EFFECTS OF EDIBLE CHITOSAN COATING ON QUALITY PARAMETERS OF POMEGRANATE (*PUNICA GRANATUM*) ARILS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

AYÇA CAN ÇETİN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

FEBRUARY 2012

Approval of the thesis:

EFFECTS OF EDIBLE CHITOSAN COATING ON QUALITY PARAMETERS OF POMEGRANATE (*PUNICA GRANATUM*) ARILS

submitted by **AYÇA CAN ÇETİN** in partial fulfillment of the requirements for the degree of **Master of Science in Food Engineering Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Alev Bayındırlı _____
Head of Department, **Food Engineering**

Prof. Dr. Fatih Yıldız _____
Supervisor, **Food Engineering Dept., METU**

Examining Committee Members:

Prof. Dr. Alev Bayındırlı _____
Food Engineering Dept., METU

Prof. Dr. Fatih Yıldız _____
Food Engineering Dept., METU

Prof. Dr. İsmail Hakkı Boyacı _____
Food Engineering Dept., Hacettepe University

Assoc. Prof. Dr. Behiç Mert _____
Food Engineering Dept., METU

Assist. Prof. Dr. Deniz Çekmecelioğlu _____
Food Engineering Dept., METU

Date: 01.02.2012

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

Name, Last name: Ayça Can Çetin
Signature :

ABSTRACT

EFFECTS OF EDIBLE CHITOSAN COATING ON QUALITY PARAMETERS OF POMEGRANATE (*PUNICA GRANATUM*) ARILS

Can Çetin, Ayça
M.Sc., Department of Food Engineering
Supervisor: Prof. Dr. Fatih Yıldız

February 2012, 97 pages

The effects of edible chitosan coating on quality factors of pomegranate (*Punica granatum*) arils were investigated in the present research. For that purpose, pomegranate arils were treated with 0% (control) and 1% chitosan (extracted from shrimp shells and deacetylated (≥75%)) solutions and stored at 4 ± 0.5 ^{o}C for 22 days. The weight loss, pH, total soluble solids, titratable acidity, total phenolic content, total radical scavenging capacity, total anthocyanin content, total mesophilic aerobic bacteria, total yeast and molds counts and sensorial properties of both chitosan coated and uncoated arils were monitored at an interval of 3 days starting from 1st day up to 22nd day and color parameters were monitored at an interval of 2 days starting from 1st day up to 21st day. Results were statistically analyzed ($p \leq 0.05$). The chitosan coating retarded weight loss, delayed the decline in sensorial quality and total anthocyanin content. Chitosan coated arils had higher contents of soluble solids, titratable acidity and pH than uncoated arils. The chitosan coating effectively delayed the microbial decay. The chitosan coating resulted in higher lightness (L*) and total color difference (ΔE) values and lower hue angle (H*) and chromaticity (C*) values. The chitosan coated arils showed lower radical scavenging capacities.

The results indicated that application of chitosan coating effectively maintained quality parameters of pomegranate arils during 22 days of storage except radical scavenging activities and chromaticity values. According to the study findings chitosan coating process extended the shelf life of refrigerated pomegranate arils from 15 days to 19 days.

Keywords: edible coating, chitosan coating, pomegranate arils, minimally processed fruit, quality

ÖZ

YENİLEBİLİR KİTOSAN KAPLAMANIN NAR (*PUNICA GRANATUM*) TANELERİNİN KALİTE PARAMETRELERİ ÜZERİNDEKİ ETKİLERİ

Can Çetin, Ayça
Yüksek Lisans, Gıda Mühendisligi Bölümü
Tez Yöneticisi: Prof. Dr. Fatih Yıldız

Şubat 2012, 97 sayfa

Çalışmada yenilebilir kitosan kaplamanın nar (*Punica granatum*) tanelerinin kalite faktörleri üzerindeki etkileri incelenmiştir. Bu amaçla, nar taneleri % 0 (kontrol) ve % 1 kitosan (karides kabuklarından üretilmiş, de-asetillenmiş (≥75%)) çözeltileriyle kaplanmış ve 22 gün boyunca 4 ± 0.5 ^{o}C'de muhafaza edilmiştir. Kitosan ile kaplanmış ve kaplanmamış nar tanelerinin ağırlık kaybı, pH, toplam çözünebilir madde miktarı, toplam titre edilebilir asitliği, toplam fenol miktarı, toplam radikal indirgeme kapasitesi, toplam antosiyanin miktarı, toplam mezofilik aerobik bakteri, küf ve maya sayımları ve duyusal özellikleri 1. depolama gününden itibaren 3 günlük aralıklarla 22. depolama gününe kadar ve renk parametreleri 1. depolama gününden itibaren 2 günlük aralıklarla 21. depolama gününe kadar gözlemlenmiştir. Sonuçlar istatistiksel olarak analiz edilmiştir ($p \leq 0.05$). Kitosan kaplama ağırlık kaybını, duyusal kalitedeki ve toplam antosiyanin miktarındaki düşüşü geciktirmiştir. Kitosan ile kaplanmış nar tanelerinin çözünebilir katı miktarı, titre edilebilir asitliği ve pH miktarları kaplanmamış nar tanelerine göre yüksek sonuçlar vermiştir. Kitosan kaplamalar aynı zamanda mikrobiyal bozulmayı geciktirmiştir. Kitosan kaplama işlemi yüksek parlaklık (L*), ve renk farkı (ΔE) ve düşük renk açısı ve türsellik

değerlerine sebep olmuştur. Kitosan ile kaplanmış nar taneleri düşük radikal indirgeme kapasitesi göstermiştir. Sonuçlar yenilebilir kitosan kaplamanın nar tanelerinin radikal indirgeme kapasitesi ve türsellik değeri dışındaki kalite parametrelerini etkili bir şekilde koruduğunu göstermektedir. Çalışma bulgularına göre kitosan ile kaplama buzdolabı sıcaklığında saklanan nar tanelerinin raf ömrünü 15 günden 19 güne artırmıştır.

Anahtar Kelimeler: yenilebilir kaplama, kitosan kaplama, nar tanesi, minimal işlem görmüş meyveler, kalite

Dedicated To My Beloved Parents

and My Dear Husband

ACKNOWLEDGEMENTS

No one walks alone and when one is walking on the journey of life just where you start to thank those that joined you, walked beside you, and helped you along the way.

I would like to express my deep and sincere gratitude to my supervisor, Professor Fatih Yıldız. His wide knowledge and his logical way of thinking have been of great value for me. His understanding, encouraging and personal guidance have provided a good basis for the present thesis.

I owe my loving thanks to my husband Doğu Çetin. Without his encouragement, understanding and love, it would have been impossible for me to finish this work.

I am greatful to my sister, Gonca Can, for her constant love, emotional support and taking care of me in many aspects.

I offer my regards and blessings to my parents Ayşe – Mehmet Can who supported me in any respect through the duration of my studies. I have always felt the privilege of having such a family.

This thesis would not have been possible without the guidance and the help of Tuğçe Şentürk who contributed and extended her valuable assistance in the preparation and completion of this study.

I am indebted to my work colleagues in PharmaCircle for supporting and understanding me during my studies.

Special thanks should be given to laboratory technicians of the department; Fatma Coşkun and Derya Eğritaş.

I would like to express my gratitude to all my student colleagues and friends; Ceyda Akhan, Fuat Gökbel, Armağan Cabadağ, Taner Öztürk, Ferhat Çelik.

Special thanks should be given to Onur Yurtsever for his assistance in formatting of my thesis.

I would like to thank to METU BAP coordination for their financial support during my studies.

Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

ANOVA: Analysis of Variance

AU: Absorbance Unit

$C_2H_3NaO_2$: sodium acetate

CFU: Colony Forming Units

DF: Dilution Factor

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DPPHH: 2,2-diphenyl-1-picrylhydrazine

FC: Folin-Ciocalteau

H_2O_2: hydrogen peroxide

HCl: hydrochloric acid

KCl: potassium chloride

NaOCl: sodium hypochlorite

PCA: Plate Count Agar

PDA: Potato Dextrose Agar

TA: Titratable Acidity

TAC: Total Anthocyanin Content

TMAC: Total Mesophilic Aerobic Count

TPC: Total Phenol Content

TSS: Total Soluble Solids

RSC: Radical Scavenging Capacity

FA: Fatty Acids

MC: methylcellulose

HPMC: hydroxypropylmethylcellulose

HPC: hydroxypropylcellulose

CMC: carboxymethylcellulose

DFE: Dietary Folate Equivalent

RAE: Retinol Activity Equivalents

CHAPTER 1

INTRODUCTION

1.1 Pomegranate Fruit

Pomegranate (*Punica granatum*) which belongs to Punicaceae family is a very special fruit as it contains essential ingredients for human health and also is being an ancient and mystic fruit that bears cultural history of its motherland; Middle East and Caucasia.

Pomegranate cultivation history dates back 3000-7000 years ago (Yılmaz, 2007). Pomegranate was mentioned as the symbol of wealth and cornucopia in Turkish, Roman and Egyptian sources and accepted as sacred in most cultures except ancient Greek Civilization. Pomegranate was taken off to America continent firstly by Spanish missioners in 1521 (Çeltikci, 2008).

Pomegranate can be cultivated in all tropical and subtropical geographies. It is also grown in warm and temperate regions limitedly. Turkey is one of the top pomegranate growing countries as being one of the native lands of the pomegranate. According to the Turkey State Statistical Institute (2002) and BATEM (2011) data, pomegranate is being cultivated in 48 provinces, particularly in provinces in Mediterranean, Aegean and South East regions in Turkey (Figure 1.1).

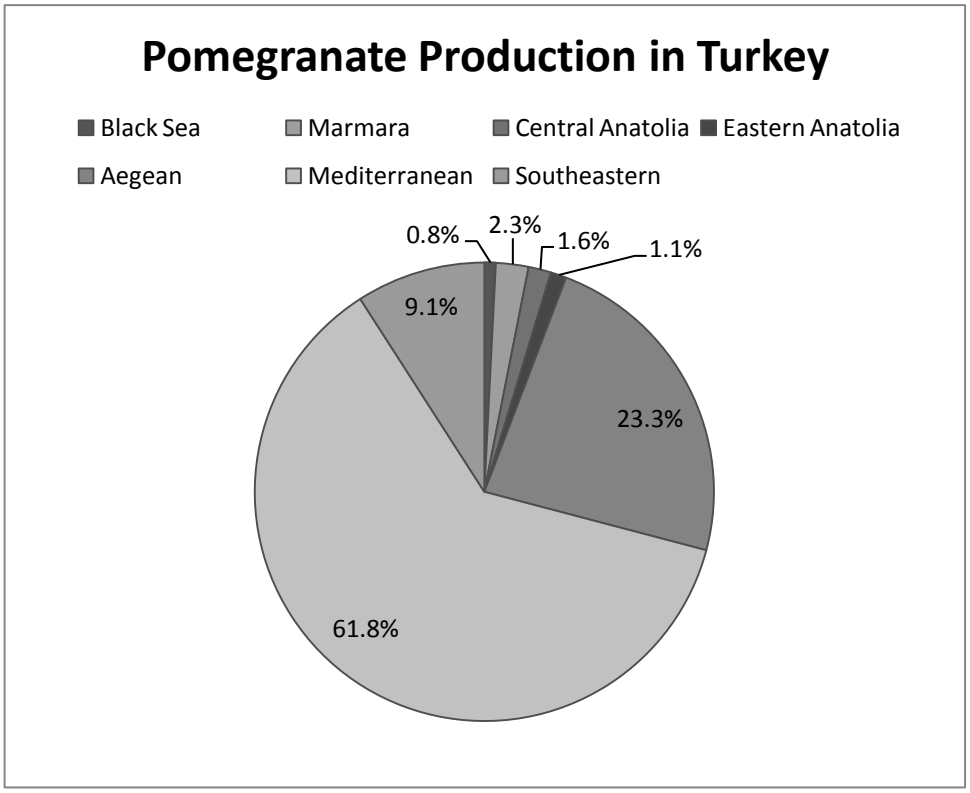

Figure 1.1 Pomegranate production ratios according to the regions in Turkey (Batem, 2011).

The production and consumption of pomegranates has been increasing in recent years in the World, also in Turkey. This increase is mainly related to consumption of pomegranate fruit in different forms such as juice, sauce, canned arils, jams, wine, drugs and cosmetics (Muradoğlu, Balta and Özrenk, 2006). The increase in pomegranate production is also related with consumers' increasing demand for the products that contains functional ingredients that are essential compounds for human health. Pomegranate fruit contains functional ingredients such as antioxidants, polyphenolic compounds, especially anthocyanins. Clinical studies have shown that polyphenols of pomegranate has prophylactic effect on cardiovascular, neurological and cancer diseases (Lansky and Newman, 2007).

Pomegranate fruit has a spherical shape. Inside of the fruit contains arils enclosed within the pericarp. Each pomegranate aril is composed of a seed which is

surrounded with juice containing outer layer. (Wetzstein, Zhang, Ravid and Wetzstein, 2011).

The edible part of the pomegranate fruit is called arils. (Kulkarni and Aradhya, 2005). Arils contain considerable amounts of acids, sugars, vitamins, polysaccharides, polyphenols and important minerals (Vardin and Fenercioğlu, 2003). The arils constitute nearly 60-80 % of the fruit. 76 - 85.5 % of the arils are composed of fruit juice. Table 1.1 shows nutritional value of 87 g of pomegranate arils (California Wonderful variety) (United States Department of Agriculture (USDA), 2011).

Table 1.1 Nutrient data in 100 g of pomegranate arils based on California Wonderful variety (USDA, 2011)

Composition	**Quantity**	**Composition**	**Quantity**
Water	77.97 g	**Riboflavin**	0.053 mg
Energy	83 kcal	**Niacin**	0.293 mg
Protein	1.67 g	**Vitamin B-6**	0.075 mg
Total lipid (fat)	1.17 g	**Folate, DFE**	38 mcg_DFE
Carbohydrate	18.71 g	**Vitamin B-12**	0.00 µg
Fiber, total dietary	4.0 g	**Vitamin A, RAE**	0 mcg_RAE
Sugars, total	13.68 g	**Vitamin A, IU**	0 IU
Phosphorus, P	36 mg	**Vitamin E (alpha-tocopherol)**	0.60 mg
Calcium, Ca	10 mg	**Vitamin D (D2+D3)**	0.0 µg
Iron, Fe	0.30 mg	**Vitamin D**	0 IU
Magnesium, Mg	12 mg	**Vitamin K (phylloquinone)**	16.4 µg
Potassium, K	226 mg	**Fatty acids, total saturated**	0.120 g
Sodium, Na	3 mg	**Fatty acids, total monounsaturated**	0.093 g
Zinc, Zn	0.35 mg	**Fatty acids, total polyunsaturated**	0.079 g
Vitamin C	10.2 mg	**Cholesterol**	0 mg
Thiamin	0.067 mg	**Caffeine**	0 mg

1.2 Edible Films and Coatings Applications on Minimally Processed Fresh Fruits

1.2.1 Minimal Processing of Food

Increasing awareness of consumers to have handy but fresh foods forced food technologists to supply food products that have less preservatives, fresh sensory properties and increased nutritional values. Although traditional food processing technologies has accomplished preserving food products, they have some adverse effects on sensory attributes and nutritional characteristics of food produce. Minimal food processing technologies began to take their place as they successfully dealt with this gap of traditional methods by conserving sensorial and nutritional attributes while preserving the food products. Huis in't Veld (1996) defined minimal processing as giving the food enough shelf life while minimally affecting the quality attributes of a produce.

The main aim of minimal processing of food is to lessen the thermal treatments that decrease the quality because of long period high temperature processes. In order to reduce loss of quality and increase the shelf life period of food, food scientists driven to study on innovative processes to equilibrate preservation and quality while extending the shelf life of the produce.

1.2.1.1 Minimal Processing of Refrigerated Fruits and Vegetables

The minimally processed refrigerated (MPR) fruits and vegetables were defined by Wiley (1994) as fruits and vegetables which are prepared by proper unit operations like peeling, shredding, slicing and juicing that does not give end point preservation treatment consisting of minimal heat, radiation or a preservative. The preservation methods may contain antioxidants, chlorinated water treatments, pH control or combination of them (Yıldız, 1994).

In the case of fruits and vegetables, it is hard to keep the quality attributes for long times as they experience some chemical and physical changes because of biochemical processes of living tissue (Kays and Paull 2004). It is hard to maintain quality of ready-to-eat, minimally processed fruits and vegetables. In the past, fresh cut and minimally processed fruits and vegetables was served only in restaurants where those fruits and vegetables were sold to customers immediately, thus, no extra effort was required to keep the quality of produce for long time periods. However nowadays, ready-to-eat, ready-to-serve and ready-to-cook fruits and vegetables have taken their places in markets in order to meet the consumer demand. Besides the consumer demand on minimally processed fruits and vegetables, consumers desire also those products to have same flavor, texture, nutrition value and as long shelf life as the original produce. It is hard to fulfill those expectations as minimally processed fruits and vegetables rapidly deteriorate, ripening is accelerated as the sugar, lipid and organic acids consumption is increased. All mentioned changes result in unwanted color and flavor changes, as well water and texture loss (Kays, 1991).

The use of edible coatings and films has been studied in order to maintain quality of fruits and vegetables for longer times as they form semi-permeable barriers to water vapor and other gases and protect those produce from harmful effects of the environment (Embuscado and Huber, 2009).

1.2.1.1.1 Edible Coatings and Films Applications for Fruits and Vegetables

There have been comprehensive studies on edible coatings and films for the last 20 years. Most of the published studies were on use of edible coatings and films on minimally processed fruits and vegetables (Olivas and Barbosa-Canovas, 2009). Although the use of edible coatings and films on fruits and vegetables have been popular for the last 20 years, the first use of them was in China where wax was used on lemon and orange fruits to preserve them in the 12th century (Hardenburg, 1967). In United States, the first filed and accepted patent on edible coatings was given to Hoffman, which used molten wax on whole fruits in order to protect them (US patent

No. 19,160,104). The main materials used as edible film and coating of fruits and vegetables are given in Figure 1.2.

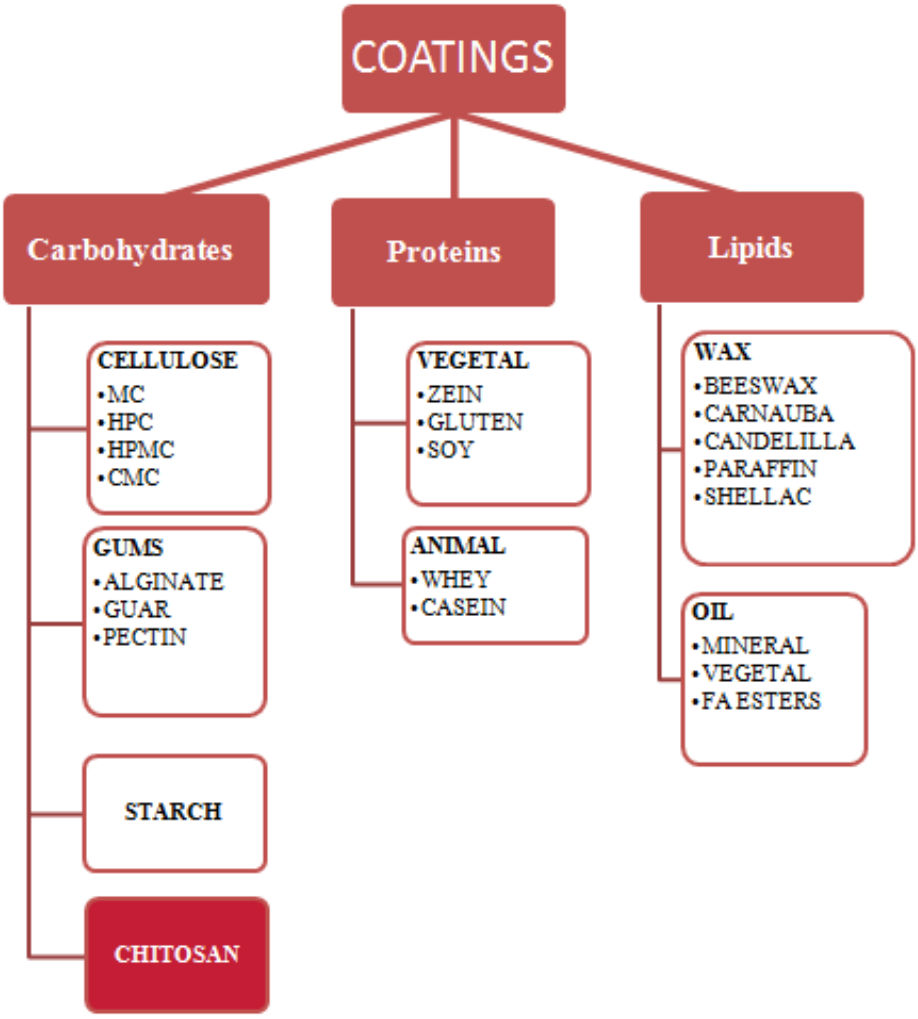

Figure 1.2 Classification of main edible coating and film materials used on fruits and vegetables

Gennadios and Weller (1990) defined edible films and coatings as “thin layers of edible material applied on foods, or between food components, by wrapping,

brushing, immersing or spraying in order to control mass transfer, provide mechanical protection or add sensory appeal to food products". Edible films are stand alone materials that are formed as separate thin layers. Edible films are generally composed of polymers. In order to set mechanical, barrier and other features of a film material, those stand alone films are used for testing purposes. If the films are used on the surface of a food material so as to protect it or develop its quality the films are called as coatings. Concordantly, coatings can be thought as a part of the food and they stay on the food during consumption (Krochta, Baldwin and Nisperos-Carriedo, 1994).

As coatings can be considered as a food component and a packaging material, they should meet with following requirements:

- environment friendliness
- easiness of application
- reasonable costs of materials and process
- acceptable sensorial attributes
- good barrier and mechanical properties
- safety and non-toxicity

1.2.1.1.2 Chitosan and Its Use As an Edible Coating

Chitosan is a natural polycationic polymer which is a derivative of chitin and is obtained by alkaline deacetylation of chitin (a N-acetylglucosamine polymer). Chitin is the main component of crustaceans such as crab and shrimps and it is also found in the wings of the butterflies, the exoskeletons of mollusks and cell wall of fungi (Rabea, Badawy, Stevens, Smagghe and Steurbaut, 2003; Koç and Özkan, 2011).

Chitin is a polysaccharide which is formed as a result of bonding of 2-acetamido-2-deoxy-β-D-glucose monomers with β(1-4) bonds and it is also called as poly β (1-4)-2-acetamido-2-deoxy-D-glucose. Chitosan is a modified natural biopolymer which possesses functional reactive amino groups. Chitosan which also named as (1-4)-2-

amino-2-deoxy-D-glucose (glucosamine) has 3 reactive functional groups which are primary and secondary hydroxyl group of C-3 and C-6 positions and amino group of C-2 position.

Following cellulose; chitin and chitosan are the most plentiful natural polymers found in nature (Aranaz, Mengibar, Harris, Panos, Miralles, Acosta, Galed and Heras, (2009) and their structure is very similar to that of cellulose. The structural difference of cellulose, chitin and chitosan is the bonding of acetamide group (–NHCOCH3) in chitin and amine group (–NH2) in chitosan instead of hydroxyl (–OH) group's bonding in second carbon of cellulose (Figure 1.3) (Koç et al., 2011; Muşabak, 2008).

In its free amine form, chitosan in insoluble in water but it is soluble in weak organic acids like acetic acid, lactic acid, formic acid, malic acid and succinic acid (Olivas et al., 2009; Rabea et al., 2003). As chitosan has a great number of amino groups through its chain it can form several complexes. In acidic mediums, $-NH_3$ groups are formed which bind on anionic sites of cell wall surface of bacteria and fungi, they can also form complexes with heavy metals at high pH's. Those characteristics of chitosan results in its extensively use as a wound healer, food preservative, artificial skin and in cosmetics and waste water treatment (Risbud, Hardikar and Bhonde, 2000; Juang and Shao 2002).

Chitosan has been widely used as an edible coating and film material to increase the shelf life and maintain quality attributes of fruits and vegetables. Chitosan can decrease respiration rates, inhibit microbial growth and delay ripening. It is thought as an ideal coating material for preservation of fruits and vegetables as its ability to form a film on their surface and to control microbial growth as the positively charged chitosan's binding on negatively charged microbial surface leading agglutination (Olivas et al., 2009). It has been reported that chitosan has indicated antimicrobial activity against *Botrytis cinerea, Bacillus cereus, Lactobacillus curvatus, Lactobacillus sakery, Listeria monocytogenes, Candida lambica, Photobacterium phosphoreum, Pseudomona fluorescens and Brochothrix thermosphacta* (Devlieghere, 2004).

Various concentration ranges of chitosan coatings were studied on various fruits and vegetables. Nongtaodum and Jangchud (2009) mentioned in their studies that increasing chitosan concentrations from 0.5 to 0.8% did not have effect the pH, microbial growth, weight loss, total soluble solid content, color and titratable acidity of mango slices. Dang, Yan, Li, Cheng, Liu and Chen, (2010) reported that 0.3, 0.5 and 1% chitosan concentrations effectively retarded water loss, titratable acidity and ascorbic acid content of sweet cherries. El-Ghaouth, Arul, Ponnampalam and Boulet (1991b) mentioned that increasing chitosan concentration from 1.0 to 1.5% did not significantly changed the effect of coating on the decay control, firmness, titratable acidity of strawberries. Jiang and Li (2001) reported in their study that increasing chitosan concentrations were effective for longer storage days. Du, Gemma and Iwahori (1997) mentioned in their study that increasing chitosan coating concentration from 1.0 to 2.0% did not significantly change firmness of peaches where 2.0% chitosan coated peaches had higher soluble solids content and lower carbon dioxide evolution. Chien, Sheu and Yang (2007) mentioned in their study that coating with 0.5, 1 and 2% chitosan did not significantly changed the sensorial quality, color, titratable acidity, ascorbic acid content, total soluble solids content and microbial growth of sliced mango where higher chitosan concentrations lead to lower water loss.

De Reuck, Sivakumar and Korsten (2009) stated that it is beneficial to use lower chitosan concentrations as it reduces production costs and also lower concentrations of chitosan has lower viscosity and it is easy to apply on the food commodity. According to the literature findings in above paragraph and also easiness of application 1% (w/v) chitosan solution was used in the present study.

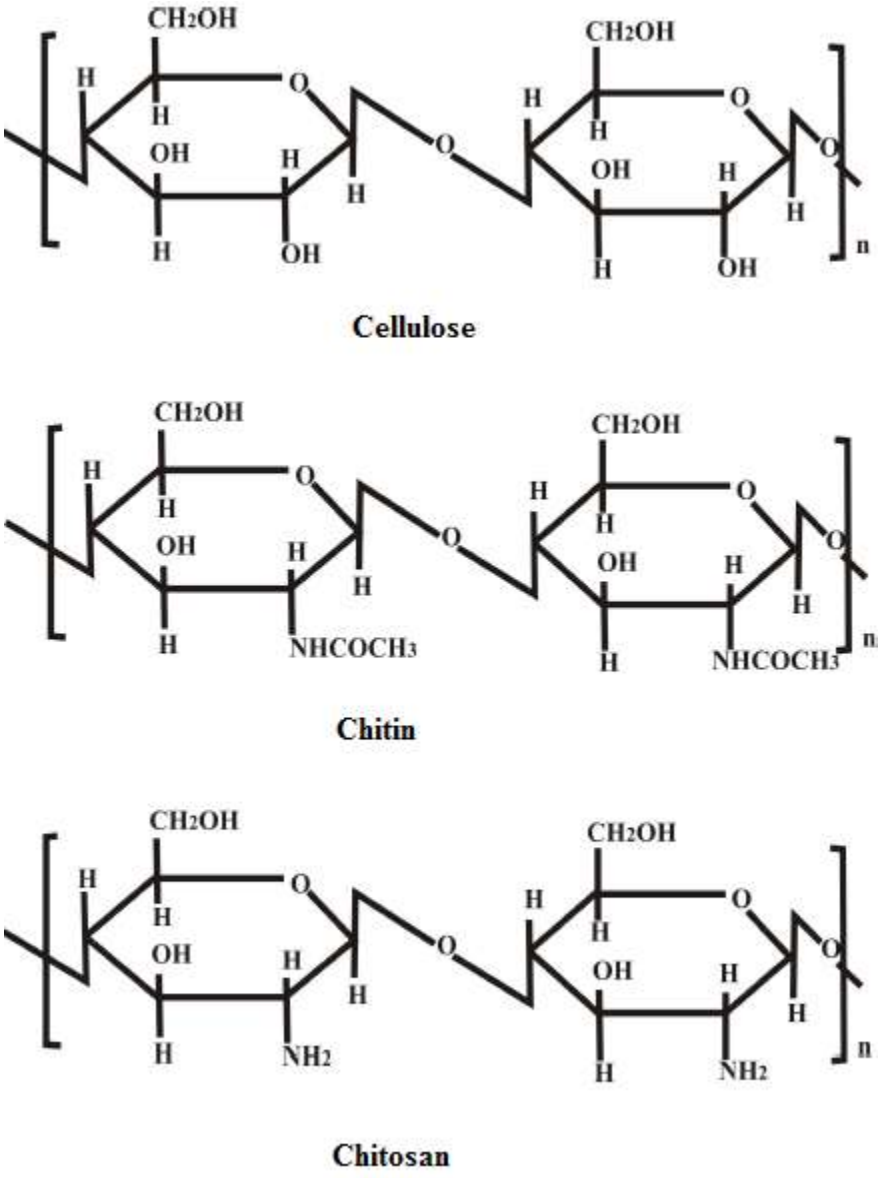

Figure 1.3 The chemical structure of cellulose, chitin and chitosan (Muşabak, 2008)

Chitosan has been approved by Food and Drug Administration (FDA) of USA for certain food applications like edible film to preserve foods. It has been approved as a food additive in Japan and Korea. *Primex*, a Norwegian company that produce chitosan has GRAS approval from FDA for chitosan (Olivas et al., 2009).

1.3 Quality Indices and Criteria of Pomegranate Fruit

In recent years, there has been an increasing trend on determining quality characteristics of red fruits and their juices, such as berry, red grape and pomegranate. This increase is directly related with increasing consumer demand for fruits that possess higher antioxidant contents as recent epidemiological studies show that consumption of red fruits reduced risk to experience cardiovascular diseases such as stroke, and also certain cancer types and aging (Gil, Barberan, Pierce, Holcroft and Kader, 2000; Malik and Mukhtar, 2006). Pomegranate is one of the most popular fruits, being an important source of phytonutritional compounds (Adams, Seeram, Aggarwal, Takada, Sand and Heber, 2006).

Pomegranate fruit contains antioxidants which are chemical compounds that are capable of affecting the generation of free radicals during free radical reactions (Bendich, Philips and Tengerdy, 1998). A free radical is an unstable and reactive molecule that has unpaired electron(s) in the outer electron orbit (Gilbert, 2000). As those unstable molecules need to stabilize themselves, they receive electrons from stable compounds and transform those stable compounds into new free radicals. A free radical triggers a chain reaction which will continue until unpaired electron of the free radical will pair up with another unpaired electron of any other free radical or the free radical will deactivate itself by antioxidants breaking the chains (Dormandy, 1983; Akay, 2001).

The method of free radical scavenging capacity (RSC) determination of pomegranate or any other fruit is based on spectrophotometrically monitoring disappearance of a free radical; 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) (Figure 1.4).

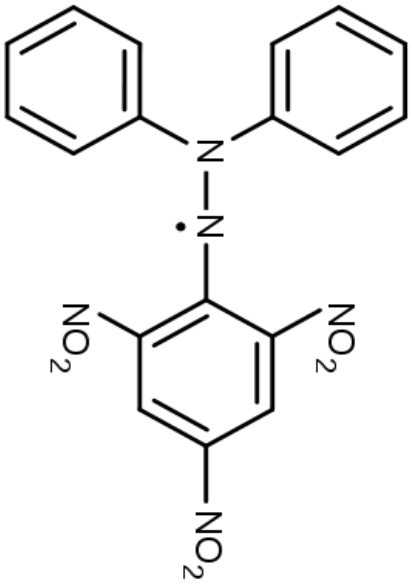

Figure 1.4 Chemical structure of DPPH radical

When mixed with the sample having antioxidant activity, the DPPH$^{\bullet}$ radical solution which has an intense purple color gives a hydrogen atom to the medium and becomes non radical DPPH form. During this change, the intense purple color disappears and yellow colored DPPHH is formed as a result of reduction (Figure 1.5). The purple colored DPPH radical exhibits a strong absorption in 515-517 nm wavelengths and the change can easily monitored in UV-Visible Spectrophotometers (Cemeroğlu, 2010).

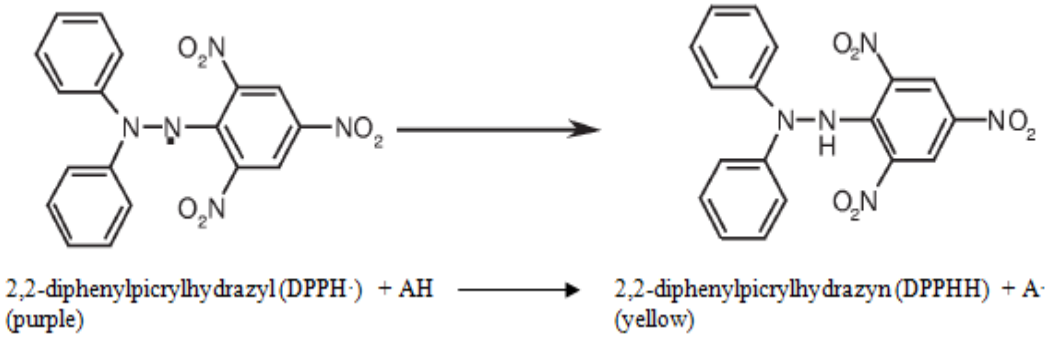

Figure 1.5 Reduction of DPPH radical

As playing a crucial role in the color quality of all fruits, vegetables and plants, anthocyanins are one of the most important quality indices of pomegranate fruit. Anthocyanins are water soluble pigments that give specific pink, red, violet, blue and purple colors to fruits, vegetables and plants (Cemeroğlu, 2010; Varasteh, Arzani, Barzegar and Zamani, 2011).

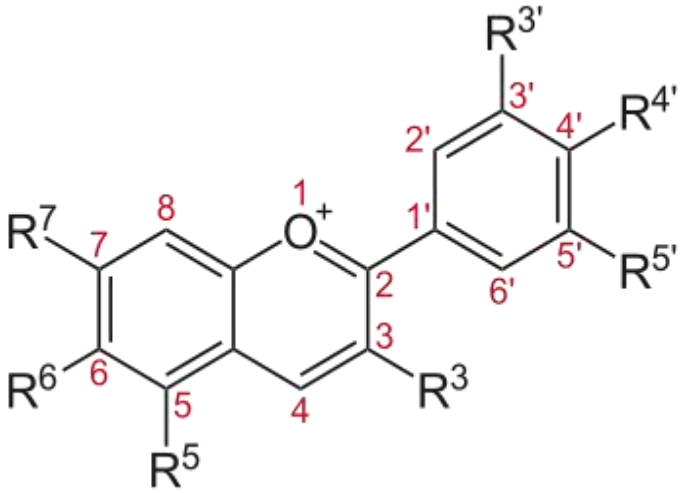

Figure 1.6 The basic structure of anthocyanins

Anthocyanin pigments are categorized as flavonoids and are polyhydroxy and polymethoxy glycoside derivatives of the 3,5,7,3-tetrahyrdoxyflavylium (Figure 1.6). As being unstable compounds their availability is highly affected by pH, temperature, light and presence of metals. Antocyanins are considered as a replacement colorants to the synthetic pigments used as additives in food products since they are natural and possess appealing and glittering colors. In addition to their colorant properties, anthocyanins demonstrated important pharmacological and biochemical activities which have been resulted in ability to scavenge of free radicals that were oxygen generated and to inhibit the lipid peroxidation, thus exhibit anti-inflammatory, anti-oxidative and chemoprotective characteristics (Da Costa, Horton and Margolis, 2000; Falcao, Chaves, Falcao, Gauche, Barreto, Bordignon-Luiz, 2008).

According to the study findings of Martí, Pérez-Vicente, and García-Viguera (2000) and Miguel, Dundlen, Antunes, Neves, and Martins (2004), delphinidin 3,5-diglucoside, delphinidin 3-glucoside, cyanidin 3,5-diglucoside, cyaniding 3-glycoside, pelargonidin 3,5-diglycoside and pelargonidin 3-glycoside are the main anthocyanins identified in pomegranate arils. As anthocyanins found in pomegranate are unstable molecules, they easily undergo some degradation reactions in course of the processing or storage (Alighourchi, Barzegar and Abbasi, 2008). Monitoring the anthocyanin content of pomegranate is important for optimizing postharvest applications and defining optimum storage conditions (Varasteh et al., 2012).

Monomeric anthocyanins behave like indicators and changing their colors in different pH medium. For instance, at pH values of 1.0, anthocyanins turn into colored oxonium form while at pH values of 4.5; uncolored carbinol pseudobase form (hemiketal form) predominates. At pH values higher than 5.0, anthocyanins convert to blue colored quinoidal anhydrous base form. The method for measuring total monomeric anthocyanin content of the fruits and vegetables is based on monitoring the mentioned color changes spectrophotometrically by relating pigment concentration with the absorbance (Cemeroğlu, 2010; AOAC 2005).

Pomegranate is a non climacteric fruit that should not be picked up from the tree before it is fully ripe (Kader, 2006). Optimum harvest time is a very critical parameter in order to provide to the customers the best eating quality. Early harvesting of pomegranates may prevent fruit to develop its characteristic aroma, color and taste whereas late harvesting may cause early decay (Kulkarni et al., 2005).

Color is another very important quality characteristic for acceptability of food products. In order to define the quality of a food product, color has a certain impact on consumers (Blendford, 1995). Color of the skin and arils is one of the quality indices of pomegranate fruits.

Sensory evaluation is an essential parameter to assess the quality of a food product. The quality of a product is determined by consumer acceptance, therefore marketability of the product. In the past, quality assessment relied only on sensory evaluation. Although development of new technologies enabled quantitative analyses on physical, chemical and microbiological parameters, it is a fact that without sensory evaluation, quantitative results are not sufficient for interpretation of acceptability. In summary, to define and control product quality, sensory evaluation cannot be overlooked (Dzung and Dzuan, n.d.).

The quality of pomegranate fruit substantially depends on the acidity and soluble solids content of its juice (Pekmezci and Erkan, n.d.). During the fruit maturation and ripening, soluble solids content of the pomegranate increases while the titratable acidity decreases. Each pomegranate cultivar has its own brix:acid ratio at harvest time. The pomegranates were classified according to soluble solid content to titratable acidity ratio in their study, the ratio between 5-7 was categorized as sour, 17-24 as sweet-sour and 31-98 was categorized as sweet varieties (Martinez, Melgarejo, Hernandez, Salazar and Martinez, 2006). Gölükcü and Tokgöz (2008) have found in their study on determining quality parameters of the important pomegranate cultivars in Turkey that the total soluble solid content of different cultivars varied from 13.00 to 17.18 ^{o}brix and titratable acidity ranges between 0.20-2.81%, whereas Kupper (1995) suggested that the total soluble solid content and

titratable acidity of pomegranate varies ranges between 8.3 to 20.5 ^{o}brix and 0.13 to 4.98 %, respectively. For acceptable flavor quality of pomegranate, minimum soluble solid content was proposed as 17% and maximum titratable acidity was proposed as 1.85 % for 'Wonderful' pomegranate variety than 1.85 percent was recommended (Ben-Arie, Segal, Guelfat-Reich, 1984). Kader (1999) has proposed that the maximum titratable acidity as 1.4 % for acceptable flavor quality of pomegranates.

Microbial quality is a crucial parameter for defining quality of pomegranate arils. Within early development of pomegranate, *Aspergillus* spp. or *Alternaria* spp. infection starts especially as a result of rain during fruit flowering and early development. The fungi result in softening of the fruit surface. The most critical postharvest decay occurs in pomegranate is gray mold which is caused by *Botrytis cinerea.* The infection result in brown and learhery skin which is followed by gray mycelial growth appearance (Kader,2006).

1.4 Objective of the Study

Pomegranate (*Punica granatum*) is a valuable fruit mainly due to its nutritional and unique sensorial properties. Although its beneficial effects on human body are well known, as it is difficult and time consuming to extract arils, its consumption is restricted. On the other side, the outer skin of pomegranate fruit is susceptible to sunburn, chilling and cracking and thus may be not attractive for fresh marketing despite their perfect internal quality. With the help of commercial aril extracting technologies that are available nowadays, demand on ready-to-eat pomegranate arils is increasing. Ready-to-eat arils have been started to take their places in supermarkets shelves, but another important point is that to maintain the fresh-like quality parameters of extracted arils as it is a major concern in minimally processed fruits. Ready-to-eat arils' coating with chitosan which is a widely used edible coating material, with its excellent antimicrobial and barrier properties, can be a solution in order to maintain the fresh like properties of ready-to-eat arils which were extracted from their natural environments.

The objective of the present study is monitoring the effects of 1% chitosan coating on some physical, chemical and microbiological and sensorial quality parameters of ready-to-eat pomegranate arils during 22 days of refrigerated storage ($4 \pm 0.5^{o}C$) in comparison with untreated arils.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Pomegranate Fruit

Pomegranate fruits (*Punica granatum*) used in experiments were obtained from the Ankara city Wholesale Market for Fresh Fruits and Vegetables in late February. This latest harvest time was preferred in order to get fully developed fruits in terms of color, taste and aroma. The exact variety of the purchased pomegranates was not known but they were sweet-sour type. Pomegranate fruits were stored at refrigerator ($4 \pm 0.5\ ^{o}C$) in the laboratory up to the time of experiments. The experiments were started to being performed 20 days after the time that they were brought to the laboratory.

2.1.2 Reagents

Chitosan (Shrimp Shell Chitosan, ≥75% (deacetylated)) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Company (St Louis, Missouri). All other chemical reagents mentioned were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical grade.

2.2 Methods

2.2.1 Washing Treatments

After discarding damaged fruits, remaining pomegranates were firstly washed with tap water and left to dry. Dried fruits were washed in chlorinated water (200 $\mu L L^{-1}$ NaOCl) (Ayhan and Esturk, 2009). Washed pomegranate fruits were carefully peeled and arils were manually extracted. Damaged arils were discarded during extraction. Extracted arils were collected in a container which was previously sanitized with hydrogen peroxide solution (3% H_2O_2) and mixed to provide uniform sample sets. Arils were dipped into chlorine solution (100 $\mu L L^{-1}$ NaOCl) for 5 min as recommended in the study of Church and Parsons (1995). Arils were drained and air-dried on a screen for 1 hour at room temperature until all arils were dried. Washing treatments mentioned above were performed both for control and coated sample groups.

2.2.2 Preparation of Chitosan Coating Solutions

In literature various different chitosan coating preparation methods were suggested. In this study the method of Park, Daeschel and Zhao (2004) was preferred due to the easiness of implementation to laboratory scale and the similarity of type of the chitosan used in experiments. In order to prepare 1 L of 1.0 % chitosan solution, 10 g of chitosan powder (shrimp shell chitosan, deacetylated (≥75%)) was dispersed in acetic acid solution (10 ml glacial acetic acid (99.85%) / 1 L distilled water) with addition of 25% food grade glycerol (w/w chitosan) (Park, et al., 2004). The film-forming chitosan solution was homogenized for 1 hour by using T-10 basic, IKA Ultra-Turrax Homogenizer (Staufen, Germany). The solution was filtered by using cheese cloth which had a mesh size of approximately 1 mm^2.

2.2.3 Coating Application

Pomegranate arils were divided into two batches. Half of arils were dipped in 1.0 % chitosan solutions for one minute. After dipping, arils were air-dried for 1 hour in order to form chitosan films on the surface of arils. The other half was dipped in acetic acid-glycerol solution without chitosan for one minute, as a control and dried in same conditions with chitosan coated arils. Dried arils were put into PP containers (100 g arils per container) that were previously sanitized with 3 % hydrogen peroxide solution (H_2O_2). Capped containers containing arils were stored at 4 ± 0.5 ^oC in a refrigerator for 22 days. The arils were reported to have tolerance to chilling rather than whole fruit so arils can be kept at 0 to 5^oC temperature range in order to maintain their chemical, sensorial and microbiological quality (Kader, 2006). The refrigerated (4 ± 0.5 ^oC) storage was preferred in the present study from the given range as it is easy to store arils in refrigerator in case of commercial usage. On storage days of 1, 4, 7, 10, 13, 16, 19, 22 sampling was done for both chitosan coated and control samples. Three replicates were performed for each analysis except sensorial and color evaluation for both control and chitosan coated sample group in each day of analysis. Sensorial evaluation was done in 5 replicates where 6 replicates were used for color measurements.

For chemical and physical analysis arils were hand pressed and filtered by using cheesecloth. In color, sensorial, microbial analysis whole arils were used.

2.2.4 Physical Analysis

2.2.4.1 Determination of pH

The pH of the samples was measured by pH meter Hanna 221 (Michigan, USA) at 20 ^oC. The pH measurements were performed at room temperature. The pH meter was calibrated before starting each measurement with pH 4.0 and pH 7.0 buffer solutions in order to achieve clear cut results.

2.2.4.2 Weight Loss

Weights of 3 containers containing arils were recorded at the initial day of experiments for both control and coated samples by Denver TP-153 precision digital balance (Goettingen, Germany) with an accuracy of 0.0001 g. Those weight measurements were performed every 3 days of storage. Weight loss for each sampling days were expressed as percentage loss of initial total weight.

2.2.4.3 Color Measurement Study with Prototype Color Measurement System

At the beginning of the study, the hand-held and bench top chromameters (Minolta Camera, Co, Japan) were used in order to determine CIELAB based color parameters. However the results were not consistent since the arils' size is smaller than the reading opening of the chromameters. As color is a very important parameter, a prototype image acquisition system was designed and the measured values were used in order to compare the color of both coated and uncoated arils. During color measurement system design, several studies were being investigated: Leon, Mery, Pedreschi and Leon (2006); Somatilake and Chalmers (2007), Kılıç, Ulusoy, Yıldırım, Boyacı (2006).

The designed image acquisition system is shown in Figure A.1 which is located in Appendix A.

The image acquisition system is composed of following parts:

- Canon EOS 350D digital camera with resolution of 8 megapixel, focal length(f) of 55 mm, shutter speed of 125 and aperture of 5.6. It was vertically placed at the top of the system. The axis of the lens and illumination sources were placed parallelly (Figure A.2). The distance between the lens of the camera and the sample was 35 cm.
- Illumination was designed by 3 Osram 11 W fluorescent lamps with 90 mm length, color temperature of 2700 K and color index of 89% (Ra).

- The illumination sources and the objective of the camera was placed top of a black plastic box, black was selected to minimize background light (Figure A.2).
- The images were taken at maximum resolution of 3456 x 2304 megapixels and transferred to Intel Core I5, 2.4 GHz PC.

R (red), G (green), B (blue) color values of the acquired pixels of the taken images were converted into L* (luminance or lightness) a* (green to red) b* (blue to yellow). This conversion is done by using Matlab R2009b (Mathworks, USA). The Matlab code given in Appendix B.1 was used for converting images from RGB color space to CIELAB color space. The white point was fixed to D65 standard (0.9642, 1, 0.8249) for CIELAB (Ruzon, 2009).

For both coated and uncoated arils, starting from the 1st day of the storage to the 21st day of the storage, 4 images were taken at an interval of 2 days. The taken images were uploaded into the computer in order to be processed on Matlab. The given Matlab code is enhanced for the current study (Appendix B.2). After the image was opened in Matlab, the program allowed selecting 3 areas (Appendix C.5) within the image and has given the LAB values for each pixel within the selected areas (Appendix C.3). For each day of measurement, 6 parallels were used in order to calculate the color parameters of both uncoated and coated arils. The results were given as L* for lightness, a* and b* which were converted to chroma ($C= (a^{*2} + b^{*2})^{1/2}$) and hue angle ($h= \arctan(b^*/a^*)$ and color difference ($\Delta E= (L^{*2} + a^{*2} + b^{*2})^{1/2}$) (Hunter, 1975; Artes, Tudela and Gil, 1998). Hue angle gives data about the color of the samples where 0^o as red-purple, 90^o as yellow, 180^o as blue-green and 270^o as blue. Chroma values show the saturation and or intensity of the color of the samples. Higher chroma values are an indication of more vivid color where lower values indicate dull colors (Ball, 1997).

It should be mentioned that in the current study, as the calibration of the given Matlab code into the current designed system was not done, the achieved L*, a* and b* values may not give the exact color values of the pomegranate arils. The designed

system is used for observing and comparing the color values of the coated and uncoated arils and also the effects of the storage time.

2.2.5 Chemical Analysis

2.2.5.1 Titratable Acidity

5 ml of extracted aril juice was diluted with 100 ml distilled water and the solution was titrated with 0.1 N NaOH to an endpoint of pH 8.1. Results were expressed as citric acid percentage.

2.2.5.2 Total Soluble Solids Content

Total soluble solids content (°Brix) of samples were measured by using Atago hand held refractometer (London, England).

2.2.5.3 Total Phenols

The amount of total phenols was determined by Folin-Ciocalteau Method (FC Method) (Singleton and Rossi, 1965). The principle of FC method is based on redox reactions in which phenolic compounds reduced FC reagent and convert themselves into oxidized form. FC reagent takes part as an oxidizing agent. By measuring the blue color of reduced FC reagent photometrically, the total phenolic compound amount calculation is made possible.

2.2.5.3.1 Determination of Total Phenol Content

20 µL of diluted sample (DF: 5) was put into 2 ml plastic cuvette and 1.58 mL water and 100 µL Folin-Ciocalteau reagent were added, respectively. The mixture was mixed thoroughly and incubated for 6 min. After incubation, 300 µL saturated

sodium carbonate solution was added. Then the cuvette content was mixed again and incubated for 2 hour at room temperature. At the end of incubation the absorbance of samples was measured at 765 nm by using UV-Visible Spectrophotometer (Analytic Jena SPECORD 50, Germany) (Singleton et al, 2002).

Distilled water was used as blank and gallic acid was used as a standard. The total phenolic compound amount of samples was expressed as milligram gallic acid equivalent in 1 L sample by using the equation obtained from the gallic acid standard graph which is mentioned in below.

2.2.5.3.2 Preparation of Gallic Acid Calibration Standard Solutions and Curve

0.5 g gallic acid was dissolved in 10 ml ethanol and diluted to 100 ml with distilled water to 5 g/liter final concentration. In order to have standards with 50, 100, 250 and 500 mg/liter gallic acid concentrations, 1, 2, 5 and 10 ml of gallic acid solutions were further diluted to 100 ml with water, respectively.

The procedure applied to samples was performed for each gallic acid concentrations. Generated standard curve was given in below figure (Figure 2.1).

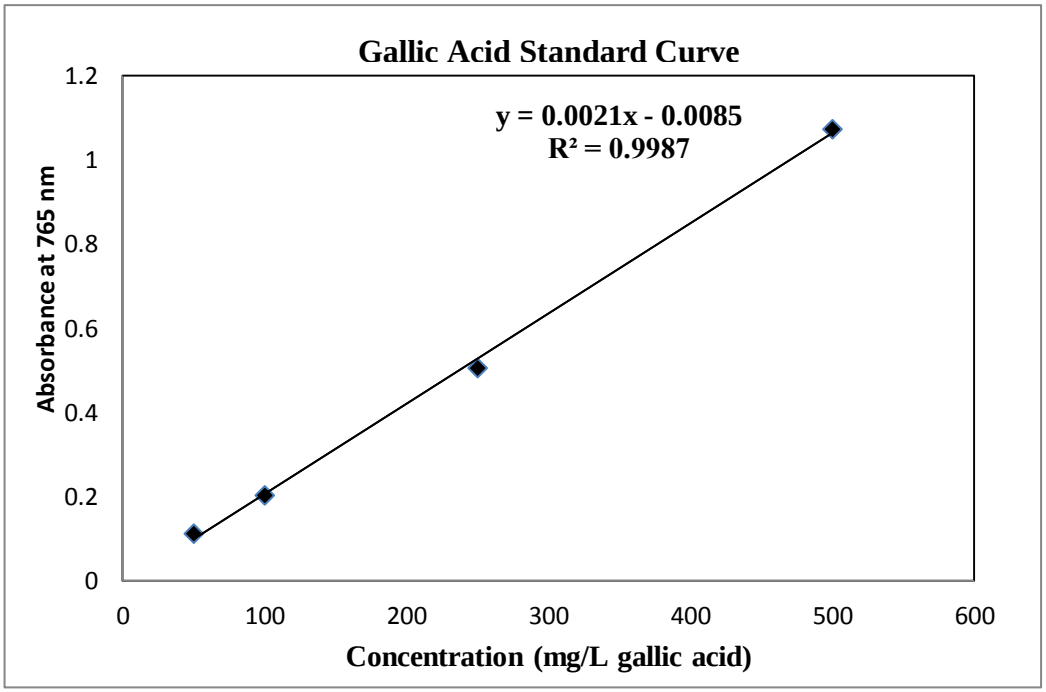

Figure 2.1 The standard curve for Folin-Ciocalteau method

The equation of gallic acid standard curve is;

$$Absorbance_{765nm} = 0.0021 \times [mg\ gallic\ acid/\ L] + 0.0085 \text{ where } R^2 = 0.9985$$

2.2.5.3.3 Preparation of Sodium Carbonate Solution

100 ml distilled water was added into 35 g of anhydrous sodium carbonate and mixed until all of sodium carbonate was dissolved. The solution was stored at room temperature for one day. A small crystal of sodium carbonate was added into the solution after 1 day, in order to trigger crystallization. Following the end of crystallization, the saturated sodium carbonate solution was filtered using glass wool. Glass wool was preferred for filtering as it is common for chemicals for being pretty much inert filtering material.

2.2.5.4 Total Anthocyanins

Total monomeric anthocyanin content of samples was determined by the pH differential method (Lee, Durst and Wrolstad, 2005).

2.2.5.4.1 Dilution Factor Determination

Dilution factor was determined by diluting the sample with pH 1.0 buffer, until read absorbance of the sample in UV-Spectrophotometer is between 0.2 and 1.4 AU, at 520 nm. 0.2 and 1.4. Determination of dilution factor is critical in order to have absorbance values within the linear range of the spectrophotometer. According to this technique, the dilution factor was determined as 40.

2.2.5.4.2 Preparation of Buffer Solutions

Preperation of pH 1.0 Buffer (potassium chloride, 0.025M) Solution

1.86 g potassium chloride (KCl) was weighed and 980 mL distilled water was added. pH of the solution was monitored and hydrochloric acid(HCl) was added in order to adjust the pH of the solution to 1.0. After 1.0 pH value was achieved, the solution was transferred into volumetric flask of 1 L and diluted to 1 L with distilled water.

Preperation of pH 4.5 Buffer (sodium acetate, 0.4M) Solution

54.43 g sodium acetate ($C_2H_3NaO_2$) was weighed and 960 mL distilled water was added. pH of the solution was monitored and hydrochloric acid (HCl) was added in order to adjust the pH of the solution to 4.5. After 4.5 pH value was achieved, the solution was transferred into volumetric flask of 1 L and diluted to 1 L with distilled water.

2.2.5.4.3 Determination of Total Anthocyanin Content of Arils

Extracted aril juice was clarified by centrifuging for 15 min at 12000 rpm. Potassium chloride pH 1.0 buffer (0.025M) and sodium acetate pH 4.5 buffer (0.4M) were used as buffer solutions. 0.1 ml sample was diluted with 3.9 ml pH 1.0 and pH 4.5 buffer solutions in different cuvettes and after 20 min absorbance was measured at 520 nm and 700 nm. Although the samples were diluted with buffer solutions, it was observed that it is unnecessary to use buffer solutions as a blank since there is no difference in absorbance readings of distilled water and buffer solutions (Cemeroğlu, B., 2010). This assumption in the literature was verified in the current study too. According to the given information in literature and verification of this assumption, distilled water was read as blank versus diluted samples in both wavelengths.

Total monomeric anthocyanin content which was expressed as cyanidin-3-glucoside equivalents, was calculated as:

$$\text{Total Monomeric Anthocyanins (mg/L)} = \frac{A \times MW \times DF \times 10^3}{(\varepsilon) \times \ell}$$

Where;

$A = (A_{520} - A_{700})_{\text{pH 1.0}} - (A_{520} - A_{700})_{\text{pH 4.5}}$

MW: Molecular weight of cyanidin-3-glucoside which is 449.2 g/mol

DF: Dilution factor, which was established as 40

ℓ : pathlength of cuvette in cm, which is 1 cm

ε : molar extinction coefficient, which is 26 900 L $mol^{-1}cm^{-1}$ for cyanidin-3-glucoside

10^3: conversion factor from g to mg

2.2.5.5 Total Radical Scavenging Activity

2.2.5.5.1 Preparation of DPPH Standard Solutions and Curve

2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was dissolved in methanol to the 15, 30, 60 and 90 µM concentrations. The absorbance of each 15, 30, 60 and 90 µM DPPH at 515 nm was measured by using UV-Visible Spectrophotometer (Analytic Jena SPECORD 50, Germany). In order to determine the equation that relates absorbance and DPPH concentration, each day before starting to the antioxidant scavenging activity measurements, absorbance versus concentration curves were generated. One of the generated DPPH standard curves was shown in Figure 2.2 as a sample graph.

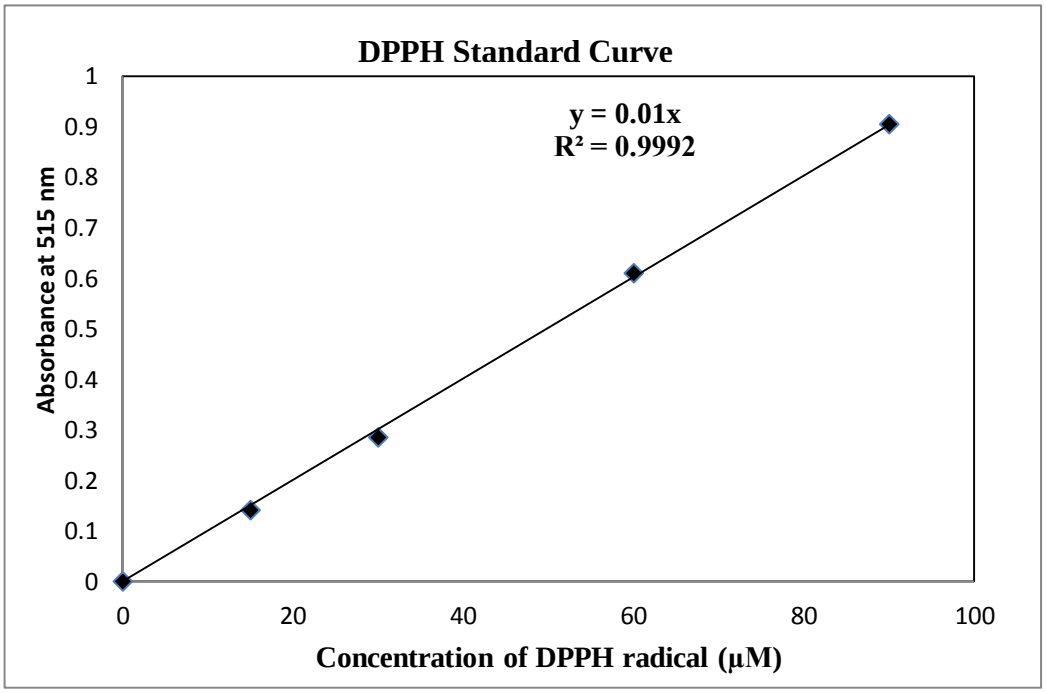

Figure 2.2 The standard curve for DPPH radical

The determined equation for the above curve is $y = 0.01x$ with $r^2 = 0.999$

2.2.5.5.2 Determination of Total Radical Scavenging Capacity of Pomegranate Arils

Free radical scavenging determination of pomegranate arils was performed by using the method of Sanchez, Larrauri and Saura-Colixto (1998).

In a test tube wrapped by aluminum foil, 0.1 mL sample solution was added to 3.9 mL DPPH radical (90μM), which was prepared daily. The sample tubes were mixed using a vortex mixer (DG-800, Donglin, Beijing, China) and incubated for 1 hour in a dark environment. After 1 hour incubation, the sample-methanol mixture was centrifuged at 4500 rpm for 5 minutes. Spectrophotometric analysis was performed at 515 nm in order to measure disappearance of DPPH radical in sample solutions.

The scavenged DPPH radical amount (μM) was determined according to the DPPH standard curve equations. For the given sample standard curve (Figure 2), the scavenged DPPH radical was determined by following equation:

$$C_{\text{DPPH Scavenged}} = 90\text{-}\left(\frac{\text{Absorbance}_{515}}{0.01}\right)$$

The percentage of scavenged DPPH was figured out by using the preceding equation as:

$$\%\ \text{DPPH Scavenged} = \left(\frac{C_{\text{DPPH Scavenged}}}{90}\right) \text{x } 100$$

2.2.6 Microbiological Analysis

10 g of arils were weighed in sterile conditions and put into a sterile stomacher bag and homogenized with Stomacher (Seward 80 Biomaster, England) in 90 ml sterile peptone water solution for 5 min. Following serial dilutions of samples by sterile peptone water, microbiological analyses was performed on Plate Count Agar (PCA, Merck) by using spread plate method for total mesophilic aerobic counts. 0.1 ml aliquot was spread on plates and the plates were incubated at 37^{o}C for 2 days.

Total yeast and mold analysis was performed on Potato Dextrose Agar (PDA, Merck) which was acidified to pH 3.5 with a sterile 10 % tartaric acid solution per liter of agar. Plates were incubated at 28^{o}C for 5 days.

Both total mesophilic aerobic bacteria and yeast and mold results were expressed as $\log_{10}$(colony forming units per gram of sample (CFU/g)). The results were achieved by using the following equation:

$$\log_{10} \mathrm{CFU/g_{arils}} = \log_{10}\left(\frac{N}{S_o V}\right)$$

Where,
N: Colony forming units per plate
So: Dilution factor
V: Volume of sample solution per plate, mL

2.2.7 Sensorial Analysis

Sensory analysis was performed by 5 panelists. The panelists were from the graduate students of Food Engineering Department of METU. The panelists evaluated the pomegranate arils for odor and appearance on a nine point hedonic scale (Peryam and Pilgrim, 1957). The used nine point hedonic scale was shown in Table 2.1.

Table 2.1 Nine Point Hedonic Scale for Sensory Evaluation

9-8.1	**Like Extremely**
8-7.1	**Like Very Much**
7-6.1	**Like Moderately**
6-5.1	**Like Slightly**
5-4.1	**Neither Like nor Dislike**
4-3.1	**Dislike Slightly**
3-2.1	**Dislike Moderately**
2-1.1	**Dislike Very Much**
1	**Dislike Extremely**

2.2.8 Data Analysis

Data were analyzed by analyses of variance (ANOVA) using IBM SPSS Statistics, Version 19 (SPSS Inc., Chicago, IL). In order to determine the significant differences for coating process and storage days, two-way ANOVA test was performed ($p < 0.05$). Tukey Post-Hoc Test was applied to storage time, in order to observe the groups that lead to significant differences.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Effect of Chitosan Coating Treatment on pH of Arils Juice

Changes in pH of both coated and uncoated pomegranate arils juice during 22 days storage at 4±0.5 ^{o}C are given in Figure 3.1. pH of both chitosan treated and untreated samples were decreased at the end of storage. Although slight changes were observed in pH, the decrease of pH during storage was found to be statistically significant ($p \leq 0.05$) (Table 3.1).

Different pH ranges of pomegranate were reported in literature. Gölükcü and Tokgöz (2008) have determined pH of some important pomegranate cultivars grown in Turkey between 2.88 and 4.01. Barone, Sottile, Caruso and Marra (2000) have found the pH range of Sicilian pomegranate varieties as 3.33 to 4.22. Martinez et al. (2006) determined the range of five pomegranate varieties as 3.35 to 4.28 where Fadavi, Barzegar, Azizi, Bayat (2005) determined pH range as 2.9 to 4.21 for the studies varieties. Ünal, Velioğlu and Cemeroğlu (1995) reported the pH values of pomegranate juice between 2.40 and 4.41. The pH ranges in the current study is found between 3.18 and 3.46 which is in accordance with the given literature ranges. The Food and Drug Administration of US (2007) has given the pH range for pomegranate arils of 2.93 to 3.20. The given FDA pH data is slightly lower (more acidic) than the current study findings. The variety of pH findings are mainly related

with factors such as variety, maturity and postharvest conditions of the pomegranates.

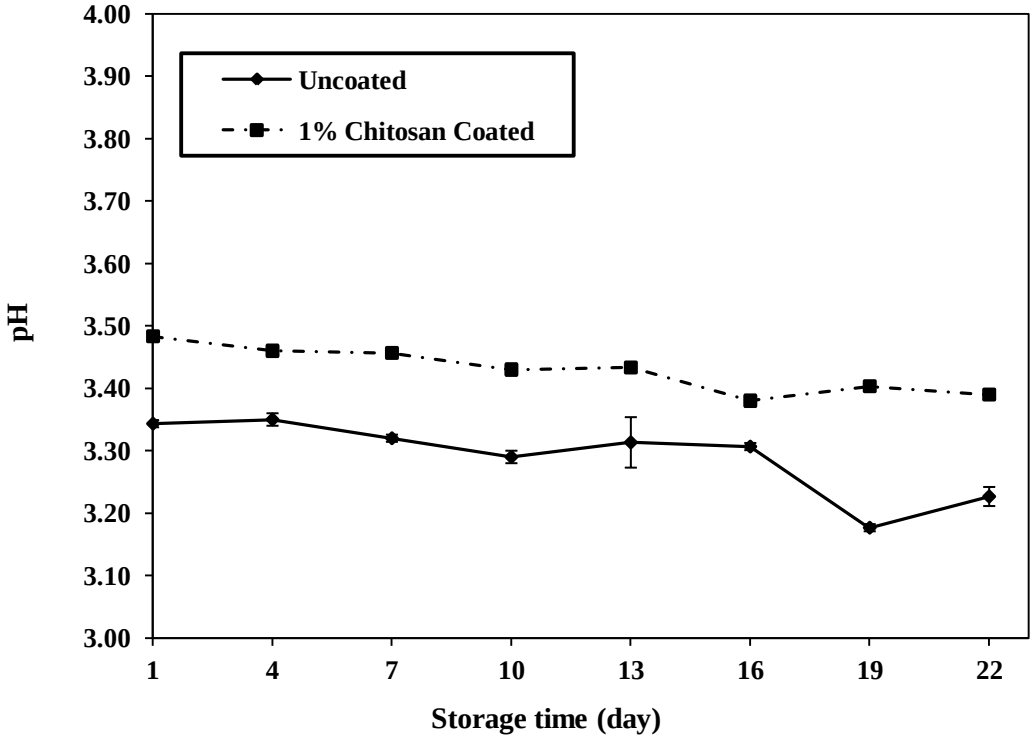

Figure 3.1 Changes in pH of pomegranate arils coated with 1% chitosan and control during storage at 4±0.5 ^{o}C. Vertical bars show standard error (n=3).

The pH values of chitosan coated arils are significantly higher that of uncoated ones ($p \leq 0.05$) (Table 3.1). The pH of chitosan coated arils is also more stable than the untreated samples. The higher and stable pH results for chitosan coated arils may be because of effect of chitosan on biochemical reactions of the arils, slower metabolic activities and respiration rates.

The change in pH is associated with number of reasons; it might be due to the effect of treatment on the biochemical condition of the fruit and slower rate of respiration and metabolic activity (Jitareerat, Paumchai and Kanlayanarat, 2007). The slower

changes in pH of chitosan coated arils presumably related with chitosan coating's modification of internal atmosphere of the arils and changing the carbon dioxide and oxygen concentrations within the fruit and thus delaying respiration process (Lowings and Cutts, 1982; Bai et al., 1988).

3.2 Effect of Chitosan Coating Treatment on Weight Loss of Pomegranate Arils

Weight loss is increased for both chitosan coated and untreated arils during storage (Figure 3.2).

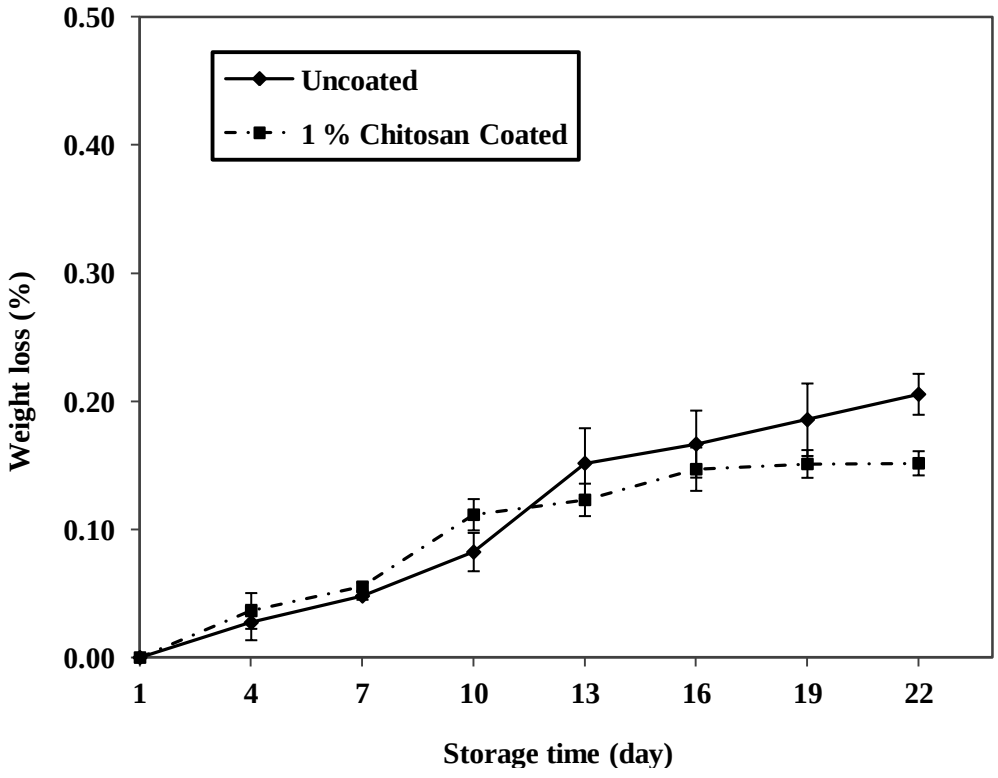

Figure 3.2 Changes in weight loss (%) of pomegranate arils coated with 1% chitosan and control during storage at 4±0.5 °C. Vertical bars show standard error (n=3).

At the end of 22 days of storage, 1 % chitosan coated arils' weight loss was reached to 0.15 % where uncoated arils have lost 0.21 % of their initial weight. Although the weight loss of both treated and untreated arils were nearly same during first 10 days of storage, after the 10 days of storage, the weight loss of coated arils was significantly lower than uncoated arils' weight loss ($p \leq 0.05$) (Table 3.1).

As the arils were extracted from the pulp, juice leaking is increased which is the main cause of weight loss (Dong, Cheng, Tan, Zheng, Jiang, 2004). Another important cause of weight loss is respiration and evaporation of water through the arils' skin. The water vapor transfer rate was mainly related with water pressure gradient between the aril tissue and its surrounding atmosphere. By binding the skin pores of arils, chitosan coating control the water vapor transfer and protect the arils' surface from mechanical damages and reduce the juice leaking as sealing the leakage wounds (Ghasemnezhad, Shiri and Sanavi, 2010; Varasteh et al., 2012).

Although the control samples were experienced the highest weight loss, it was lower than the critical limit which was determined by Kays (1991) as 4-6 percent of the total weight. Exceeding this limit cause visible discoloration and shriveling of the surface of the produce (Bico, Raposo, Morais and Morais, 2009). Kader (2006) mentioned in his study that the pomegranate arils have lower rates of respiration and ethylene production than whole fruit. This may be the reason of lower weight loss results observed in the current study.

The positive effect of chitosan coating on weight loss of other fruits and vegetables has been reported, including cucumber and bell pepper (El-Ghaouth, Arul, Ponnampalam and Boulet, 1991a), longan fruit (Jieng and Li, 2001), litchi (Dong et al., 2004), sliced and whole mango fruit (Chien et al. 2007; Abbasi, Iqbal, Maqbool and Hafiz, 2009), citrus fruit (Chien, Sheu and Lin, 2007a), strawberry (Riberio, Vicente, Teixeria and Miranda, 2007), grape fruit (Meng, Li, Lui and Tian, 2008), apricot (Ghasemnezhad et al., 2010), banana (Malmiri, 2011), whole pomegranate fruit (Varasteh et al., 2012).

3.3 Effect of Chitosan Coating Treatment on Total Soluble Solids of Aril Juice

Changes in total soluble solid contents (TSS) of both coated and uncoated pomegranate aril juice during 22 days storage at 4±0.5 ^{o}C are given in Figure 3.3.

Gölükcü et al. (2008) have found TSS contents of some important pomegranate cultivars grown in Turkey between 13.00 to 17.8%. Bilişli and Çevik (2000) have reported that the TSS contents of pomegranate varieties are changing between 14.5 to 16.7%. Vardin (2000) given the TSS range as 13.5 to 15.1 ^{o}Brix for varieties grown in south eastern region of Turkey. The TSS contents of 10 pomegranate cultivars grown in Iran were given as 10.0 to 16.5 ^{o}Brix (Fadavi et al., 2005) where Al Maiman and Ahmad (2000) has reported the TSS range for pomegranates as 16.3 to 16.9 ^{o}Brix. Although the findings in this study were in accordance with most of the literature data, given TSS content values for some pomegranate varieties has higher than our findings. The changes in TSS values can be related with lots of factors such as differences in studied varieties and also climate, region, soil varieties, harvest time of the produce (Gölükcü et al., 2008).

The Codex Alimentarus Commission established minimum total soluble solids content for pomegranate juice as 11.2% (Anonymous, 2005). Findings in this study were in accordance with the given limit for TSS of pomegranate juice.

Total soluble solid content of pomegranate arils increases during fruit development. The reason of this increase may be predicated on starch hydrolysis process which forms simple sugars, which is a desired experience for pomegranate fruits (Kulkarni and Aradhya, 2005). In the current study, TSS content of both uncoated and coated pomegranate arils fell significantly after 22 days of storage ($p \leq 0.05$). The decline in total soluble solid contents of fruits was explained by Abbasi et. al (2009) as a result of respiration process that cause decrease of carbohydrate and pectins amounts, partial protein hydrolysis, and degredation of glycosides into their sub units. The

pomegranates used in this study were fully mature; in order to get fully developed fruits in terms of color, taste and aroma. It was mentioned that, TSS content of a fruit is decreased if the fruit is over-mature (Hardy and Sanderson, 2010). Using fully mature fruits may be the reason of decrease in TSS concentration of the pomegranate arils. The reason of decrease in TSS concentration can be related with also conversion of simple sugars to water and CO_2 in late stages of storage (Saira, Rathore, Majeed, Awan and Shah, 2009).

Statistical results showed that juice of arils that have been treated with chitosan had significantly higher total soluble solids content during storage ($p \leq 0.05$) (Table 3.1). This finding is in parallel with studies on effect of chitosan coating on different fruits. Chitosan coating application showed advantageous effects on total soluble solids content of peach (Du, Gemma, Iwahori, 1997), longan fruit (Jiang et al., 2001), Chinese water chestnut (Pen and Jiang, 2003), peeled litchi fruit (Dong et al., 2004), sliced mango fruit (Chien et al., 2007), citrus fruit (Chien et al., 2007a), sliced red pitayas (Chien et al., 2007b), grape (Meng et al., 2008), sweet cherries (Dang, et al., 2010). The higher TSS concentration may be attributed to lower respiration rates of chitosan coated fruits (Du et al., 1997; Jiang et al., 2001).

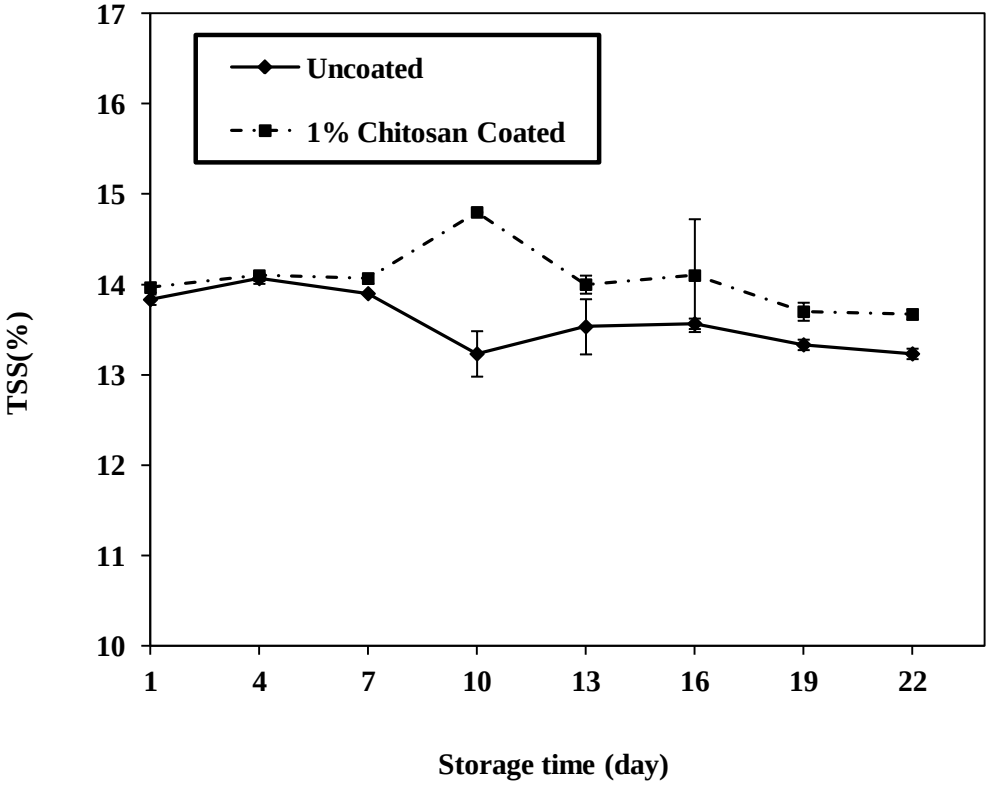

Figure 3.3 Changes in total soluble solids (%) of pomegranate arils coated with 1% chitosan and control during storage at 4±0.5 ^{o}C. Vertical bars show standard error (n=3).

3.4 Effect of Chitosan Coating Treatment on Titratable Acidity (TA) of Aril Juice

Changes in titratable acidity (TA) of both coated and uncoated pomegranate aril juice during 22 days storage at 4±0.5 ^{o}C are given in Figure 3.4 in terms of percentage citric acid. Slight but not significant effects were observed in titratable acidity of both chitosan coated and uncoated arils' juice along with increased storage time ($p>0.05$) (Table 3.1).

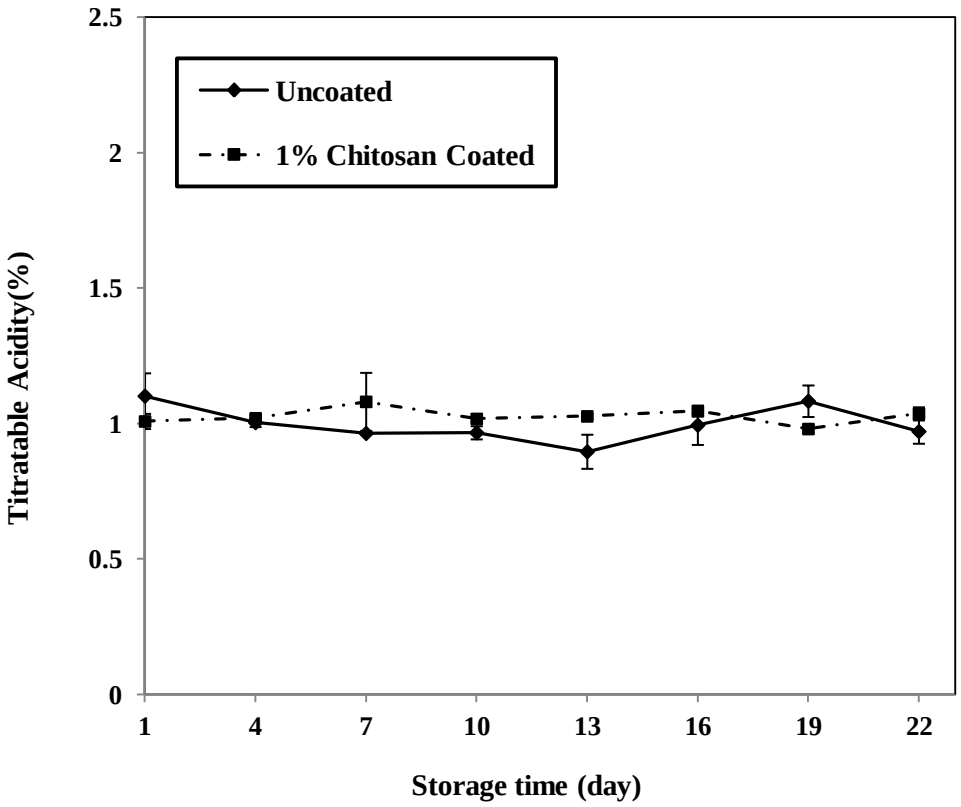

Figure 3.4 Changes in titratable acidity (% citric acid) of pomegranate arils coated with 1% chitosan and control during storage at 4±0.5 ^{o}C. Vertical bars show standard error (n=3).

Pomegranate fruit is rich in organic acids, mainly citric, acetic, tartaric, fumaric, malic and lactic acids. Citric acid is the predominant organic acid found in all pomegranate cultivars (Melgarejo, Salazar and Artes, 2000).

Studies showed that titratable acidity contents of pomegranates have a wide range that differs according to the cultivar. Onur and Kaska (1985) mentioned in their study that, pomegranate cultivars can be classified according to their titratable acidity contents; if the titratable acidity is below 1% pomegranates are classified as 'sweet', if titratable acidity ranges between 1 to 2% classified as 'sweet-sour' and if titratable acidity is higher than 2% classified as sour cultivars.

Sood, Dhindsa and Wagle (1982) determined in their study that four different pomegranate cultivars' titratable acidity range changes through a narrow range like 0.44 to 0.47% where Ünal *et al.* (1995) determined a wider range of 0.20 to 5.52% for several pomegranate cultivars in terms of citric acid. Vardin (2000) also set a wider range of titratable acidity values which are between 0.42% and 3.12% for the investigated pomegranate cultivars. Poyrazoğlu, Gökmen and Artık (2002) determined the titratable acidity of the analyzed pomegranates as 0.46% to 1.73%. Fadavi et al. (2005) designated the titratable acidity of different cultivars as 0.40% to 2.45%. Gölükcü et al. (2008) have studied the titratable acidity contents of some important pomegranate cultivars grown in Turkey. The maximum TA content was found as 2.81% of İzmir-1499 cultivar and the minimum TA content was observed in 'seedless' cultivar as 0.20%. Özgen, Durgaç, Serçe and Kaya (2008) detected titratable acidity of pomegranate cultivars between 0.20 to 3.20% in terms of citric acid. Those varieties in titratable acidity ranges can be attributed mainly on the investigational cultivar and also other factors such as climate, soil type and differences in the applied cultural process (Gölükcü et al., 2008). Those findings are in accordance with the present study findings.

For 'Wonderful' cultivar which is grown in California, titratable acidity values lower than 1.85 percent was recommended (Ben-Arie et al., 1984). Kader (1999) has proposed that the maximum titratable acidity as 1.4 % for acceptable flavor quality of pomegranates. Those findings are in accordance with the present study findings. During 22 days of storage, titratable acidity content of the both untreated and coated samples did not exceed the given limits.

There was a significant effect of coating application on titratable acidity of pomegranate arils at the $p \leq 0.05$ level. During storage, titratable acidity of coated pomegranate arils was significantly higher that of untreated arils (Table 3.1). Higher titratable acidity content was also observed on other fruits and vegetables that were treated with chitosan. Chitosan coating application resulted in higher titratable acidity contents of strawberries (El-Ghaouth, Arul, Ponnampalam and Boulet 1991b), peeled litchi fruit (Dong et al., 2004), Chinese water chestnut (Pen et al.,

2003), sliced mango fruit (Chien et al., 2007), citrus fruit (Chien et al., 2007a), mango fruit (Abbasi et al., 2009), sweet cherries (Dang et al., 2010).

As being an important indice of quality and acceptability, it is not desired for fruits to have very high or very low acidity values. The decrease in titratable acidity is an indication of fruit senescence. This decrease with increased storage time can be a consequence of conversion of organic acids into sugars in respiratory process and metabolic reactions causing further usage of them (Abbasi et al., 2009; Echeverria and Valich, 1989).

Chitosan coating effectually retarded fruit senescence as the application resulted in slower changes on titratable acidity. It is a certain fact that if a fruit has a lower respiration rate, usage of the organic acids in enzymatic reactions are also lowly (Bico et al., 2009). The advantageous effect chitosan coating presumably related with its modification of internal atmosphere of the arils and changing the carbon dioxide and oxygen concentrations within the fruit and thus delaying respiration process (Lowings & Cutts, 1982; Bai et al., 1988).

Table 3.1 The effect of chitosan coating on pH, weight loss (%), total titratable acidity (%) and total soluble solid content (%) of pomegranate arils during refrigerated storage at $4 \pm 0.5^{o}C$.[1,2]

Coating Process	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 22
pH								
Control	$3.34 \pm 0.01_a$	$3.35 \pm 0.01_a$	$3.32 \pm 0.01_a$	$3.29 \pm 0.01_b$	$3.31 \pm 0.04_b$	$3.31 \pm 0.01_c$	$3.18 \pm 0.01_d$	$3.23 \pm 0.01_d$
Coated	$3.48 \pm 0.01_a$	$3.46 \pm 0.01_a$	$3.46 \pm 0.01_a$	$3.43 \pm 0.01_b$	$3.43 \pm 0.01_b$	$3.38 \pm 0.01_c$	$3.40 \pm 0.01_d$	$3.39 \pm 0.01_d$
Weight Loss								
Control	$0.00 \pm 0.00_a$	$0.03 \pm 0.01_b$	$0.05 \pm 0.00_b$	$0.08 \pm 0.02_c$	$0.15 \pm 0.03_d$	$0.17 \pm 0.03_d$	$0.19 \pm 0.03_d$	$0.21 \pm 0.02_d$
Coated	$0.00 \pm 0.00_a$	$0.04 \pm 0.01_b$	$0.06 \pm 0.00_b$	$0.11 \pm 0.01_c$	$0.12 \pm 0.01_d$	$0.15 \pm 0.02_d$	$0.15 \pm 0.01_d$	$0.15 \pm 0.00_d$
Total titratable acidity								
Control	$1.10 \pm 0.08_a$	$1.00 \pm 0.02_a$	$0.96 \pm 0.00_a$	$0.97 \pm 0.02_a$	$0.90 \pm 0.63_a$	$0.99 \pm 0.07_a$	$1.08 \pm 0.06_a$	$0.97 \pm 0.05_a$
Coated	$1.01 \pm 0.03_a$	$1.02 \pm 0.01_a$	$1.08 \pm 0.10_a$	$1.02 \pm 0.01_a$	$1.03 \pm 0.01_a$	$1.05 \pm 0.01_a$	$0.98 \pm 0.01_a$	$1.04 \pm 0.02_a$
Total soluble solid								
Control	$13.83 \pm 0.06_a$	$14.06 \pm 0.06_a$	$13.90 \pm 0.00_a$	$13.23 \pm 0.25_a$	$13.53 \pm 0.31_a$	$13.57 \pm 0.06_a$	$13.33 \pm 0.06_a$	$13.23 \pm 0.06_a$
Coated	$13.97 \pm 0.06_a$	$14.10 \pm 0.00_a$	$14.06 \pm 0.06_a$	$14.80 \pm 0.00_a$	$14.00 \pm 0.10_a$	$14.10 \pm 0.62_a$	$13.70 \pm 0.10_a$	$13.67 \pm 0.06_a$

1 For each parameter, similar small letters (subscript) in rows are not significantly different at ($p \leq 0.05$).
2 All given data are the mean ± standard deviation of three replicates (n=3)

3.5 Effect of Chitosan Coating Treatment on Color of Pomegranate Arils

Color is a critical factor for the quality perception and freshness indication of pomegranate arils. Figure 3.5 to 3.8 shows the changes in surface color of both chitosan coated and uncoated pomegranate arils which were stored at $4 \pm 0.5^{o}C$ for 21 days. The color changes were investigated as L* (lightness), C* (chromaticity), h* (hue angle) and ΔE for euclidean distance between two color points (total color difference).

The L*, lightness of the samples, is an indicator of fruit darkening (Munoz, Almenar, Valle, Velez and Gavara, 2008). Figure 3.5 shows the changes in L* values of both coated and uncoated samples within 21 days of storage. The L* values of both chitosan coated and uncoated samples showed a slight but not significant decrease with increasing storage time ($p > 0.05$) (Table 3.2). The L* values of 1% chitosan coated samples were significantly higher than the uncoated samples ($p \leq 0.05$), which indicated that the uncoated arils were significantly darker than coated ones throughout the storage period. The darkening of the arils mainly related with browning of the tissues. These results also showed that chitosan resulted in more shiny surfaces of arils.

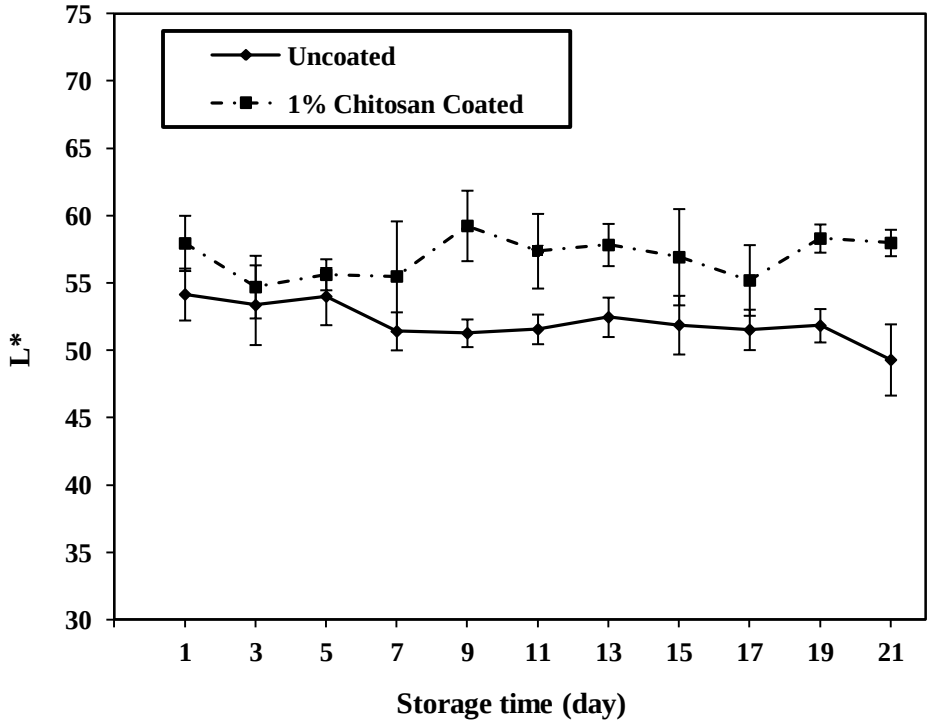

Figure 3.5 Changes in L* (lightness) of pomegranate arils coated with 1% chitosan and control during storage at 4±0.5 ^{o}C. Vertical bars show standard error (n=6).

The C*, chromaticity of the samples shows the saturation degree of the color and is proportional to the color strength (Bico, Raposo, Morais and Morais, 2009). Figure 3.6 shows the changes in C*, chromaticity values of the chitosan coated and uncoated samples during 21 days of storage. The C* values of both coated and uncoated arils were decreased significantly with the increasing storage time ($p \leq 0.05$) which indicated that with increasing storage pomegranate arils' brightness was decreased. The chitosan coated samples' chromaticity values were significantly lower than that of uncoated samples ($p \leq 0.05$) which indicated that coated arils exhibited a duller appearance than untreated arils(Table 3.2). The lower chromaticity values of chitosan coated arils also showed that the coated arils' colors was less vivid than uncoated ones (Munoz et al., 2008).

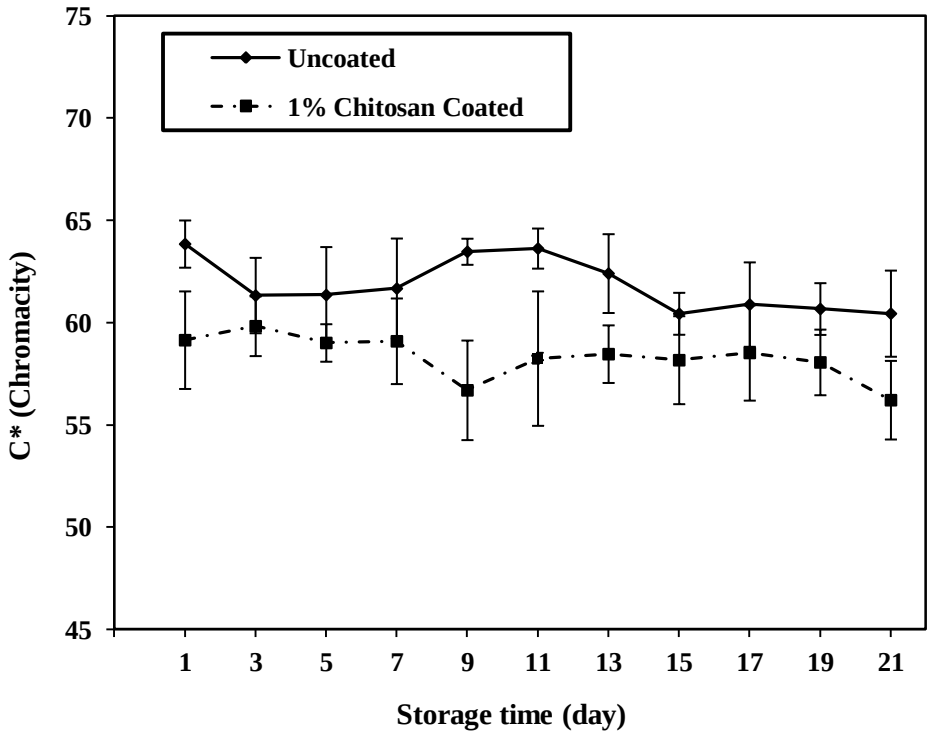

Figure 3.6 Changes in C* (chromaticity) of pomegranate arils coated with 1% chitosan and control during storage at 4±0.5 ^{o}C. Vertical bars show standard error (n=6).

The H*, hue angle shows the color nuance of the samples (where 0^{o} red-purple, 90^{o} yellow). The H* values of the chitosan coated and uncoated arils were changed significantly throughout the storage ($p \leq 0.05$). The significant effect of storage time was observed in ANOVA tests however performed Tukey tests was not able to show the groups that lead to significant differences (Table 3.2). The observed H* values were located on red to purple range; therefore a red color was indicated. The chitosan coated arils' hue angle values were significantly lower than uncoated ones ($p \leq 0.05$. These results indicated that chitosan coated arils maintained their red color than uncoated ones during storage.

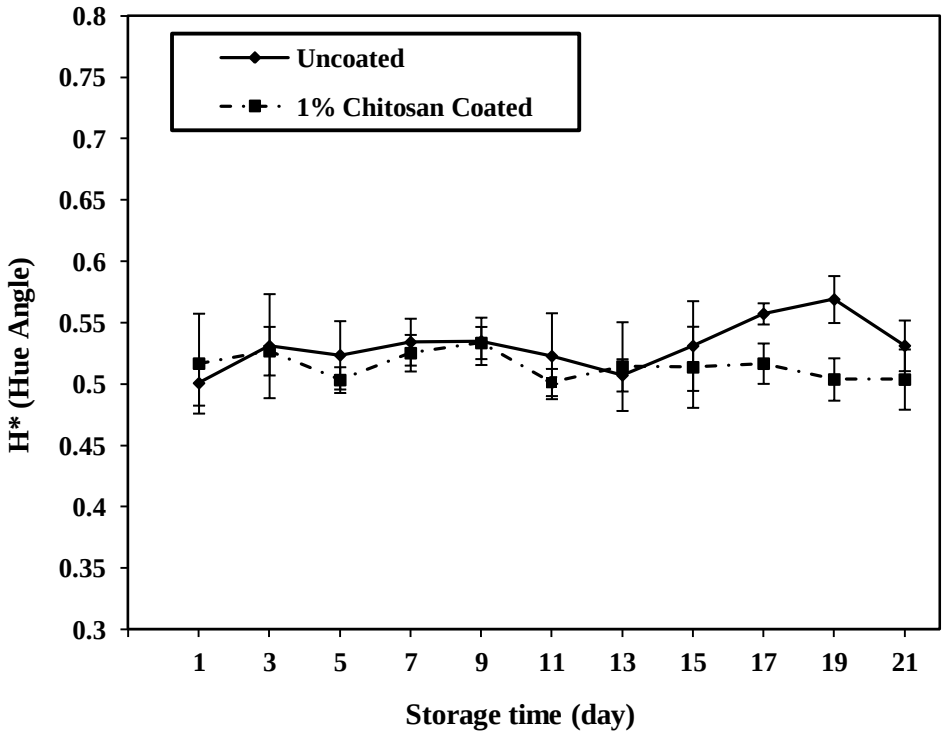

Figure 3.7 Changes in H* (hue angle) of pomegranate arils coated with 1% chitosan and control during storage at 4±0.5 ^{o}C. Vertical bars show standard error (n=6).

The change in total color (ΔE) of both chitosan coated and uncoated arils during storage are shown in Figure 3.8. ΔE values of chitosan coated arils were significantly higher than ΔE values of uncoated arils ($p \leq 0.05$), which indicated that coated arils has higher L*, a* and b* values. The ΔE values were decreased significantly with increasing storage time ($p \leq 0.05$) (Table 3.2). The change in ΔE value of chitosan coated arils was smaller as it was observed that ΔE value of coated arils decreased 2.5% of its initial value where uncoated arils' ΔE value decreased 6.8% of its initial value.

To sum up, the chitosan coated arils' lightness and redness were significantly higher than that of uncoated samples where the chitosan coating resulted in more dull surface color than the uncoated samples ($p \leq 0.05$). In overall color difference (ΔE), chitosan coating resulted in significantly higher ΔE values throughout the storage

(Table 3.2). The chitosan coating application maintained the red color of the arils. This may be related with chitosan coating's prevention of oxygen exposure to the arils (Ball, 1997). Chitosan coating also resulted in more shiny surface color. The only drawback was observed on chromaticity values of chitosan coated arils, which can be attributed the changes of the light reflection on the fruit surface because of the formed chitosan film. The observed results were represented in Table 3.2.

The effects of chitosan on color parameters of variety of fruits and vegetables were studied with different authors: Aguilar et al. (2008) observed higher L* and b* values on chitosan coated papaya fruit, Zhang et al. (1997) reported that chitosan coating's effect on decreasing browning of litchi fruit. Jiang and Li (2001) reported that longan fruits coated with 2% chitosan remained their bright color during 30 days of storage. Djioua, Charles, Freire, Filgueiras, Collin and Sallanon (2010), studied the effects of chitosan on color quality of fresh-cut mangoes and they observed the chitosan's beneficial effect on decreasing the loss on color parameters.

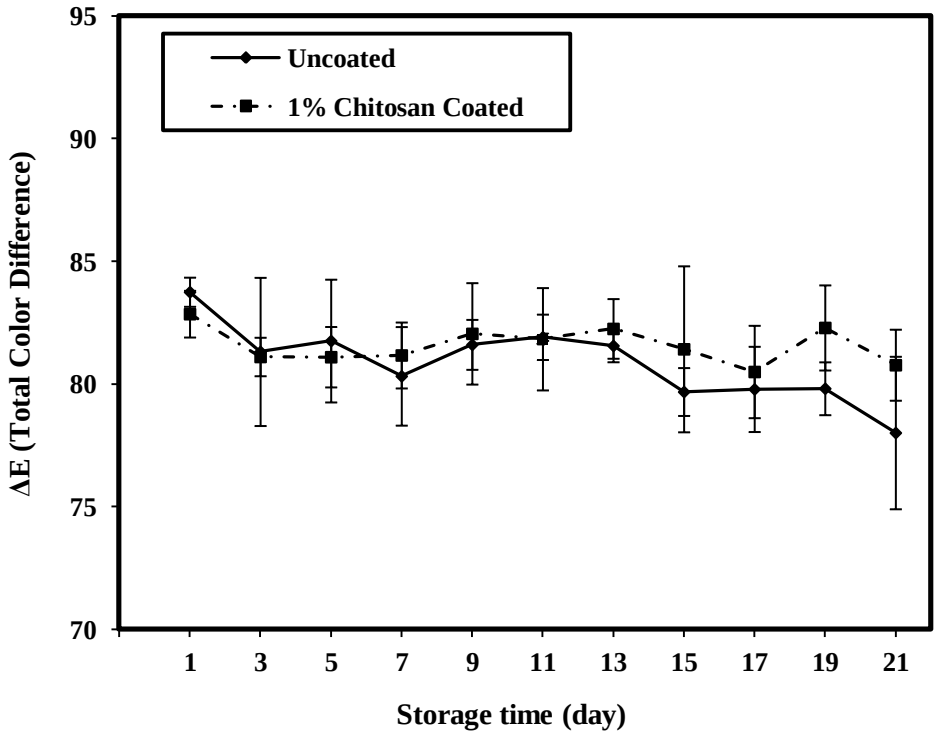

Figure 3.8 Changes in ΔE (total color difference) of pomegranate arils coated with 1% chitosan and control during storage at 4±0.5 ^{o}C. Vertical bars show standard error (n=6).

Table 3.2 The effect of chitosan coating on Lightness (L*), Chromaticity (C*), Hue angle and Total color difference (ΔE) of pomegranate arils during refrigerated storage at 4 ± 0.5°C.[1,2]

Coating Application	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
L* (Lightness)											
L* Control	$54.2 \pm 1.9_a$	$53.4 \pm 3.0_a$	$54.0 \pm 2.1_a$	$51.4 \pm 1.4_a$	$51.3 \pm 1.0_a$	$51.6 \pm 1.1_a$	$52.5 \pm 1.5_a$	$51.9 \pm 2.2_a$	$51.5 \pm 1.5_a$	$51.8 \pm 1.2_a$	$49.3 \pm 2.6_a$
L* Coated	$57.9 \pm 2.0_a$	$54.7 \pm 2.3_a$	$55.6 \pm 1.2_a$	$55.5 \pm 2.6_a$	$59.2 \pm 2.6_a$	$57.4 \pm 2.8_a$	$57.8 \pm 1.6_a$	$56.9 \pm 3.6_a$	$55.2 \pm 2.6_a$	$58.3 \pm 1.0_a$	$58.0 \pm 1.0_a$
C* (Chromaticity)											
C* Control	$63.9 \pm 1.2_a$	$61.3 \pm 1.8_a$	$61.4 \pm 2.3_a$	$61.7 \pm 2.5_a$	$63.5 \pm 0.6_a$	$63.6 \pm 1.0_a$	$62.4 \pm 1.9_b$	$60.4 \pm 1.0_b$	$60.9 \pm 2.1_b$	$60.7 \pm 1.3_b$	$60.5 \pm 2.1_b$
C* Coated	$59.2 \pm 2.4_a$	$59.8 \pm 1.5_a$	$59.0 \pm 0.9_a$	$59.1 \pm 2.1_a$	$56.7 \pm 2.4_a$	$58.3 \pm 3.3_a$	$58.5 \pm 1.4_b$	$58.2 \pm 2.2_b$	$58.5 \pm 2.3_b$	$58.1 \pm 1.6_b$	$56.2 \pm 1.9_b$
H* (Hue Angle)											
H* Control	$0.50 \pm 0.02_a$	$0.53 \pm 0.04_a$	$0.52 \pm 0.03_a$	$0.53 \pm 0.02_a$	$0.53 \pm 0.02_a$	$0.52 \pm 0.04_a$	$0.51 \pm 0.01_a$	$0.53 \pm 0.04_a$	$0.56 \pm 0.01_a$	$0.57 \pm 0.02_a$	$0.53 \pm 0.02_a$
H* Coated	$0.52 \pm 0.04_a$	$0.53 \pm 0.02_a$	$0.50 \pm 0.02_a$	$0.53 \pm 0.02_a$	$0.53 \pm 0.01_a$	$0.50 \pm 0.01_a$	$0.51 \pm 0.04_a$	$0.51 \pm 0.03_a$	$0.52 \pm 0.01_a$	$0.50 \pm 0.02_a$	$0.50 \pm 0.02_a$
ΔE (Total Color Difference)											
ΔE Control	$83.7 \pm 0.6_a$	$81.3 \pm 3.0_a$	$81.8 \pm 2.5_a$	$80.3 \pm 2.0_b$	$81.6 \pm 1.0_b$	$81.9 \pm 0.9_b$	$81.6 \pm 0.7_b$	$79.7 \pm 1.0_c$	$79.8 \pm 1.7_c$	$79.8 \pm 1.1_c$	$78.0 \pm 3.1_c$
ΔE Coated	$82.9 \pm 1.0_a$	$81.1 \pm 0.8_a$	$81.1 \pm 1.2_a$	$81.2 \pm 1.3_b$	$82.1 \pm 2.1_b$	$81.8 \pm 2.1_b$	$82.3 \pm 1.2_b$	$81.4 \pm 3.4_c$	$80.5 \pm 1.9_c$	$82.3 \pm 1.7_c$	$80.8 \pm 1.5_c$

1 For each parameter, similar small letters (subscript) in rows are not significantly different at ($p \leq 0.05$).
2 All given data are the mean ± standard deviation of six replicates (n=6)

3.6 Effect of Chitosan Coating Treatment on Total Phenolic Content of Pomegranate Aril Juice

Pomegranate is an important source of phenolic compounds. Those phenolic compounds, especially the high molecular weight ones were mainly found in the husk of pomegranates. Guo Yang, Wei, Li, Xu and Jiang (2003) have found that the extract of pomegranate husk contains 10 fold higher phenolic compound amounts than the extract of the pulp.

The changes of the total phenolic content in pomegranate arils treated with chitosan coating and untreated during 22 days of storage at 4±0.5 ^{o}C are shown in Figure 3.9. The total phenolic content was expressed in milligrams gallic acid equivalent per liter of fruit juice. There were significant effects of chitosan coating process and storage day on the total phenol content of arils ($p \leq 0.05$) (Table 3.3).

Al-Maiman and Ahmad (2002) has studied physical and chemical changes of 'Taifi' pomegranate cultivars during maturation. They classified the fruits as unripe, semi ripen and fully ripen stages and observed that the phenolic content of the fully ripen pomegranates has the lowest phenol contents. The total phenolic content was determined as 3.65 mg/100g, 3.22 mg/100g and 1.90 mg/100g for unripe, semi ripen and fully ripen pomegranates respectively (Uzuner, 2008). Gölükcü et al., (2008) found the total phenolic content of some cultivars grown in Turkey as changing between 1535 mg/kg to 3701 mg/kg. Özgen *et al*. (2008) reported that the total phenol content of pomegranate cultivars grown in the Mediterranean region of Turkey varied between 1245 and 2076 mg gallic acid equivalent per liter of aril juice. Sepulveda, Saenz, Pena, Robert, Bartolome and Cordoves (2010) have reported the total phenol content range of Chilean pomegranates between 676 and 1280 mg gallic acid equivalent per liter of aril juice. The current study results of phenolic contents were generally lower than the found data in literature. A wide variation was observed for phenolic compounds of different cultivars. There may be lots of factors for this

variety such as location of plantation (Wang, Zheng and Galletta, 2002; Hakkinen and Torronen, 2003), ripening status (Raffo, Leonardi, Fogliano, Ambrosino, Salucci, Gennaro, Bugianesi Giuffrida and Quaglia, 2002), the condition and season of harvest (Wu, Gu, Holden, Haytowitz, Gebhardt, Beecher and Prior, 2004).

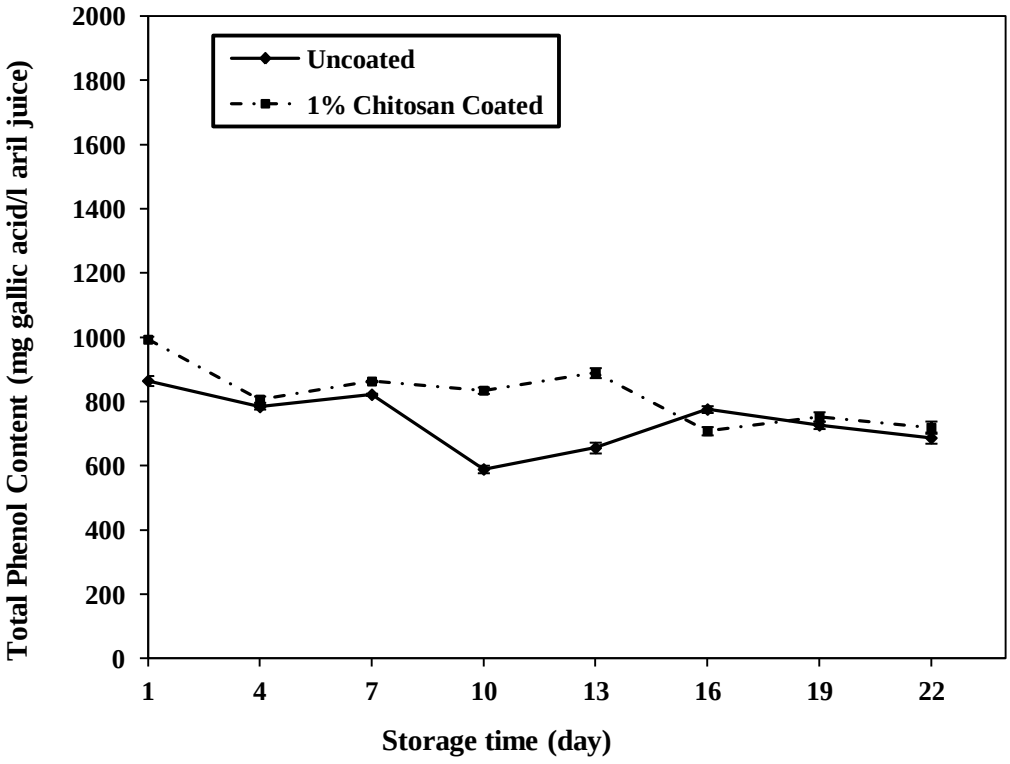

Figure 3.9 Changes in total phenol content (mg gallic acid/L of aril juice) of pomegranate arils coated with 1% chitosan and control during storage at 4±0.5 ^{o}C. Vertical bars show standard error (n=3).

The chitosan coated arils demonstrated a significantly higher total phenol contents during storage ($p \leq 0.05$) (Table 3.3). The highest phenol content was observed with 1% chitosan coated samples (992.86 mg gallic acid equivalent per liter of aril juice) on the first day of storage and the lowest one was observed in untreated samples

(588.89 mg gallic acid equivalent per liter of aril juice) on the 10th day of storage. Kulkarni et al. (2005), studied the total phenol content change during pomegranate maturation and observed the decrease of phenolic contents during maturation. They recorded the highest phenolic content amount in 20th day old fruit as 506 mg per 100 g arils. The total phenolic content of the pomegranate arils show a 73.9% decrease from 20 to 140 days of development. Benhamou (1996) reported in his study that chitosan had the potential to induce phenolics in plants. Increase of phenolic compound production of tomato plants coated with chitosan was also reported by Benhamou and Theriault (1992). Ben Shalom, Ardi, Pinto, Aki, Fallik (2003) showed that chitosan treatment of cucumber elicited the POD activity. In present study, although the phenolic content of chitosan treated arils were higher than untreated ones, the phenolic content amounts of both untreated and treated arils fluctuated along the storage time and decreased at the end of the 22 days of storage ($p \leq 0.05$). Those fluctuating values of phenolic content might be due to the changes in both total acidity and total soluble solids content which also affected the total anthocyanin and antioxidant activity contents (Ayhan and Esturk, 2009). The decrease in phenol content of fruits during storage was explained by Macheix, Fleuriet and Billot (1990) as a result of cell structure breakdown due to senescence phenomena during storage (Ghasemnezhad et al., 2010). On the other hand, further studies are needed as the storage time of the present study was limited with 22 days, the effect of chitosan on further trend of total phenolic content of pomegranate arils after 22 days of storage is not known.

3.7 Effect of Chitosan Coating Treatment on Radical Scavenging Capacity of Pomegranate Aril Juice

There is an increasing trend in food science research to determine fruit and vegetable antioxidant contents and activities during postharvest storage (Fernando, Ayala-Zavala, Wang, Wang and González-Aguilar, 2004). In present study, the effect of chitosan coating on radical scavenging capacity of pomegranate arils was examined

and the results were expressed in terms of the percentage of free radical scavenging capacity.

The changes of the radical scavenging capacities (%) of in pomegranate arils treated with chitosan coating and untreated during 22 days of storage at $4 \pm 0.5\ ^{o}C$ are shown in Figure 3.10.

Ayhan et al. (2008) reported that pomegranate arils' antioxidant activity increased until the 9^{th} day and a decrease at the end of the storage under passive, low and no oxygen atmospheres where they observed an increase along with increasing time of storage in oxygen enriched atmosphere. D'Aquino, Palma, Schirra, Continella, Tribulato and La-Malfa (2010) reported decreasing antioxidant activity of film wrapped pomegranates with increasing time while an increase was observed in control fruit. In current study the radical scavenging activities of chitosan coated arils were significantly lower than that of uncoated ones ($p \leq 0.05$). It may be related with chitosan coating's modification of internal atmosphere of the arils. Changing carbon dioxide and oxygen concentrations within the fruit may be resulted in lower radical scavenging values.

The radical scavenging capacities of both chitosan treated and untreated arils were increased along with increased storage time ($p \leq 0.05$) (Table 3.3). Although the total phenolic content of arils for both treatments was decreased with increasing storage time, the radical scavenging capacity of arils was increased. Some previous studies showed a correlation between the total phenolic content and antioxidant capacity of some food assays. The current study findings are not in agreement with those findings but other studies that found no correlation (Bajpai, Pande, Tewari and Prakash, 2005). This can be attributed to the reason that the observed antioxidant capacity was not purely from the phenolic content but due to other contents such as ascorbic acid and pigments and also their synergistic effects that can make a contribution to the antioxidant capacity of pomegranate arils (Wu, Beecher, Holden, Haytowitz, Gebhardt and Prior, 2004; Şengül, Yıldız, Gungör, Çetin, Eser and Ercişli, 2009).

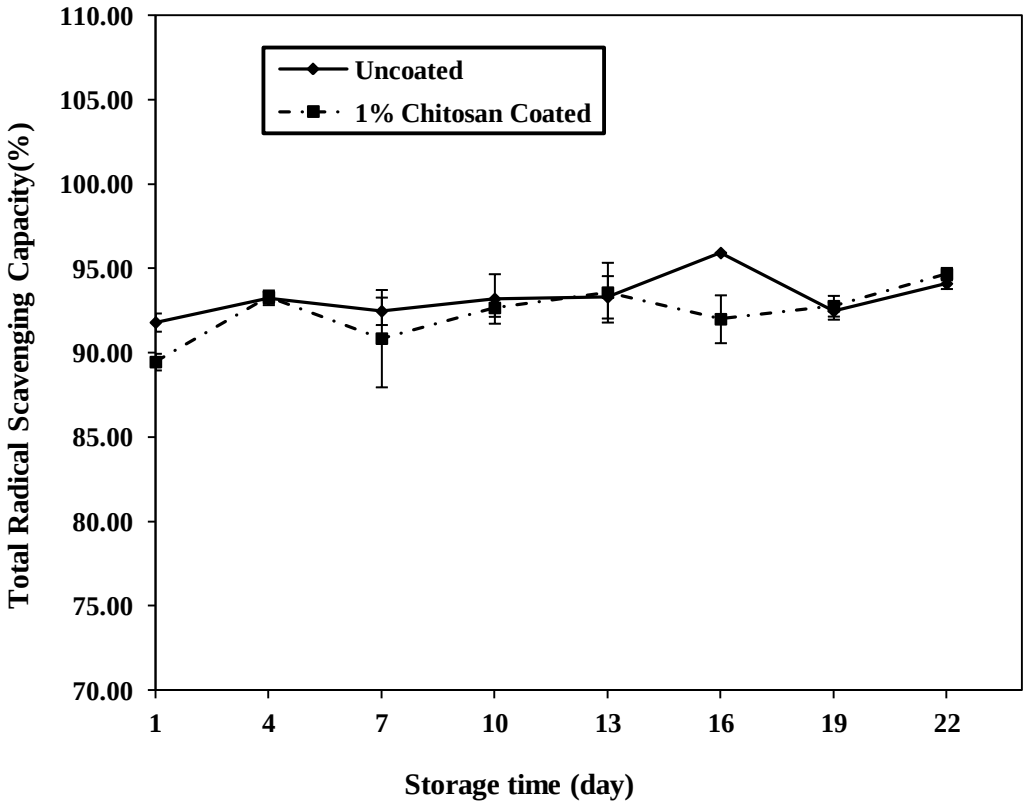

Figure 3.10 Changes in total radical scavenging capacities (%) of pomegranate arils coated with 1% chitosan and control during storage at 4±0.5 ^{o}C. Vertical bars show standard error (n=3).

Another reason is related with the method used in total phenolic content which is Folin Ciocalteu Method. This method is not capable of measuring an absolute amount of different phenolic compounds. Based on their specific structure, different phenolic compounds have different antioxidant activities, thus those phenolic compounds that could not quantified with Folin Ciocalteu Method may contribute to the antioxidant activity values (Şengül et al., 2009).

3.8 Effect of Chitosan Coating Treatment on Total Anthocyanin Content of Pomegranate Aril Juice

Total anthocyanin content of extracted pomegranate aril juice coated with and without chitosan during 22 days of storage period is shown in Figure 3.11. The total anthocyanin content was given in terms of mg mg cyanidin-3- glucoside equivalent per liter of aril juice. The anthocyanin content is significantly decreased with increasing storage time for both chitosan treated and untreated samples ($p \leq 0.05$) (Table 3.3).

The total anthocyanin content of different pomegranate cultivars was studied in literature. It was reported that the total anthocyanin content of Rabbab-e-Neyriz' cultivar as 935 mg/L (Varasteh et al., 2012), Wonderful cultivar as 306 mg/L (Gil et al., 2000), Mollar cultivar as 250 mg/L (Perez-Vicente, Serrano, Abellan and Garcia-Viguera, 2004), Primosole as 33.8mg/100g (D'Aquino et al., 2010). Sepulveda et al., (2010) studied the anthocyanin content of different Chilean pomegranate cultivars and reported a range of 170 mg/L to 1342 mg/L. The reason of those variations within different studies is related with the studied cultivars, maturity of the fruit, preharvest and postharvest conditions (Varasteh et al., 2012). The present study findings for anthocyanin contents were ranged between 166 mg/L to 85 mg/L.

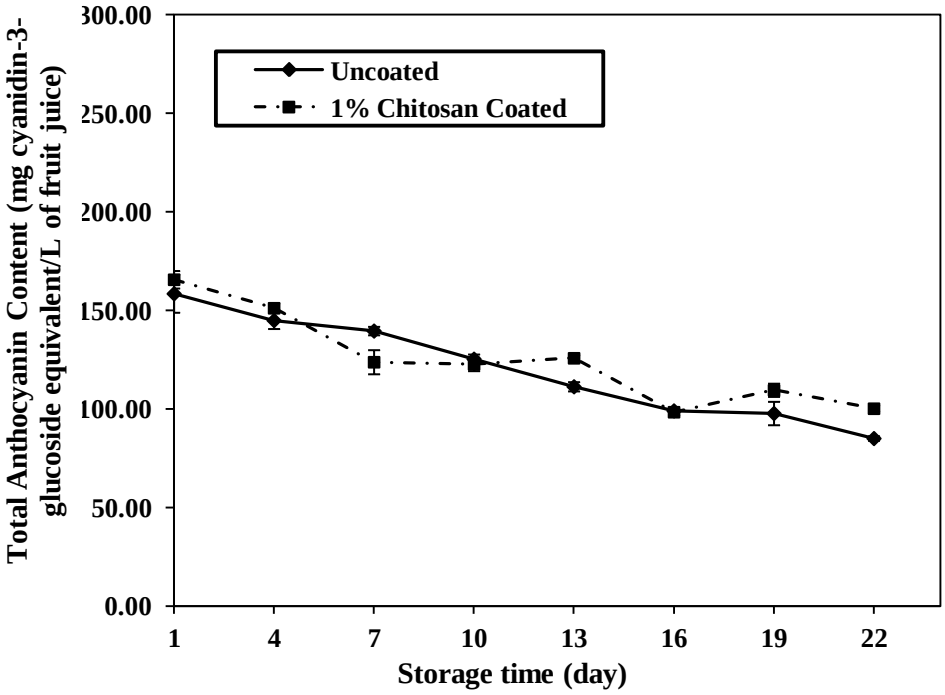

Figure 3.11 Changes in total anthocyanin content (mg cyanidin-3- glucoside equivalent/L of aril juice) of pomegranate arils coated with 1% chitosan and control during storage at 4±0.5 ^{o}C. Vertical bars show standard error (n=3).

The previous studies showed that chitosan application has beneficial effects on preserving anthocyanin content of pomegranate arils. Higher anthocyanin contents were observed on chitosan coated litchi fruit (Zhang et. al., 1997; De Reuck et al., 2009), strawberries (El Ghaouth, 1991; Zhang and Quantick, 1998) and raspberries (Zhang et al., 1998) and also pomegranate fruit coated with chitosan (Varasteh et al., 2012). As chitosan forms a coating on the fruit surface, it modifies the CO_2 and O_2 levels within the inner atmosphere. This could result in reduced O_2 supply which is a co substrate required for enzymatic oxidation reaction of the anthocyanin (Zhang et al., 1998). The present study findings were also accordance with above studies; total anthocyanin content of 1% chitosan coated arils was significantly higher than the uncoated arils during the storage time ($p \leq 0.05$).

Table 3.3 The effect of chitosan coating on total phenolic content (TPC) (mg gallic acid equivalent/L of juice), radical scavenging capacity (RSC) (%) and total anthocyanin content (TAC) (mg cyanidin-3-glucoside equivalent/L of fruit juice) of pomegranate arils during refrigerated storage at 4 ± 0.5°C.[1,2]

Coating Application	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 22
Total Phenol Content								
TPC Control	864.29 ± 15.62$_a$	783.33 ± 7.93$_b$	821.43 ± 3.31$_b$	588.89 ± 11.73$_c$	655.56 ± 16.83$_c$	775.40 ± 10.59$_c$	726.19 ± 11.43$_c$	686.51 ± 17.36$_c$
TPC Coated	992.86 ± 55.38$_a$	806.35 ± 79.76$_b$	862.70 ± 51.20$_b$	834.13 ± 4.96$_c$	888.89 ± 11.00$_c$	707.94 ± 17.87$_c$	752.38 ± 34.59$_c$	719.05 ± 54.08$_c$
Radical scavenging capacities								
RSC Control	91.80 ± 0.54$_a$	93.23 ± 0.39$_b$	92.47 ± 0.00$_b$	93.20 ± 1.47$_b$	93.30 ± 1.27$_b$	95.92 ± 0.01$_b$	92.48 ± 0.51$_b$	94.10 ± 0.31$_b$
RSC Coated	89.46 ± 0.49$_a$	93.32 ± 0.39$_b$	90.85 ± 2.88$_b$	92.67 ± 0.52$_b$	93.57 ± 1.77$_b$	92.00 ± 1.42$_b$	92.77 ± 0.61$_b$	94.67 ± 0.38$_b$
Total anthocyanin content								
TAC Control	165.65 ± 9.63$_a$	151.18 ± 4.29$_b$	123.79 ± 2.15$_c$	122.68 ± 2.35$_d$	125.80 ± 2.35$_d$	98.41 ± 1.02$_e$	109.55 ± 5.94$_e$	100.19 ± 1.16$_f$
TAC Coated	158.53 ± 4.45$_a$	144.95 ± 2.41$_b$	139.60 ± 6.12$_c$	125.35 ± 3.43$_d$	111.33 ± 1.54$_d$	99.08 ± 1.54$_e$	92.77 ± 3.36$_e$	85.05 ± 1.39$_f$

1 For each parameter, similar small letters (subscript) in rows are not significantly different at ($p \leq 0.05$).
2 All given data are the mean ± standard deviation of three replicates (n=3)

3.9 Effect of Chitosan Coating Treatment on Microbial Quality of Pomegranate Arils

The microbiological quality of pomegranate arils coated with and without chitosan during 22 days of storage period is shown in Table 3.4.

As can be seen from the Table 3.4, total mesophilic aerobic counts of both uncoated and samples were under detectable limits up to 16^{th} day of storage. The viable mesophilic aerobic counts of uncoated samples increased from 4.21 and reach 6.44 log CFU/g at the end of the 22 days of storage. The chitosan coated arils' mesophilic aerobic count increased from 3.01 and reach 3.80 log CFU/g at the end of the storage. The maximum aerobic bacteria limit established by Spanish legislation is 7 log CFU/g (Lopez-Rubira, Conesa, Allende and Artes, 2005). Even at the end of the storage, the given threshold limit for total mesophilic aerobic bacteria was not exceeded for both coated and uncoated arils. Chitosan coating on arils significantly inhibited microbial growth.

Chitosan coating application is significantly inhibited also the yeast and mold counts of the pomegranate arils ($p \leq 0.05$). The yeast and mold counts of uncoated samples were under detectable limits up to 13^{th} day of storage and the counts increased from 3.73 and reach 5.67 log CFU/g at the end of 22 days of storage. The chitosan coated arils' yeast and mold counts were undetectable until 16^{th} day of storage and increased from 2.73 and reach 4.49 log CFU/g at the end of the storage.

Chitosan has been reported as an effective edible coating material for controlling the microbial decay of litchi fruit (Zhang and Quantick, 1997), , papaya (Bautista-Banos, Hernandez-Lopez, Bosquez-Molina and Wilson, 2003; Hewajulige, Sivakumar, Sultanbawa, Wijeratnam and Wijesundera, 2006), fresh cut broccoli (Moreira, Roura and Ponce, 2011), cucumbers (E1-Ghaouth et al., 1991a), strawberries (E1-Ghaouth

et al., 1991b), peach, japanese pear and kiwifruit (Du *et al*., 1997) and apple fruit (Du, Gemma and Iwahori, 1998).

It has been shown that chitosan is a natural fungicide against variety of fungi (Benhamou, 1996). Ben-Shalom et al. (2003) and El Ghaouth et al. (1992) reported the chitosan is effective on inhibition of spore germination, germ tube elongation and radial growth. Chitosan is also extend the shelf life of fruits by decreasing decay (Reddy, Belkacemi, Corcuff, Castaigne and Arul, 2000; Bautista-Banos et al., 2003). The mechanism behind the growth inhibition of chitosan has been proposed as positively charged chitosan's binding on negatively charged microbial surface leading agglutination. This mechanism result in leakage of proteinaceous and other intracellular components (Meng et al., 2008; Rabea et al., 2003). Another mechanism reported by Hadwiger, Kendra, Fristensky and Wagoner (1986) was the effect of interference between bacterial DNA with diffused hydrolysis products on protein synthesis and mRNA. The most important decay of pomegranate is the gray mold caused by *Botrytis cinerea.* Chitosan effectively damages the plasma membrane of B. *cinerea* by inhibiting spore germination, mycelial growth, germ tube elongation (Liu, Tian, Meng, and Xu, 2007). As chitosan forms a film on the arils surface, it protects the fruit from pathogens and reduces the decay during storage (Meng et al., 2008). Those study results support the present study results that suggested chitosan's effect on decreasing incidence of decay on pomegranate arils during storage. The arils were washed with chlorine solution before coating. The chlorine sanitization may also have beneficial effect on inhibition of decay.

Table 3.4 The effect of chitosan coating on microbial quality (log CFU/g of arils) of pomegranate arils during refrigerated storage at $4 \pm 0.5^{o}C$.[1,2,3]

Coating Application	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 22
Total Mesophilic Aerobic Counts								
TMAC Control	n.d.	n.d.	n.d.	n.d.	n.d.	$4.21 \pm 0.13_{b}$	$5.15 \pm 0.03_{c}$	$6.44 \pm 0.01_{d}$
TMAC Coated	n.d.	n.d.	n.d.	n.d.	n.d.	$3.01 \pm 0.13_{b}$	$3.45 \pm 0.03_{c}$	$3.80 \pm 0.06_{d}$
Total Yeast and Mold Counts								
TYMC Control	n.d.	n.d.	n.d.	n.d.	$3.73 \pm 0.07_{b}$	$4.14 \pm 0.07_{c}$	$5.00 \pm 0.08_{d}$	$5.67 \pm 0.05_{e}$
TYMC Coated	n.d.	n.d.	n.d.	n.d.	n.d.	$2.73 \pm 0.07_{b}$	3.73 ± 0.07c	$4.49 \pm 0.2_{d}$

1 For each parameter, similar small letters (subscript) in rows are not significantly different at ($p \leq 0.05$).
2 All given data are the mean ± standard deviation of three replicates (n=3)
3 n.d.: under the limit of detection

3.10 Effect of Chitosan Coating Treatment on Sensorial Quality of Pomegranate Arils

Table 3.5 indicates the effects of chitosan coating on the appearance and odor attributes and acceptance of arils during storage at $4 \pm 0.5^{o}C$. The acceptable score was set as score of 5 of above out of 9 for both appearance and odor parameters.

The chitosan coated and uncoated arils showed a significant decrease on sensory scores of appearance and odor with increasing storage time ($p \leq 0.05$) (Table 3.5). The sensorial results were in accordance with the results of the color, chemical and microbiological analysis.

The appearance scores of chitosan coated arils were significantly higher than that of uncoated arils within cold storage ($p \leq 0.05$) (Table 3.5). Even at the 22^{nd} day of storage chitosan coated arils' appearance scores were above the acceptable score where the appearances of uncoated arils were not acceptable starting from the 19^{th} day of storage. Chitosan coating delayed the drop on appearance quality. The similar results were also observed in color measurements. The images of both treated and untreated arils during storage were represented in Appendix C.

The odor scores of chitosan coated arils were significantly higher than the scores of uncoated arils throughout cold storage ($p \leq 0.05$) (Table 3.5). Until the 22^{nd} day of storage the odor scores were above the acceptable limit for both treated and untreated arils. At the 22^{nd} day of storage odor score of uncoated arils were under acceptable limits where the scores of coated arils were on the limit. The odor scores were started from a lower value when compared to maximum score for the 1^{st} day. The reason of lower odor scores of samples at the 1^{st} day may be attributed to the effect of acetic acid dipping treatments.

The beneficial effect of chitosan on sensorial quality of minimally processed fruits and vegetables were observed on other studies also. Pen et al. (2003) reported that chitosan treatment delayed development of discoloration and maintained the appearance of Chinese water chestnut. Dong et al. (2004) mentioned in their study that chitosan treatments delayed the decline in sensory quality of peeled litchi fruit Chien et al. (2007) emphasized in their study that chitosan coating delayed the drop in sensory quality and prevented surface cracking of sliced mango fruit (2007). Chien et. al. (2007) observed in their study delaying effect of chitosan coating on decrease in sensory quality of sliced red pitayas. Dang et al. (2010) reported in their study also that chitosan coating was effective on delaying the drop of sensory attributes of sweet cherries.

3.11 Shelf Life Determination of 1% Chitosan Coated Pomegranate Arils

The pomegranate arils were reported in literature as they can be stored for 21 days at $0^{\circ}C$, 18 days at $2^{\circ}C$ and 14 days at $5^{\circ}C$ if they are not contaminated and damaged (Kader, 2006).

The critical limit of weight loss was determined as 4-6 percent of total weight of minimally processed fruits (Kays, 1991). In the present study the given critical limit was not exceeded for both chitosan coated and uncoated arils during 22 days of storage.

The Codex Alimentarus Commision determined the minimum total soluble solids content of pomegranate as 11.2 percent (Anonymous, 2005). In the current study the TSS contents of both chitosan coated and uncoated arils were never reached to the given threshold values during 22 days of storage.

Table 3.5 The effect of chitosan coating on sensory evaluation of appearance and odor of pomegranate arils during refrigerated storage at $4 \pm 0.5^{o}C$.[1,2,3]

Coating Application	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 22
Appearance								
Appearance Control	$8.6 \pm 0.07_{a}$	$8.2 \pm 0.08_{b}$	$7.4 \pm 0.06_{c}$	$7.2 \pm 0.08_{d}$	$6.3 \pm 0.08_{e}$	$5.8 \pm 0.09_{f}$	$4.6 \pm 0.06_{g}$	$4.5 \pm 0.08_{g}$
Appearance Coated	$8.7 \pm 0.07_{a}$	$8.4 \pm 0.08_{b}$	$7.6 \pm 0.07_{c}$	$7.4 \pm 0.08_{d}$	$6.9 \pm 0.24_{e}$	$6.4 \pm 0.07_{f}$	$5.2 \pm 0.21_{g}$	$5.2 \pm 0.05_{g}$
Odor								
Odor Control	$8.1 \pm 0.05_{a}$	$7.9 \pm 0.05_{a}$	$7.8 \pm 0.05_{a}$	$7.8 \pm 0.05_{a}$	$6.6 \pm 0.06_{b}$	$6.3 \pm 0.05_{c}$	$5.3 \pm 0.05_{d}$	$4.9 \pm 0.05_{e}$
Odor Coated	$7.9 \pm 0.05_{a}$	$7.9 \pm 0.05_{a}$	$7.8 \pm 0.05_{a}$	$7.8 \pm 0.05_{a}$	$6.8 \pm 0.06_{b}$	$6.4 \pm 0.05_{c}$	$6.0 \pm 0.05_{d}$	$5.0 \pm 0.06_{e}$

1 For each parameter, similar small letters (subscript) in rows are not significantly different at ($p \leq 0.05$).
2 All given data are the mean ± standard deviation of five replicates (n=5)
3 The score under 5.0 was denoted as unacceptable for both analysis

Kader (1999) proposed the maximum titratable acidity as 1.4 percent for acceptable flavor quality. The given limit was not exceeded for both chitosan coated and uncoated arils through the 22 days of storage.

According to the Turkish Food Codex Communiqué on Determining the Maximum Levels of Certain Contaminants in Foodstuffs (2011) the limit for total mesophilic aerobic bacteria was given as 5 log CFU/g for fruits and vegetables and limit for total yeasts and molds was given as 4 log CFU/g. The threshold value given for total mesophilic aerobic bacteria was not exceeded within 22 days of storage for chitosan coated arils where it was exceeded after 16^{th} day of storage for uncoated arils. The given limit for total yeasts and molds were exceeded after 19^{th} day of storage for chitosan coated arils where the threshold value was exceeded after 15^{th} day of storage for uncoated arils.

In terms of sensorial analysis, the appearance scores were not acceptable after 16^{th} day of storage for uncoated arils and were acceptable for chitosan coated arils even at the end of the 22 days of storage. The odor scores were not acceptable after 19^{th} day of storage for uncoated arils and were within the acceptable limits during 22 days of storage for chitosan coated arils.

According to the given critical limits and best line fitting for the critical parameters the shelf life of uncoated arils were determined as 15 days where the shelf life of 1% chitosan coated arils were determined as 19 days under refrigerated ($4 \pm 0.5°C$) temperatures. The results showed that chitosan coating treatment extended the shelf life of pomegranate arils for 4 days.

CHAPTER 4

CONCLUSIONS

The aim of the present study was to observe influence of chitosan coating treatment on sensorial, physical, chemical and microbiological characteristics of pomegranate (*Punica granatum*) arils in refrigerated storage ($4 \pm 0.5^{o}C$). To that end, manually extracted pomegranate arils were treated with 0% (control) and 1% aqueous chitosan solutions and the change in some quality parameters were monitored during 22 days of refrigerated storage.

The results presented in this study showed that besides its negative effects on radical scavenging capacity and chromaticity parameters, chitosan is a viable alternative as a commercial edible coating on refrigerated ready-to-eat pomegranate arils. The use of chitosan may contribute to improve quality parameters of ready-to-eat pomegranate arils and have potential to prolong its shelf life. According to the study findings 1% chitosan coating application extended the shelf life of refrigerated pomegranate arils from 15 days to 19 days (an additional 4 days).

CHAPTER 5

RECOMMENDATIONS

The results indicated that by treating pomegranate arils with 1% chitosan coating, quality parameters can be maintained during postharvest refrigerated storage and thus senescence can be retarded. In addition to studied parameters, it is advisable to conduct further postharvest tests in order to assess feasibility of chitosan coatings' commercial usage. Further research is needed to compare the effects of different concentrations of chitosan coating on quality parameters of pomegranate arils in order to set an optimum coating concentration. It is also advisable to study on different biomaterials as edible coating on pomegranate arils for comparing their efficiency with chitosan. The designed color measurement system used in the present study should be also further studied in order to achieve more precise results which can be an alternative for conventional color measurement instruments.

REFERENCES

Abbasi, N.A., Iqbal, Z., Maqbool, M., Hafiz, I.A. (2009). Postharvest Quality of Mango (*Mangifera Indica L.*) Fruit As Affected by Chitosan Coating. *Pakistan Journal of Botany, 41*(1), 343-357.

Adams, L. S., Seeram, N. P., Aggarwal, B. B., Takada, Y., Sand, D., Heber, D. (2006). Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. *Journal of Agriculture and Food Chemistry, 54,* 980-985.

Akay, F. (2001). *Comparison of radical scavenging capacities of selected mediterranean foods (Published master's thesis).* Middle East Technical University, Ankara, Turkey.

Alighourchi, H., Barzegar, M., and Abbasi, S. (2008). Effect of gamma irradiation on the stability of anthocyanins and shelf-life of various pomegranate juices. *Food Chemistry, 110,* 1036–1040.

Al-Maiman, S.A., Ahmad, D. (2002). Changes in physical and chemical properties during pomegarnate (Punica granatum) fruit maturation. *Food Chemistry, 76,* 437-441.

Anonymous. (2005). *Codex general standard for fruit juices and nektars (CODEX STAN 247-2005)*. FAO/WHO Codex Alimentarius Commission, Rome.

Aranaz, I., Mengibar, M., Harris, R., Panos, I., Miralles, B., Acosta, N., Galed, G., Heras, A. (2009). Functional Characterization of Chitin and Chitosan. *Current Chemical Biology, 3,* 203-230.

Artes, F., Tudela, J.A., Gil, M.I. (1998). Improving the keeping quality of pomegranate fruit by intermittent warming. *Z Lebensm Unters Forsch A, 207,* 316-321.

Ayhan Z., Esturk O. (2009). Effects of modified atmosphere packaging (MAP) on the quality attributes and shelf-life of "ready-to-eat" pomegranate arils. *Journal of Food Science, 74,* 399-405.

Bai, R.K., Huang, M.Y., Jiang, Y.Y. (1988). Selective permeability of chitosan-acetic acid complex membrane and chitosan-polymer complex membrane for oxygen and carbon dioxide. *Polymer Bulletin*, *20*, 83.

Bajpai M, Pande A, Tewari, S.K., Prakash, D. (2005). Phenolic contents and antioxidant activity of some food and medicinal plants. *International Journal of Food Sciences and Nutrition*, *56*(4), 287-291.

Ball, J.A. (1997). *Evaluation of Two Lipid-Based Edible Coatings For Their Ability to Preserve Post Harvest Quality of Green Bell Peppers (Published master's thesis)*. Virginia Polytechnic Institute and State University. Blacksburg, Virginia.

Barone, E., Sottile, F., Caruso, T., Marra, F.P. (2000). Preliminary observations on some Sicilian pomegranate (Punica granatum L.) varieties. In: P. Melgarejo-Moreno, J.J. Martinez-Nicolas, J. Martinez-Tome (Ed.s) *Production, Processing and Marketing of Pomegranate in The Mediterranean Region: Advances in Research and Technology*, pp:137-141. Ciheam-Iamz, Zaragoza.

Bautista-Banos, S., Hernandez-Lauzardo, A. N., Velazquez-del Valle, M. G., Hernandez-Lopez, M., Ait Barka, E., Bosquez-Molina, E., et al. (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Protection*, *25*, 108–118.

BATEM, (n.d.). Dünyada Ve Türkiye'de Nar Yetiştiriciliği, Batı Akdeniz Tarımsal Araştırma Enstitüsü Müdürlüğü. Retrieved September 18, 2011, from http://www.batem.gov.tr/urunler/meyvelerimiz/nar/nar.htm.

Ben-Arie, R., Segal, N. and Guelfat-Reich, S. (1984). The maturation and ripening of the Wonderful pomegranate. *Journal of the American Society for Horticultural Science, 109*, 898-902.

Bendich, A., Philips, M., Tengerdy, R. (1998). *Antioxidant Nutrients and Immune Functions*. New York: Plenium Press.

Benhamou, N., 1996. Elicitor-induced plant defence pathways. *Trends in Plant Science, 1*, 233–240.

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 qualityof fresh cut banana. *Food Control. 20*, 508–514.

Bilişli, A., Çevik, D. (1997). Bazı Nar Çeşitlerinin Dondurularak Değerlendirilmesi Üzerine Araştırmalar. T.C. Tarım ve Köyişleri Bakanlığı Tarımsal Araştırmalar Genel Müdürlüğü. *TAGEM-GY-04-M-3, 52.*

Blendford, D. (1995). Colouring consumer's perceptions. *Food Ingredients and Analysis International*, *17*(2), 10-11.

Çeltikci, N.P. (2008). Pomegranate and Pomegranate Derivatives In Turkish Economics. *(Published master's thesis).* Akdeniz University. Antalya, Turkey.

Cemeroğlu, B. (Ed.). (2010). *Gıda Analizleri*. Ankara: Gıda Teknolojisi Derneği Yayınları.

Cemeroğlu, B., Artik, N., Erbaş, S. (1992). Extraction and composition of pomegranate juice. *Fluessiges-Obst*, *59*(6), 335-340.

Chien, P.J., Sheu F., Lin, H. R. (2007a). Coating citrus (Murcott tangor) fruit with low molecular weight chitosan increases postharvest quality and shelf life. *Journal of Food Chemistry, 100,* 1160–1164.

Chien, P.J., Sheu F., Lin, H. R. (2007b). Quality assessment of low molecular weight chitosan coating on sliced red pitayas. *Journal of Food Engineering, 79,* 736–740

Chien, P.J., Sheu, F.,Yang, F.H. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering, 78,* 225–229.

D'Aquino, S., Palma, A., Schirra, M., Continella, A., Tribulato, E.,La- Malfa S. (2010). Influence of film wrapping and fludioxonil application on quality of pomegranate fruit *Postharvest Biology and Technology*, *55*, 121-128.

Da Costa, C.T., Horton, D. and Margolis, S.A. (2000). Analysis of anthocyanins in foods by liquid chromatography, liquid chromatography–mass spectrometry, and capillary electrophoresis. The Journal of *Chromatography A, 881,* 403–410.

Dang Q.F., Yan J.Q., Li Y., Cheng X.J., Liu C.S., Chen X.G. (2010). Chitosan Acetate as an Active Coating Material and Its Effects on the Storing of *Prunus avium L. Journal of Food Science, 75* (2), 125-131.

De Reuck, K., Sivakumar, D., Korsten, L. (2009). Effect of integrated application of chitosan coating and modified atmosphere packaging on overall quality retention in litchi cultivars. *Journal of the Science of Food and Agriculture*, *89,* 915–920.

Devlieghere, F., Vermeulen, A., Debevere, J. (2004). Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables . *Food Microbiology*, *21*, 703–714.

Djioua, T., Charles, F., Freire, M., Filgueiras, H., Ducamp-Collin, M., Sallanon, H. (2010). Combined effects of postharvest heat treatment and chitosan coating on quality of fresh-cut mangoes (Mangifera indica L.). *International Journal of Food Science and Technology*, *45*, 849–855.

Dong, H., Cheng, L., Tan, J., Zheng, K., & Jiang, Y. (2004). Effect of chito san coating on quality and shelf life of peeled litchi fruit. *Journal of Food Engineerin*g, *64*, 355–358.

Dormandy, T. L. (1983). Approach to free radicals. *The Lancet, 8357,* 1010-1014.

Du, J., Gemma, H., Iwahori, S. (1997). Effects of chitosan coating on the storage of peach, Japanese pear and kiwifruit. *Japanese Society for Horticultural Science, 66*(1), 15-22.

Du, J., Gemma, H., Iwahori, S. (1998). Effects of Chitosan coating on the storability and on the ultrastructural changes of 'Jonagold' apple fruit in storage. *Food Preservation Science, 1,* 23-29.

Dzung, N.H., Dzuan, L. (n.d.). *The Role Of Sensory Evaluation In Food Quality Control, Food Research And Development: A Case Of Coffee Study*, HoChiMinh city University of Technology, Hanoi, Vietnam.

Echeverria, E., Valich, J. (1989). Enzymes of sugar and acid metabolism in stored Valencia organs. *Journal of the American Society for Horticultural Science*, *114*, 445-449.

El Ghaouth, A., Arul, J., Ponnampalam, R., Boulet, M. (1991a). Use of chitosan coating to reduce water-loss and maintain quality of cucumber and bell pepper fruits. *Journal of Food Processing and Preservation*, *15*, 359–368.

El Ghaouth, A., Arul, J., Ponnampalam, R. and Boulet, M. (1991b). Chitosan coating effect on storability and quality of strawberries. *Journal of Food Science, 56*, 1618–1620.

El Ghaouth, A., Arul, J., Grenier, J., Asselin, A. (1992). Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. *Phytopathology, 82*, 398–402.

Embuscado, M.E., Huber, K.C. (Ed.s). (2009). *Edible Films and Coatings for Food Applications*. New York, USA: Springer Science+Business Media, LLC.

Erkan Koç, B., Özkan, M. (2011). Utilization of Chitosan In Food Industry. *Gıda, 36*(3), 161-168.

Fadavi, A., Barzegar, M., Azizi, M.H., Bayat, M.. (2005). Physicochemical Composition of Ten Pomegranate Cultivars (Punica granatum L.) grown in Iran. *International Food Science and Technology, 11*(2), 113-119.

Falcao, A. P., Chaves, E. S., Falcao, L. D., Gauche, C., Barreto, P. L. M., Bordignon-Luiz, M. T. (2009). Rheological behavior and color stability of anthocyanins from Merlot (*Vitis vinifera L*.) and Bordô (*Vitis labrusca L*.) grapes in a jam model system. *Ciência e Tecnologia de Alimentos, 29*, 857-862.

FDA. (2007). Approximate pH of foods and food products. *US Food and Drug Administration Center for Food Safety and Applied Nutrition*. Retrieved January 11, 2012, from http://foodscience.caes.uga.edu.

Fernando, J.Ayala-Zavalaa, Wang, Y.S., Wang, Y.C. and González-Aguilar, A.G. (2004). Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *Lebensmittel*-Wissenschaft und-Technologie, *37*, 87–695.

Gennadios, A., Weller, C.L. (1990). Edible films and coatings from wheat and corn proteins . *Food Technology, 44*, 63 – 69.

Ghasemnezhad, M., Shiri, M. A., Sanavi, M. (2010). Effect of chitosan coatings on some quality indices of apricot (Prunus armeniaca L.) during cold storage. *Caspian Journal of Environmental Sciences, 8*, 25-33.

Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B. Holcroft, D. M. and Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agriculture and Food Chemistry, 48*, 4581-4589.

Gilbert, D. L. (2000). *Fifty years of radical ideas.* Annals of the New York Academy of Sciences; 899(1).

Gölükcü, M., Tokgöz H. (2008). Some quality parameters in parameters in pomegranate juice of important pomegranates cultivars (Punica granatum) grown in Turkey. *Hasad Gıda, 23 (274),* 26-31.

Guo, C., Yang, J., Wei, J., Li, Y., Xu, J., Jiang, Y. (2003). Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research*, *23*, 1719-1726.

Hadwiger, L. A., Kendra, D. F., Fristensky, B. W., & Wagoner, W. (1986). Chitosan both activates genes in plants and inhibits RNA synthesis in fungi. In G. Muzarelli, C. Jeuniaus, & G. W. Graham (Eds.), *Chitin in nature and technology* (pp. 209–214). New York:Plenum Press.

Hakkinen, S.H., Torronen, A.R. (2003). Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: influence of cultivar, cultivation site and technique. *Food Research International*, *33*, 517–524.

Hardenburg, R.E. (1967). Wax and related coatings for horticultural products. A bibliography. *Agricultural Research Service Bulletin*, USDA, Washington, DC, 51–15.

Hardy, S., Sanerson, G. (2010). Citrus maturity testing. *Primefact, 980*, 1-6.

Hewajulige, I.G.N., Sivakumar, D., Sultanbawa, Y., Wijeratnam R.S.W., Wijesundera, R.L.C. (2006). Effect of Chitosan Coating on Postharvest Life of Papaya (Carica papaya L.) var. Rathna Grown in Sri Lanka. *Tropical Agriculture Research.,18*, 135-142.

Hoffman, A.F. (1916). U.S. Patent No. 19,160,104. Washington, DC: U.S. Patent and Trademark Office.

Huis in't Veld, J.H.J. 1996. Minimal Processing of Foods: Potential, Changes and Problems. A paper presented to the EFFoST Conference on the Minimal Processing of Food, Cologne, 6–9 November.

Hunter, R. S. (1975). Scales for measurements of color differences. In Measurement of Appearance. *Wiley-Interscience*, NY, p. 133.

Jiang, Y.,Li, Y. (2001). Effect of chitosan coating on the post harvest life and quality of longan fruit. *Journal of Food Chemistry, 73*, 139-143.

Jitareerat, P., Paumchai, S., and Kanlayanarat, S. (2007). Effect of chitosan on ripening enzymatic activity, and disease development in mango (Mangifera indica L.) fruit. *New Zealand Journal of Crop & Horticultural Science., 35*, 211-218.

Juang, R.S., Shao, H.J. (2002). A simplified equilibrium model for sorption of heavy metal ions from aqueous solutions on chitosan . *Water Resources, 36* (12), 2999 – 3008.

Kader, A.A. (1999). Fruit Maturity, Ripening, and Quality Relationships. *Acta Horticulturae (ISHS), 485*, 8.

Kader, A.A. (2006). Postharvest biology and technology of pomegranates. Chapter 14, in: Seeram, N.P. et al (eds). Pomegranates: ancient roots to modern medicine. CRC Press, Boca Raton, FL.

Kays, S.J. (1991). Metabolic processes in harvested products. *In: Postharvest. Physiology of Perishable Plant Products*. Van Nostrand Reinhold , New York , 75 – 142.

Kays, S.J., Paul, R.E. (2004). Stress in harvested products. In: *Postharvest Biology*. Exon Press Athens, GA, 355 – 414.

Kılıç, K., Ulusoy, B.O., Yıldırım, M., Boyacı, İ.H. (2007). Scanner-based color measurement in L∗a∗b∗ format with artificial neural networks (ANN). *European Food Research and Technology*, 226, 121-126.

Krochta, J.M., Baldwin, E.A., Nisperos-Carriedo, M.O. (1994). *Edible Coatings and Films to Improve Food Quality*. Lancaster , PA: Technomic Publishing Company, pp 1 – 379.

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

Kupper, W., Pekmezci, M., Henze, J. (1995). Studies on CA-storage of pomegranate (Punica granatum L., cv. Hicaz), *Acta Horticulture*, *398*, 101.

Lansky, E., Shubert, S., Neman, I., 1998, Pharmological and Therapeutic properties of pomegranate. I. *International Symposium of Pomegranate*, Orihuela (Alicante) Spain, 231-235.

Lansky, E.P., Newman, R.A. (2007). *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethno-Pharmacology*, *109*, 177-206.

Lee, J., Durst, R. W., Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of the AOAC International, 88*, 1269–1278.

Leon, K., Mery, D., Pedreschi, F., Leon, J. (2006). Color measurement in L*a*b* units from RGB digital images. *Food Research International, 39*, 1084-1091.

Liu, J., Tian, S. P., Meng, X. H., Xu, Y. (2007). Control effects of chitosan on postharvest diseases and physiological response of tomato fruit. *Postharvest Biology and Technology*, *44*, 300–306.

Lowings, P.H., Cutts, D.F. (1982). The preservation of fresh fruit and vegetables. *Proc. Institute* of *Food Science* and *Technology Annu. Symp*., Nottingham, UK.

Macheix, J.J., Fleuriet, A. and Billot, J. (1990). Fruit phenolics. Florida: *CRC Press, Inc.*

Malik, A., Mukhtar, H. (2006). Prostate cancer prevention through pomegranate fruit. *Cell Cycle, 5*, 371-373.

Malmiri, J., Osman, H., Tan, A., C.P., Abdul Rahman, R. (2011). Development of an edible coating based on chitosan-glycerol to delay 'Berangan' banana (Musa sapientum cv. Berangan) ripening process. *International Food Research Journal, 18*(3), 989-997.

Martí, N., Pérez-Vicente, A., García-Viguera, C. (2000). Influence of storage temperature and ascorbic acid addition on pomegranate juice. *Journal of the Science of Food and Agriculture*, *82*(2), 217–221.

Martinez, J.J., Melgarejo, P., Hernandez, F., Salazar, D.M., Martinez, R., (2006). Seed characterisation of five new pomegranate (Punica granatum L.) varieties. Scientia Horticulturae, *110*, 241–246.

Melgarejo, P., Salazar, D.M., Artes, F. (2000). Organic acids and sugars composition of harvested pomegranate fruits. *European Food Research Technology*, *211*, 185-190.

Meng, X. H., Li, B. Q., Liu, J., Tian, S. P. (2008). Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage. *Food Chemistry, 106*, 501–508.

Miguel, G., Dundlen, S., Antunes, D., Neves, A., Martins, D. (2004). The effect of two methods of pomegranate juice extraction on quality during storage at 4°C. *Journal of Biomedicine and Biotechnology, 5*, 332–337.

Moreira M.R., Roura, S.I., Ponce, A. (2011). Effectiveness of chitosan edible coatings to improve microbiological and sensory quality of fresh cut broccoli. *Food Science and Technology*, *44* (10), 2335-2341.

Munoz P.H., Almenar, E., Valle, V.D., Velez, D., Gavara, R. (2008). Effect of chitosan coating combined with postharvest calcium treatment on strawberry (Fragaria x ananassa) quality during refrigerated storage. *Food Chemistry*, *110*, 428-435.

Muradoğlu, F., Balta, M.F., Özrenk, B.K., 2006, Pomegranate (Punica granatum L.) genetic resources from Hakkari, Turkey, *Research Journal of Agriculture and Biological Sciences*, *2(*6), 520-525.

Muşabak, C. (2008). *Effect Of Chitosan Coating And Modified Atmosphereackaging On Chemical Properties Of Atlantic Bonito (Sarda Sarda) Fillets (Published master's thesis).* Atatürk University Graduate School of Agriculture Faculty Department of Fishery Sciences, Erzurum, Turkey.

Nongtaodum S., Jangchud A. (2009). Effects of Edible Chitosan Coating on Quality of Fresh-Cut Mangoes (Fa-lun) During Storage. *Kasetsart Journal Natural Science,43*, 282-289.

Olivas, G.I., Barbosa-Canovas, G. (2009). Edible Films and Coatings for Fruits and Vegetables. In M.E. Embuscado, K.C. Huber (Ed.s), *Edible Films and Coatings for Food Applications* (pp.211-244). New York, USA: Springer Science+Business Media, LLC.

Onur, C., N. Kaska. (1985). Akdeniz bölgesi narlarının (Punica granatum L.) seleksiyonu (Selection of Pomegranate of Mediterranean region). *Turkish Journal of Agriculture and Forestry, D2, 9*(1), 25-33.

Özgen, M., Durgaç, O., Serçe S., Kaya, C. (2008). Chemical and antioxidant properties of pomegranate cultivars grown in mediterranean region of Turkey. *Food Chemistry, 111*(3), 703-706.

Park, S.I., Daeschel, M.A., Zhao, Y. (2004). Functional Properties of Antimicrobial Lysozyme–Chitosan Composite Films. *Journal of Food Science, 69*(8), 215-221.

Pavlath, A.E., Orts W. (2009). Edible Films and Coatings: Why, What, and How. In Edible Films and Coatings for Food Applications (p.5). New York,:Springer.

Pekmezci, M., Erkan, M. (n.d.). Pomegranate. *United States Department Of Agriculture, Beltsville Agriculture Research Center*. Retrieved on September 12, 2011, from http://www.ba.ars.usda.gov/hb66/113pomegranate.pdf.

Pen, L.T., Jiang, Y.M. (2003). Effects of chitosan coating on shelf life andquality of fresh-cut Chinese water chestnut. *Lebensmittel-Wissenschaft und-Technologie, 36,* 359-364.

Perez-Vicente, A., Serrano, P., Abellan, P., Garcia-Viguera, C. (2004). Influence of packaging material on pomegranate juice colour and bioactive compounds during storage. *Journal of the Science of Food and Agriculture, 84*, 639–644.

Peryam, D.R., Pilgrim, F.J. (1957). Hedonic Scale Method of Measuring Food Preferences. *Food Technology, 11*, 9-14.

Poyrazoğlu, E., Gökmen, V., Artık, N. (2002). Organic acid and phenolic compounds in pomegranates (Punica granatum L.) grown in Turkey. *Journal of Food Composition and Analysis, 15*(5), 567-575.

Rabea, E. I., Badawy, M. E. T., Stevens, C. V., Smagghe, G., Steurbaut, W. (2003). Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules, 4*(6), 1457–1465.

Raffo, A., Leonardi, C., Fogliano, V., Ambrosino, P., Salucci, M., Gennaro, L., Bugianesi R., Giuffrida F., Quaglia G. (2002). Nutritional value of cherry tomatoes (Lycopersicon esculentum Cv. Naomi F1) harvested at different ripening stages. *Journal of Agricultural and Food Chemistry, 50*, 6550–6556.

Reddy, B. M. V., Belkacemi, K., Corcuff, R., Castaigne, F., Arul, J. (2000). Effect of pre-harvest chitosan sprays on post-harvest infection by Botrytis cinerea and quality of strawberry fruit. *Postharvest Biology and Technology*, *20*, 39–51.

Riberio, C., Vicente, A. A., Teixeria, J.A., Miranda, C. (2007). Optimization of edible coating composition to retard strawberry fruit senescence. *Postharvest Biology and Technology. 44*, 63–70.

Risbud M., Hardikar A., Bhonde R. (2000). Growth modulation of fibroblast by chitosan-polyvinyl pyrrolidone hydrogel: implications for wound management. *Journal of Bioscience, 25* (1), 147 – 159.

Ruzon, M.A. (2009). *RGB to LAB Code for Converting Images In MATLAB From RGB to CIELAB Color Space*. Retrieved August, 24, 2011, from http://ai.stanford.edu/~ruzon/software/rgblab.html.

Saira, I., Rathore, H.A., Majeed, S., Awan, S., Shah, S. Z. A. (2009). The studies on the physico-chemical and organoleptic characteristics of apricot (Prunus armeniaca L.) produced in Rawalakot, Azad Jammu and Kashmir during storage. *Pakistan Journal of Nutrition, 8* (6), 856-860.

Sanchez M., Larrauri J., Saura-Colixto P. (1998). A Procedure to Measure the Antioradical Efficiency of PolyPhenols. *Journal of the Science of Food Agriculture, 76*, 270-276.

Şengül, M., Yıldız, H., Güngör, N., Çetin, B., Eser, Z., Ercişli, S. (2009). Total Phenolic Content, Antioxidant and Antimicrobial Activities of Some Medicinal Plants. *Pakistan Journal of Pharmaceutical Sciences, 22*, 102-106.

Singleton, V. B., Rossi, J. A. (1965). Colorimetry of total phenolics with phoshomolybdic-phosphotungustic acid reagents. *American Journal of Enology and Viticulture, 16*, 144-158.

Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. *Methods in Enzymology, 299*, 152-178.

Somatilake, S., Chalmers, A.N. (2007). An Image-Based Food Classification System. *Proceedings of Image and Vision Computing New Zealand,* 260-265.

Sood, D.R., Dhindsa, K.S., Wagle, D.S. (1982). Studies on the nutritive value of pomegranate (Punica granatum). *Haryana Journal of Horticultural Sciences, 11*(3-4): 175-179.

TGKY. (2011). Turkish Food Codex. *T.C. Resmi Gazete*. December, 29 1997. Ankara. Sayı: 28157.

Ünal, C., Velioğlu, S., Cemeroğlu, B. (1995). Türk nar sularının bilesim ögeleri. *Gıda, 20*(6), 339-345.

USDA Nutrient Database for Standard Reference, Release 24. US Department of Agriculture, Agricultural Research Service; 1999. Nutrient Data Laboratory Home Page. Retrieved December 24, 2011, from http://ndb.nal.usda.gov/ndb/foods/show/2460.

Uzuner, S. (2008). Changes In Ellagic Acid Content And Total Antioxidant Activity During Different Production Methods And Storage Conditions Of Pomegranate Juice, (*Published doctorate thesis*), Hacettepe University, Ankara, Turkey.

Varasteh, F., Arzani, K., Barzegar, M., Zamani Z. (2012). Changes in anthocyanins in arils of chitosan-coated pomegranate (Punica gratanum L. cv. Rabbab-e-Neyriz) fruit during cold storage. *Journal of Food Chemistry, 130*, 267-272.

Vardin, H, Fenercioğlu, H. (2003). Study on the development of pomegranate juice processing technology: Clarification of pomegranate juice. *Nahrung, 47,* 300-303.

Vardin, H. (2000). Harran Ovasında Yetişen Değişik Nar Çeşitlerinin Gıda Sanayinde Kullanım Olanakları Üzerine Bir Çalışma. (*Published doctorate thesis*), Çukurova University Natural Sciences Institute, Adana, Turkey.

Wang, S.Y., Zheng, W., Galletta, G.J. (2002). Cultural system affects fruit quality and antioxidant capacity in strawberries. *Journal of Agricultural and Food Chemistry 50*, 6534–6542.

Waterhouse, A.L. (2001). *Determination of total phenolics, in current protocols in food analytical chemistry*, NJ: John Wiley and Sons Inc.

Wetzstein, H.Y., Zhang, Z., Ravid, N., Wetzstein, M.E. (2011). Characterization of Attributes Related to Fruit Size in Pomegranate. *Hortscience, 46*(6), 908-912.

Wiley, R.C. (1994). *Minimally Processed Refrigerated Fruits and Vegetables*. London, UK: Chapman & Hall.

Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt S.E., Prior, R.L. (2004). Lipophilic and Hydrophilic Antioxidant Capacities of Common Foods in the United States. *Journal of Agricultural and Food Chemistry, 52*, 4026-4037.

Wu, X., Gu, L., Holden, J., Haytowitz, D.B., Gebhardt, S.E., Beecher, G., Prior, R.L. (2004). Development of a database for total antioxidant capacity in foods: a preliminary study. *Journal of Agricultural and Food Chemistry, 17*, 407–422.

Yıldız, F. (1994). Initial Preparation, Handling, and Distribution of Minimally Processed Refrigerated Fruits. In R.C., Wiley, *Minimally Processed Refrigerated Fruits and Vegetables* (pp.15-49). London, UK: Chapman & Hall.

Yılmaz, C. (2007). Nar. *Hasad Yayıncılık*, 10-176.

Zhang, D., Quantick, P.Q. (1997). Effects of chitosan coating on enzymatic browning and decay during postharvest storage of litchi (Litchi chinensis Sonn.) fruit. *Postharvest Biology and Technology, 12*, 195-202.

Zhang, D., Quantick, P. C. (1998). Antifungal effects of chitosan coating on fresh strawberries and raspberries during storage. *The Journal of Horticultural Science and Biotechnology, 73,* 763–767.

APPENDIX A

PROTOTYPE IMAGE BASED COLOR MEASUREMENT SYSTEM

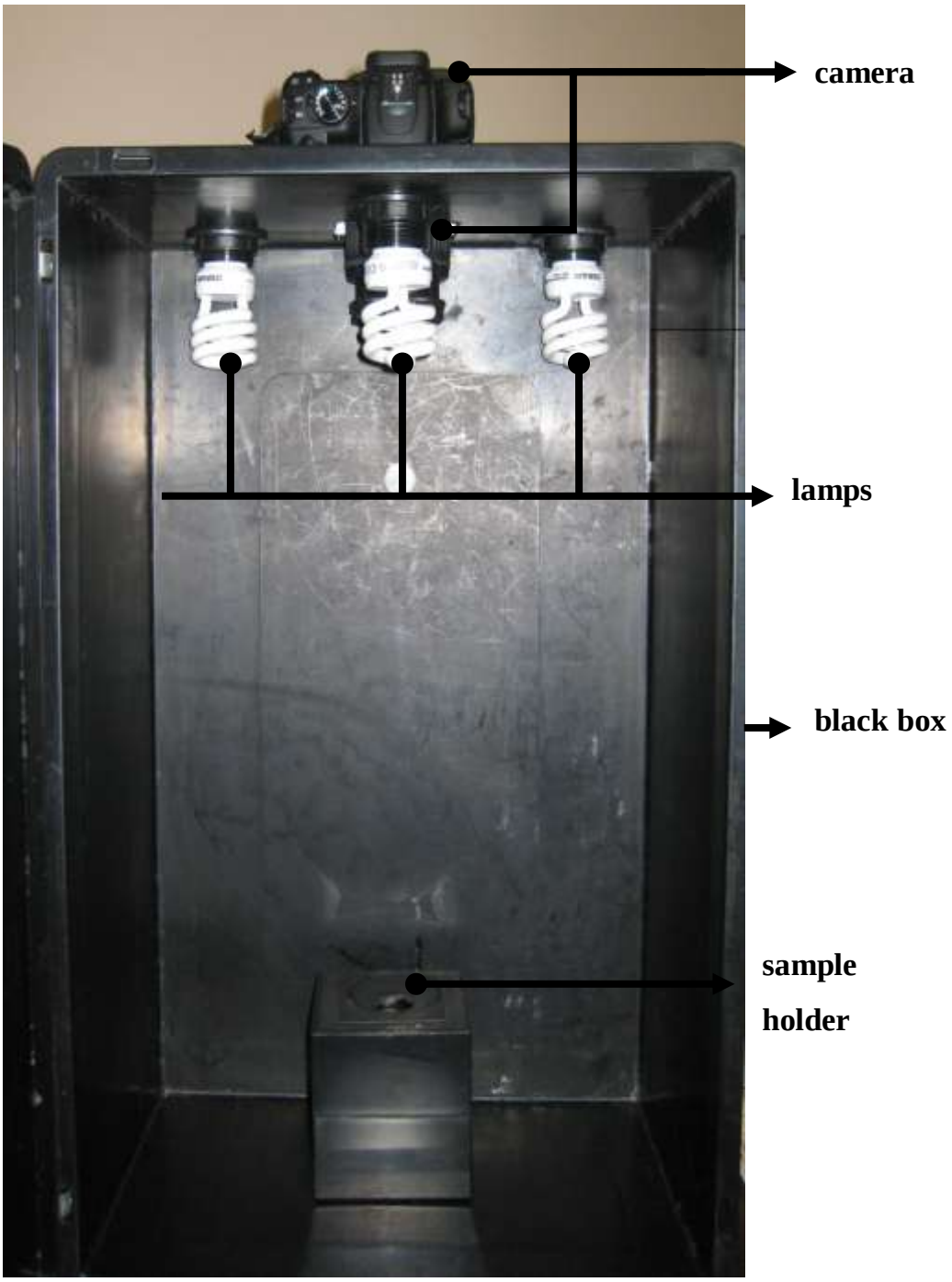

Figure A.1 Image acquisition system

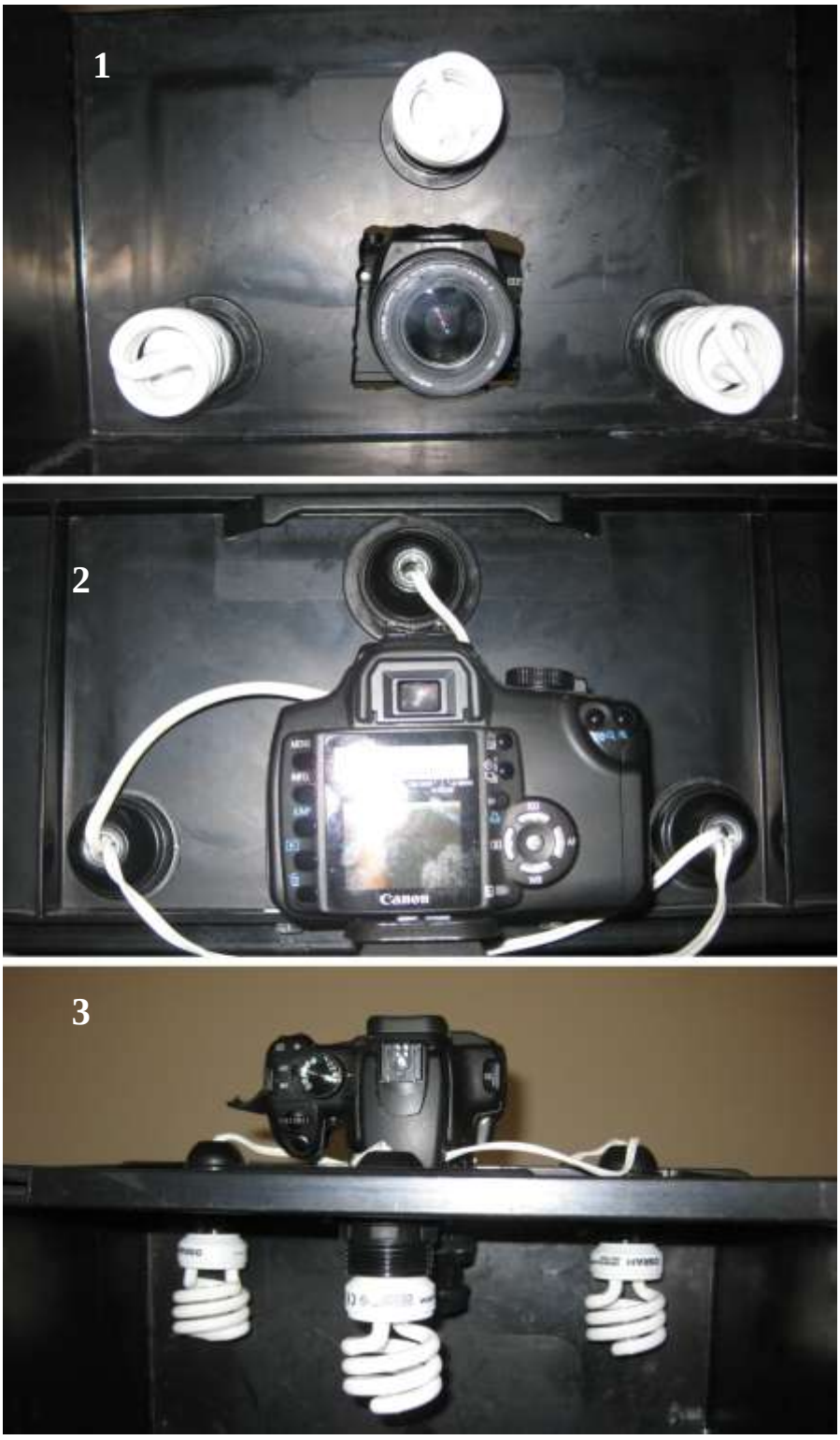

Figure A.2 Closer view of the image acquisition system: (1) inner view of the lamps and the objective of the camera (2) outer view of the camera (3) side view of the lamps and the camera

APPENDIX B

THE MATLAB CODES USED FOR COLOR MEASUREMENTS

B.1 The Matlab Code Used For Converting RGB Color Space To LAB Color Space

```
function [L,a,b] = RGB2Lab(R,G,B)
%RGB2LAB Convert an image from RGB to CIELAB
%
% function [L, a, b] = RGB2Lab(R, G, B)
% function [L, a, b] = RGB2Lab(I)
% function I = RGB2Lab(...)
%
% RGB2Lab takes red, green, and blue matrices, or a single M x N x 3 image,
% and returns an image in the CIELAB color space.  RGB values can be
% either between 0 and 1 or between 0 and 255.  Values for L are in the
% range [0,100] while a and b are roughly in the range [-110,110].  The
% output is of type double.
%
% This transform is based on ITU-R Recommendation BT.709 using the D65
% white point reference. The error in transforming RGB -> Lab -> RGB is
% approximately 10^-5.
%
% See also LAB2RGB.

% By Mark Ruzon from C code by Yossi Rubner, 23 September 1997.
% Updated for MATLAB 5 28 January 1998.
% Updated for MATLAB 7 30 March 2009.

if nargin == 1
  B = double(R(:,:,3));
  G = double(R(:,:,2));
  R = double(R(:,:,1));
end
```

```
if max(max(R)) > 1.0 || max(max(G)) > 1.0 || max(max(B)) > 1.0
  R = double(R) / 255;
  G = double(G) / 255;
  B = double(B) / 255;
end

% Set a threshold
T = 0.008856;

[M, N] = size(R);
s = M * N;
RGB = [reshape(R,1,s); reshape(G,1,s); reshape(B,1,s)];

% RGB to XYZ
MAT = [0.412453 0.357580 0.180423;
       0.212671 0.715160 0.072169;
       0.019334 0.119193 0.950227];
XYZ = MAT * RGB;

% Normalize for D65 white point
X = XYZ(1,:) / 0.950456;
Y = XYZ(2,:);
Z = XYZ(3,:) / 1.088754;

XT = X > T;
YT = Y > T;
ZT = Z > T;

Y3 = Y.^(1/3);

fX = XT .* X.^(1/3) + (~XT) .* (7.787 .* X + 16/116);
fY = YT .* Y3 + (~YT) .* (7.787 .* Y + 16/116);
fZ = ZT .* Z.^(1/3) + (~ZT) .* (7.787 .* Z + 16/116);

L = reshape(YT .* (116 * Y3 - 16.0) + (~YT) .* (903.3 * Y), M, N);
a = reshape(500 * (fX - fY), M, N);
b = reshape(200 * (fY - fZ), M, N);

if nargout < 2
  L = cat(3,L,a,b);
end
```

B.2 The Matlab Code Used For Selecting Region and Calculating Color Parameters

```
function [L, a, b] = nar(file)
numPoints = 10;
[a b] = strread(file, '%s %s', 'delimiter','.');
tifffile = strcat(a, '.tiff');
handle = figure;
image = imread(file);
            imshow(image);
            hold
for p2=1:3,
   labfile = strcat(a, '_');
   labfile = strcat(labfile, int2str(p2));
   labfile = strcat(labfile, '.txt');
   fd = fopen(char(labfile),'w');

   k = waitforbuttonpress;
   point1 = get(gca,'CurrentPoint');    % button down detected
   finalRect = rbbox;                   % return figure units
   point2 = get(gca,'CurrentPoint');    % button up detected
   point1 = point1(1,1:2);              % extract x and y
   point2 = point2(1,1:2);
   p1 = min(point1,point2);             % calculate locations
   offset = abs(point1-point2);         % and dimensions
   x = [p1(1) p1(1)+offset(1) p1(1)+offset(1) p1(1) p1(1)];
   y = [p1(2) p1(2) p1(2)+offset(2) p1(2)+offset(2) p1(2)];
   hold on
   axis manual
   plot(x,y)

   for i = p1(1):2:p1(1)+offset(1),
```

```
        for j = p1(2):2:p1(2)+offset(2),
            rgb = image(ceil(j), ceil(i), :);
            lab = RGB2Lab(rgb);
            deltae = sqrt((lab(:,:,1)*lab(:,:,1)) + (lab(:,:,2)*lab(:,:,2)) + (lab(:,:,3)*lab(:,:,3)));
            hue=atan(lab(:,:,3)/lab(:,:,2));
            chroma = sqrt((lab(:,:,2)*lab(:,:,2)) + (lab(:,:,3)*lab(:,:,3)));
            fprintf(fd, '%f %f %f %f %f %f\n', lab(:,:,1), lab(:,:,2), lab(:,:,3), deltae, hue, chroma);
        end
    end

    lab = load(char(labfile));
    fprintf(fd, '\n');
    mean(lab)
    fprintf(fd, '%f %f %f %f %f %f\n', ans(:,1), ans(:,2), ans(:,3), ans(:,4), ans(:,5), ans(:,6));
    std(lab)
    fprintf(fd, '%f %f %f %f %f %f\n', ans(:,1), ans(:,2), ans(:,3), ans(:,4), ans(:,5), ans(:,6));
end
print(handle, '-dtiff', char(tifffile));
fclose('all');
```

APPENDIX C

IMAGES OF POMEGRANATE ARILS DURING STORAGE

C.1 Images of Uncoated Arils During Storage

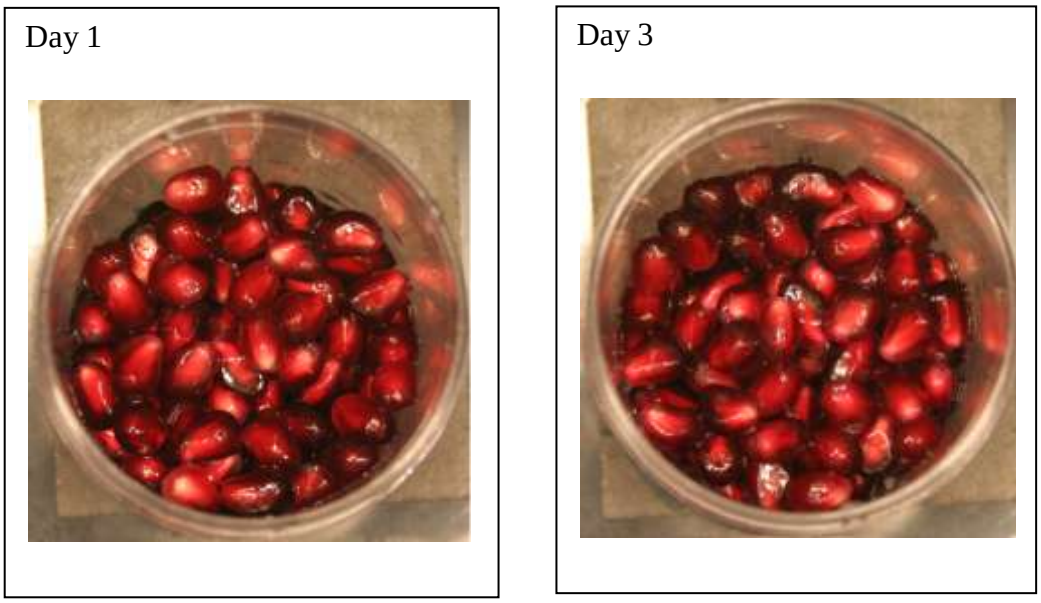

Figure C.1 Images of Uncoated Arils During Storage Days: 1,3

Figure C.1 (*Continued*) Images of Uncoated Arils During Storage Days: 5,7,9,11

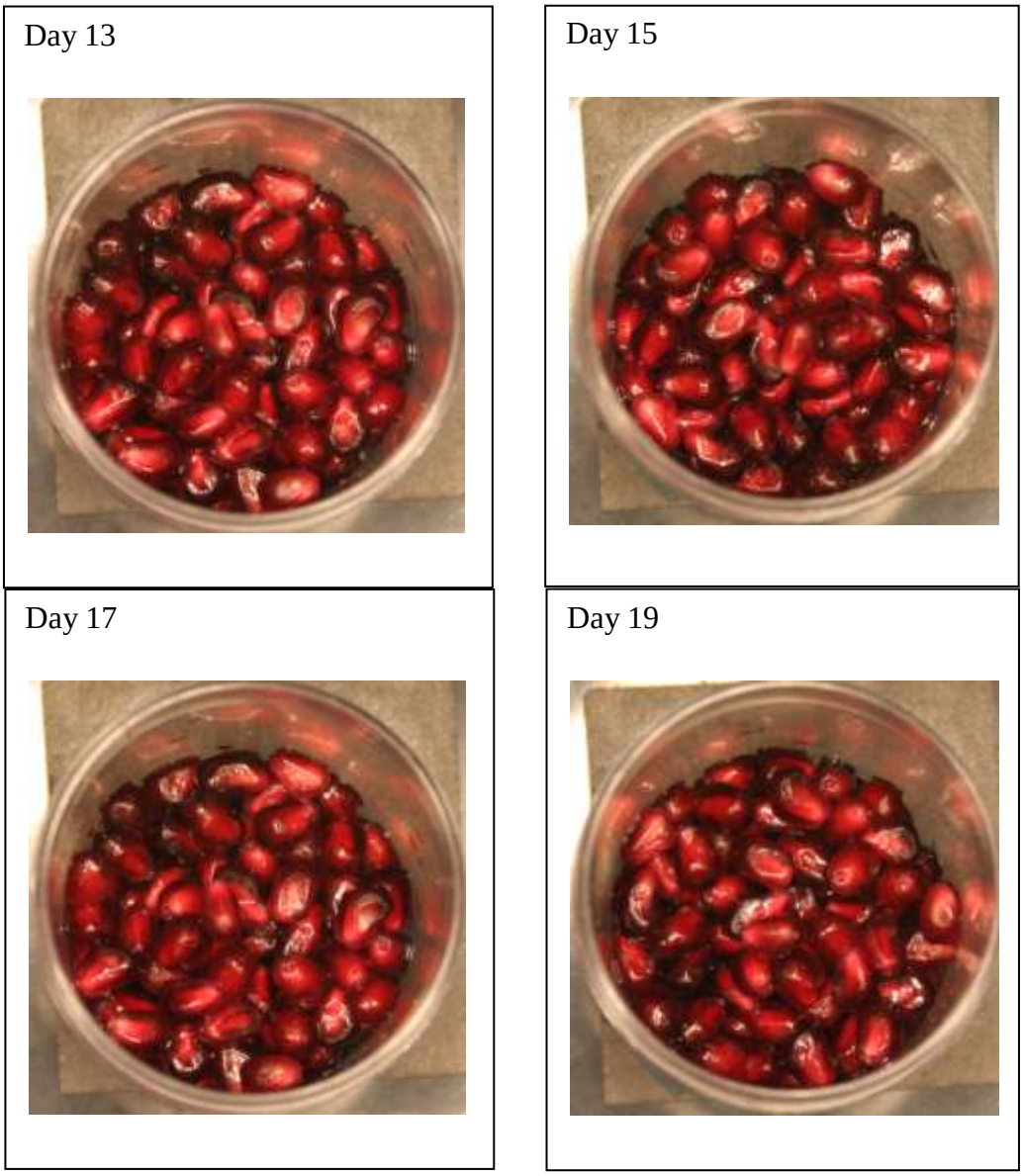

Figure C.1 (*Continued*) Images of Uncoated Arils During Storage Days 13, 15, 17, 19

Day 21

Figure C.1 (*Continued*) Images of Uncoated Arils During Storage Day: 21

C.2 Images of Chitosan Coated Arils During Storage

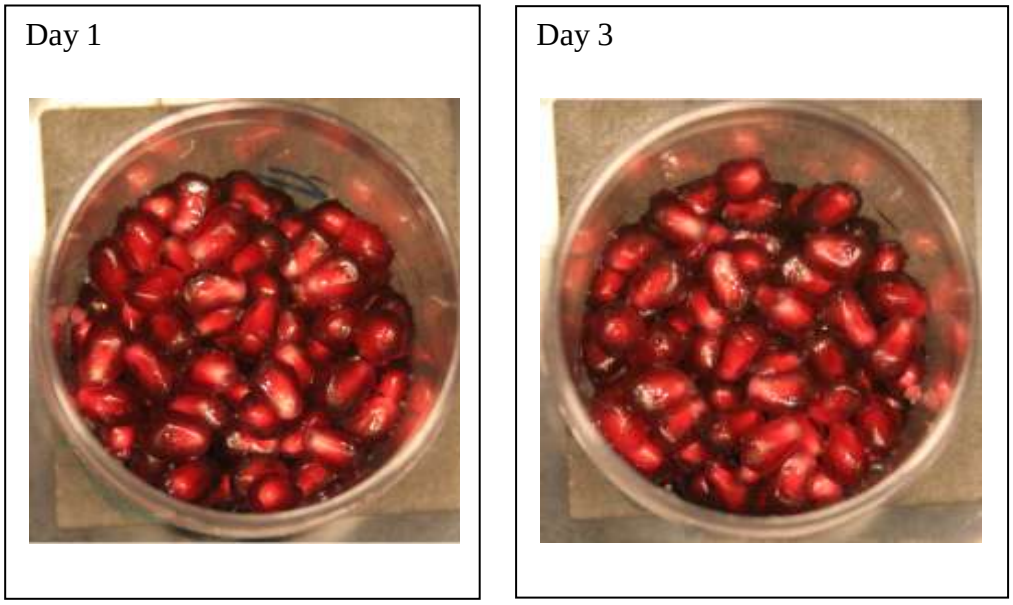

Figure C.2 Images of Chitosan Coated Arils During Storage Days: 1,3

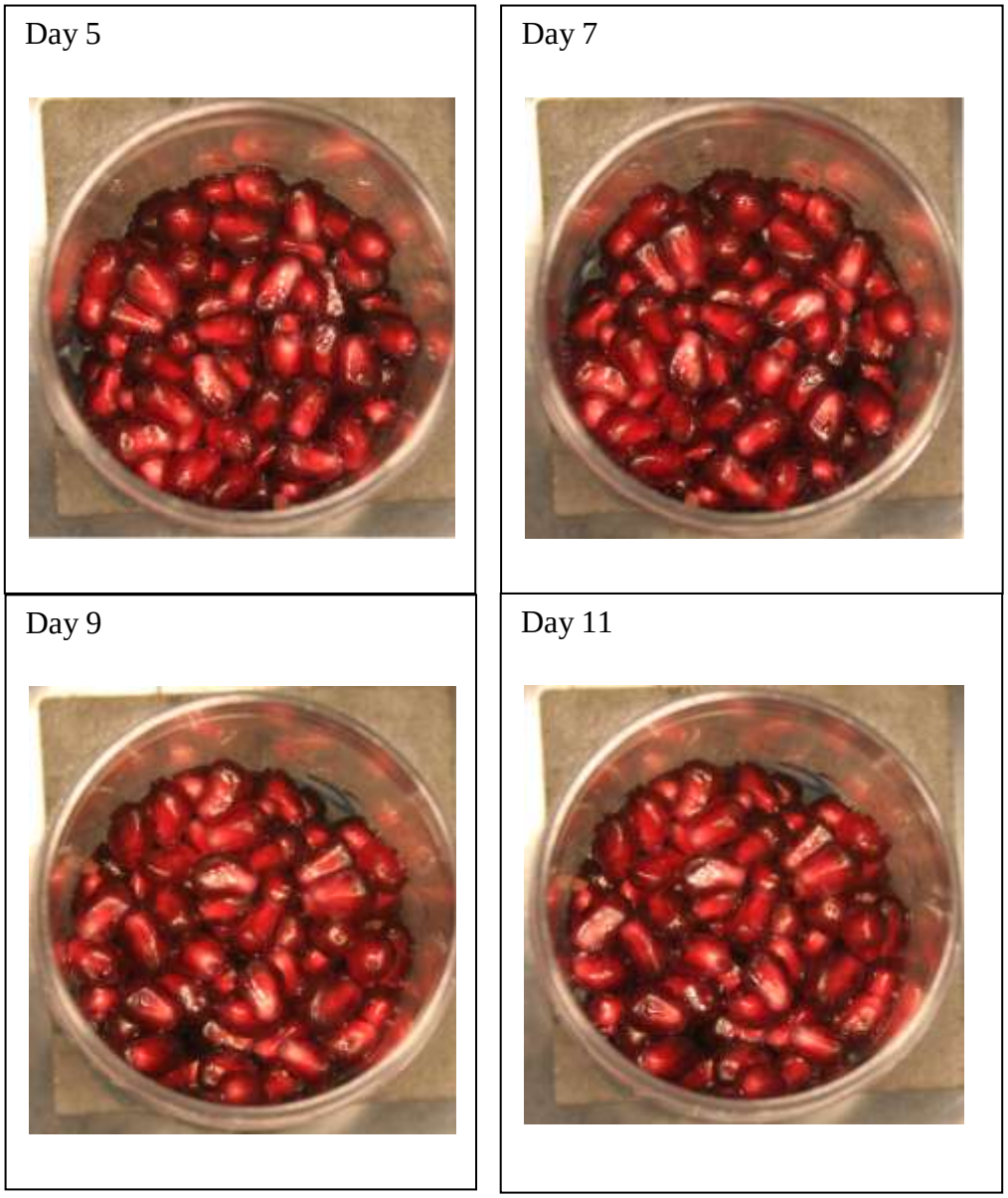

Figure C.2 (*Continued*) Images of Chitosan Coated Arils During Storage Days: 5, 7, 9, 11

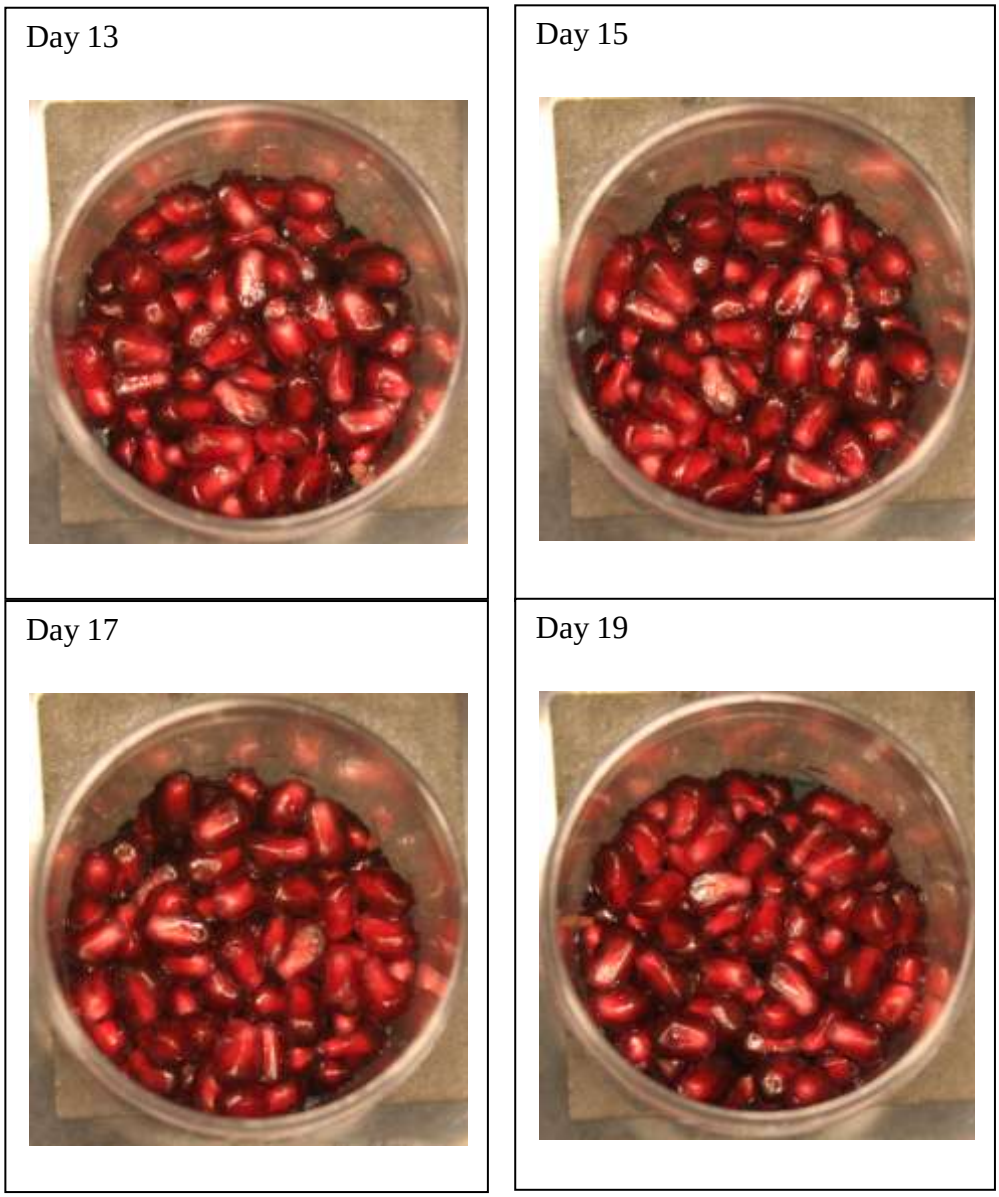

Figure C.2 (*Continued*) Images of Chitosan Coated Arils During Storage Days: 13, 15, 17, 19

Day 21

Figure C.2 (*Continued*) Images of Chitosan Coated Arils During Storage Day: 21

C.3 Comparison of Images of Chitosan Coated Arils Taken at the 1st and 21st Days of Storage

Chitosan Coated Arils Day 1	Chitosan Coated Arils Day 21
	

Figure C.3 Comparision of Images of Chitosan Coated Arils Taken At the 1st and 21st Days of Storage

C.4 Comparison of Images of Uncoated Arils Taken at The 1st and 21st Days of Storage

Uncoated Arils Day 1	Uncoated Arils Day 21
	

Figure C.4 Comparision of Images of Uncoated Arils Taken At the 1st and 21st Days of Storage

C.5 Image Sampling Example Performed By Matlab

Figure C.5 Image Sampling Example Performed By Matlab