cond. no. =	1,0256	
	DF = 33	R2 =	0,147	Y-miss =	0	
	Comp. = 3	R2 Adj. =	-0,008	RSD =	2,0416	

Table A.6. ANOVA Table for Tensile Strenght Results of Corn-zein Coated PP Films
for GLY Plasticization

Tensile Strength	DF	SS	MS (variance)	F	p	SD
Total	40	5762,24	144,056			
Constant	1	5723,58	5723,58			
Total Corrected	39	38,6592	0,991261			0,995621
Regression	6	4,7384	0,789733	0,768296	**0,600**	0,888669
Residual	33	33,9208	1,0279			1,01386
Lack of Fit (Model Error)	1	19,5658	19,5658	43,616	**0,000**	4,42333
Pure Error (Replicate Error)	32	14,355	0,448593			0,669771
	N = 40	Q2 =	0,000	Cond. no. =	1,0256	
	DF = 33	R2 =	0,123	Y-miss =	0	
	Comp. = 3	R2 Adj. =	-0,037	RSD =	1,0139	

Table A.7. The Coefficient Table Tensile Strength Results of Corn-zein Coated PP Films for PEG Plasticization

Tensile Strength	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	11,4771	0,322806	6,85679e-028	0,656753
Solvent Concentration	-0,295263	0,326918	0,372981	0,66512
Corn Concentration	0,374367	0,326918	**0,260389**	0,66512
Plasticizer Concentration	-0,0656658	0,326918	**0,842038**	0,66512
Solvent-Corn Interaction	0,161393	0,331083	0,629148	0,673593
Solvent-Plasticizer Interaction	0,149247	0,331083	0,655093	0,673593
Corn- Plasticizer Interaction	0,582273	0,331083	0,0879011	0,673593
N = 40	Q2 =	0,008	Cond. no. =	1,0256
DF = 33	R2 =	0,147	Y-miss =	0
Comp. = 3	R2 Adj. =	-0,008	RSD =	2,0416
			Conf. lev. =	0,95

Table A.8. The Coefficient Table Tensile Strength Results of Corn-zein Coated PP Films for GLY Plasticization

Tensile Strength	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	11,962	0,160305	0	0,326142
Solvent Concentration	-0,159511	0,162347	0,332988	0,330297
Corn Concentration	-0,0168359	0,162347	**0,918031**	0,330297
Plasticizer Concentration	0,0851094	0,162347	**0,603612**	0,330297
Solvent-Corn Interaction	-0,188984	0,164415	**0,25864**	0,334505
Solvent-Plasticizer Interaction	0,169784	0,164415	0,309273	0,334505
Corn- Plasticizer Interaction	0,162006	0,164415	0,331623	0,334505
N = 40	Q2 =	0,000	Cond. no. =	1,0256
DF = 33	R2 =	0,123	Y-miss =	0
Comp. = 3	R2 Adj. =	-0,037	RSD =	1,0139
			Conf. lev. =	0,95

Table A.9. ANOVA Table for Elongation at Break Results of Corn-zein Coated PP Films for PEG Plasticization

Elongation at Break	DF	SS	MS (variance)	F	p	SD
Total	40	901769	22544,2			
Constant	1	882023	882023			
Total Corrected	39	19745,6	506,296			22,501
Regression	6	7176,29	1196,05	3,14017	**0,015**	34,5839
Residual	33	12569,3	380,887			19,5163
Lack of Fit (Model Error)	1	975,284	975,284	2,69184	**0,011**	31,2295
Pure Error (Replicate Error)	32	11594	362,312			19,0345
	N = 40	Q2 =	0,081	Cond. no. =	1,0256	
	DF = 33	R2 =	0,363	Y-miss =	0	
	Comp.=3	R2 Adj. =	0,248	RSD =	19,5163	

Table A.10. ANOVA Table for Elongation at Break Results of Corn-zein Coated PP Films for GLY Plasticization

Elongation at Break	DF	SS	MS (variance)	F	p	SD
Total	40	984176	24604,4			
Constant	1	977679	977679			
Total Corrected	39	6496,69	166,582			12,9067
Regression	6	2167,33	361,221	2,75336	**0,028**	19,0058
Residual	33	4329,36	131,193			11,4539
Lack of Fit (Model Error)	1	374,878	374,878	3,03355	**0,091**	19,3618
Pure Error (Replicate Error)	32	3954,48	123,578			11,1165
	N = 40	Q2 =	0,145	Cond. no. =	1,0256	
	DF = 33	R2 =	0,334	Y-miss =	0	
	Comp.=3	R2 Adj. =	0,212	RSD =	11,4539	

Table A.11. The Coefficient Table Elongation at Break Results of Corn-zein Coated PP
Films for PEG Plasticization

Elongation at Break	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	148,494	3,0858	3,79393e-032	6,27811
Solvent Concentration	-1,24697	3,12511	**0,692453**	6,35809
Corn Concentration	-3,91507	3,12511	**0,219092**	6,35809
Plasticizer Concentration	9,60543	3,12511	0,00422248	6,35809
Solvent-Corn Interaction	5,72965	3,16492	**0,0793546**	6,43909
Solvent-Plasticizer Interaction	-0,34435	3,16493	**0,91402**	6,43909
Corn- Plasticizer Interaction	-6,6205	3,16493	0,0442273	6,43909
N = 40	Q2 =	0,081	Cond. no. =	1,0256
DF = 33	R2 =	0,363	Y-miss =	0
Comp. = 3	R2 Adj. =	0,248	RSD =	19,5163
			Conf. lev. =	0,95

Table A.12. The Coefficient Table Elongation at Break Results of Corn-zein Coated PP
Films for GLY Plasticization

Elongation at Break	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	156,339	1,81103	0	3,68456
Solvent Concentration	-1,9823	1,8341	**0,287621**	3,7315
Corn Concentration	-2,29667	1,8341	**0,219297**	3,7315
Plasticizer Concentration	4,50268	1,8341	0,0195291	3,7315
Solvent-Corn Interaction	-0,634526	1,85746	**0,73481**	3,77904
Solvent-Plasticizer Interaction	-2,57826	1,85746	**0,17442**	3,77904
Corn- Plasticizer Interaction	-4,43989	1,85746	0,0227	3,77904
N = 40	Q2 =	0,145	Cond. no. =	1,0256
DF = 33	R2 =	0,334	Y-miss =	0
Comp. = 3	R2 Adj. =	0,212	RSD =	11,4539
			Conf. lev. =	0,95

Table A.13. ANOVA Table for Young Modulus Results of Corn-zein Coated PP Films for PEG Plasticization

Young Modulus	DF	SS	MS (variance)	F	p	SD
Total	40	6,6e+007	1,663e+006			
Constant	1	6,3e+007	6,373e+007			
Total Corrected	39	2,8e+006	72150,8			268,609
Regression	6	1,8e+006	310940	10,8211	**0,000**	557,62
Residual	33	948243	28734,6			169,513
Lack of Fit (Model Error)	1	8478,69	8478,69	0,288709	**0,095**	92,0798
Pure Error (Replicate Error)	32	939764	29367,6			171,37
	N = 40	Q2 =	0,514	Cond. no. =	1,0256	
	DF = 33	R2 =	0,663	Y-miss =	0	
	Comp.= 3	R2 Adj. =	0,602	RSD =	169,5129	

Table A.14. ANOVA Table for Young Modulus Results of Corn-zein Coated PP Films for GLY Plasticization

Young Modulus	DF	SS	MS (variance)	F	p	SD
Total	40	5,103e+007	1,275e+006			
Constant	1	5,001e+007	5,001e+007			
Total Corrected	39	1,018e+006	26114,3			161,599
Regression	6	546323	91053,9	6,36427	**0,000**	301,751
Residual	33	472133	14307			119,612
Lack of Fit (Model Error)	1	17343,9	17343,9	1,22036	**0,078**	131,696
Pure Error (Replicate Error)	32	454789	14212,1			119,215
	N = 40	Q2 =	0,225	Cond. no. =	1,0256	
	DF = 33	R2 =	0,536	Y-miss =	0	
	Comp.=3	R2 Adj. =	0,452	RSD =	119,6121	

Table A.15. The Coefficient Table Young Modulus Results of Corn-zein Coated PP Films for PEG Plasticization

Young Modulus	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	1262,25	26,8023	7,65453e-032	54,5298
Solvent Concentration	95,0766	27,1438	0,00134549	55,2245
Corn Concentration	86,5158	27,1438	0,00313625	55,2245
Plasticizer Concentration	132,792	27,1438	2,53367e-005	55,2245
Solvent-Corn Interaction	103,248	27,4896	0,000668914	55,928
Solvent-Plasticizer Interaction	-27,874	27,4896	**0,317968**	55,928
Corn- Plasticizer Interaction	50,913	27,4896	**0,0729775**	55,928
N = 40	Q2 =	0,514	Cond. no. =	1,0256
DF = 33	R2 =	0,663	Y-miss =	0
Comp. = 3	R2 Adj. =	0,602	RSD =	169,5129
			Conf. lev. =	0,95

Table A.16. The Coefficient Table Young Modulus Results of Corn-zein Coated PP Films for GLY Plasticization

Young Modulus	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	1118,22	18,9123	4,577e-035	38,477
Solvent Concentration	29,5987	19,1533	**0,131796**	38,967
Corn Concentration	53,8637	19,1533	0,00821999	38,967
Plasticizer Concentration	-5,19706	19,1533	**0,787819**	38,967
Solvent-Corn Interaction	102,291	19,3973	8,232e-006	39,464
Solvent-Plasticizer Interaction	-0,652186	19,3973	**0,973374**	39,464
Corn- Plasticizer Interaction	-1,27125	19,3973	**0,948146**	39,464
N = 40	Q2 =	0,225	Cond. no. =	1,0256
DF = 33	R2 =	0,536	Y-miss =	0
Comp. = 3	R2 Adj. =	0,452	RSD =	119,6121
			Conf. lev. =	0,95

Table A.17. ANOVA Table for Transparency Results of Corn-zein Coated PP Films for PEG Plasticization

Transparency	DF	SS	MS (variance)	F	p	SD
Total	40	135148	3378,7			
Constant	1	90820,9	90820,9			
Total Corrected	39	44327,1	1136,59			33,714
Regression	6	43863,6	7310,6	520,5	**0,000**	85,502
Residual	33	463,496	14,0453			3,7477
Lack of Fit (Model Error)	1	83,0963	83,0963	6,9902	**0,013**	9,1157
Pure Error (Replicate Error)	32	380,4	11,8875			3,4478
	N = 40	Q2 = 0,980		Cond. no. = 1,0256		
	DF = 33	R2 = 0,990		Y-miss = 0		
	Comp. = 3	R2 Adj. = 0,988		RSD = 3,7477		

Table A.18. ANOVA Table for Transparency Results of Corn-zein Coated PP Films for GLY Plasticization

Transparency	DF	SS	MS (variance)	F	p	SD
Total	40	144730	3618,25			
Constant	1	100200	100200			
Total Corrected	39	44529,9	1141,79			33,7904
Regression	6	43177,9	7196,32	175,655	**0,000**	84,8312
Residual	33	1351,96	40,9684			6,40066
Lack of Fit (Model Error)	1	1233,96	1233,96	334,633	**0,000**	35,1277
Pure Error (Replicate Error)	32	118	3,6875			1,92029
	N = 40	Q2 = 0,957		Cond. no. = 1,0256		
	DF = 33	R2 = 0,970		Y-miss = 0		
	Comp. = 3	R2 Adj. = 0,964		RSD = 6,4007		

Table A.19. The Coefficient Table Transparency Results of Corn-zein Coated PP Films
for PEG Plasticization

Transparency	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	47,65	0,592565	0	1,20558
Solvent Concentration	32,9778	0,600114	**5,0074e-034**	1,22094
Corn Concentration	-2,78492	0,600115	**5,297e-005**	1,22094
Plasticizer Concentration	-3,92952	0,600115	1,941e-007	1,22094
Solvent-Corn Interaction	-1,60446	0,607759	**0,012564**	1,2365
Solvent-Plasticizer Interaction	0,300944	0,607759	0,623761	1,2365
Corn- Plasticizer Interaction	-3,41591	0,607759	2,95328e-006	1,2365
N = 40	Q2 =	0,980	Cond. no. =	1,0256
DF = 33	R2 =	0,990	Y-miss =	0
Comp. = 3	R2 Adj. =	0,988	RSD =	3,7477
			Conf. lev. =	0,95

Table A.20. The Coefficient Table Transparency Results of Corn-zein Coated PP Films
for GLY Plasticization

Transparency	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	50,05	1,01203	1,55836e-032	2,059
Solvent Concentration	31,7986	1,02493	**5,41482e-026**	2,08523
Corn Concentration	3,22548	1,02493	0,00348649	2,08523
Plasticizer Concentration	4,70169	1,02493	6,18934e-005	2,08523
Solvent-Corn Interaction	-2,18197	1,03798	0,0432556	2,11179
Solvent-Plasticizer Interaction	-6,55768	1,03798	**3,79875e-007**	2,11179
Corn- Plasticizer Interaction	4,16255	1,03798	0,000326823	2,11179
N = 40	Q2 =	0,957	Cond. no. =	1,0256
DF = 33	R2 =	0,970	Y-miss =	0
Comp. = 3	R2 Adj. =	0,964	RSD =	6,4007
			Conf. lev. =	0,95

Table A.21. ANOVA Table for Contact Angle Results of Corn-zein Coated PP Films for PEG Plasticization

Contact Angle	DF	SS	MS (variance)	F	p	SD
Total	40	59863	1496,57			
Constant	1	52056,2	52056,2			
Total Corrected	39	7806,77	200,174			14,1483
Regression	6	5015,53	835,921	9,88282	**0,000**	28,9123
Residual	33	2791,25	84,5832			9,19691
Lack of Fit (Model Error)	1	2404,8	2404,85	199,159	**0,000**	49,0392
Pure Error (Replicate Error)	32	386,4	12,075			3,47491
	N = 40	Q2 =	0,513	Cond. no. =	1,0256	
	DF = 33	R2 =	0,642	Y-miss =	0	
	Comp. = 3	R2 Adj. =	0,577	RSD =	9,1969	

Table A.22. ANOVA Table for Contact Angle Results of Corn-zein Coated PP Films for GLY Plasticization

Contact Angle	DF	SS	MS (variance)	F	p	SD
Total	40	106419	2660,48			
Constant	1	103531	103531			
Total Corrected	39	2888,38	74,0609			8,60586
Regression	6	2665,44	444,24	65,7595	**0,000**	21,077
Residual	33	222,932	6,75553			2,59914
Lack of Fit (Model Error)	1	81,3324	81,3324	18,3802	**0,000**	9,01845
Pure Error (Replicate Error)	32	141,6	4,425			2,10357
	N = 40	Q2 =	0,897	Cond. no. =	1,0256	
	DF = 33	R2 =	0,923	Y-miss =	0	
	Comp. = 3	R2 Adj. =	0,909	RSD =	2,5991	

Table A.23. The Coefficient Table Contact Angle Results of Corn-zein Coated PP Films for PEG Plasticization

Contact Angle	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	36,075	1,45416	6,45146e-023	2,95851
Solvent Concentration	7,63513	1,47268	1,0706e-005	2,9962
Corn Concentration	-4,8008	1,47269	**0,002588**	2,9962
Plasticizer Concentration	-1,23347	1,47269	0,4083	2,9962
Solvent-Corn Interaction	-3,80134	1,49145	0,0156465	3,03437
Solvent-Plasticizer Interaction	-1,24469	1,49145	**0,409972**	3,03437
Corn- Plasticizer Interaction	-5,55989	1,49145	0,000723304	3,03437
N = 40	Q2 = 0,513		Cond. no. =	1,0256
DF = 33	R2 = 0,642		Y-miss =	0
Comp. = 3	R2 Adj. = 0,577		RSD =	9,1969
			Conf. lev. =	0,95

Table A.24. The Coefficient Table Contact Angle Results of Corn-zein Coated PP Films for GLY Plasticization

Contact Angle	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	50,875	0,41096	0	0,836104
Solvent Concentration	2,8782	0,416195	6,70192e-008	0,846756
Corn Concentration	-5,19997	0,416195	**4,6607e-014**	0,846756
Plasticizer Concentration	4,33631	0,416195	5,73597e-012	0,846756
Solvent-Corn Interaction	3,68006	0,421497	4,31693e-010	0,857543
Solvent-Plasticizer Interaction	-0,00747	0,421497	**0,98608**	0,857543
Corn- Plasticizer Interaction	-1,01932	0,421497	0,021272	0,857543
N = 40	Q2 = 0,897		Cond. no. =	1,0256
DF = 33	R2 = 0,923		Y-miss =	0
Comp. = 3	R2 Adj. = 0,909		RSD =	2,5991
			Conf. lev. =	0,95