ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

QUALITY EVALUATION OF POMEGRANATE ARILS WITH CHICKPEA STARCH BASED EDIBLE FILM

M.Sc. THESIS

Tayfun YAMAN

Department of Food Engineering
Food Engineering Program

JUNE 2013

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Tayfun YAMAN
(506091532)

Department of Food Engineering
Food Engineering Program

Thesis Advisor: Dr. F. Ebru FIRATLIGIL DURMUS

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

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YÜKSEK LİSANS TEZİ

Tayfun YAMAN
(506091532)

Gıda Mühendisliği Anabilim Dalı
Gıda Mühendisliği Programı

Tez Danışmanı: Dr. F. Ebru FIRATLIGİL DURMUŞ

HAZİRAN 2013

Tayfun Yaman, a **M.Sc.** student of **ITU Graduate School of Science Engineering and Technology** student ID **506091532**, successfully defended the thesis entitled "**QUALITY EVALUATION OF POMEGRANATE ARILS WITH CHICKPEA STARCH BASED EDIBLE FILM**", which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor : **Dr. F. Ebru FIRATLIGIL DURMUS** .....
İstanbul Technical University

Jury Members : **Prof. Dr. Beraat ÖZÇELİK** .....
İstanbul Technical University

Yrd.Doç.Dr. Derya KAHVECİ .....
Yeditepe University

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To my parents and brother,

FOREWORD

Shelf life of the food products depends on the surrounding atmosphere with relative humidity, oxygen, light etc. If the food interacts directly with surrounding environment, the quality and shelf life decreases. It loses/gains moisture or aroma, takes oxygen (results in oxidative rancidity) or is contaminated by microorganisms. In food system, edible films which adjust the transfer of water vapor, oxygen, carbon dioxide and lipid, are good solution to these problems. Moisture, aroma and fat contents and their transfers are important in food products which prepared with mixture of different materials. As a solution to these problems, different packaging techniques have been developed. Nowadays, there are more research about edible film and coatings.

Most of the starch based edible films are made from corn, wheat, pea and potato. Chickpea as a rich in starch content is a promising material for starch based edible films. In this study, edible films made from chickpea is used and proporties of edible films are determined. Pomegranate arils are used to coat with chickpea starch based edible film and quailty evaluation is investigated.

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ABBREVIATIONS

ABTS	**:** 2,2'azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
CUPRAC	**:** Cupric reducing antioxidant capacity
DPPH	**:** 1,1-diphenhenyl-2-picrylhydrazyl
OP	**:** Oxygen Permeability
RH	**:** Relative Humidity
TSS	**:** Total Soluble Solid
WVP	**:** Water Vapor Permeability

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QUALITY EVALUATION OF POMEGRANATE ARILS WITH CHICKPEA STARCH BASED EDIBLE FILM

SUMMARY

An edible film is defined as a thin layer made of edible material which is formed on a food as a coating..

Edible films can regualte the transfer of moisture, oxygen, carbon dioxide, lipid, aroma, and flavor compounds in food systems and can improve shelf life of food product and food quality. Although the usage of edible films and coatings in food products is applied lately, the idea of edible films and coating has intrigued packaging and food engineers for a long time. For example, during the twelfth and thirteenth centuries, dipping of oranges and lemons in wax to prevent water loss was applied in China. Edible films and coatings have an use in lots of applications including casings for sausages, chocolate coatings for nuts and fruits and wax coatings for fruit and vegetables.

The use of starch based edible film is increasing because of its functional properties in food processing and storage. To be low cost source, to be rich in many foods like corn, pea, potato, chickpea etc., to be easy to process and to use as natural are increasing its importance.

Objectives of this research were to (1) to determine the water vapor permeability and oxygen permeability of starch based edible films to select the best formulation for coating pomegranate arils, (2) to obtain information about the sorption isotherms of the edible film at different temperatures and modelling with different sorption isotherm models for predicting stability and quality changes during the packaging, (3) to investigate the effect of coating pomegranate arils on physcial and chemical changes (weight loss, titratable acidity, pH, total soluble solids content), total phenolic content, total flavonoid content and antioxidant activity.

For these objectives, chickpea purchased from central supermarket and pomegranate obtained from greengrocer in Istanbul province is used. Chickpea was directly used to produce starch. 4% starch solution was used to form film forming solution and 5 different plasticizer content was used: glycerol, sorbitol and three different ratios of them (1:1, 1:3, 3:1). Then water vapor permeability and oxygen permeability of these edible films were determined. According to results, three best formulation was choosen to coat pomegranate arils. Coated arils were used to observe physical and chemical changes during storage at 4 ^{o}C 10 days : weight loss, total soluble solids content, titratable acidity and pH. Then pomegranate arils were used to observe their total phenolic contents, total flavonoid contents and total antioxidant activity by three different methods: 1,1-diphenhenyl-2-picrylhydrazyl (DPPH) radical scavenging

activity, Cupric Reducing Antioxidant Capacity (CUPRAC) and 2,2'azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) analysis method.

The low cost, abundance of source, good mechanical and barrier properties of chickpea starch potentiate their applications for food preservation. The water vapor annd oxygen permeability of edible films from chickpea starch plasticized by glycerol and sorbitol were evaluated. The results obtained show that different ratios of plasticizer affected WVP and OP of edible films. The addition of plasticizer increased the water vapor permeability of starch films. The highest WVP value was observed in 90% glycerol plasticized edible film, while the lowest in 70% sorbitol added film. 70% Sorbitol plasticized film has better moisture barrier property with having lower WVP value.

Oxygen permeability of 90% sorbitol added edible film was significantly lower than that of 90% glycerol plasticized edible film. Increasing the percentage of plasticizer sorbitol results in decreasing the oxygen permeability thus can improve oxygen barrier properties. It has reverse effect on glycerol. Increased level of glycerol had higher oxygen permeability.

When it is compared the sorption isotherms of the edible films, the hygroscopic properties from higher to lower is respectively edible film plasticized by 90% sorbitol, 80% sorbitol and 70% sorbitol. The slope of the curve of edible films is increased after the water activity value 0.6. In this study, sorption models for applicablity are investigated for different edible films. As a result, sorption equilibrium can be well described with Halsey equation. Because it is applicable for all edible film samples.

The minimum weight loss was obtained in arils with 90% sorbitol plasticized coatings, while the maximum weight loss was observed in uncoated arils. In this term, coating improves the quailty of pomegranate arils.

All in all, the chickpea starch-based film coatings maintained the quality of pomegranate arils and prolonged shelf life, compared to uncoated fruit. Starch-based solutions can be alternative to conserve pomegranate arils.

NOHUT NİŞATASI BAZLI YENİLEBİLİR FİLM İLE KAPLANMIŞ NAR TANELERİNİN KALİTE DEĞERLENDİRİLMESİ

ÖZET

Yenilebilir filmler ve kaplamalar gıdanın dış yüzüne kaplanan ve gıdanın raf ömrünü ve kalitesini arttırmaya yönelik uygulanan yenilebilir malzemeler olarak tanımlanırlar.

Meyve ve sebzeler yapılarında kabuk ve mum gibi doğal olarak koruyucu bir tabakaya sahiptirler. Bu doğal tabakanın, oksijen, karbondioksit gibi çeşitli gaz ve nem kaybını düzenlediği, aroma ve lezzet kaybını azalttığı bilinmektedir. Gıda sistemlerinde nem, oksijen, karbondioksit, lipid, aroma ve tat bileşenlerinin geçirgenliğini düzenleyen yenilebilir filmler, gıdanın yapısnı ve raf ömrünü geliştirir.

Yenilebilir film ve kaplamalar olarak, polisakkaritler, proteinler ve lipidler gibi biyopolimerlerden yararlanılmaktadır. Yenilebilir filmler ve kaplama konusu son yıllarda artarak devam etmekteyse de, bu yöntem geleneksel olarak, gıdayı korumak veya yeni tat kazandırmak için asırlardan beri kullanılmakta olduğu bilinmektedir. En çok bilinen örnekleri, meyvelerin mum ile, şekerlemeler, badem, üzüm, fındık, fıstık gibi meyvelerin ve fırıncılık ürünlerinin çikolata ile, et ürünlerinin yağ bazlı filmlerle kaplanmasıdır.

Yenilebilir filmler, birçok kullanım alanı ile gıdanın besin değerini kaybetmeden, güvenilir ve yüksek kaliteli olarak pazarlanmasını sağlar. Yenilebilir film veya kaplamanın en önemli kullanım alanları arasında, kütle transferini kontrol etmesi, mekanik koruma ve duyusal çekicilik sağlaması sayılabilir.

Gıdalardaki ve depolama koşullarındaki fonskiyonel özellikleri nedeniyle nişasta bazlı yenilebilir filmlerin kullanımı gün geçtikçe artmaktadır. Düşük fiyatlı olması, mısır, bezelye, patates, nohut gibi birçok gıdada bolca bulunması, kolay işlenmesi ve doğal olarak kullanılması nişastanın önemini arttırmaktadır.

Bu çalışmanın amaçları: (1) nar tanelerini kaplamak için en uygun formülasyonu seçmek için nişasta bazlı yenilebilir filmlerin su buharı geçirgenliğini ve oksijen geçirgenliğini belirlemek, (2) paketleme süresince stabilite ve kalite değişikliklerini tahmin etmek için yenilebilir filmlerin farklı sıcaklılarda ve modellerde sorpsiyon izotermleri hakkında bilgi edinmek (3) kaplamanın nar taneleri üzerindeki etkilerini (fiziksel ve kimyasal değişiklikler, toplam fenolik içeriği, toplam flavonoid içeriği, toplam antioksidan aktivitesi) incelemek.

Bu amaçla, İstanbul manavından alınan nar ve süpermarketten alınan nohutlar kullanılmıştır. Nohut doğruca nişasta üretimi için kullanıldı. %4 lük nişasta çözeltisi film oluşturan çözelti eldesinde kullanıldı. Film oluşturan çözeltiye beş farklı

plastikleştirici ajan eklendi. Bunlar; gliserol, sorbitol ve gliserolle sorbitolün farklı oranlarda karışımından elde edilen plastikleştiriciler (Gliserol:Sorbitol, 1:1, 1:3, 3:1). Farklı bileşimlerde yenilebilir filmler elde edildikten sonra bu filmlerin su buharı geçirgenlikleri ve oksijen geçirgenlikleri belirlendi. Sonuçlara göre, nar tanelerini kaplamak için en iyi üç formülasyon seçildi. Seçilen bu formülasyonlarla nar taneleri kaplandı. Kaplanan nar tanelerinin 4 ^{o}C'de 10 gün boyunca depolanması sırasındaki fiziksel ve kimyasal değişiklikleri incelendi: ağırlık kaybı, toplam çözünebilir katı madde içeriği, titrasyon asitliği ve pH. Ayrıca nar tanelerinin depolama sırasında her gün yapılan analizlerle toplam fenolik içeriği, toplam flavonoid içeriği ve toplam antioksidan aktivitesi araştırıldı. Toplam antioksidan aktivitesi için 3 farklı metoddan yararlanıldı:1,1-diphenhenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, Cupric Reducing Antioxidant Capacity (CUPRAC) ve 2,2'azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) analiz metodları.

Gliserol ve sorbitolün plastikleştirici ajan olarak kullanıldığı nohut nişastasından elde edilen yenilebilir filmlerim su buharı geçirgenlikleri ve oksijen geçirgenlikleri değerlendirildi. Elde edilen sonuçlar farklı oranlarda ilave edilen plastikleştiricilerin su buharı ve oksijen geçirgenliklerini etkilediğini göstermiştir. Plastikleştirici ajan ilavesi nişasta bazlı filmlerin su buharı geçirgenliklerini arttırmıştır. En yüksek su buharı geçirgenlik değeri %90 gliserolle plastikleştirilmiş yenilebilir filmde (8,7458 ± 0,7a g.mm/day.m^2.kPa) gözlenirken, en düşük su buharı geçirgenlik değeriyse %70 sorbitol ilave edilmiş filmde (0,7733 ± 0,3h g.mm/day.m^2.kPa) gözlendi. %70 sorbitol içeren yenilebilir film düşük su buharı geçirgenliğe sahip olmasıyla nem bariyer özellikleri diğer filmlere göre daha iyidir.

%90 sorbitol (0,885 ml/day.m^2.kPa) ilave edilmiş yenilebilir filmin oksijen geçirgenliği %90 gliserolle (8,040 ml/day.m^2.kPa) plastikleştirilmiş yenilebilir filminkinden önemli derecede düşüktür. Yenilebilir filme katılan sorbitolün yüzdesinin arttırılması filmin oksijen geçirgenlinin azalmasına yani daha iyi oksijen bariyer özelliklerine nedenolmuştur. Gliseroldeyse durum sorbitolün gösterdiği etkinin zıttıdır. Filmdeki gliserol yüzdesi arttıkça, oksijen geçirgenliği de artmaktadır.

Yenilebilir filmlerin sorpsiyon izotermleri karşılaştırıldığında, higroskopik özellikleri en yüksekten en düşüğe doğru sırasıyla, %90, %80 ve %70 sorbitolle plastikleştirilmiş yenilebilir filmlerdir. Su aktivitesi değeri 0,6'dan sonra yenilebilir film eğrilerinin eğimi artmıştır. Bu çalışmada sorpsiyon modellerine uygunluğun belirlenmesi için 3 farklı sıcaklıkta (20 ^{o}C, 30 ^{o}C, 40 ^{o}C) 3 farklı yenilebilir film (%70, %80 ve %90 sorbitol ilave edilmiş) kullanılmıştır. Sonuç olarak, Halsey denklemi tüm film örneklerine uygunluk göstererek sorpsiyon eşitliğini iyi bir şekilde tanımlıyor.

Minimum ağırlık kaybı %90 sorbitol ilave edilmiş yenilebilir filmle kaplanmış nar tanelerinde (0,71 % weight loss) gözlenirken, maksimum ağırlık kaybı kaplanmamış nar tanelerinde (1,17 % weight loss) gözlendi. Bu açıdan, kaplama nar tanelerinin kalitesini geliştirir.

Antioksidan aktiviteleri incelendiğinde her üç metodla (DPPH, ABTS, CUPRAC) da 4 ^{o}C'de 10 günlük depolama süresi boyunca kaplanmamış nar tanelerinin antioksidan içeriği farklı konsantrastonlarda ilave edilmiş (70%, 80% ve 90%) sorbitol ile plastikleştirilmiş yenilebilir filmle kaplanmış nar tanelerinin antioksidan içerğinden daha düşük olduğu gözlenmiştir.

Kısacası, nohut nişastası bazlı yenilebilir filmle kaplama işlemi kaplanmamış örneklerle karşılaştırıldığında nar tanelerinin kalitesini devam ettirdi ve raf ömrünü uzattı. Nişasta bazlı çözeltiler nar tanelerini muhafaza etmek için alternatif olabilir.

1. INTRODUCTION

Edible films are thin layers made from natural resources to coat food products for preventing them and extending their shelf life (Falguera et al., 2011). Although usage of edible films seem lately, this application have been using for many years. In 12th century, waxes has been used to retard dehidration of sour fruits in China. Fats and oils have been using to prevent meats from shrinking till 16th century. In 19th century, sucrose as an edible preservative, have been used to prevent oxidation and spoilage of nuts, almonds and walnuts during storage (Dursun and Erkan, 2009).

Food products expose to many changes during storage like physical, chemical and microbiological. Suitable coating can be vital for food products to prevent food from detrimental effects and to improve its quality and shelf life (Cha and Chinnan, 2004). For all food products, to have long shelf life properties are related with conditions (humidity, oxygen, light etc.) in which food have been stored. To extend shelf life of foods, new packaging materials have been developed. Because of the waste of packaging materials environment have been polluting. Edible films by being environmentally friendly and renewable, are alternative to packaging materials to reduce usage of plastic material. It can also help to reduce the carbon emission to the air (Wang et al., 2011).

Various combinations of proteins, polysaccharides and lipids are used to produce edible films. Edible films can have different properties as mass transfer barriers to moisture, oxygen, carbon dioxide, lipid, flavor and aroma between food and the surrounding atmosphere depending on the material to form edible film (Hassan and Norziah, 2012). Food scientists and engineers are focused on edible films which has new properties as a coating material and new materials.

In this study, chickpea starch is used to produce edible film. There have been many research about starch based edible films made from corn, potato, pea, tapioca, cassava etc. But there are not many studies about chickpea starch based edible films. The first aim of our study is to determine the water vapor permeability and oxygen permeability of starch based edible films to select the best formulation for coating

pomegranate arils. Second aim of this study is to obtain information about the sorption isotherms of the edible film at different temperatures and modelling with different sorption isotherm models for predicting stability and quality changes during the packaging. Third aim of this study is to investigate the effect of coating pomegranate arils on physcial and chemical changes (weight loss, titratable acidity, pH, total soluble solids content), total phenolic content, total flavonoid content and antioxidant activity.

2. LITERATURE REVIEW

2.1 General Characteristics of Edible Films

Edible films and coatings are thin layers obtained by natural and synthetic subtances which can be added in food systems as a selective barriers to moisture transfer, oxygen uptake, lipid oxidation and the loss of volatile aromas and flavors. Therefore, the edible films and coatings can be used to improve shelf life and food quality of many products such as fruits and vegetables (Tapia et al., 2007, Bastos et al., 2009).

Edible films and coatings by having one or more of the functions listed in Table 2.1 are nutritious, safe, stable and economic and have been used in the storage and marketing of foods (Pareta et al., 2006). Some of the most significant uses of edible films are to control mass transfer between the product and the environment, to maintain structural integrity and to improve mechanical properties (Garcia et al., 2000, Sablani et al., 2009). The Possible uses of edible films and coatings are listed in Table 2.1 (Chiumarelli and Hubinger, 2012, Garcia et al., 1999, Chamorro et al., 2011, Garcia et al., 2000, Tapia et al., 2007).

Table 2.1: Possible uses of edible films and coatings.

Possible Uses
Control mass transfer
- Barrier to moisture migration
- Barrier to oxygen and carbon dioxide transmission
- Retard oil and fat migration
- Retard solute migration
Provide mechanical protection
- Improve structural integrity (tensile strength, flexibility, extensibility)
Carry food additives
- Antibrowning agents, antioxidants, antimicrobials, colorants, flavors, nutrients and spices
Improve food quailty and sensory properties

The uses of edible films change depending on many parameters. Parameters which affects the uses of edible films are given in the Table 2.2 (Ghanbarzadeha et al., 2011, Javanmardi et al., 2011, Oses et al., 2009, Sablani et al., 2009, Vargas et al., 2008, Falguera et al., 2011).

As it is seen in the Table 2.2, the usage of edible films is based on the formulation of the film, film forming conditions and the temperature and the relative humidity of environment in which edible coated food is stored.

Table 2.2: Parameters that affects uses of edible films.

Formulation	- Type of components and concentration - pH - Additives (antimicrobials, antioxidants, plasticizers)
Film Forming Conditions	- Temperature - Film thickness - pH - Drying conditions
Film Using Conditions	- Temperature - Relative Humidity

The interest about the edbile films and coatings have been increased because of using them in food packaging and food protection. Edible films became alternative method of packaging because of its benefits. The benefits of edible films different from other traditional packages are listen in Table 2.3 (Campos et al., 2011, Fontes et al., 2011, Falguera et al., 2011, Navarro-Tarazaga et al., 2011).

Table 2.3: Benefits of edible films.

Environmental benefits	- Less amount of waste traditional packaging - Lower environmental contamiantion
Product benefits	- Improve mechanical properties - Enhance optical and organoleptic properties - Preserve the nutritional quality - Longer shelf life
Economical benefits	- Reduce the cost of packaging - Cheap raw material
Use benetifs	- Convey food additives and nutrients - Respond to consumer demand for more natural products - Protect the product from mechanical damage, chemical, physical and microbiological activities. - Provide a protective barrier between product and environment - Improve the handling properties of the food

2.1.1 Properties of edible films

Edible films and coatings should be odourless, tasteless, colourless and transparent not to have adverse effect on consuming. Films generally should be flexible and resistant to cohesion. They should meet the different necessities like moisture barrier, gas barrier, solubility in water and lipid, colour and appearance, mechanical properties and etc. Besides, the surface appearance of edible films should be improved and adhesiveness should be decreased (Dursun and Erkan, 2009).

To produce a good quality of edible films and coatings, they should have some properties as in listed below (Dursun and Erkan, 2009, Cerquiera et al., 2011, Falguera et al., 2011):

- Raw materials which are used in edible film forming, must be generally recognized as safe (GRAS) compounds so to ensure consumer demands for healty food.
- It must provide structural integrity and improve handling properties.
- It must provide controlled respiration depending on their gas exchange properties (mainly water vapour, oxygen and carbon dioxide permeabilities).
- It must improve mechanical properties especially their resistance to stretching and rupture.
- It must meet requirements related with optical properties (opacity and color) and flavor (in most cases flavorless coatings are needed).
- It must extend shelf life and delay the growth of contaminating microorganisms.

2.2 Film Components

Edible films and coatings are generally made from three different components: proteins, lipids and polysaccharides. This components can be used alone or together to have the desired film property.

2.2.1 Proteins

Protein derived films can be made from animal proteins (caseine, whep protein, collagen, gelatine etc.) or vegetable proteins (zein, soy protein, gluten etc.) (Dursun and Erkan, 2009).

Proteins have good film forming properties. Protein based edible films have better mechanical resistance and oxygen, lipid and aroma barrier properties than polysaccharide based edible films. On the other hand, protein films have poor barrier to moisture becuase of their hydrophilic nature (Kim and Ustunol, 2001). To have better moisture barrier properties, hydrophobic compounds can be added to protein films but it can decrease the mechanical strength (Fadini et al., 2013).

Khwaldia et al. (2004) used milk proteins for edible films and coatings and they showed that these films and coatings may retard moisture loss and they are excellent barriers to oxygen with showing good tensile strength and moderate elongation. The films made by milk proteins are also flexible and with having no flavor and taste, they have good organoleptic properties.

Ghanbarzadeh et al. (2006) investigated the effect of plasticizing sugars (fructose, galactose and glucose) on mechanical properties of zein films. For this reason, zein (20%) was dissolved in warm aqueous ethanol (80%) and sugars were added to the solution (50%, 70%, 100% w/w, plasticizer/zein) and stirred for 10 minute in a mixer. Then cold water was added to precipitate zein-plasticizer dispersions. Resins were collected as soft solids. This study showed that galactose containing films had better tensile properties with higher tensile strength, strain at break and Young Modulus that film containing fructose and glucose (Ghanbarzadeh et al., 2006).

Pochat-Bohatier et al. studied the influence of relative humidity on carbon dioxide sorpition in wheat gluten films. The film forming solution (100 g) was prepared using wheat gluten (15 g), sodium sulfite (0,03 g), ethanol (32 ml), glycerol (3 g) and distilled water and pH of the solution was adjusted to 4 using acetic acid. This research indicated that the increase in the water content of wheat gluten improves the affinity between carbon dioxide and the protein matrix, leading to outstanding sorption values for high RH.

Gounga et al. (2007) prepared edible films from different concentration of whey protein isolate (WPI) and different ratio of glycerol (Gly) to choose a best combination. 5%, 7% and 9% (w/v) WPI were used at three WPI:Gly ratios (3.6:1; 3:1; and 2:1). The best film combination in aspect of water vapor permeability was the 5% WPI with a 3.6:1 WPI:Gly ratio, while the 9% WPI with 3.6:1 WPI:Gly showed the best result for the oxygen permeability.

2.2.2 Lipids

When you compare with protein and polysaccharide films, lipid films are better barriers to moisture but their appearances are opaque and their flexibilities are low. They also give an after taste, in this view they influence the organoleptic characteristics of the food products (Fontes et al., 2011). Formerly, waxes and lipids were used alone, but nowadays they are mixed with solvents, emulgators, surfactants, plasticizers and etc.

Acetylated monoglycerides, natural waxes and various oils and fats are used for lipid based edible films. This kind of materials are used because they are excellent barrier to moisture. This properties of lipid films make them generally used for coating to meat products. Lipid films lower the respiration of product and it results in extended

shelf life. Moreover, lipid films are used to make the appearance of fruits and vegetables glossy. It is also known that they are efficient on protecting fruits from mold growing. However, this films posses poor mechanical properties and chemical stability (Dursun and Erkan, 2009).

Jimenez et al. (2011) invesitgated the effect of saturated and unsaturated fatty acids on hydroxypropyl-methylcellulose (HPMC) based films. Lauric (LA), miristic (MA), palmitic (PA), stearic (SA) and oleic (OA) acids are used with HPMC with ratio 1:0.15 polymer:lipid. Saturated LA, MA and PA formed bigger lipid micellar structures than SA and OA in the HPMC aqueous system, which grew notably during film drying, giving rise to crystallized lipid layers in the films which were not observed for SA and OA. Laminar structures improved the moisture barrier properties, but resulted in more brittle, less stretchable, more opaque and less glossy films, depending on the particle size.

2.2.3 Polysaccharides

Starch (potato, corn, wheat, chickpea, rice and other derivatives), cellulose (cotton, wood and other derivatives), gums (guar, locust bean, alginate, karregenan, pectin and other derivatives) and chitin/chitosan can be used to produce a polysaccharide edible film (Dursun and Erhan, 2009) Polysaccharides have an improtant role in food industry as they are cheap, abundant in nature, untoxic and can be produced from various resources (Li et al., 2011).

Zhang et al. (2006) studied the effect of plasticizers on mechanical properties of pea starch films. They used monosaccharides (mannose, galactose and fructose) and polyols (glycerol and sorbitol) as a plasticizer to form a different edible films. This research suggest that monosaccharide-plasticized films have better plasticizing effect in terms of physical properties than polyol-plasticized films. Because edible films plasticized with monosaccharides had better mechanical properties (higher tensile strength and elongation) and lower water vapor permeability.

Maran et al. (2013) made research about tapioca starch based edible films to develop a model for barrier and optical properties. They used tapioca starch, glycerol, agar and span to produce film forming solution. They have seen that water vapor permeability, oxygen permeability, moisture content, solubility and swelling capacity

of the films increase with the plasticizing effect of glycerol because of its hydrophillic nature.

Ghanbarzadeh et al. (2006) studied the physical properties of edible modified starch/ carboxymethyl cellullose (CMC) films. They showed that starch-CMC films are better mechanical properties and enchanced moisture resistance thus it can be replaced of starch films.

2.3 Water Vapor Permeability

ASTM E96-80 defines permeability as the rate of water vapor transmission through a unit area of flat material of unit thickness induced by a unit vapor pressure difference between two specific surfaces, under specified temperature and humidity conditions (ASTM, 1980). Permeability consists of a process of solution and diffusion where the vapor dissolves on one side of the film and then diffuses through to the other side. Water vapor permeability results provide us an information about possible mass transfer mechanism and solute and polymer interactions in edible films (Bertuzzi et al., 2007).

2.3.1 Parameter that affect water vapor permeability

The factors which affect water vapor permeability are (Bertuzzi et al., 2007):

- Temperature,
- Relative humidity gradients,
- Film thickness,
- Plasticizer content.

Generally the temperature is in inverse proportion with the solubility so with increased temperature and slightly decreased solubility results in increased diffusion of water vapor through edible films. Enchanced movement of polymer segments and increased energy levels of permeating molecules causes increase in diffisuvity with increasing temperature. In a short, permeability is a positive function of the temperature. (Bertuzzi et al., 2007).

Bertuzzi et al. (2007) made a research about the effect of thickness and relative humidity on high amylose corn starch film WVP and showed that the increase in film

thickness is significantly dependent upon relative humidity and original film thickness.

Ghasemlou et al. (2010) studied the effect of various concentrations of plasticizer on film properties and showed that there is an important difference between the WVP values of edible films made with different plasticizer concentrations.

2.3.2 Water vapor permeability values of some of the edible films

The water vapor permeability values of some of the edible films are given in the Table 2.4.

Table 2.4: WVP values of some of the edible films.

Film	Thickness (mm)	Condition	WVP ($g.mm/m^2 \cdot d.kPa$)	Reference
Gelatin:Sorbitol	0.043	22°C-0/100% RH	3.2	Sorbal et al., 2001
Peanut protein-Glycerol	0.125	23°C-0/50% RH	9.03	Jangchud and Chinnan, 1999
Amaranth Flour-Glycerol	0.080	25 °C-0/100% RH	3.8	Tapia-Blacido et al., 2011
Soy protein Isolate	0.116	25 °C-0/50% RH	0.94	Cho et al., 2010
Whey Protein Isolate: Gly	0.13	25 °C-0/50% RH	11.92	Ramos et al., 2013
Starch-Glycerol	0.060	25 °C-0/100% RH	1.8	Rodriguez et al.,2006
Manoic Starch: Sorbitol	0.034	25 °C-0/52% RH	3.91	Bertuzzi et al., 2007
High Amylose Corn Starch: Gly	0.040	25 °C-0/75% RH	1.17	Fakhoury et al., 2012
Tapioca Starch: Glycerol	0.038	25 °C-0/75% RH	4.82	Maran et al., 2013
Whey Protein: Sorbitol	-	25 °C-0/100% RH	9.64	Ozdemir and Floros, 2008
Sweet Potato Starch	-	23°C-0/50% RH	0.3	Ehivet et al., 2011

2.4 Pomegranate

The scientific name of pomegranate is *Punica granatum L.*. It is belong to Punicacea family. *Punica granatum L.* is grown well in tropical and subtropical regions. The climate is important for pomegranate growing. The summers should be hot and

winters should be cool because ripening of pomegranate necessitate hot and dry air. Turkey, Greece, Spain, Egypt, Russia, The United States (California), Japan, China, India, Iran and Afghanistan have best region over the world to produce pomegranate (Saad et al., 2012). Although pomegranate fruits are mostly consumed fresh they are also used for to produce juice, jelly, wine, jams, syrup, grenadine and sauce (Tehranifar et al., 2010, Al-Said et al., 2009).

Pomegranate is a round fruit which has partly yellow and light and deep pink and red colour is dominant over the skin. Pomegranate mainly has three parts: Peel, seeds, and arils. The edible part of pomegranate is called as arils which has yellow to deep red colour. Half of the whole fruit constitute from arils. 22% by weight of aril is seed and the rest 78% by weight is juice (Ozgen et al., 2008). 85.4% of the fresh juice is moisture and the rest of juice is comrised of total soluble solids, total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid and proteins (Kulkarni and Aradhya, 2005).

Pomegrante is a popular fruit to be on studies. Because it is rich in antioxidants and its health benefits (Tezcan et al., 2009). There are many reports about the positive health effects of consuming pomegranate (Zaouay et al., 2012). It is a nutritious fruit source with consisting of important chemical compounds and minerals. Pomegranate have carbohydrates, minerals, crude fibres, and many natural antioxidants such as vitamin C and phenolic compounds (Zaouay et al., 2012). Polyphenols that pomegranate consists are flavoids (flavanols, anthocyanins etc.), tannins (condensed-proanthocyanidins and hydrolyzable tannins-ellagitannins and gallotannins). Organic and phenolic acids, sterols and triterpenoids, fatty acids, triglycerides, and alkaloids are other phytochemicals in pomegranate (Varela-Santos et al., 2012).

Some of the known medical properties of pomegranate fruits are listed in below (Qua et al., 2013, He et al., 2011, Caliskan and Bayazit, 2012, He et al., 2012, Legua et al., 2012, Viuda-Martos et al., 2010) :

- Reduced oxidative stress,
- Anticarcinogenic,
- Antibacterial,
- Antiviral,
- Antidiarrhoeal,

- Hypoglycaemic,
- Decrease the risk of cardio and cerebrovascular diseases,
- Cure to atherosclerosis, diarrhea, gastric ulcers, venereal disease, and estrogen-related diseases,
- Reduce the blood pressure
- Antiatherosclerotic
- Improve skin health
- Anti-inflammatory
- Antioxidant
- Antidiabetic
- Improve oral health

2.4.1 Chemical composition of pomegranate

There are several factors that affects the chemical composition of pomegranate. These are the cultivar, growing region, climate, maturity of fruit, cultivation practice and storage conditions. (Viuda-Martos et al., 2010) All of three parts of pomegrante (juice, seed, peel) has different chemical composition. It is reported in some research that pomegranate peel has luteolin, quercetin, kaempferol, gallagic, glycosides, punicalagin, punicalin (Van Elswijk et al., 2004, Amakura et al., 2000, Seeram et al., 2005). Pomegranate juice has various compounds such as anthocyanins, glucose, organic acid, ascorbic acid, gallic acid, caffeic acid, catechin, quercetin, rutin and minerals (Poyrazoglu et al., 2002, Ignarro et al., 2006, Lansky and Newman, 2007, Heber and et al. 2007, Jaiswal et al., 2010). Some of the research are made in seed oil of pomegranate. Different acids have been reported in seed oil such as conjugated linolenic acid, linoleic acid, oleic acid, stearic acid, punicic acid, eleostearic acid and catalpic acid (Ozgul et al, 2005, Fadavi et al., 2006, El-Nemr et al, 2006, Sassano et al., 2009).

Table 2.5: Chemical composition of pomegranate (Adsule and Patil, 1995; Dallas, 2003).

Component	Content		
	Jagtap et al.,1992	Sood et al.,1982	Dallas, 2003
Moisture (%)	78	77.0-78.2	80.97
Carboydrates (%)	14.6	17.5-20.0	17.17
Crude fiber (%)	5.1	-	0.6
Protein (%)	1.6	1.78-1.96	0.95
Fat (%)	0.1	1.72-2.11	0.3
Ash (%)	0.7	0.66-0.76	0.61
Pectin (%)	0.27	0.47-0.55	-
Total sugar (%)	-	6.2-9.0	-
Reducing sugar (%)	-	5.6-7.5	-
Nonreducing sugar (%)	-	0.1-3.3	-
Energy value (kcal/100g)	65	-	68

Pomegranate is a nutritious fruit source due to its important chemical composition and mineral content. Pomegranate has carbohydrates and minerals (calcium, phosphorus, iron, magnesium, copper, sodium, potassium, sulfur) as important nutrients. It consist of several vitamins such as thiamine, riboflavin, niacin and vitamin C. Table 2.6 shows vitamin and mineral contents of pomegranate.

Table 2.6: Vitamin and mineral contents of pomegranate (Adsule and Patil, 1995, Dallas, 2003).

Component	**Content (mg/100 g)**		
	Jagtap et al., 1992	Sood et al., 1982	Dallas, 2003
Thiamine	0.06	-	0.03
Riboflavin	0.1	-	0.03
Niacin	0.3	-	0.3
Vitamin C	16.0	5.3-7.7	6.1
Calcium	10.0	24-145	-
Phosphorus	70.0	33-44	8
Iron	0.30	0.62-0.69	-
Magnesium	12.0	-	3
Copper	0.17	-	-
Sodium	4.0	-	-
Potassium	17.1	-	259
Sulfur	-	25-28	-

3. MATERIAL AND METHOD

3.1 Materials

Chickpeas (*Cicer arietinum*, kocbasi) used in this study were taken from the market of Istanbul. Pomegranates were taken from the greengrocer of Istanbul province. The fruits were selected for uniform size, maturity and appearance and freedom from defects. The samples quickly were transferred to the laboratory and processed on the same day.

3.2 Chemicals

Two different plasticizers were used to make film forming solution: glycerol and sorbitol. Both of the plasticizers were purchased from Kimetsan (Ankara, Turkey).

For moisture sorption, nine different salt solution were used: LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, NaBr, NaCl, KCl, and $BaCl_2$. The salts were obtained from Merck KGaA (Darmstadt, Germany).

For extraction and spectrophotometric analysis, methanol (≥99.9%), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), hydrochloric acid (HCl, 37%), ammonium acetate (NH_4Ac), Copper (II) chloride ($CuCl_2$), dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogenphosphate (KH_2PO_4) were obtained from Merck KgaA (Darmstadt, Germany).

Folin-Ciocalteu phenol reagent, gallic acid (≥98%), quercetin, catechin (≥98%), neocupraine (Nc), ethanol (≥99.8%), 1,1-diphenhenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany); Aluminum chloride ($AlCl_3$) and Trolox were obtained from Fluka Chemie (Buchs, Switzerland); ABTS from Applichem GmbH (Darmstadt, Germany) and ferric chloride ($FeCl_3$) from Lachema (Czech Republic).

3.3 Methods

3.3.1 Starch isolation

One kg of chickpea were put into a container and 3 liters of distilled water added. Chickpea were steeped in water for 24 hours at 4 oC. Then grains wereground in a blender. The ground slurry was screened through sieve (120 mesh). The left over the sieve was washed throughly with distilled water. The filtrate slurry was allowed to stand for 1 h. The supernatant was removed and the settled starch layer was collected and dried in an oven at 40 oC for 12 h.

3.3.2 Film forming

The film forming solutions were prepared using 4% (w/w) chickpea starch with five different composition of plasticizers: glycerol, sorbitol and three different ratio of glycerol:sorbitol (1:3, 1:1, 3:1).

Firstly, 4% (w/v) starch solutions were heated to boiling temperature for 30 min under magnetic stirring to make the starch granules gelatinized, which were called as the film forming solution. Four different plasticizers were added into the starch solutions at 70%, 80%, 90% ratios in 20 min. Film forming solution then was transferred to ultrasonic bath for 5 min to degas the solution. After degassing, solution was cooled 50-60 ^{o}C was poured on 14-cm dia petri dishes. Dishes were placed in an air-circulating oven at 60 ^{o}C to be dried. Then films were peeled off from the petri dishes and placed into desiccator at 51% relative humidity (RH) and room temperature (~25 ^{o}C) to be conditioned for 2 days before testing.

3.3.3 Thickness

The thickness of film was measured with an electronic digital micrometer (0,001 mm accuracy, Torq, Australia). Five different positions of the samples were measured and mean thickness values were used for calculating water vapor permeability of the film.

3.3.4 Water vapor permeability

Water vapor permeability (WVP) of films were determined gravimetrically at room temperature according to the ASTM E96-00 method. Film specimens were conditioned for 48 h in a desiccator at room temperature and 52% relative humidity ($Mg(NO_3)_2.6H_2O$ saturated solution) to ensure equilibrium condition before being analyzed. The conditioning is done because of eliminating the water absorption possibility of edible film in order to measure just the water vapor diffusion through the film.

After conditioning, films were sealed on cups with diameter 7,0 cm and height 3,0 cm containing unhydrated $CaCl_2$. Test cups were then placed in a desiccator at room temperature (~25 ^oC) and 51% relative humidity.

The cups were weighed at 1 h intervals over a 8 h period and recorded values were used in calculation of water vapor permeability using ASTM method. The results were expressed as g.mm/day.m^2.kPa.

3.3.5 Oxygen permeability

The oxygen permeability of edible films were determined according to ASTM D-3985 method at 23 °C. Analyses were done by the Scientific and Technological Research Council of Turkey (TUBITAK). The results were expressed as ml/m^2/day.

3.3.6 Sorption isotherms

The sorption isotherm of three selected films (70%-80%-90% sorbitol containing films) were determined gravimetrically at 20°C, 30°C and 40°C. Measurements were replicated three times for each films.

Saturated solutions were used to adjust the environment around the films with desired relative humidity or water activity (a_w). To obtain different relative humidity, nine different saturated salt solutions were prepared: LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, NaBr, NaCl, KCl and $BaCl_2$; and placed in 9 sealed glass jars at 20°C, 30°C and 40°C. Table 3.1 shows water activity for all saturated salt solutions at different temperatures.

The film samples which were conditioned inside the desiccator filled with silicagel for 5 days, were cut into pieces approximately 2 cm^2 (1x2 cm). Samples were

weighed and placed on small cups made from aluminium folio. These pieces were placed on small petri dishes by holding it on a tripod inside the glass jars that contain the saturated salt solutions. The glass jars were kept inside an environmental chamber maintained at constant temperature. Films sampled were equilibrated in the glass jars over a week. Then the relative humidity and water activitiy of film samples were measured. The moisture isotherm curves with different temperatures were created by plotting moisture content to a_w.

Table 3.1: Water activity values of saturated salt solutions at different temperatures.

			Water Activity		
Salt Solution	Water (ml)	Salt (g)	20 °C	30 °C	40 °C
LiCl	42.5	75	0.1131	0.1128	0.1121
CH_3COOK	37.5	100	0.2311	0.2161	0.2010
$MgCl_2$	12.5	100	0.3307	0.3244	0.3160
K_2CO_3	45	100	0.4316	0.4317	0.4230
$Mg(NO_3)_2$	15	100	0.5438	0.5140	0.4842
NaBr	35	100	0.5914	0.5603	0.5317
NaCl	30	100	0.7547	0.7509	0.7468
KCl	40	100	0.8511	0.8362	0.8232
$BaCl_2$	35	125	0.9100	0.8980	0.8910

3.3.7 Water activity

The water activity (a_w) values of films were measured with a water activity meter (Protimeter Plc, England) at 25$\pm$2 °C.

3.3.8 Moisture content

For moisture content measurement, the conditioned films samples were weighed before and after drying at 80 °C till the films reach constant weight. Moisture content determination was studied with three paralel and the mean values were used in calculations.

3.3.9 Mathematical methods

3.3.9.1 Modelling of water vapor permeability

The water vapor permeability of films were determined by using ASTM method. The reason why we prefered this method is because many studies is done with this method and we can easily compare our results with other researches. According to this, water vapor transmission rate (WVTR) is calculated with equaiton 3.1.

$$WVTR = \frac{m}{t.A} \quad \textbf{(3.1)}$$

m/t is the slope of mass change over time ; A is the area of exposed film. The second parameter which is determined by ASTM method is Permeance shown in equation 3.2.

$$Permeance = \frac{WVTR}{(P_{A1} - P_{A2})} \quad \textbf{(3.2)}$$

ASTM E96 assume that P_{A1} is equal to P_{A0} ($P_{A1=}$ P_{A0}). This generally wrong assuming for edible films. Therefore, Schwartzberg and confirmation of water vapor permeability method can be used as an alternative method. Transmission term is an objective value for comparing the barrier properties of films having same thickness.

At last, Permeability is calculated by using equation 3.3. Permeability is related with the thickness of film.

$$Permeability = Permeability\ constant\ x\ Thickness \quad \textbf{(3.3)}$$

3.3.9.2 Modelling of sorption isotherm

Experimental sorption values of films at three different temperatures were modelled with eleven equations as shown in Table 3.2.

Parameters of sorption models (A, B, C and M_0) were determined from experimental datas with nonlinear regression analysis.

Experimental sorption data were fitted using eleven equations and models were checked if it is applicable or not with regression coefficient, mean relative error (MRE) and standard error of the mean (SEM) of predicteds. For the applicability of the model MRE values is used. This value should be lower than 10% and R^2 should be more than 0,90 to accept the model applicable for sorption isotherm.

$$\mathrm{MRE} = \frac{100}{\mathrm{N}} \sum \left| \frac{\mathrm{M_{exp}} - \mathrm{M_{pre}}}{\mathrm{M_{pre}}} \right| \quad \textbf{(3.4)}$$

$$\mathrm{SEM} = \sqrt{\frac{\sum(\mathrm{M_{exp}} - \mathrm{M_{pre}})}{\mathrm{df}}} \quad \textbf{(3.5)}$$

In these equations, M_{exp} and M_{pre} are respectively moisture contents of experimental and predicted, N is a number of experimental data and df is degrees of freedom (number of experimental data-number of constant in model).

Table 3.2: Sorption isotherm models and equations.

Model	**Equation**
BET	$M = \frac{Mo \cdot A \cdot a_w}{[(1 - a_w) + (A - 1) \cdot (1 - a_w) \cdot a_w]}$
Henderson	$M = \left(\frac{\ln(1 - a_w)}{-A}\right)^{1/B}$
Iglesias and Chirife'78	$M = \exp[A + BC \ln(C - lna_w)]$
Iglesias and Chirife'81	$M = A \cdot \left(\frac{a_w}{1 - a_w}\right) + B$
Oswin	$M = A \cdot \left(\frac{a_w}{1 - a_w}\right)^B$
Smith	$M = A + B.(1 - a_w)$
Halsey	$M = \left(-\frac{A}{lnaw}\right)^{1/B}$
Bradley	$M = A + B \cdot \ln(-\ln a_w)$
Kuhn	$M = A + \frac{B}{\ln a_w}$
Chung ve Pfost	$M = -\frac{1}{B} \ln\left(-\frac{\ln a_w}{\mathrm{A}}\right)$
Harkins-Jura	$lna_w = B - \frac{A}{M^2}$

3.3.10 Quality evaluation of pomegranate arils

3.3.10.1 Coating formulation and application

Pomegranate samples were washed, rinsed and dried prior to cutting process. Subsequently, pomegranates were peeled. Peeling operation was done by using knife. Pomegranate was cut into half and then arils were removed manually. Arils were collected in a tray and then stored at 4 °C till the operation of coating.

Three selected film formulation was used in coating of pomegrante arils: 4% chickpea starch solution plasticized with sorbitol in a ratio of dry basis of starch 70%, 80% and 90%. Film forming solutions were prepared by dissolving starch (4g /100 ml water) in distilled water and heating at boiling temperature for 30 min. Sorbitol was added as plasticizer at 2,8g /100 ml, 3,2g /100 ml, and 3,6g / 100ml starch solution. When film forming solution was ready, the solution degassed in an ultrasonic bath for 5 min. Then, arils were coated.

Arils were dipped in three different film forming solution in turn. Arils were hold inside the solution for 5 min. Then excess of film forming solution was allowed to drip off from arils with sieves for 2 min. Subsequently, laminar flow was applied to the aril for drying and drying was accomplised at room temperature. Samples were put into polypropylene bags to store at 4 °C till testing.

3.3.10.2 Weight loss

Weight loss of pomegranate arils during cold storage was determined by the difference between the initial weight of the samples at every day during 10 days. For this reason, 100 g of aril samples were weighed before storage. Weight loss value was calculated according to equation 3.6:

$$\mathrm{WL} = \frac{\mathrm{Wo}-\mathrm{Wf}}{\mathrm{Wo}} \mathrm{x}100 \qquad \textbf{(3.6)}$$

Where WL is the weight loss (%), Wo is the inital weight (g) and Wf is the final weight (g). Weight loss were expressed as percentage loss of inital weight.

3.3.10.3 Titratable acidity and pH

For determining titratable acidity, 1 mL of pomegranate juice was homogenized in 25 mL distilled water. Then phenolphthalein was added as an indicator to see the end-point of pH 8.1. Subsequently, 0,1N NaOH was used for titration. Titratable acidity was expressed as percentage of citric acid. The pH of pomegranate juice was measured by Hanna Instruments (UK).

3.3.10.4 Total soluble solids content

The total soluble solids content of pomegranate juice was determined by refractometer. The results were expressed as ^{o}Brix at 20 ^{o}C.

3.3.10.5 Extraction

Each day during 10 days, 10g of samples from different coated arils and control were removed and stored at -80 ^{o}C till the extraction process. Prior to extraction, samples were milled under liquid nitrogen using grinder (IKA, Germany).

0,5 g of freeze-dried samples were weighed under liquid nitrogen for extraction. They were moved into test tubes and 3 ml of 75% methanol was added. Then samples were sonicated for 15 min at 4 ^{o}C in ultrasonic bath (Ultrasonic Cleaner-VWR). After sonication the samples were centrifuged at 2500 rpm under 4 ^{o}C for 10 min with Universal 32 (Tuttlingen, Germany). Then supernatant liquid is removed and extracts were stored at -20 ^{o}C until the analysis. The pellet was used again to be extracted. This time extraction procedure was done with 2 ml of 75% methanol. The same procedure was applied and 2 ml of supernant was collected and total fresh solvent was fulfilled to 5 ml.

3.3.10.6 Total phenolic content

Folin-Ciocalteau is a method regularly used to determine total phenolics in pomegrante as well as in other fruits and vegetables, however there are also some interfering substances which can react with this agent, such as ascorbic acid, aromatic amines, sulfur dioxide and Fe(II).

With modification of the method of Velioglu et al. (1998), the total phenolic content of edible film coated and uncoated pomegranate arils were measured. 100 µl of sample extract was mixed with 900 µl of distilled water in a test tube. Then 5 ml of

0.2 N Folin-Ciocalteu reactive added. After waiting 3 minutes, 4 ml of saturated Na_2CO_3 was added. Then test tube was vortexed by vorteks mini shakers (IKA, Germany) and was incubated for 2 hours at room temperature ina dark place. Absorbance measurement was done at 765 nm by using SP-3000 nano spectrophotometer (OPTIMA, Japan).

Gallic acid standard solutions were used to prepared the calibration curve. The results were calculated in terms of mg gallic acid equivalents (GAE) per 100 g of dry weight (DW) and reported as mean value ± SD.

3.3.10.7 Total flavonoid content

The method of Dewanto et al. (2002) was used to determine the total flavonoid content of samples. 250 µl of extract was put into test tubes and 1.25 ml distilled water was added. Subsequently, 75 µl of 5% of $NaNO_2$ was added. After 6 minutes, 150 µl of 10% $AlCl_3.6H_2O$ was added. 5 minutes later, 1M 0.5 ml NaOH was added and volum was adjusted 2.5 ml with distilled water. Then mixture was vortexed for 10 seconds. The absorbance was measured without waiting at 510 nm by using SP-3000 nano spectrophotmeter (OPTIMA, Japan) against a reagent blank.

The calibration curve was prepared by using catechin standard solutions. The results were expressed as catechin equivalents (CE) per 100 g of dry weight and reported as mean value ± SD.

3.3.10.8 Total antioxidant activity analysis

To measure the total antioxidant activity of pomegranate, three different method was used: DPPH (1,1-diphenhenyl-2-picrylhydrazyl) radical scavenging method, Cupric reducing antioxidant capacity (CUPRAC) analysis method, and ABTS (2,2'azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) analysis method.

3.3.10.9 DPPH (1,1-diphenhenyl-2-picrylhydrazyl) radical scavenging method

The total antioxidant activity measured by DPPH method adapted from Kumaran et al. (2006). 100 µl sample extract was mixed with 2 ml of 0.1 mM DPPH (in methanol). Then the mixture was vortexed for 10 seconds and kept in the dark at

room temperature for 30 minutes. Absorbance was read at 517 nm against methanol by SP-3000 nano spectrophotmeter (OPTIMA, Japan).

Trolox in 75% methanol was used to prepare calibration curve. The results were expressed as Trolox equivalents (TEAC) per 100 g of dry weight and reported as mean value ± SD.

3.3.10.10 Cupric reducing antioxidant capacity (CUPRAC) analysis method

For CUPRAC analysis, 100 µl of sample was put into a test tube. Then, respectively, 1 ml of $CuCl_2$, 1 ml of neocuproine solution ($7.5x10^{-3}$ M), and 1 ml of ammonium acetate buffer solution at pH 7.0 were added. Subsequently, 1 ml of distilled water was added to make the volume 4.1 ml and all the solution was mixed. The mixture was kept in a dark for 30 minutes and absorbance was measured at 450 nm against a reagent blank SP-3000 nano spectrophotmeter (OPTIMA, Japan).

The calibration curve was prepared with Trolox in 75% methanol and the results were expressed as Trolox equivalents (TEAC) per 100 g of dry weight and reported as mean value ± SD.

3.3.10.11 ABTS (2,2'azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) analysis method

The antioxidant activity of pomegranate by ABTS method was determined by the adopted method of Miller and Rice-Evans (1997). ABTS reagent was prepared by weighing 220 mg ABTS in 200 ml and potassium persulfate solution ($K_2S_2O_8$) was prepared by dissolving 38 mg of $K_2S_2O_8$ in 2 ml water. These solutions were mixed and let to form the radical for a night.

0.05 M KPi buffer solution at pH 8.0 was prepared by mixing 0.05 M potassium dihydrogen phosphate (KH_2PO_4) and 0.05 M dipotassium hydrogen phosphate ($KHPO_4$). ABTS reagent mixture was prepared by mixing ABTS and KPi solution until its absorbance reachs 0.9±0.2. The pH of the mixture had to be 7.4 at the end.
100 µl extract was put into test tube and 1 ml of ABTS reagent was added and vortexed for 10 seconds. After waiting for 45 seconds, absorbance was measured at 734 nm by spectrophotometer (Shimadzu UV-1700 UV Vis) against water blank.

The calibration curve was prepared with Trolox in 75% methanol and results were expressed as Trolox equivalents (TEAC) per 100 g of dry weight and reported as mean value ± SD.

3.3.11 Statistical analyses

In this study, Minitab 16 was used to analyze all the data. Analyses of variance (ANOVA) and Tukey's means comparison test with a significance level of 0.05 were applied. Values followed by the same letter are not significantly different.

4. RESULTS AND DISCUSSION

4.1 Water Vapor Permeability of Films

The water vapor permeability of films were calculated in a condition which the inner place of the test cup has 0% RH (provided by $CaCl_2$) and the outer space has 51% RH (provided by $MgCl_2.6H_20$) at room temperature. Table 4.1 shows the water vapor permeability values of different starch films. WVP values are expressed as g.mm/day.m^2.kPa.

Table 4.1: WVP values of edible film samples.

Film	Thickness (mm)	WVP(g.mm/day.m^2.kPa)
Starch + 70% Glycerol	0.09 ± 0.006	3.7570 ± 0.6def
Starch + 80% Glycerol	0.10 ± 0.007	6.5911 ± 0.2bc
Starch + 90% Glycerol	0.09 ± 0.008	8.7458 ± 0.7a
Starch + 70% Sorbitol	0.09 ± 0.009	0.7733 ± 0.3h
Starch + 80% Sorbitol	0.09 ± 0.006	1.9565 ± 0.2fgh
Starch + 90% Sorbitol	0.09 ± 0.007	2.9609 ± 1.3efg
Starch + 70% Gly1:S1	0.10 ± 0.008	2.7321 ± 0.7efg
Starch + 80% Gly1:S1	0.10 ± 0.008	5.2008 ± 0.3bcd
Starch + 90% Gly1:S1	0.10 ± 0.008	6.5032 ± 0.6bc
Starch + 70% Gly1:S3	0.09 ± 0.006	1.7167 ± 0.4gh
Starch + 80% Gly1:S3	0.09 ± 0.007	4.4874 ± 0.2de
Starch + 90% Gly1:S3	0.09 ± 0.006	5.0887 ± 0.6cd
Starch + 70% Gly3:S1	0.09 ± 0.010	3.8208 ± 0.4de
Starch + 80% Gly3:S1	0.09 ± 0.008	6.4068 ± 0.9bc
Starch + 90% Gly3:S1	0.09 ± 0.010	6.9584 ± 0.6ab

[1] Mean values ± Standard deviation

[2] Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).

The water vapor permeability values vary from 0.7733 g.mm/d.m^2.kPa (Starch + 70% Sorbitol) to 8.7458 g.mm/d.m^2.kPa (Starch + 90% Glycerol). As we can see from the Table 6.1, there was a significant difference between the WVP values of films made with different plasticizers which are glycerol, sorbitol and their mixture with different ratios (Glycerol:Sorbitol; respectively, 1:1; 1:3; 3:1).

Starch is a good source for producing edible film but starch-based edible films without plasticizers are brittle. To overcome film brittleness plasticizing agents are added to formulation. The brittleness caused by extensive intermolecular forces. Plasticizers reduce these forces and improve flexibility and extensibility of the films. Because plasticizers extend, dilute and soften the structure, the chain mobility is increased and difussion coefficient for gas and water significantly increase (Bertuzzi et al., 2007). In this study, glycerol, sorbitol and the mixture of glycerol and sorbitol in different ratios were used as plasticizer to overcome the brittleness of chickpea starch film. This study investigated the WVP values of different content of plasticizers.

As it can be observed in Table 4.1 and Figure 4.1, the concentration of plasticizer regardless of the type increased the water vapor permeability of films. WVP values were always higher with increased concentration of plasticizer. The study of Talja et al. (2007) also proves that WVP of plasticized films increases with increasing plasticizer content.

It can be easily seen in Figure 4.1 that WVP of starch film plasticized by glycerol was higher than that of starch film plasticized with binary plasticizer mixtures and sorbitol. I.e. permeability was influenced by glycerol, being higher as plasticizer concentration increase. It maybe the reason for that is glycerol is a relatively small hydrophilic molecule, which can be inserted between adjacent polymeric chains, decreasing intermolecular forces and increasing molecular mobility in the film matrix. The increased mobility results in greater free volume and segmental motions, which facilitates the migration of water vapor molecules through the film (Rodriguez et al., 2006). The same results were obtained in research by Ghasemlou et al. (2011) and Chillo et al. (2008).

As it is seen in Figure 4.1, when the concentration of glycerol of film was increased, water vapor permeability value of the film linearly increased (R^2 = 0.9939). In this view, this study has common results with the study of Bertuzzi et al. (2006). Their

study shows that WVP increased linearly as the concentration of glycerol increased in the range of glycerol content studied (R^2 = 0.90). These results could be related to structural modifications of the starch network produced by the plasticizer and to the hydrophilic character of glycerol, which favors the absorption and desorption of water molecules (Bertuzzi et al., 2007).

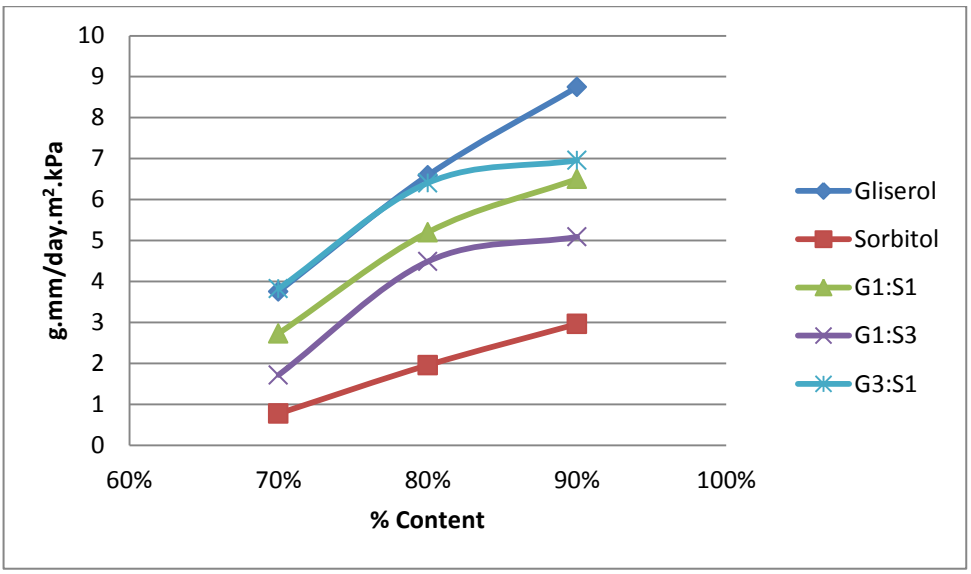


Figure 4.1: Comparison of WVP of edible films plasticized with different agents.

This situation is valid for the edible film prepared with sorbitol too. Because WVP value of the film was linearly increased with the increasing sorbitol concentration (R^2 = 0.9978). But the WVP of films plasticized with sorbitol is lower than glycerol-plasticized film. This difference may be because of different hygroscopicity of these plasticizers. Glycerol exhibits greater hydrophilic character than sorbitol. Kowalczyk et al. (2011) made research about pea protein isolate and investigate the effect of plasticizers. Their findings support my results.

The WVP values of at lowest concentration (70% w/w) of glycerol and sorbitol plasticized films are, respcetively, 3.7570 g.mm/d.m^2.kPa and 0.7733 g.mm/d.m^2.kPa. Kowalczyk and Baraniak (2011) reported the WVP values of glycerol and sorbitol (4% w/w) prepared pea protein isolate films was 14.31 g.mm/d.m^2.kPa and 1.08 g.mm/d.m^2.kPa. The WVP of edible film made from glycerol is four times higher than that observed in our study. This difference probably caused by different material used for edible film forming.

Sorbitol plasticized edible films has better water vapor barrier properties by having lower water vapor permeability values.

4.2 Oxygen Permeability

The oxygen permeability (OP) of film samples were determined according to ASTM D-3985 method at 23 ^{o}C. Table 4.2 shows the oxygen permeability of different formulated films. The oxygen permeability values of films are expressed as ml/day.m^2.kPa.

Oxygen permeability plays an important role in food packaging. Because oxygen permeability affects food oxidation rate. When the oxygen transmission rate is increased, food oxidation is accelerated. By reducing the oxygen transmission rate, you can decrease the development of off-flavors, off-odors and nutritional loss associated with oxidation in foodstuffs. In a short, reducing oxygen permeability of films can be alternative method to reduce food oxidation (Fabra et al., 2011). Here in this study, the starch edible film with different composition are investigated in terms of oxygen permeability to pick up the best formulation for pomegranate's arils coating.

Table 4.2: OP values of edible film samples.

Film	Thickness (mm)	OP (ml/day.m^2.kPa)
Starch + 70% Glycerol	0.09 ± 0.006	2.720
Starch + 80% Glycerol	0.10 ± 0.007	4.780
Starch + 90% Glycerol	0.09 ± 0.008	8.040
Starch + 70% Sorbitol	0.09 ± 0.009	3.430
Starch + 80% Sorbitol	0.09 ± 0.006	3.350
Starch + 90% Sorbitol	0.09 ± 0.007	0.885
Starch + 70% Gly1:S1	0.10 ± 0.008	1.140
Starch + 80% Gly1:S1	0.10 ± 0.008	1.380
Starch + 90% Gly1:S1	0.10 ± 0.008	1.580
Starch + 70% Gly1:S3	0.09 ± 0.006	0.953
Starch + 80% Gly1:S3	0.09 ± 0.007	1.130
Starch + 90% Gly1:S3	0.09 ± 0.006	0.780
Starch + 70% Gly3:S1	0.09 ± 0.010	2.960
Starch + 80% Gly3:S1	0.09 ± 0.008	4.648
Starch + 90% Gly3:S1	0.09 ± 0.010	6.300

As it is seen in Table 4.2, the OP values of films was changed from 0.780 ml/d.m^2.kPa to 8.040 ml/d.m^2.kPa. When the Figure 4.2 is observed, it can be obviously said that oxygen permeability values were increased with increasing concentration of glycerol in edible film. On the other hand, the OP values were decreased with increasing sorbitol concentration in the edible films. The lowest oxygen permeability value was obtained from the film plasticized with 1:3 mixture of Glycerol:Sorbitol.

Glycerol plasticized starch films had higher oxygen permeability than sorbitol plasticized starch films. The reason is maybe due to glycerol as a plasticizer has more effect than sorbitol on decreasing the intermolecular attractions between adjacent polymeric chains, which in turn facilitates the penetration of gas molecules through that network (Ribiero et al., 2007).

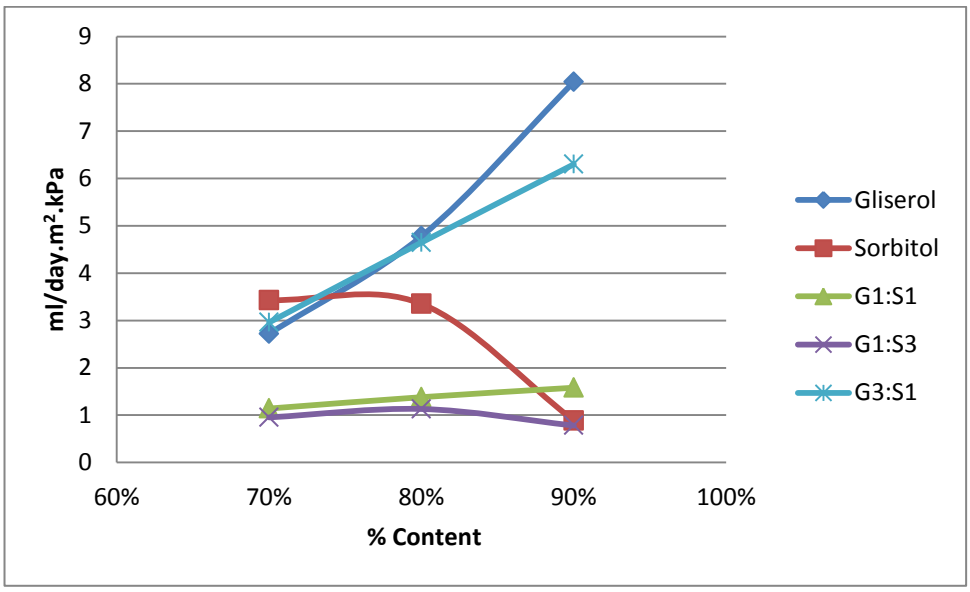


Figure 4.2: Comparison of OP of different edible film formulations.

4.3 Sorption Isotherm

After evaluation of fifteen different film compositions in terms of water vapor permeability and oxygen permeability, three film formulation was chosen to coat the pomegranate's arils. These are starch edible film plasticized with 70%, 80% and 90% sorbitol. These films were investigated for their sorption isotherm at 20, 30 and 40 ^{o}C. Table 4.3a, Table 4.3b and Table 4.3c shows the moisture contents % (M) of films in different water activity (a_w) values. Moisture content of edible film

plasticized with 70% sorbitol at 20, 30 and 40 ^{o}C, in the water activity range from 0.27 to 0.82, are respectively, 4.28-37.09%; 3.39-32.34%; 3.31-30.02% (d.b.). In the same conditions, moisture content of film prepared with 80% sorbitol are respectively, between 7.83-42.15%; 5.61-38.77%; 4.23-34.77% (d.b.). Edible film added 90% sorbitol in same conditions has respectively 7.23-37.76%; 6.32-37.41%; 5.47-36.32% (d.b.) mositure.

Table 4.3a: Mositure content of 70% sorbitol plasticized edible film in different water activity values and temperatures.

20 ^{o}C		**30 ^{0}C**		**40 ^{0}C**	
a_w	**M**	**a_w**	**M**	**a_w**	**M**
0.27	4.28	0.29	3.39	0.31	3.31
0.35	4.90	0.37	3.98	0.39	3.33
0.45	6.94	0.45	5.16	0.48	4.81
0.48	8.12	0.50	6.32	0.51	5.86
0.56	12.71	0.56	8.24	0.57	7.76
0.60	14.63	0.61	12.20	0.63	11.49
0.65	17.79	0.66	14.32	0.68	14.13
0.71	24.65	0.71	21.58	0.73	19.98
0.76	37.09	0.77	32.34	0.78	30.02

Table 4.3b: Mositure content of 80% sorbitol plasticized edible film in different water activity values and temperatures.

20 ^{o}C		**30 ^{0}C**		**40 ^{0}C**	
a_w	**M**	**a_w**	**M**	**a_w**	**M**
0.28	7.83	0.32	5.61	0.33	4.23
0.35	8.48	0.36	5.95	0.39	4.58
0.39	9.85	0.41	6.90	0.43	5.84
0.44	12.22	0.45	8.16	0.47	6.59
0.51	14.32	0.53	11.12	0.57	10.32
0.54	16.23	0.60	15.44	0.65	15.33
0.61	22.62	0.67	22.96	0.69	19.57
0.68	32.09	0.70	27.18	0.74	27.25
0.74	42.15	0.76	38.77	0.78	34.77

Table 4.3c: Mositure content of 90% sorbitol plasticized edible film in different water activity values and temperatures.

20 °C		**30 °C**		**40 °C**	
a_w	**M**	**a_w**	**M**	**a_w**	**M**
0.27	7.23	0.28	6.32	0.29	5.47
0.33	8.95	0.33	7.03	0.34	6.35
0.43	11.62	0.44	8.43	0.48	7.78
0.46	12.53	0.48	9.99	0.50	8.67
0.53	15.70	0.55	12.46	0.57	10.19
0.55	17.23	0.57	14.12	0.61	11.88
0.61	23.12	0.64	18.05	0.73	17.49
0.66	28.78	0.7	26.32	0.78	25.20
0.72	37.76	0.74	37.41	0.82	36.32

The sorption isotherms of edible films plasticized with 70%, 80%, and 90% sorbitol are shown in respectively, Figure 4.3a, Figure 4.3b, Figure 4.3c. These figures shows that all of 3 sorption profiles slightly followed by the Type II isotherm by showing almost sigmodial sorption isotherm. Polysaccharide films with high sugar contents had long flat segment up to 0.6 water activities followed by rapid increase in water sorption because of differences in water sorption affinities of polysaccharide and sugars. Such polysaccharide films adsorb more water than sugar at low water activities up to 0,6 (Talja et al., 2008).

As it can be seen in the figures, there was a slight slope at low water activity values and an exponential increase at high water activity. This behaviour can be because of the structural changes in the films which is caused by swelling of hydrophillic matrix (Al-Hassan and Norziah, 2012).

There is an increase in moisture content of edible films when you increased the concentration of plasticizer in edible film. Water content of all films increased with increased RH. Similar results were given in the research of Talja et al. (2007).

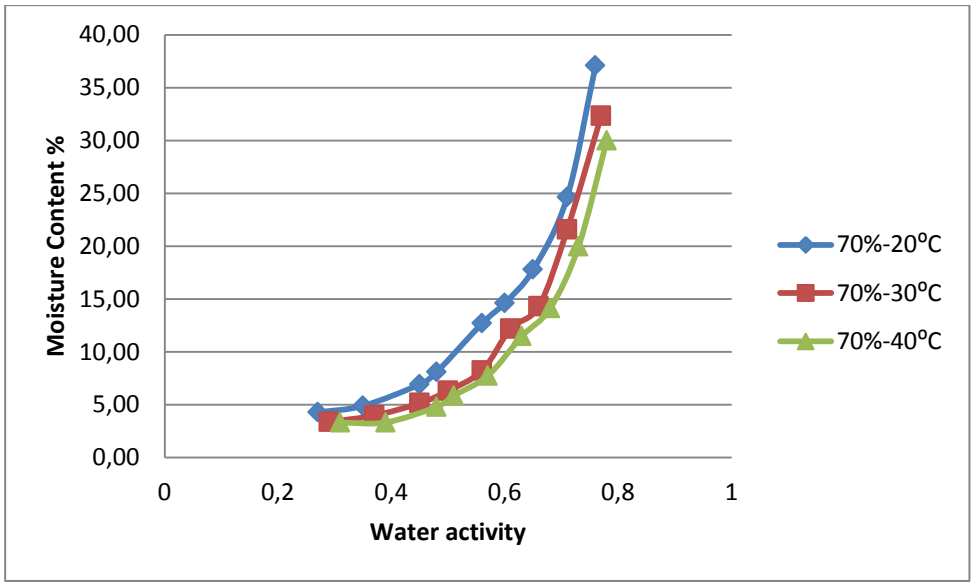


Figure 4.3a: Moisture content of 70% sorbitol plasticized film samples as a function of water activity at different temperatures.

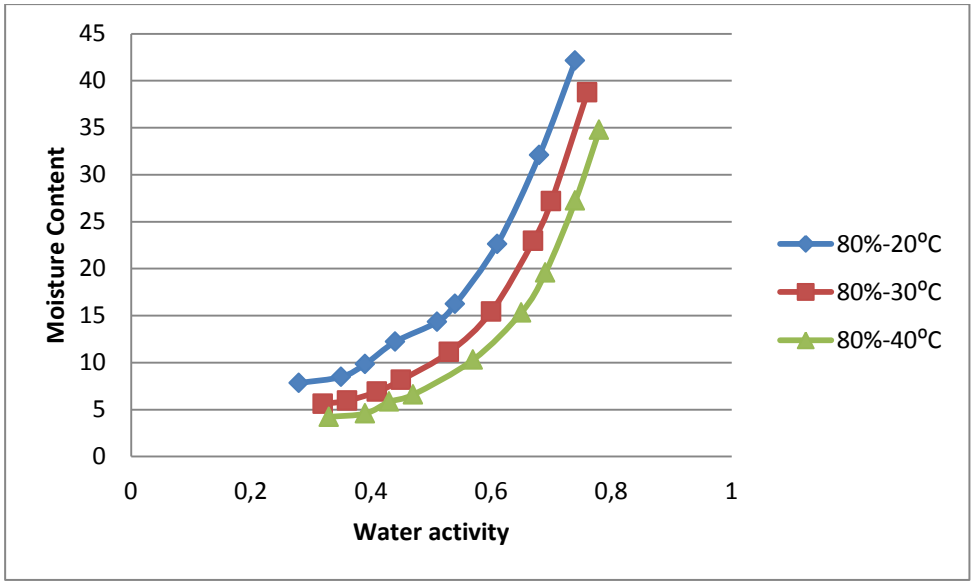


Figure 4.3b: Moisture content of 80% sorbitol plasticized film samples as a function of water activity at different temperatures.

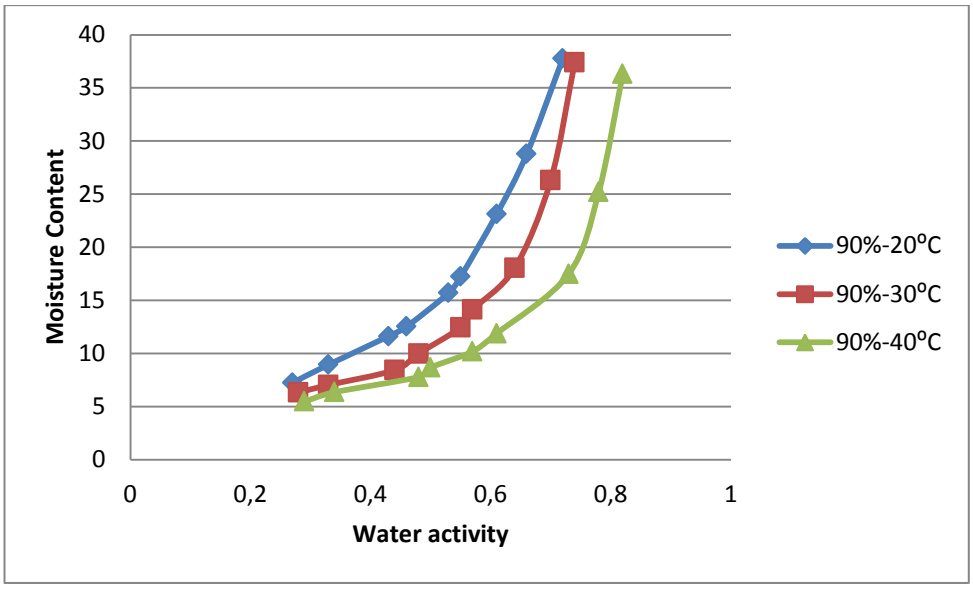


Figure 4.3c: Moisture content of 90% sorbitol plasticized film samples as a function of water activity at different temperatures.

There are many equations about sorption isotherms in literature. In this study, Smith, Oswin, Henderson, BET, Chung and Pfost, Iglesias and Chirifie'78, Iglesias and Chirifie'84, Bradley, Kuhn and Harkins-Jura models were used. The equations of these models are shown in Table 3.2. The values of A, B and MRE for the applicability of the model was calculated by using linear and multiple regression. The values obtained from 70%, 80% and 90% sorbitol plasticized edible films are listed respectively in Table 4.4a, Table 4.4b and Table 4.4c.

Table 4.4a: Sorption isotherm model constants and applicability values of model for 70% sorbitol plasticized edible film at different temperatures.

		Constants of Model		Applicability Values of Model		
Model	**T (ºC)**	**A**	**B**	**R^2**	**MRE**	**SEM**
Smith	20	42.019	59.245	0.815	36.365	4.939
	30	36.354	53.837	0.789	45.481	4.728
	40	33.449	51.110	0.797	43.999	4.329
Oswin	20	9.788	1.033	0.978	7.736	1.964
	30	7.281	1.116	0.970	10.614	1.934
	40	6.170	1.140	0.967	10.548	1.624
Henderson	20	0.138	0.691	0.955	11.587	3.118
	30	0.191	0.632	0.943	14.569	3.033
	40	0.223	0.606	0.941	14.620	2.646
Halsey	20	3.337	0.704	0.991	5.817	1.054
	30	2.491	0.660	0.986	7.491	1.070
	40	2.228	0.657	0.983	7.595	0.855
Harkins-Jura	20	17.176	-0.386	0.953	11.758	1.713
	30	10.335	-0.368	0.963	9.112	1.321
	40	8.067	-0.353	0.920	13.107	1.434
Chung & Pfost	20	1.258	0.051	0.874	31.583	4.074
	30	1.139	0.057	0.857	39.303	3.890
	40	1.080	0.061	0.867	37.280	3.504
Bradley	20	1.145	0.956	0.874	39.863	4.358
	30	1.034	0.953	0.857	48.837	4.202
	40	0.986	0.948	0.867	46.609	3.763
Kuhn	20	10.216	29.859	0.587	52.750	7.377
	30	10.023	26.308	0.568	61.031	6.767
	40	10.437	25.268	0.588	60.795	6.161
Iglesias & Chirifie'78	20	4.254	0.870	0.959	55.822	15.914
	30	4.564	0.428	0.942	73.266	13.056
	40	4.707	0.194	0.937	79.822	11.993
Iglesias & Chirifie'81	20	11.521	-1.939	0.981	11.274	1.570
	30	9.966	-2.878	0.977	16.519	1.568
	40	8.681	-2.755	0.979	15.202	1.393
BET	20	0.110	-0.015	0.051	7.568	2.006
	30	0.169	-0.062	0.232	10.524	2.122
	40	0.209	-0.091	0.293	10.372	1.781

Table 4.4b: Sorption isotherm model constants and applicability values of model for 80% sorbitol plasticized edible film at different temperatures.

		Constants of Model		Applicability Values of Model		
Model	**T (ºC)**	**A**	**B**	**R^2**	**MRE**	**SEM**
Smith	20	58.988	80.291	0.824	26.984	6.113
	30	47.731	68.450	0.877	22.833	4.939
	40	41.796	62.705	0.873	25.360	4.728
Oswin	20	15.615	0.936	0.968	8.199	3.121
	30	10.740	1.053	0.988	8.892	1.964
	40	8.041	1.117	0.992	9.278	1.934
Henderson	20	0.081	0.769	0.942	11.301	4.362
	30	0.140	0.661	0.974	9.871	3.118
	40	0.189	0.608	0.979	10.117	3.033
Halsey	20	5.599	0.771	0.985	5.552	2.008
	30	3.603	0.706	0.996	9.468	2.286
	40	2.780	0.679	0.997	9.043	1.547
Harkins-Jura	20	55.008	-0.366	0.968	5.763	1.073
	30	25.170	-0.356	0.967	18.810	1.956
	40	14.587	34.159	0.728	37.649	7.103
Chung & Pfost	20	1.356	0.037	0.875	23.543	5.150
	30	1.230	0.045	0.922	19.770	4.054
	40	1.131	0.051	0.923	22.758	3.804
Bradley	20	1.240	0.968	0.875	30.882	5.505
	30	1.163	0.959	0.922	26.055	3.823
	40	1.069	0.954	0.923	32.321	3.570
Kuhn	20	14.258	42.146	0.613	40.617	9.075
	30	14.747	37.500	0.725	34.074	7.322
	40	14.587	34.159	0.728	37.649	7.103
Iglesias & Chirifie'78	20	3.883	1.510	0.955	36.409	21871
	30	4.361	0.908	0.976	51.032	17,518
	40	4.655	0.484	0.976	63.398	15,831
Iglesias & Chirifie'81	20	16.575	-1.248	0.978	10.813	2.171
	30	12.424	-1.751	0.993	12.080	2.163
	40	10.148	-2.157	0.993	13.589	1.533
BET	20	0.057	0.016	0.114	7.833	3.061
	30	0.103	-0.020	0.168	9.380	3.130
	40	0.154	-0.059	0.536	10.133	2.788

Table 4.4c: Sorption isotherm model constants and applicability values of model for 90% sorbitol plasticized edible film at different temperatures.

Model	T (ºC)	Constants of Model		Applicability Values of Model		
		A	B	R^2	MRE	SEM
Smith	20	49.255	63.148	0.876	65.862	4.329
	30	42.864	57.532	0.778	1.178	0.814
	40	35.283	48.505	0.768	1.981	0.638
Oswin	20	15.531	0.853	0.983	59.347	1.624
	30	11.959	0.868	0.944	6.448	0.020
	40	9.309	0.753	0.957	6.751	0.234
Henderson	20	0.064	0.858	0.963	1.764	0.756
	30	0.085	0.835	0.911	6.146	0.028
	40	0.087	0.908	0.917	6.489	0.186
Halsey	20	6.609	0.832	0.994	2.246	0.690
	30	5.216	0.826	0.969	6.831	0.081
	40	6.268	1005	0.978	7.084	0.294
Harkins-Jura	20	52.296	-0.394	0.963	2.905	0.598
	30	38.424	-0.322	0.990	7.790	0.232
	40	32.264	-0.222	0.986	7.945	0.452
Chung & Pfost	20	1.545	0.047	0.916	-3.161	1.437
	30	1.391	0.052	0.835	1.703	0.731
	40	1.361	0.067	0.853	2.452	0.552
Bradley	20	1.439	0.958	0.916	-5.365	1.742
	30	1.218	0.958	0.835	-0.792	1.125
	40	1.183	0.945	0.853	0064	0.988
Kuhn	20	10.466	34.880	0.669	-7.896	2.092
	30	9.922	30.764	0.564	-1.256	1.199
	40	9.543	27.682	0.547	0.516	0.906
Iglesias & Chirife'78	20	3.510	1.688	0.976	4.759	0.732
	30	3.585	1.394	0.926	4.306	0.647
	40	3.228	1.337	0.923	4.321	0.746
Iglesias & Chirife'81	20	14.026	1.122	0.994	0.455	0.937
	30	12.105	-0.699	0.958	5.198	0.178
	40	7.045	1.423	0.974	5.522	0.009
BET	20	0.046	0.037	0.613	2.002	0.723
	30	0.063	0.043	0.263	6.380	0.009
	40	0.050	0.122	0.749	6.683	0.221

Applicability of sorption models for edible films prepared with different formulations are investigated taking consideration to the MRE value and R^2 . The

model has been chosen as applicable when the MRE value is lower than 10 and R^2 is higher than 0.90. Applicable models for different edible films were indicated in Table 4.5.

Table 4.5: Applicable models for sorption isotherms.

	70% Sorbitol			**80% Sorbitol**			**90% Sorbitol**		
Model	**20 °C**	**30 °C**	**40 °C**	**20 °C**	**30 °C**	**40 °C**	**20 °C**	**30 °C**	**40 °C**
Smith									
Oswin	X	X	X	X	X	X		X	X
Henderson					X	X	X	X	X
BET									
Halsey	X	X	X	X	X	X	X	X	X
Chung & Pfost							X		
Iglesias & Chirifie'78							X	X	X
Iglesias & Chirifie'81							X	X	X
Bradley							X		
Kuhn									
Harkins-Jura		X		X			X	X	X

4.4 Weight Loss

Figure 4.4 shows weight loss (%) results at 4 ^{o}C storage during 10 days. Pomegranate aril weight constantly decreased with all samples and control. Control group did not had significant difference than coated samples. Weight loss did not exceed 0.80% for 70, 80 and 90% sorbitol plasticized edible film coated samples during 10 days storage at 4 ^{o}C. But control had almost 1.2% weight loss at the end of storage. It is seen in Figure 4.4 that all of starch-based coatings reduced wieght loss during storage when compared with the control. This has a good agreement with the results of Vina et al. (2007).

Water loss directly affects the quality of minimally processed fruits and is in charge of decreased freshness of fruit because of the absence of protective tissues of peel and exposure of internal tissues (Conesa et al., 2007). It was reported that edible

coatings reduced weight loss of fresh strawberries and raspberries compared to control, because the edible film formed over the fruit and delayed the migration of moisture from fruit to environment therefore reducing weight loss during storage (Ayranci and Tunc, 2004).

If the weight loss is between 4 and 6% of the total fresh weight, it would be critical for the product. Because the values above the critical limits is the factor of deterioration or deformation of the food product as mentioned by Bico et al. (2009). In this study, the weight loss reached up to 1.2% in control arils at the end of 10 days storage. It is under the critical limit.

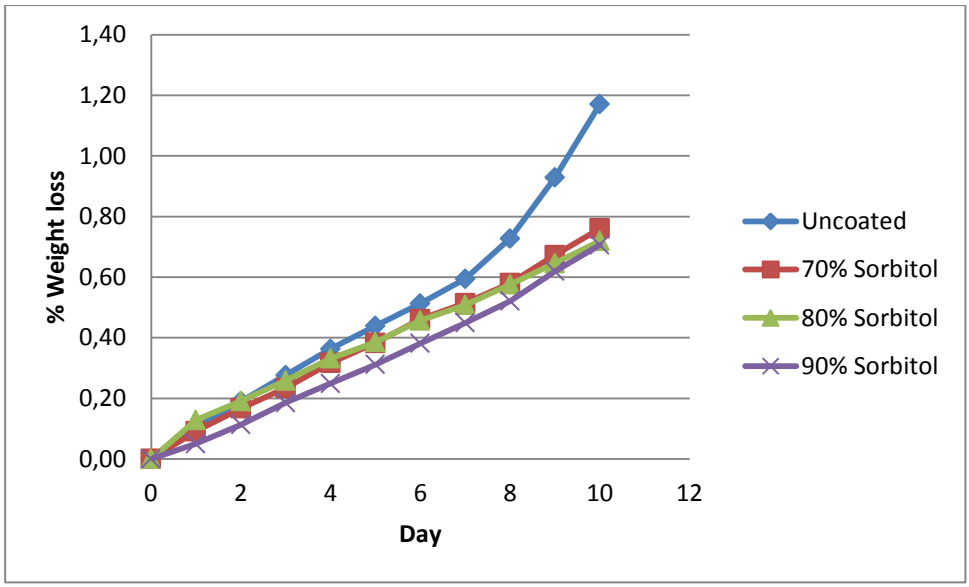


Figure 4.4: Effect of coating on weight loss (%) of Pomegranate arils during storage at 4 ^{o}C for 10 days.

4.5 Total Soluble Solids Content

Total soluble solid content for control group was determined as 14 ^{o}Brix. Figure 4.5 shows the changes in TSS value of control and different edible films during 10 days. As it can be clearly seen from Figure 4.5, all of TSS contents including control sample were decreased. Zaouay et al. (2012) made a study about physico-chemical properties of different pomegranate cultivars grown in Tunisia and our control sample had the same TSS value with their cultivars. Turkmen and Eksi (2011) investigate the Brix degree of pomegranate juice and their result was between 12.2 and 17.8. This results seems parallel with our study.

On the other hand, Oz and Ulukanlı (2011) studied the edible starch-based coating glycerol and oleum nigella on pomegranate arils. Their storage time for coated arils was 12 days and day by day their TSS value increased from 14.8 ^{o}Brix to 17 ^{o}Brix. This difference may be caused because of using different film formulation.

At the end of storage, TSS value decreased to 13.25 ^{o}Brix in control, 12 ^{o}Brix in 70% and 90% sorbitol coated film and 12.5 ^{o}Brix 80% sorbitol coated film. It is clear that edible film plasticized with sorbitol coated arils had effect on reducing TSS content.

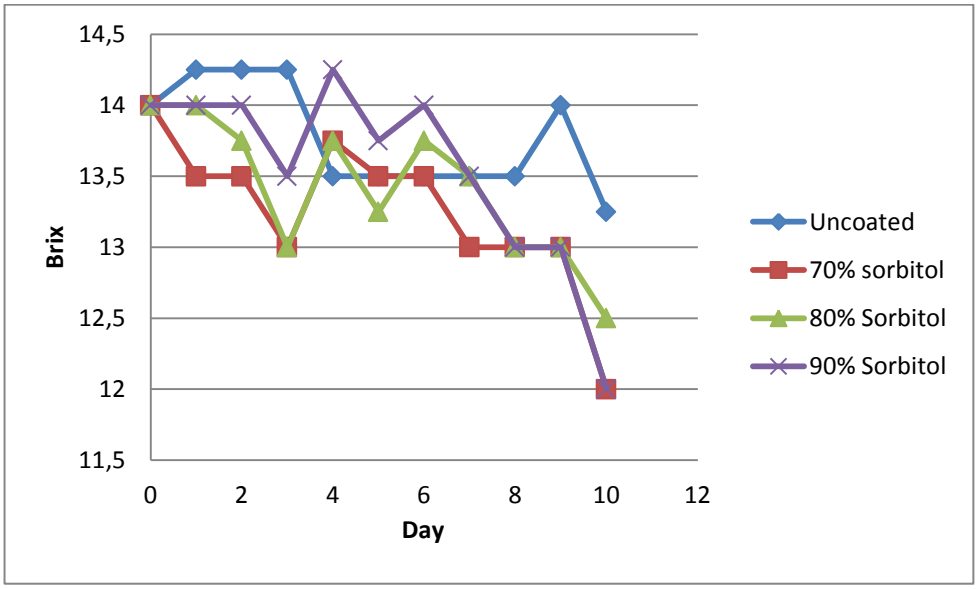


Figure 4.5: Changes in TSS content of Pomegranate arils coated with different concentrations of sorbitol plasticized edible film and control stored at 4 ^{o}C for 10 days.

4.6 pH and Titratable Acidity

Figure 4.6 and Figure 4.7 shows the changes in values of pH and titratable acidity in pomegranate arils which are coated with edible film during 10 days at 4 ^{o}C storage. The pH of control and coated arils increased as it can be seen in Figure 4.6, while the titratable acidity decreased. The pH of all samples and control slightly decreased during 4 days and following days increased. But when you compare with the initial day, pH value did not increase a lot.

It is observed that titratable acidiy of coated arils decreased in two days but TA of control increased during 3 days and following days decreased as shown in Figure 4.7. Our results is supported by study of Han et al. (2005). There was a significant

decrease in TA on 2 day for 70% sorbitol-plasticized edible film coated arils, although it did not change rest of the storage days.

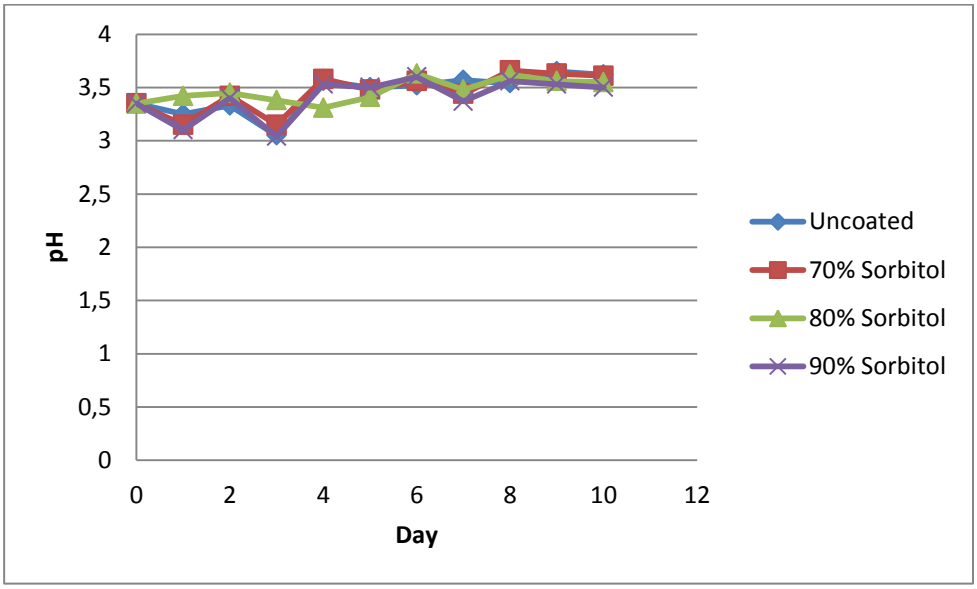


Figure 4.6: Changes in pH content of Pomegranate arils coated with different concentrations of sorbitol plasticized edible film and control stored at 4 ^{o}C for 10 days.

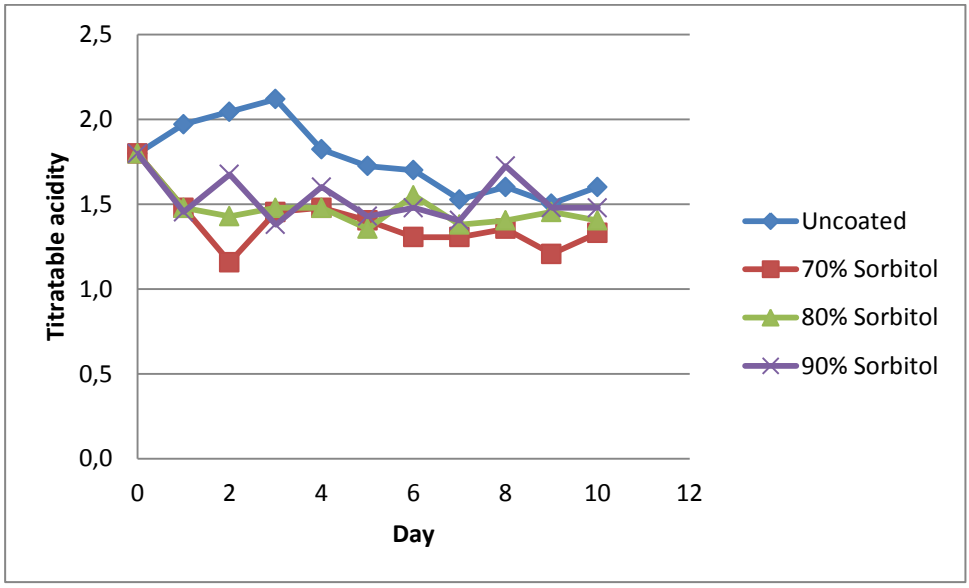


Figure 4.7: Changes in TA content of Pomegranate arils coated with different concentrations of sorbitol plasticized edible film and control stored at 4 ^{o}C for 10 days.

4.7 Total Phenolic Content

Total phenolic content results were expressed as mg gallic acid equivalents (GAE)/100 g DW. Standard calibration curve of gallic acid is seen in Figure 4.8. The standard curve was prepared between 0.04-0.5 mg/ml concentration and the equation was used to calculate the absorbance values of the samples measured by spectrophotometer.

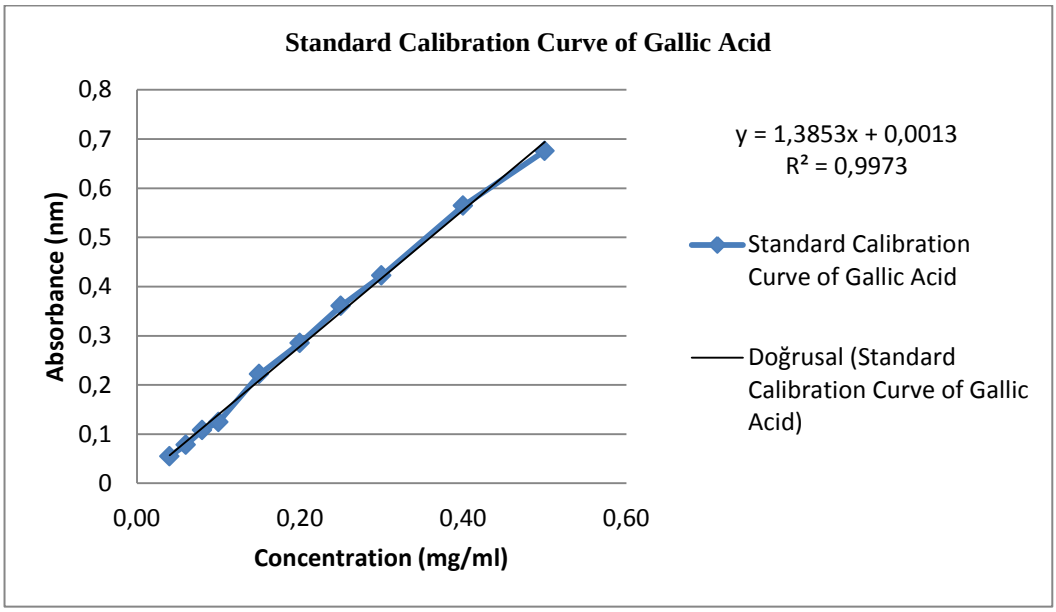


Figure 4.8: Standard calibration curve of gallic acid

Table 4.6 shows the results and statistical evaluation of total phenolic contents of pomegranate aril samples both coated and uncoated. As it can be seen in the table 4.6, 0. day samples has showed the highest total phenolic value. At the end of 10 days total phenolic contents of pomegranate arils decreased.

Table 4.6: Total phenolic contents for all samples during 10 day storage.

	Film Sampe (mg GAE/100mg DW)			
Day	Uncoated	70% Sorbtitol	80% Sorbitol	90% Sorbitol
0	153.060 ± 10.27a	153.060 ± 10.27a	153.060 ± 10.27a	153.060 ± 10.27a
1	145.600 ± 11.62ab	134.532 ± 9.61abc	126.832 ± 8.04bc	142.232 ± 10.27ab
2	130.200 ± 2.32bcd	128.275 ± 17.07abc	134.532 ± 3.98ab	143.194 ± 14.62ab
3	130.200 ± 2.32bcd	131.644 ± 6.67bc	125.629 ± 15.84bc	120.576 ± 11.51bc
4	139.825 ± 9.44abc	123.704 ± 8.61bc	120.576 ± 2.08bcd	132.847 ± 9.39ab
5	122.741 ± 1.82cd	114.801 ± 5.32bc	111.913 ± 3.56cde	136.938 ± 19.41 ab
6	118.169 ± 5.77d	115.041 ± 9.53bc	113.598 ± 3.41cde	137.419 ± 6.71ab
7	146.081 ± 8.46ab	132.847 ± 8.55ab	109.748 ± 2.08cde	117.688 ± 7.92bc
8	116.726 ± 3.61d	113.598 ± 4.58bc	114.560 ± 5.21bcde	116.244 ± 2.54bc
9	153.300 ± 8.46a	105.657 ± 5.83c	102.048 ± 3.26de	129.960 ± 7.23ab
10	114.079 ± 5.83d	110.951 ± 5.05bc	97.476 ± 4.64e	96.995 ± 6.67c

[1] Mean values ± Standard deviation

[2] Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).

Caliskan and Beyazit (2012) investigated the total phenolic contents of 76 different pomegranate accessions and the values has varied from 108.0 ± 4.1to 564.9±10.0 27 mg GAE/100mg DW. Our pomegranate arils had total phenolic content value as 153.060 ± 10.27 mg GAE/100mg DW. Most of the pomegranate arils had the total phenolic content values close to our sample.

Al-Weshahy et al. (2013) reported that total phenolic content of freeze-dried potato peel has decreased during 2 weeks. Initial decrease in the polyphenolic compounds may be related to their stability under storage. Polyphenols are known to undergo oxidative and enzymatic breakdown to their subunits.

As observed in Figure 4.9, during 10 day storage at 4 ^{o}C, total phenolic content of all samples decreased. 80% and 90% sorbitol plasticized edible film coated aril samples had the most reduction on total phenolic content at the end of 10 days. 70% sorbitol added edible film coated aril sample had almost same total phenolic content with control.

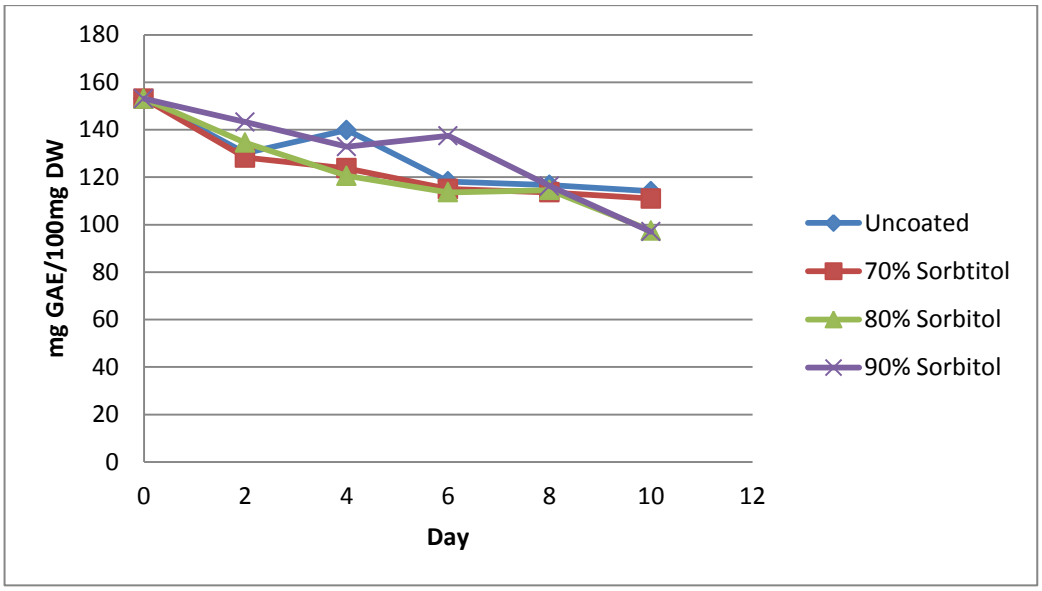


Figure 4.9: Change in total phenolic content during 10 day storage at 4 ^{o}C.

4.8 Total Flavonoid Content

Total flavonoid content results were expressed as mg quercetin equivalents (QE) /100 g DW. Standart calibration curve of quercetin is shown in Figure 4.10. The standard calibration curve prepared between 0.01-0.5 mg/ml concentration and the equation was used to calculate the absorbance values of the samples measured by spectrophotometer.

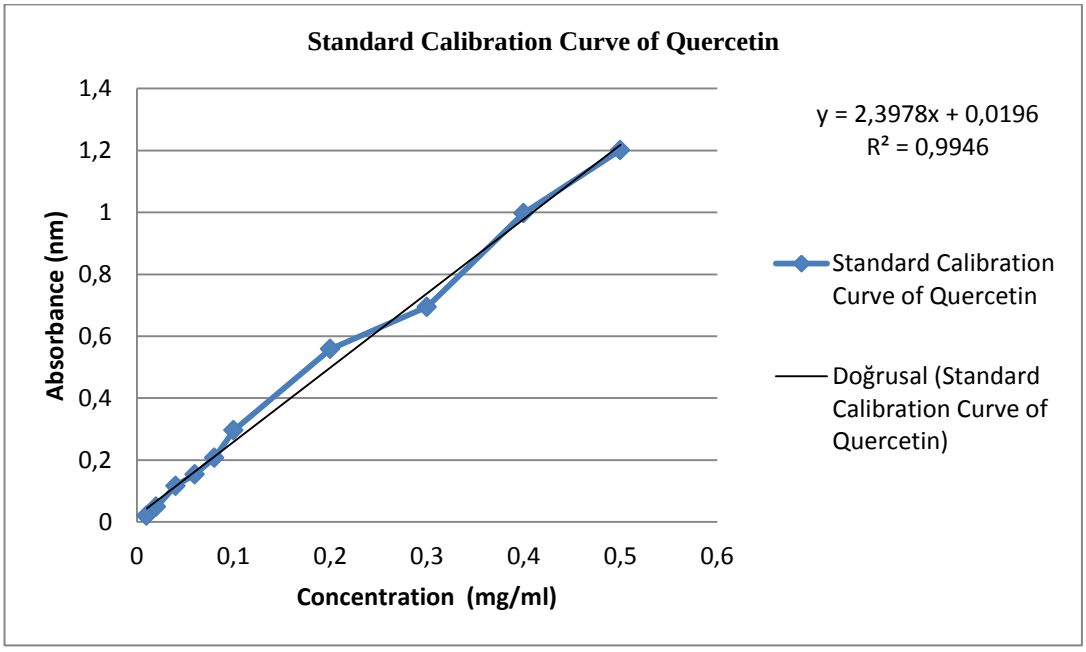


Figure 4.10: Standard calibration curve of quercetin.

The results and statistical evaluation of total flavonoid contents of all samples are listed in Table 4.7. The 0 day had the highest total flavonoid content value (18.934 ± 2.09 mg QE/100mg DW).

Table 4.7: Total flavonoid contents for all samples during 10 day storage.

	Film Sampe (mg QE/100mg DW)			
Day	Uncoated	70% Sorbtitol	80% Sorbitol	90% Sorbitol
0	18.934 ± 2.09a	18.934 ± 2.09a	18.934 ± 2.09ab	18.934 ± 2.09ab
1	9.898 ± 5.75a	2.669 ± 0.72a	7.813 ± 3.02ab	18.100 ± 16.14ab
2	12.261 ± 9.54a	12.956 ± 5.92a	12.539 ± 8.28ab	10.315 ± 3.60b
3	9.064 ± 5.18a	8.925 ± 11.59a	0.723 ± 0.48b	5.310 ± 5.69b
4	1.,288 ± 3.47a	6.423 ± 6.50a	7.257 ± 5.06ab	7.571 ± 2.48a
5	4.754 ± 2.60a	4.893 ± 4.22a	2.947 ± 2.37b	10.315 ± 9.78b
6	7.396 ± 7.25a	8.230 ± 0.64a	8.369 ± 10.72ab	6.284 ± 2.93b
7	9.481 ± 3.07a	11.427 ± 7.99a	2.252 ± 1.67b	5.727 ± 0.87b
8	6.145 ± 1.88a	4.615 ± 7.35a	5.727 ± 4.42b	5.588 ± 6.23b
9	8.369 ± 2.06a	3.086 ± 3.31a	2.530 ± 0.64b	1.974 ± 2.06b
10	4.893 ± 5.04a	5.727 ± 2.30a	6.580 ± 1.28a	2.252 ± 2.54b

[1] Mean values ± Standard deviation
[2] Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).

It can easily seen from statistical evaluation that there has not been observed any significant difference for uncoated and 70% sorbitol added edible film coated arils. Figure 4.11 shows the change in total flavonoid content during 10 days cold storage of pomegranate aril samples. There have been decrease in total flavonoid content of all samples. 90% sorbitol plasticized edible film coated arils had the most decrease on total flavonoid content during 10 days (2.252 ± 2.54 mg QE/100mg DW). 80% and 70% sorbitol added coatings had higher total flavonoid content than control.

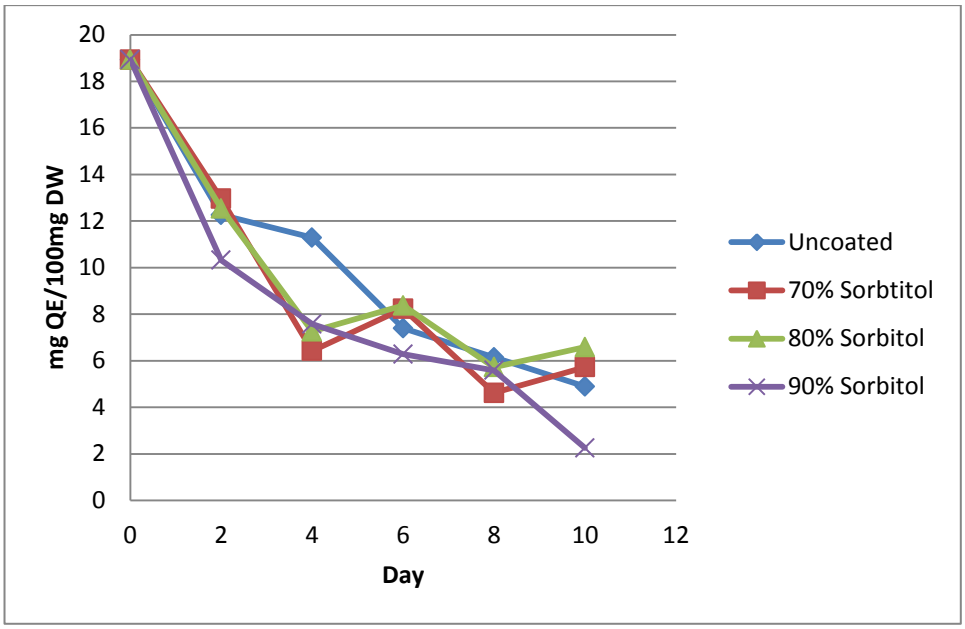


Figure 4.11: Change in total flavonoid content during 10 day storage at 4 ^{o}C.

4.9 Total Antioxidant Activity

4.9.1 Total antioxidant activity by DPPH method

Total antioxidant activity results by DPPH method were expressed as mg trolox (TEAC) /100 g DW. Standart calibration curve of Trolox prepared for DPPH method is shown in Figure 4.12. The standard calibration curve prepared between 0.01-0.1 mg/ml concentration and the equation was used to calculate the absorbance values of the samples measured by spectrophotometer.

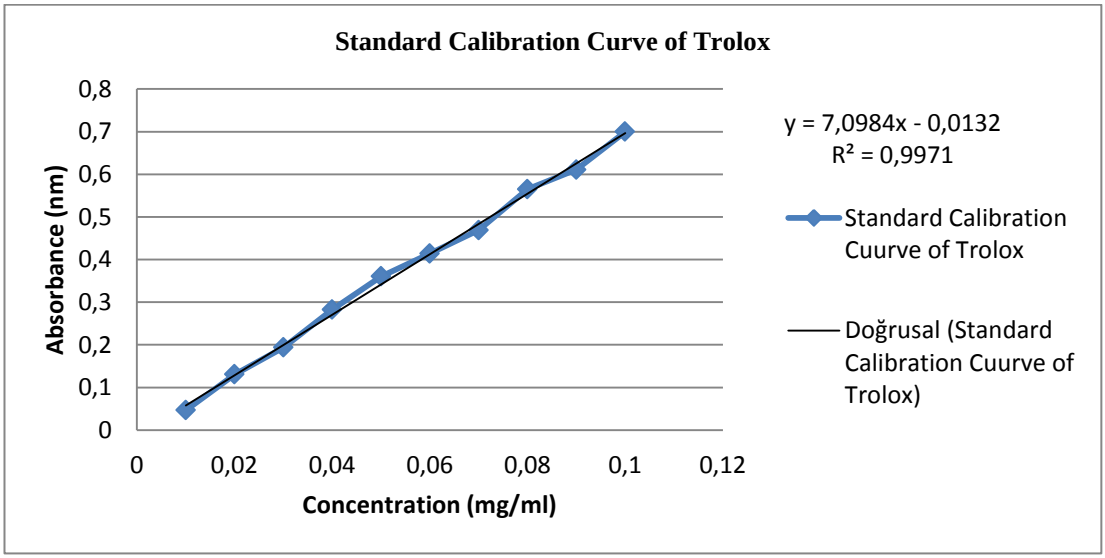


Figure 4.12: Standard calibration curve of Trolox for DPPH method.

Table 4.8 shows the results and statistical evaluation of total antioxidant activity by DPPH method of pomegranate aril samples both coated and uncoated during 10 days. 90% sorbitol coated aril had the highest as 357.968 ± 89.84 mg TEAC/100g DW on 1st day and 80% sorbitol plasticized edible film coated aril had the lowest as 67.762 ± 35.94 mg TEAC/100g DW on 5th day as presented in Table 4.8:

Table 4.8: Total antioxidant activity analysis by DPPH for all samples during 10 day storage.

	Film Sampe (mg TEAC/100g DW)			
Day	Uncoated	70% Sorbtitol	80% Sorbitol	90% Sorbitol
0	176.237 ± 57.31abc	176.237 ± 57.31ab	176.237 ± 57.31abcd	176.237 ± 57.31a
1	146.418 ± 17.22bc	268.746 ± 112.16ab	294.573 ± 110.08ab	357.968 ± 89.84b
2	244.093 ± 31.36abc	171.071 ± 14.37ab	175.533 ± 26.39bcd	112.138 ± 11.64b
3	232.118 ± 71.63abc	182.576 ± 28.62ab	178.820 ± 67.38abcd	154.636 ± 88.68b
4	114.016 ± 34.80c	125.521 ± 83.93ab	86.311 ± 50.27d	96.407 ± 60.52b
5	169.663 ± 8.10bc	180.698 ± 18.17ab	282.129 ± 54.12abc	67.762 ± 35.94ab
6	308.661 ± 37.41a	115.895 ± 13.44ab	353.742 ± 44.93a	193.847 ± 151.91b
7	222,726 ± 77.48abc	96.876 ± 84.04b	156.045 ± 29.40bcd	148.531 ± 28.36ab
8	258.885 ± 34.94ab	199.012 ± 143.84ab	307.722 ± 63.26ab	245.501 ± 27.28ab
9	137.965 ± 28.47bc	324.627 ± 58.47a	107.677 ± 60.45cd	251.371 ± 26.00ab
10	216.387 ± 61.36abc	230.005 ± 26.96ab	244.562 ± 63.50abcd	265.694 ± 54.73ab

[1] Mean values ± Standard deviation
[2] Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).

Change in total antioxidant activity by DPPH method during 10 days storage at 4 ^{o}C is shown in Figure 4.13. It is seen that antioxidant activity of control sample increased in 2 days while, antioxidant activity of all coated samples either decrease or maintained. The early increase in antioxidant activity during storage might be due to the production of glycoalkaloids in response to the stress of uncoated sample. Another explanation may be related to the possible breakdown of the polyphenolic compound during early stages of storage resulting in pronounced decrease in their levels. Such multiple breakdown products may still possess potent antioxidant capacity. (Al-Weshashy et al., 2013). Coating may decrease the stress for the first days of storage.

At the end of 10 days storage, 90% sorbitol added coating had the highest antioxidant activity by DPPH method, following that 80% and %70 plasticized edible film coating and then control sample.

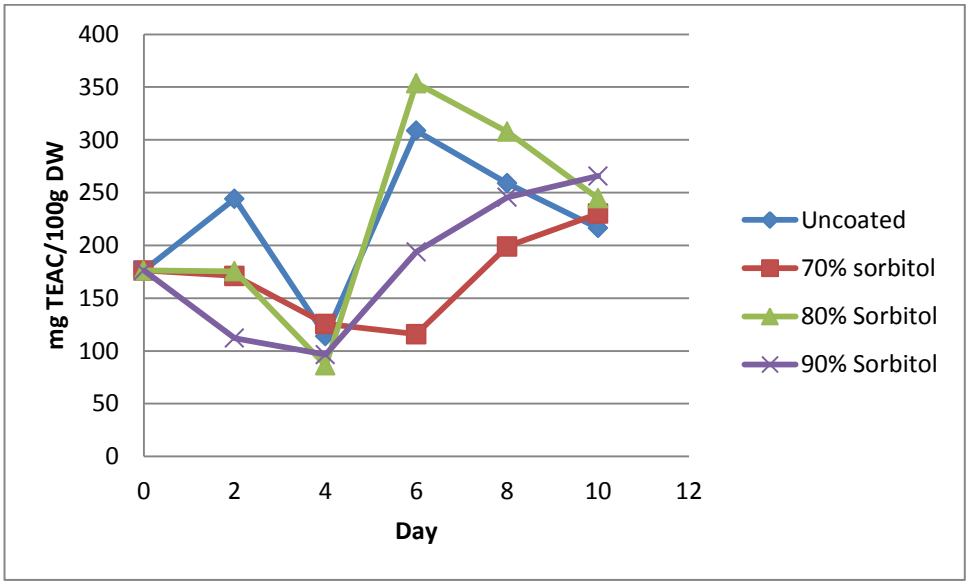


Figure 4.13: Change in total antioxidant activity of all samples by DPPH method during 10 days storage at 4 ^{o}C.

4.9.2 Total antioxidant activity by CUPRAC method

Total antioxidant activity results by CUPRAC method were expressed as mg trolox (TEAC) /100 g DW. Standart calibration curve of Trolox prepared for CUPRAC method is shown in Figure 4.14. The standard calibration curve prepared between 0,01-0,8 mg/ml concentration and the equation was used to calculate the absorbance values of the samples measured by spectrophotometer.

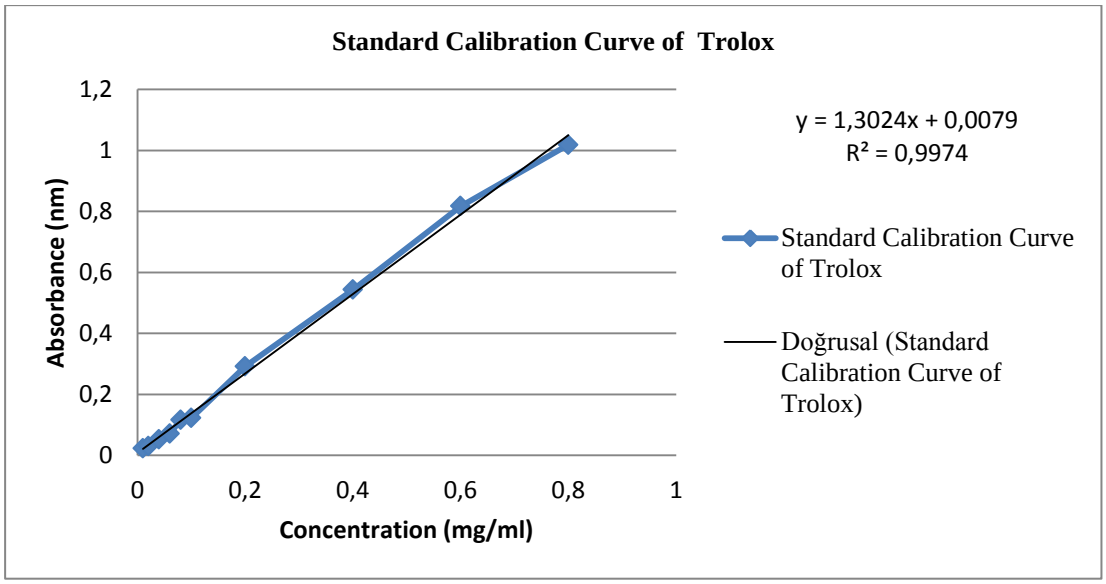


Figure 4.14: Standard calibration curve of Trolox for CUPRAC method.

Table 4.9 shows the results and statistical evaluation of total antioxidant activity by CUPRAC method of pomegranate aril samples both coated and uncoated during 10 days. 90% sorbitol added coating had the highest as 741.273 ± 20.96 mg TEAC/100g DW on 5th day and 90% sorbitol added coating had the lowest as 201.756 ± 28.79 mg TEAC/100g DW on 3rd day as presented in Table 4.9.

Table 4.9: Total antioxidant activity analysis by CUPRAC for all samples during 10 days storage.

	Film Sample (mg TEAC/100g DW)			
Day	Uncoated	70% Sorbtitol	80% Sorbitol	90% Sorbitol
0	623.029 ± 22.16a	623.029 ± 22.16a	623.029 ± 22.16a	623.029 ± 22.16b
1	502.994 ± 20.87b	566.723 ± 29.93ab	451.039 ± 58.36bc	507.857 ± 53.64c
2	321.023 ± 64.12e	371.187 ± 2.47de	370.675 ± 34.83cd	458.973 ± 28.16cd
3	370.931 ± 14.22de	376.305 ± 17.66de	323.326 ± 36.21d	201.756 ± 28.79e
4	432.100 ± 41.45bcd	417.511 ± 31.79cd	469.467 ± 28.03b	435.171 ± 16.28cd
5	418.023 ± 1.93bcd	407.530 ± 13.66d	412.648 ± 16.40bcd	741.273 ± 20.96a
6	451.551 ± 16.34bcd	399.084 ± 67.29d	424.166 ± 57.33bc	467.931 ± 13.53cd
7	475.609 ± 32.14bc	495.828 ± 17.80bc	401.899 ± 6.71bcd	440.290 ± 44.15cd
8	370.931 ± 31.51de	416.488 ± 33.03cd	358.390 ± 3.94cd	528.844 ± 61.87bc
9	396.780 ± 19.15cde	295.685 ± 25.96e	326.909 ± 22.81d	390.382 ± 51.65d
10	316.416 ± 11.59e	384.751 ± 14.65d	396.268 ± 16.84bcd	399.852 ± 3.63d

[1] Mean values ± Standard deviation
[2] Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).

As it is seen in Figure 4.15. Antioxidant activity by CUPRAC of all samples decreased in 2 days. At the end of 10 days storage, the highest to lowest antioxidant activity was observed respectively 90% sorbitol, 80% sorbitol, 70% sorbitol added coating and uncoated samples. It can be concluded that coating has a positivie effect in terms of antioxidant activity.

Nath et al. (2011) made a research about changes in post-harvest phytochemical qualities of broccoli florets during ambient and refrigerated storage and they found that antioxidant activity decreased by the end of 144 h storage in both ambient and refrigirated atmospheres. Although our samples are different, we had the similiar results in antioxidant activity by CUPRAC method.

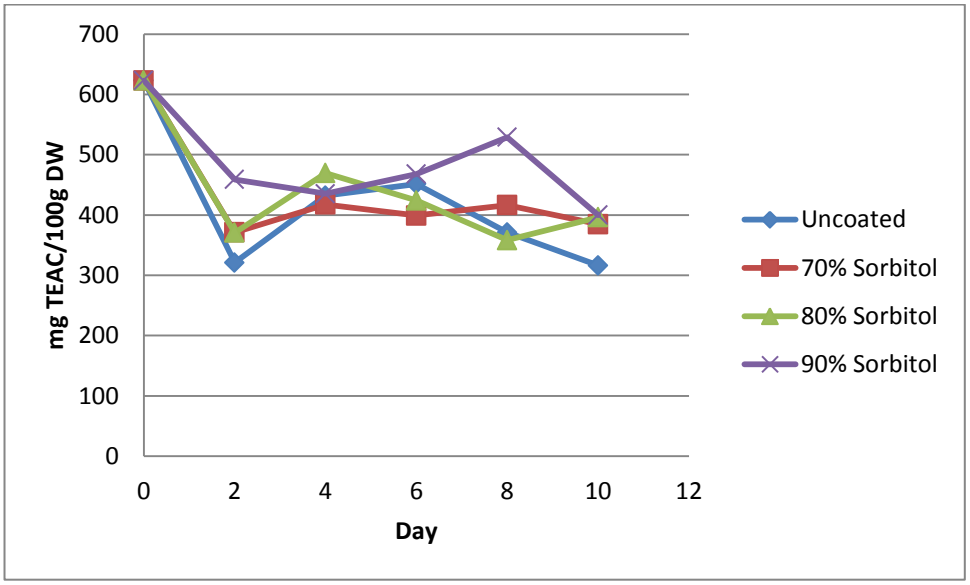


Figure 4.15: Change in total antioxidant activity of all samples by CUPRAC method during 10 days storage at 4 ^{o}C.

4.9.3 Total antioxidant activity by ABTS method

Total antioxidant activity results by ABTS method were expressed as mg trolox (TEAC) /100 g DW. Standart calibration curve of Trolox prepared for ABTS method is shown in Figure 4.16. The standard calibration curve prepared between 0.01-0.1 mg/ml concentration and the equation was used to calculate the absorbance values of the samples measured by spectrophotometer.

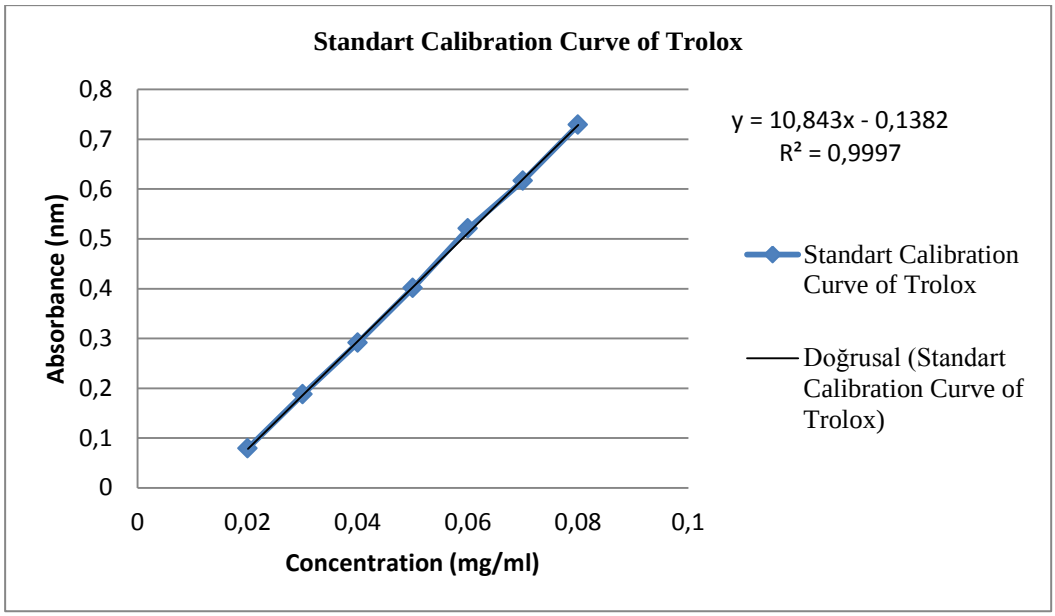


Figure 4.16: Standard calibration curve of Trolox for ABTS method.

Table 4.10 shows the results and statistical evaluation of total antioxidant activity by ABTS method of pomegranate aril samples both coated and uncoated during 10 days. Coating with 70% sorbitol had the highest as 547.081 ± 8.20 mg TEAC/100g DW on 7th day and uncoated aril had the lowest as 392.450 ± 84.43 mg TEAC/100g DW on 0 day as presented in Table 4.10.

Table 4.10: Total antioxidant activity analysis by ABTS for all samples during 10 day storage.

	Film Sample (mg TEAC/100g DW)			
Day	Uncoated	70% Sorbtitol	80% Sorbitol	90% Sorbitol
0	392.450 ± 84.43b	392.450 ± 84.43d	392.450 ± 84.43b	392.450 ± 84.43b
1	452,.396 ± 14.12ab	507.732 ± 2.82abc	498.202 ± 6.46a	466.845 ± 5.76ab
2	465.615 ± 17.46ab	546.159 ± 9.76a	522.795 ± 15.82a	517.569 ± 37.61a
3	458,237 ± 6.48ab	497.894 ± 9.24abc	500.661 ± 22.14a	422.269 ± 5.56ab
4	470.227 ± 6.28ab	476.990 ± 34.15abcd	510.806 ± 59.49a	457.008 ± 15.36ab
5	469.304 ± 51.85ab	483.753 ± 13.50abcd	506.502 ± 16.32a	485.905 ± 9.86ab
6	478.219 ± 8.32ab	523.102 ± 27.54ab	464.386 ± 13.69ab	502.813 ± 34.42ab
7	496.665 ± 12.04a	547.081 ± 8.20a	451.474 ± 4.73ab	430.570 ± 14.71ab
8	436.411 ± 13.65ab	476.682 ± 14.99abcd	463.156 ± 42.90ab	424.421 ± 25.47ab
9	463.156 ± 42.90ab	424.421 ± 25.47cd	477.912 ± 9.63ab	461.311 ± 28.05ab
10	442.866 ± 9.08ab	443.789 ± 31.32bcd	447.478 ± 23.93ab	490.209 ± 65.70ab

[1] Mean values ± Standard deviation

[2] Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).

The antioxidant activity by ABTS method of all samples increased in two days. But the following two days decreased. . During 10 days storage antioxidant activity increased. Al-Weshashy et al. (2013) investigated the antioxidant activity of freeze-dried potato peels during 8 weeks storage at different temperatures. They found the increased antioxidant activty. This study has a good agreement with their results although the samples are different.

Sayyari et al. (2011) observed the antioxidant activity during postharvest storage of pomegranate and they found that during two days storage, antioxidant activity of pomegranate has increased. This is a similar result to our study.

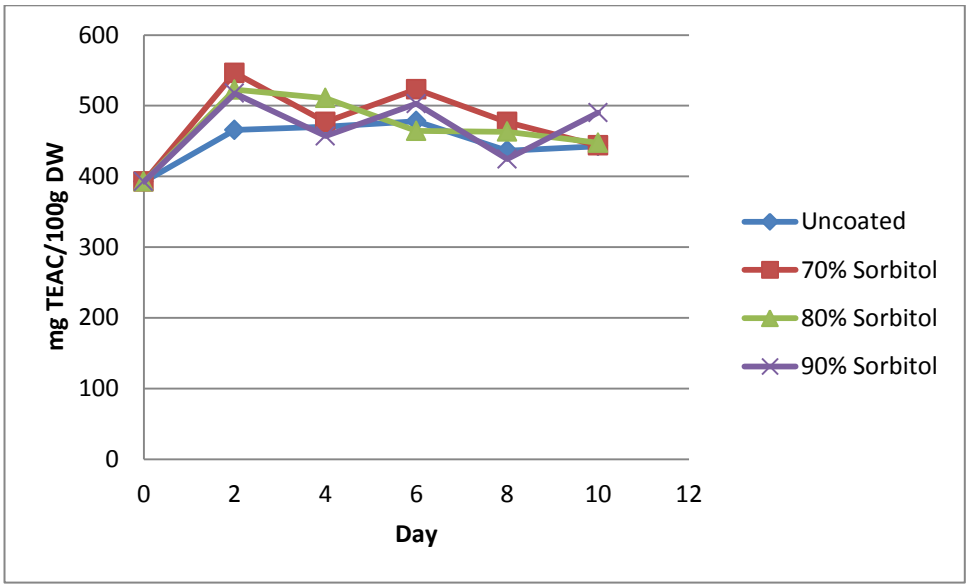


Figure 4.17: Change in total antioxidant activity of all samples by ABTS method during 10 days storage at 4 ^{o}C.

5. CONCLUSIONS

The low cost, abundance of source, good mechanical and barrier properties of chickpea starch potentiate their applications for food preservation. The water vapor and oxygen permeability of edible films from chickpea starch plasticized by glycerol and sorbitol were evaluated. The results obtained show that different ratios of plasticizer affected WVP and OP of edible films. The addition of plasticizer increased the water vapor permeability of starch films. The highest WVP value was observed in 90% glycerol plasticized edible film, while the lowest in 70% sorbitol added film. 70% Sorbitol plasticized film has better moisture barrier property with having lower WVP value.

Oxygen permeability of 90% sorbitol added edible film was significantly lower than that of 90% glycerol plasticized edible film. Increasing the percentage of plasticizer sorbitol results in decreasing the oxygen permeability thus can improve oxygen barrier properties. It has reverse effect on glycerol. Increased level of glycerol had higher oxygen permeability.

When it is compared the sorption isotherms of the edible films, the hygroscopic properties from higher to lower is respectively edible film plasticized by 90% sorbitol, 80% sorbitol and 70% sorbitol. The slope of the curve of edible films is increased after the water activity value 0.6. In this study, sorption models for applicablity are investigated for different edible films. As a result, sorption equilibrium can be well described with Halsey equation. Because it is applicable for all edible film samples.

The minimum weight loss was obtained in arils with 90% sorbitol plasticized coatings, while the maximum weight loss was observed in uncoated arils. In this term, coating improves the quailty of pomegranate arils.

In conclusion, the chickpea starch-based film coatings maintained the quality of pomegranate arils and prolonged shelf life, compared to uncoated fruit. Starch-based solutions can be alternative to prevent pomegranate arils.

REFERENCES

Al-Said, F.A., Opara, L.U., Al-Yahyai, R.A. (2009). Physico-chemical and textural quality attributes of pomegranate cultivars (*Punica granatum* L.) grown in the Sultanate of Oman. *Journal of Food Engineering*, ***90***, 129–134.

Al-Hassan, A.A., Norziah, M.H.(2012). Starch-gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids*, ***26***, 108-117.

Al-Weshahy, A., El-Nokety, M., Bakhete, M., Rao, V. (2013). Effect of storage on antioxidant activity of freeze-dried potato peels. *Research International*, ***50***, 507–512.

Ayranci, E., Tunc, S. (2004). The effect of edible coatings on water and vitamin C loss of apricots (Armeniaca vulgaris Lam.) and green peppers (Capsicum annuum L.). *Food Chem.*, ***87***, 339-342.

Bastos, D.S., Araujo, K.G, Leaoa, M.H. (2009)**.** Ascorbic acid retaining using a new calcium alginate-Capsul based edible film. *Journal of Microencapsulation*, ***26(2),*** 97–103.

Bertuzzi, M.A., Castro Vidaurre, E.F., Armada, M., Gottifredi, J.C. (2007) Water vapor permeability of edible starch based films. *Journal of Food Engineering*, ***80***, 972–978.

Bico, S.L.S., Raposo, M.F.J., Morais, R.M.S.C, Morais, A.M.M.B. 2009. Combined effects of chemical dip and/or carrageenan coating and/or controlled atmosphere on quality of fresh cut banana. *Food Cont.*, ***20***, 508-514.

Caliskan, O. and Bayazit, S. (2012). Phytochemical and antioxidant attributes of autochthonous Turkish pomegranates. *Scientia Horticulturae*, ***147***, 81–88.

Campos, C.A., Gerschenson, L.N., Flores, S.K. (2011) Development of edible films and coatings with antimicrobial activity. *Food Bioprocess Technol*, ***4***, 849–875.

Cerqueira, M.A., Bourbon, A.I., Pinheiro, A.C., Martinsa, J.T., Souza, B.W.S., Teixeira, J.A., Vicente, A.A. (2011). Galactomannans use in the development of edible films/coatings for food applications. *Trends in Food Science & Technology*, ***22***, 662-671.

Cha, D.S. and Chinnan, M.S. (2004). Biopolymer-Based Antimicrobial Packaging. *Critical Reviews in Food Science and Nutrition*, ***44***, 223–237.

Chamorro, S.A.V., Palou, L., Del Rio, M.A., Perez, M.B. (2011). Antimicrobial Edible Films and Coatings for Fresh and Minimally Processed Fruits and Vegetables. *Food Science and Nutrition*, ***51***, 872–900.

Chillo, S., Flores, S., Mastromatteo, M., Gerschenson, M.C.L.,Del Nobile, M.A. (2008). Influence of glycerol and chitosan on tapioca starch-based edible film properties. *Journal of Food Engineering*, ***88***, 159–168.

Chiumarelli, M. and Hubinger, M.D. (2012) Stability, solubility, mechanical and barrier properties of cassava starch-Carnauba wax edible coatings to preserve fresh-cut apples. *Food Hydrocolloids*, ***28***, 59-67.

Cho, S.Y., Lee, S.Y., Rhee, C. (2010).Edible oxygen barrier bilayer film pouches from corn zein and soy protein isolate for olive oil packaging. *Food Science and Technology*, ***43***, 1234-1239.

Conesa, A., Artes-Hernandez, F., Geysen, S., Nicolai, B., Artes, F. (2007). High oxygen combined with high carbon dioxide improves microbial and sensory quality of fresh-cut peppers. *Postharvest Biol. Technol.*, ***43***, 230-237.

Dursun, S. and Erkan, N. (2009) Yenilebilir protein filmler ve su ürünlerinde kullanımı. *Journal of FisheriesSciences.com*, ***3(4),*** 352-373.

Ehivet, F.E., Min, B., Park, M., Oh, J.(2011). Characterization and Antimicrobial Activity of Sweetpotato Starch-Based Edible Film Containing Origanum (*Thymus capitatus*) Oil. *Journal of Food Science*, ***76(1),*** 179-184.

Erdohan, Z.O. and Turhan, K.N. (2005). Barrier and Mechanical Properties of Methylcellulose–Whey Protein Films. *Packag. Technol. Sci*, ***18***, 295–302.

Fabra, M.J., Hambleton, A., Talens, P., Debeaufort, F., Chiralt, A. (2011). Effect of ferulic acid and a-tocopherol antioxidants on properties of sodium caseinate edible films. *Food Hydrocolloids*, ***25***, 1441-1447.

Fadini, A.L., Rocha, A.L., Alvim, I.D., Sadahira, M.S., Queiroz, M.B., Alves, R.M.V., Silva, L.B. (2013). Mechanical properties and water vapour permeability of hydrolysed collagenecocoa butter edible films plasticised with sucrose. *Food Hydrocolloids*, ***30***, 625-631.

Fakhoury, F.M., Martelli, S.M., Bertan, L.C., Yamashita, F., Mei, L.H.I., Queiroz, F.P.C. (2012). Edible films made from blends of manioc starch and gelatin Influence of different types of plasticizer and different levels of macromolecules on their properties. *Food Science and Technology*, ***49***, 149-154.

Falguera, V., Quintero, J.P., Jimenez, A., Ibarz, A. (2011). Edible films and coatings: Structures, active functions and trends in their use. *Trends in Food Science & Technology*, ***22***, 292-303.

Garcia, M.A., Martino, M.N., Zaritzky, N.E. (1999).Edible Starch Films and Coatings Characterization: Scanning Electron Microscopy, Water Vapor, and Gas Permeabilities. *Scanning*, ***21***, 348–353.

Garcia, M.A., Martino, M.N., Zaritzky, N.E. (2000). Lipid Addition to Improve Barrier Properties of Edible Starch-based Films and Coatings. *Journal of Food Science*, ***65(6),*** 941-947.

Ghanbarzadeh, B., Oromiehie, A.R., Musavi, M., Jomeh, Z.E., Rad, E.R., Milani, J. (2006). Effect of plasticizing sugars on rheological and thermal properties of zein resins and mechanical properties of zein films. *Food Research International*, ***39***, 882–890.

Ghanbarzadeh, B., Almasi, H., Entezami, A. (2011) Improving the barrier and mechanical properties of corn starch-based edible films: Effect of citric acid and carboxymethyl cellulose. *Industrial Crops and Products*, ***33***, 229–235.

Ghasemlou M., Khodaiyan, F., Oromiehie, A., Yarman, M.S. (2011). Development and characterisation of a new biodegradable edible film made from kefiran, an exopolysaccharide obtained from kefir grains. *Food Chemistry*, ***127***, 1496–1502.

Ghasemloua, M., Khodaiyana, F., Oromiehie, A. (2011). Physical, mechanical, barrier, and thermal properties of polyol-plasticized biodegradable edible film made from kefiran. *Carbohydrate Polymers*, ***84***, 477–483.

Gounga, M.E., Xu, S.Y., Wang, Z. (2007). Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formation. *Journal of Food Engineering*, ***83***, 521–530.

Hana, C., Zhao, Y., Leonard, S.W., Traber, M.G. (2004) Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria* × *ananassa*) and raspberries (*Rubus ideaus*). *Postharvest Biology and Technology*, ***33***, 67–78.

He, L., Xu, H., Liu, X., He, W., Yuan, F., Hou, Z., Gao, Y. (2011).Identification of phenolic compounds from pomegranate (Punica granatum L.) seed residues and investigation into their antioxidant capacities by HPLC–ABTS+ assay. *Food Research International, **44**,* 1161–1167.

He, L., Zhang, X., Xu, H., Xu, C., Yuan, F., Knez, Z., Novak, Z. (2012). Subcritical water extraction of phenolic compounds from pomegranate (*Punica granatum* L.) seed residues and investigation into their antioxidant activities with HPLC–ABTS•+ assay. *Food And Bioproducts Processing, **90**,* 215–223.

Jagtap, D.B., Desai, U.T., Kale, P.N. (1992). Chemical composition of some indigenous and exotic cultivars of pomegranate. *Maharashtra J. Hort., **6(1),*** 10-12.

Jangchud, A., Chinnan, M.S.(1999). Properties of Peanut Protein Film: Sorption Isotherm and Plasticizer Effect. *Lebensm Wiss. u.Technol*., ***32**,* 89-94.

Javanmardi, M., Ahangari, R., Tavakolipour, H. (2011). Effects of wheat starch edible films on rancidity and moisture uptake of pistachio kernels as a new package. *Journal of Food Process Engineering, **34**,* 1156–1171.

Khwaldia, K., Perez, C., Banon, S., Desobry, S., Hardy, J. (2004). Milk proteins for edible films and coatings. *Food Science and Nutrition, **44**,* 239–251.

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. *J. Agric. Food Chem.**, 49**,* 4388-4391.

Kowalczyk, D. and Baraniak, B. (2011). Effects of plasticizers, pH and heating of film-forming solution on the properties of pea protein isolate films. *Journal of Food Engineering, **105**,* 295–305.

Kulkarni, A.P. and Aradhya, S.M. (2005).Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chemistry, **93**,* 319–324.

Kumaran, A., Karunakaran, R.J. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus. Food Chemistry, **97,*** 109–114.

Legua, P., Melgarejo, P., Abdelmajid, H., Martinez, J.M., Martinez, R., Ilham, H., Hafida, H., Hernandez, F. (2012). Total Phenols and Antioxidant Capacity in 10 Moroccan Pomegranate Varieties. *Journal of Food Science, **71(1),*** 155-120.

Li, M., Liu, P., Zou, W., Yu, L., Xie, F., Pu, H., Liu, H., Chen, L.(2011). Extrusion processing and characterization of edible starch films with different amylose contents. *Journal of Food Engineering*, ***106***, 95–101.

Maran, J.P., Sivakumar, V., Sridhar, R., Thirugnanasanbandham, K. (2013). Development of model for barrier and optical properties of tapioca starch based edible films. *Carbohydrate Polymers*, ***92***, 1335– 1347.

Nath, A., Bagchi, B., Misra, L.K., Bidyut, C.D. (2011). Changes in post-harvest phytochemical qualities of broccoli florets during ambient and refrigerated storage. *Food Chemistry*, ***127***, 1510–1514.

Navarro-Tarazaga, M.L., Massa, A., Pérez-Gago, M.B. (2011). Effect of beeswax content on hydroxypropyl methylcellulose-based edible film properties and postharvest quality of coated plums (Cv. Angeleno). *Food Science and Technology*, ***44***, 2328-2334.

Oses, J., Niza, S., Ziani, K., Mate, J.I. (2009). Potato starch edible films to control oxidative rancidity of polyunsaturated lipids: effects of film composition, thickness and water activity. *International Journal of Food Science and Technology*, ***44***, 1360–1366.

Ozdemir, M. and Floros, J.D. (2008). Optimization of edible whey protein films containing preservatives for water vapor permeability, water solubility and sensory characteristics. *Journal of Food Engineering*, ***86***, 215–224.

Ozgen, M., Durgac, C., Serce, S., Kaya, C. (2008). Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. *Food Chemistry*, ***111,*** 703–706.

Pareta, R., Edirisinghe, M.J. (2006). A novel method for the preparation of starch films and coatings. *Carbohydrate Polymers*, ***63,*** 425–431.

Ramos, O.L., Reinas, I., Silva, S.I., Fernandes, J.C., Cerqueira, M.A., Pereira, R.N., Vicente, A.A., Poças, M.F., Pintado, M.E., Malcata, F.X.(2013). Effect of whey protein purity and glycerol content upon physical properties of edible films manufactured therefrom. *Food Hydrocolloids*, ***30***, 110-122.

Ribiero, C., Vicente, A.A., Teixeira, J.A., Miranda, C. (2007). Optimization of edible film coating composition to retard strawberry fruit scenescence. *Postharvest Biology and Technology*, ***44***, 63-70.

Rodriguez, M.,Oses, J., Ziani, K., Mate, J.I. (2006). Combined effect of plasticizers and surfactants on the physical properties of starch based edible films. *Food Research International*, ***39,*** 840–846.

Qua, W., Pan, Z., Ma, H. (2010). Extraction modeling and activities of antioxidants from pomegranate marc. *Journal of Food Engineering*, ***99,*** 16–23.

Saad, H., Bouhtoury, F.C., Pizzi, A., Rode, K., Charrier, B., Ayed, N. (2012) Characterization of pomegranate peels tannin extractives. *Industrial Crops and Products*, ***40***, 239– 246.

Sablani, S.S., Dasse, F., Bastarrachea, L., Dhawan, S., Hendrix, K.M., Min, S.C. (2009). Apple Peel-Based Edible Film Development Using a High-Pressure Homogenization . *Journal of Food Science*, ***74(7),*** 372-381.

Sayyaria, M., Castillo, S., Valero, D. Serrano, M. (2011). Acetyl salicylic acid alleviates chilling injury and maintains nutritive and bioactive compounds and antioxidant activity during postharvest storage of pomegranates. *Postharvest Biology and Technology*, ***60***, 136–142.

Sobral, P.J.A., Menegalli, F.C., Hubinger, M.D., Roques, M.A. (2001). Mechanical, water vapor barrier and thermal properties of gelatin based edible films. *Food hydrocolloids*, ***15,*** 423-432.

Sood, D.R., Dhindsa, K.S., Wagle, D.S. (1982). Studies on the nutritive value of pomegranate. *Haryana J. Hort. Sci.*, ***11(3),*** 177.

Talja, R.A., Helen, H., Roos, Y.H., Jouppila, K. (2008). Effect of type and content of binary polyol mixtures on physical and mechanical properties of starch-based edible films. *Carbohydrate Polymers*, ***71,*** 269–276.

Tapia, M.S., Rojas-Grau, M.A., Rodriguez, F.J., Ramirez, J., Carmona, A., Martin-Belloso, O.(2007). Alginate- and Gellan-Based Edible Films for Probiotic Coatings on Fresh-Cut Fruits. *Journal of Food Science*, ***72(4),*** 190-196.

Tapia-Blacido, D.R., Sobral, P.J.A., Menegalli, F.C.(2011).Optimization of amaranth flour films plasticized with glycerol and sorbitol by multi-response analysis. *Food Science and Technology*, ***44***, 1731-1738.

Tehranifar, A., Zarei, M., Nemati, Z., Esfandiyari, B., Vazifeshenas, M.R. (2010) Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. *Scientia Horticulturae*, ***126***, 180–185.

Tezcan, F., Ozguven, M.G., Diken, T., Ozcelik, B., Erim, F.B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry*, ***115***, 873–877.

Tulin, A. and Ulukanli, Z. (2012) Application of edible starch-based coating including glycerol plus oleum nigella on arils from long-stored whole pomegranate fruits. *Journal of Food Processing and Preservation*, ***36***, 81–95.

Turkmen, I. and Eksi, A. (2011). Brix degree and sorbitol/xylitol level of authentic pomegranate (*Punica granatum*) juice. *Food Chemistry*, ***127,*** 1404–1407.

Vargas, M., Pastor, C., Chiralt, A., McClements, D. J., González-Martínez, C. (2008). Recent advances in edible coatings for fresh and minimally processed fruits. *Critical Reviews in Food Science and Nutrition*, ***48(6),*** 496–511.

Varela-Santos, E., Ochoa-Martinez, A., Tabilo-Munizaga, G., Reyes, J.E., Pérez-Won, M., Briones-Labarca, V., Morales-Castro, J. (2012). Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. *Innovative Food Science and Emerging Technologies*, ***13,*** 13–22.

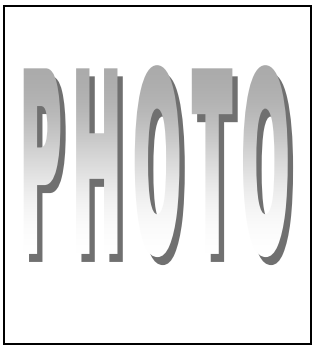
Vina, S.Z., Mugridge, A., Garcia, M.A., Ferreyra, R.M., Martino, M.N., Chaves, A.R., Zaritzky, N.E. (2007). Effect of polyvinylchloride films and edible starch coatings on quality aspects of refrigerated Brussels sprouts. *Food Chem.*, ***103***, 701-709.

Viuda-Martos, M., J., Lopez, F., Perez, J.A. (2010). Pomegranate and its Many Functional Components as Related to Human Health. *Comprehensive Reviews in Food Science and Food Safety*, ***9***, 635-654.

Zaouay, F., Mena, P., Garcia-Viguera, C., Mars, M. (2012). Antioxidant activity and physico-chemical properties of Tunisian grown pomegranate (*Punica granatum* L.) cultivars. *Industrial Crops and Products*, ***40,*** 81– 89.

Zhang, Y. and Han, H. (2006). Mechanical and thermal characteristics of pea starch films plasticized with monosaccharides and polyols. *Journal of Food Science*, ***71(2),*** 109-118.

CURRICULUM VITAE



Name Surname: Tayfun YAMAN

Place and Date of Birth: BURSA / 21.10.1987

Address: Barbaros Mah., Sırmaperde Sok., Saadettin Bey Apt., No:10/2, D:5
Altunizade, Uskudar/ISTANBUL

E-Mail: tayfunyaman87@gmail.com

B.Sc.: Yildiz Technical University / Chemistry Department

M.Sc.: Istanbul Technical University / Food Engineering Department