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MASTER THESIS

NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

TC
NİĞDE ÖMER HALİSDEMİR UNİVERSİTY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

EFFECTIVENESS OF CHITOSAN TREATMENT ON MAINTAINING QUALITY PARAMETERS IN RED GLOBE CULTIVAR (*Vitis vinifera* L.) DURING THE STORAGE PERIOD

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Supervisor
Assoc. Professor Dr. Mustafa ÖZDEN

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Rohullah QADERİ tarafından **Doç. Dr. Mustafa ÖZDEN'nin** danışmanlığında hazırlanan "**Effectiveness of chitosan treatment on maintaining quality parameters in Red Globe grape cultivar (*Vitis vinifera* L.) during the storage period**" adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü **Bitkisel Üretim ve Teknolojileri** Ana Bilim Dalı'nda Yüksek Lisans tezi olarak kabul edilmiştir.

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Doç. Dr. Murat BARUT
MÜDÜR V.

THESIS CERTIFICATION

I hereby declare that this thesis has been written by me and that, to the best of my knowledge and belief. All information presented as part of this thesis is scientific and in accordance with the academic rules. Any help I have received in preparing the thesis, and all sources used, have been acknowledged in the thesis.

Signature

Rohullah QADERI

SUMMARY

EFFECTIVENESS OF CHITOSAN TREATMENT ON MAINTAINING QUALITY PARAMETERS IN RED GLOBE CULTIVAR (*Vitis vinifera* L.) DURING THE STORAGE PERIOD

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In this study, the effects of chitosan (CT), with Salicylic acid (SA) on prolonging shelf life and keeping quality of Red Globe cultivar during storage at ± 0.5^{o}C with at ± 90–95% humidity were investigated. For this purpose, 1% CT and different concentrations of SA (1mM, 2mM) were applied on grapes. Fruit decay, weight loss, color, total soluble solids,pH, titratable acidity (TA), fruit flesh firmness and bioactive compounds such as total phenolic contents, total flavonoids, total anthocyanins, and total antioxidant capacity were analyzed followed by FRAP and DPPH assays for every 15 days intervals. At the end of storage period fruit decay was 3.196% in control group where the decay rate in CT+2mM SA 0.887% was found. At the end of storage weight loss in control, CT, CT+1mM SA and CT+2mM SA were 0.145%, 0.142%, 0.085% and 0.081%, respectively. There were no significant on total soluble solids and total phenolic contents. However, there were significant effects on fruit decay, weight loss, TA, pH, Chroma index, and fruit flesh firmness, which indicates quality parameters. On the other hand, Chitosan coating was also found promising treatments in phytochemical compounds and total antioxidant capacity.

Keywords: chitosan, salicylic acid, red globe, storage.

ÖZET

RED GLOBE ÜZÜM ÇEŞİDİNDE (*Vitis vinifera* L.) KİTOSAN UYGULAMASININ DEPOLAMA SÜRESİNCE KALİTE PARAMETRELERİ ÜZERİNE ETKİNLİĞİ

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Bitkisel Üretim ve Teknolojileri Anabılım Dalı

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Sunulan bu çalışmada, hasat sonrası Kitosan (KT) ve Salisilik Asit (SA) uygulamalarının depolama süresince Red Globe üzüm çeşidinin raf ömrü ve kalite özellikleri üzerine etkileri incelenmiştir. Bu amaç doğrultusunda, %1 KT ve farklı konsantrasyonlardaki SA (1 mM ve 2mM) kaplama solüsyonları salkımlara daldırma yöntemi ile uygulanmıştır. 60 günlük muhafaza süresi boyunca her 15 günde bir olmak üzere Meyve Bozulma Oranı (%), Ağırlık Kaybı (%), pH, Titreedilebilir Asitlik (TA), Meyve Eti Sertliği (N) ve ayrıca Toplam Fenolik Madde İçeriği, Toplam Flavonoid İçeriği, Toplam Antosiyanin Miktarı ile Toplam Antioksidan Kapasitesi gibi fitokimyasal analizler gerçekleştirilmiştir. Deneme sonucunda, Meyve Bozulma Oranı, Kontrol grubu meyvelerinde %3.196 olarak belirlenirken, KT+ 2mM SA uygulamasında bu oran %0.887 olarak belirlenmiştir. Ağırlık Kaybı (%) değerleri ise depolama süresi sonunda, sırasıyla %0.145 (Kontrol), %0.142 (KT), %0.085 (KT+ 1mM SA) ve %0.081 (KT+ 2mM SA) olarak bulunmuştur. Suda Çözünebilir Kuru Madde (SÇKM) ve Toplam Fenolik Madde İçeriği sonuçları arasında istatiksel olarak farklılık bulunmamaktadır. Fakat Ağırlık Kaybı, TA, pH, Kroma İndeksi ve Meyve Eti Sertliği özellikleri üzerinde kaplama uygulamaları arasındaki fark önemli bulunmuştur. KT kaplama uygulaması, depolama süresince meyve fitokimyasal bileşikleri ve ayrıca Toplam Antioksidan Kapasitesi üzerinde ümit var bir hasat sonrası uygulama olarak belirlenmiştir. T.A.

Anahtar Sözcükler: Kitosan, salisilik asit, red globe, depolama.

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SYMBOLS AND ABBREVIATION

Symbols	Description
g	Gram
mL	Milliliter
mM	MilliMolar
L	Litre
°C	Centigrade
pH	Alkalinity and acidity factor
v:v	Volume:volume
w:v	Weight:volume
%	Percentage
V	Volume
mm	Millimeter
mg	Milligram
nM	Nanomolar
µg	Microgram
N	Newton
Kg	Kilogram

Abbreviation	Description
MAP	Modified Atmosphere
GRAS	Generally Recognized as Safe
USFDA	United State Food and Drug as Safe
SA	Salicylic Acid
SPS	Sucrose Phosphate Synthase
TSS	Total Soluble Solids
TA	Titratable Acidity
TPP	Tri Polyphosphate
DPPH	2, 2-diphenyl-1-picrylhydrazyl
CT	Chitosan

CE	Catechin Equivalents
FRAP	Ferric Reducing Power Assay
BHT	Butylhydroxytolune
LSD	Least Significant Difference
GAE	Gallic Acid Equivalent
FAO	Food and Agricultural Organization
Malvidin	Malvidin-3-O-Glucoside
FW	Fresh Weight

CHAPTER I

INTRODUCTION

Grapes are one of the most popular among the commonly featuring fresh fruits. Grapes have been widely regarded as "fruit queens" since ancient times. These small European and Mediterranean origin berries are the source of many nutritional health boosters such as polyphenolics antioxidants, vitamins, and minerals. No wonder why most of us often include them in our diet. Every year more than 27million tons (FAO, 2016) is the production of table grapes in the world, and Turkey is one of the top producing countries in the world with a production amount of 2 million ton (FAO, 2016).

Table grapes are highly perishable non-climacteric. Quality of table grapes is mainly based on their chemical contents (sugar contents, taste, flavor, nutritional value and etc.) or physical characteristics (texture, appearance) or composition of these two factors.
Grapes post-harvest decay can be because of physical, physiological or pathological factors that may happens in pre-harvest or in post-harvest (Shiri et al., 2013); and its shelf life is diminishing by firmness loss, berry drop, discoloration of the stem, desiccation and fungal rots(Sousa et al., 2013).

The use of sulfur dioxide (SO_2) during cold storage of grapes either by fumigation or generators is the most common commercial method for keeping quality after harvest (Crisosto et al., 2002). Despite of its effect in decay controlling and prevent stem browning, the application of SO_2 has been restricted in most countries. Residues of SO_2 is very noxious for fresh fruits and vegetables, because it causes phyto-toxicity symptoms, including bleach of the berries and browning the rachis (Smilanick et al., 1990). As an alternate to SO_2, use of the modified atmosphere packing (MAP) technique for table grapes has been recorded (Hernandez.A et al., 2006). For storability of perishable fruits and vegetables application of a semi permeable coating with modified atmosphere of CO_2/O_2, under small scale storage conditions has been shown good results (Valle et al., 2005). For maintain quality, use of edible coatings are one of the best methods. Edible coatings have been commonly used to improve food appearance and preserve the quality due to their environmentally friendly role (Vu, K. Detal, 2010; Khwaldia et al., 2004).They could work as obstacles to moisture and oxygen during processing,

transportation and storage (Vu et al., 2010: Xu et al., 2007). In addition, they could postpone food decay by inhibiting the growth of microorganisms due to their natural activity or combination of antimicrobial compounds (Vu et al., 2010; Cha and Chinnan, 2004). Edible coating usually made up of proteins and polysaccharides that can also help to control moisture, by that improve shelf life. They help to preserve perishable food crops from decay by deferring dehydration, suspending respiration, boost textual quality, aid to maintain unstable flavor compounds and decreasing microbial growth (Debeaufort et al.1998).Today many studies about chitosan coating usually focus on varieties of fruits and vegetables or mixture coating based on chitosan (Jiangilan and Shaoying, 2013).Chitosan is a deactylated derived from chitin, it is a high molecular-weight cationic linear polysaccharide which composed of D-glucosamine and, to lesser extent, N-acetyl-D-glucosamine with B-1, 4-linkage (Rinaudo, 2006; Shahidi, 2007; Baez sanudo et al., 2009; Petriccione et al., 2015; Romanazzi et al., 2017). Chitosan is a main component of the shells of crustacean such as crab, shrimp, crawfish (Romanazzi et al., 2017), exoskeletons of shellfish or the cell wall of some microorganisms and fungi (Hirano et al., 1976). Chitosan coatings are the best edible and biological protective coatings for different types of foods, because of their lack of toxicity (Jayakumar et al., 2005), biodegradability (Arvanitoyannis, 1999), film form properties (Arvanitoyannis et al., 1998), antimicrobial action (No et al., 2007; Aider, 2010; Yang et al., 2012) and also modify easy by physical and chemical methods (Le Tien et al., 2003). In additions several investigators found that a chitosan coating has potential to prevent decay and therefore extent the storage life of fruits and vegetables (Chien et al., 2007). Chitosan coating have been used to improve storage life and increase shelf life of some fruits like table grapes (Romanazzi et al., 2012; shiri et al., 2013), strawberry (VU et al., 2010; Wang and Gao, 2013), sweet cherry (Petriccione, 2015), litchi fruit, apple (El Ghaouth et al., 2000) and etc. Chitosan has been certified as a “Generally Recognized as safe” (GRAS) food additive by the United State food and Drug Administration (USFDA) (USFDA, 2013).Salicylic acid (SA) is endogenous signal molecule that play an important role in regulating stress responses and plant growth process, such as heat production or thermogenesis, stomatal guidance transpiration, ion observation and transport, disease resistance, seed germination, sexual polarization, yield crop and glycolysis (Klessing and Malamy, 1994; Asghari, M and Aghdam, M, S, 2010). Srivastava and Dwivedi, (2000) has reported that salicylic acid delaying ripening of fruit, maybe by restricting biosynthesis of ethylene or action and preserve postharvest quality. SA can be introduce

as an effective alternate to chemicals (Asghari, M and Aghdam, M, S, 2010). Asghari et al. (2009) mentioned that post-harvest treatment of table grapes with SA before chitosan coating significantly increased the effect of coating and decreased fruit decay. In other study Zeng et al. (2006) found that the level of hydrogen per oxide (H_2O_2) and rate of (O_2^-) generation after 8 days in SA-treated fruits were higher than controls.SA reaction leads to high level of (H_2O_2) accumulation in cells that persuades fruit resistance. Chilling injury is a kind of low temperature damage due to oxidative burst and SA treatment is an inexpensive, easy to set up and applicable to horticulture crops (Ding et al., 2001). Plants in response to abiotic stresses synthesis a group of proteins known as heat shock proteins. SA treatments induce heat shock protein prior to low temperature storage (Asghari, M and Aghdam, M, S, 2010). Fruit ripening is accompanied by change in several aspects of quality like softening, decrease color development, aroma production and etc. (Wills et al., 1998). Asghari, M and Aghdam, M, S (2010) reported that SA delays fruit ripening, and Zhang et al. (2003) demonstrated that SA decrease ethylene production and degrading cell wall and membrane enzymes and leads to decrease fruit softening rate. Total soluble solids (TSS) and soluble sugars increase during fruit ripening because of the action of sucrose phosphate synthase (SPS), which is a key enzyme in sucrose biosynthesis (Hubbard et al., 1994). SPS is activate by ethylene and ripening process itself while storage (Langenkemper et al., 1998). On other hand, cell walls have large amounts of polysaccharides, mainly pectins and cellulose, and are consumed because of the activity of the cell wall degrading enzymes which significantly increase in TSS content. SA dramatically protect cell walls by decreasing degrading enzymes, and as a result SA prevents from dramatic increase TSS content of cells. In recent years consumers interest in nutraceuticals or functional foods which are specific foods or physically active food components (Hasler, 1998). In plant foods phenolic compounds are widely distributed and because of that they are valuable part of human diet. Phenolic compounds might work as an antioxidant and maintain foods from oxidative deterioration. In current years, studying of determining antioxidant activity of phenolic compounds have increased because of the possible role of reactive oxygen species against pathogenesis of degenerative diseases such as cancer. In grapes, phenolic compounds contribute to the desired color, corpulence, astringency, flavor and vitamins; aid in oxido reduction and condensation reactions; and bactericidal features (Shiri et al., 2013).

The objective of this study is to examine the effect of chitosan coating on Red Globe grape cultivar. In addition, the effective of chitosan on quality parameters of grape berries nutritional quality, especially total phenolic contents and total anti-oxidant capacity, were determined.

CHAPTER II

LITRETURE REVIEW

The fast growing world population needs to produce healthy and adequate food, and the protection of produced food is also important. Food and Agriculture Organization (FAO, 2011) has reported that in developing countries 14% to 50% of fruit and vegetables produced on the overall rate is lost after harvest. Mainly it is because of microbiological decay (Kader, 2005). This percentage is extremely increased in developing countries, because of sufficient harvest and postharvest technologies of fruits and vegetables (FAO, 2011).

Fresh produce sensitivity to postharvest diseases and quality characteristics increases after harvest because of physiological and biochemical changes in the products (Romanazzi et al., 2017). Dutta et al. (2009) described that amount of fresh fruits and vegetables consumption increases on global scale due awareness of healthy eating in recent years. Lin and Zhao (2007) mentioned that postharvest losses in nutritional quality of fruits and vegetables are particularly reduction in vitamin C and polyphenols content, which occurs due to physiological changes during storage and decrease the health benefits from the consumption of fruits and vegetables. For this reason, the development of packing and storage technologies to increase shelf life and marketing period life of fruit and vegetables are one of the priorities for food industry (Han and Gennadius, 2005).

Edible coating is one of the most important development in recent years (Baldwin et al., 2011). Edible coating of fruit and vegetables during storage control the moisture loss, respiration rate, oxidation process and extend the shelf life. Edible coating could also give similar effect as modified atmosphere storage by modifying the internal gas composition (Kerch, 2015). Edible coating have showed positive effect on color (Xu et al., 2007), soluble solids content (Ali et al., 2011) and antioxidant capacity (Lin et al., 2008).

Chitosan based edible coating and films have been recently reviewed (Dhall, 2013; Shiekh et al., 2014; Kerch, 2015; Romanazzi et al., 2017) and it has been concluded that chitosan could effectively maintain the fruit and vegetables quality, and can prevent their

postharvest decay during storage and shelf life. Chitosan which is a polysaccharide and commonly could found in nature, and has become important day to day because of its biodegradability and its non-toxic structure. However, it is an indispensable edible coating for fruits and vegetables (Koç ve Özkan, 2011).

Chitosan coatings delay the respiration rate, decrease the weight loss and prolong the shelf life of fruit and vegetables during storage. The impact of chitosan coating on shelf life, microbiological quality and biochemical process during storage of fruit and vegetables have described in a number of recent publications (Kerch, 2015). Chitosan has filmogenic property and with this property minimize water and weight loss of fruits during storage (Bourliew et al., 2009).

The chitosan coating treatment minimized weight loss of stored apples, showed less respiration rate when it combined with heat treatment and significantly reduced pH and increased titratable acidity(TA) (Shao et al., 2012). Pears reduced their vital activities when it coated with chitosan during storage, which maintained the food quality and prolonged the shelf life. Compared with control group, chitosan coated pears showed reduced weight loss (Zhou et al., 2008). Chitosan-treated peaches than controls showed lower respiration rates and higher titratable acidity (Li and Yu, 2001).

Chitosan forms a coating on the surface of sweet cherries and effectively delayed water loss and promoted changes in titra table acidity and total soluble solids of sweet cherries (Dang et al., 2010). In other study Hernandez-munoz et al. (2008) treated the strawberries with chitosan and reported that chitosan reduced the weight loss and respiration rate of strawberries and added calcium to chitosan coating and found coated strawberries had higher titra table acidity and lower pH and TSS. Similarly weight loss reduction observed in table grapes when it coated by chitosan (Shiri et al., 2012) and Chitosan with grape seed extract (Xu et al., 2007).

In some other studies, chitosan maintained the firmness of table grapes during storage (Xu et al., 2007; Sanchez—Gonzalez et al., 2011). Chitosan coating maintain the firmness and color of table grapes during storage was reported (Shiri et al., 2012; Gao et al., 2013). Diaz-Mula et al. (2012) applied coating material based on sodium alginate at different concentrations (1%, 3%, 5% w/v) to cherry fruits. They reported that the coated fruits had

positive effects on the preservation quality criteria such as color, firmness, acidity and respiration rate. Furthermore, according to controls, coated groups had a positive effect on the total phenolic contents and total anti-oxidant capacity.
Chitosan+ bergamot oil and biocompatible hydroxyl cellulose coating applied on table grapes. During storage, weight loss, TSS, total phenolic contents, antioxidant activities, color and texture were determined and found that chitosan+ bergamot oil caused the lowest respiration rate and higher quality criteria during storage (Sanchez- Gonzalez et al., 2011).

(Shiri et al. (2013) coated the table grapes with 0.5% and 1% chitosan and then stored at 0^{o}C for 60 days and reported that changes of the total phenolic contents, total flavonoids contents and total antioxidant capacity of the chitosan coated berries were delayed, while quercetin 3-galactoside and total quercetin were higher in the control group.

Salicylic acid can be introduce as an effective alternate to chemicals (Asghari, M and Aghdam, M, S, 2010). Asghari et al., (2009) said that post-harvest treatment of table grapes with salicylic acid before chitosan coating significantly increased the effect of coating and decreased fruit decay.

Asghari, M and Aghdam, M, S (2010) reported that salicylic acid delays fruit ripening, and Zhang et al. (2003) demonstrated that salicylic acid decreased ethylene production and degrading cell wall and membrane enzymes and leaded to decrease in fruit softening rate.

Perdones et al. (2012) applied chitosan-based coatings 3% (w / v) with lemon essential oil and 1% (w / v) concentration on strawberries and examined the effect of these coatings on some quality criteria during storage. As a result, they reported that there was no statistical difference between treated and non-treated grapes in respect of pH, acidity and quality of TSS quality parameters of chitosan based coating. In addition, researchers have shown that chitosan-based coating material containing lemon volatile oil reduces respiration rate during storage in strawberry (Perdones et al., 2012).

Al-Qurashi and Awad (2015) studied the effects of chitosan coating on the quality criteria, antioxidant capacities, antioxidant compounds and some related enzyme activities at 1%,

1.5 and 2% chitosan concentrations in "El-Bayadi" table grape. As a result of the study, only 1% chitosan coating had positive effects on weight loss, whereas the effect of applications on quality criteria such as TSS, TA and pH was not significant. While the total phenolic content decreases with the applied chitosan concentration, the total flavonoid and ascorbic acid content increases. In addition, researchers have shown that the antioxidant capacity of coated grapes is higher than the antioxidant capacity of controls.

Koçak and Honey (2017) on '0900 Ziraat' cherry cultivar were used in separate and combined combinations of MAP, UV-C , alginate (1% w / v) and chitosan (1% w / v) coating application, and determined fruit quality during storage period. As a result of the study, it has been found that applications had positive effects on different levels of quality criteria in the cherry during storage. In particular, combinations of edible coatings with UV-C maintain the phytochemical properties in fruit during storage (Koçak and Bal, 2017).

Velickova et al. (2013) examined the effectiveness of four different coating formulations during shelf life in strawberries. Strawberries were covered with chitosan, chitosan-beeswax, tri polyphosphate (TPP) and composite and then stored at 20 ° C for 30 days at 30-40 % conditions for 7 days. Weight loss, respiration rate, fruit skin color, flesh firmness, pH, TA and TSS were analyzed after storage. At the end of the study, the researchers stated that the chitosan coating had positive effects on all these quality parameters (Velickova et al., 2013).

Petriccione et al. (2015) applied chitosan at two different concentration (1% and 2%) in three strawberry cultivars such as Candonga Jonica and SabrinaResearchers have reported that chitosan treatments limited changes in total phenolic, anthocyanin, flavonoid contents and reductions in antioxidant capacity during storage.

CHAPTER III

MATERIALS AND METHOD

3.1 Materials

3.1.1 Plant material

'Red Globe' table grape clusters (*Vitis vinifera* L.) were grown according to standard cultural practices in a commercial vineyard located in Manisa, Turkey. Grapes were harvested from local grower's vineyard at the commercial maturity on 21 September 2017 and immediately transported to the laboratory of Ayhan Şahenk Agriculture and Technologies Faculty, Niğde Ömer Halisdemir University (Photo 3.1). The clusters were selected on the basis of uniform color, size, firmness and the absence of blemishes or diseases and were randomly distributed into batches.

a **b**

Photo 3.1. An image from the grower's vineyard (a) and Harvested Red globe (b)

3.1.2 Chemicals

Low molecular weight chitosan (SIGMA ALDRICH, Lot # STBG9041), SA and Modified Atmosphere packing (MAP) material (Life Pack Co.), produced for the preservation of grape berries, was obtained from the respective commercial companies. The pore size of the MAP material used was 0.016mm.

3.2 Method

3.2.1 Chitosan treatment, packing and storage

For experimental use, 1 % of Chitosan (CT), was prepared by dissolving in 0.5% (v/v) glacial acetic acid under continuous stirring. When it dissolved, the pH value of the chitosan solution was adjusted to 5.2 using 1 M NaOH (Photo 3.2). After that sterilized the grapes, were distributed into four groups. Each group had at least six clusters per treatment. Each treatment was repeated at least three times. The treatment groups were as follow;

1- Control (DDW)
2- CT
3- CT+ 1 mM Salicylic acid (SA)
4- CT+ 2 mM Salicylic acid (SA)

Photo 3.2. Coating solutions preparing

First grape clusters were dipped in sterile distilled water for five minutes as control, second group was dipped a solution containing chitosan, and third group was dipped into solution containing chitosan + 1mM SA plus five minute waiting, the last treatment group was dipped in to solution containing chitosan + 2mM SA along with five minute waiting. After treatments had done, all samples were dried under room temperature for 2 hours (Photo 3.3). Finally, the treated and controls clusters were packaged in modified atmosphere plastic boxes (MAP) to maintain the relative humidity (RH) at ± 90–95%, and finally they were stored at ± 0.5^{o}C (Photo 3.4).

a b

Photo 3.3. An image during coating application (a), and drying the coated grape cluster on the filter papers (b)

a b

Photo 3.4. An image from cold storage (a) and an image from packed sample with MAP during storage (b)

During cold storage period of 60 days, after each 15 days, including the day of the sample, samples were taken out of the storage and kept at room temperature for one night to stimulate the shelf life. Following this study, for each application, the pomological measurements and phytochemical analyzes detailed below were performed after each 15 days for each application (Photo 3.5).

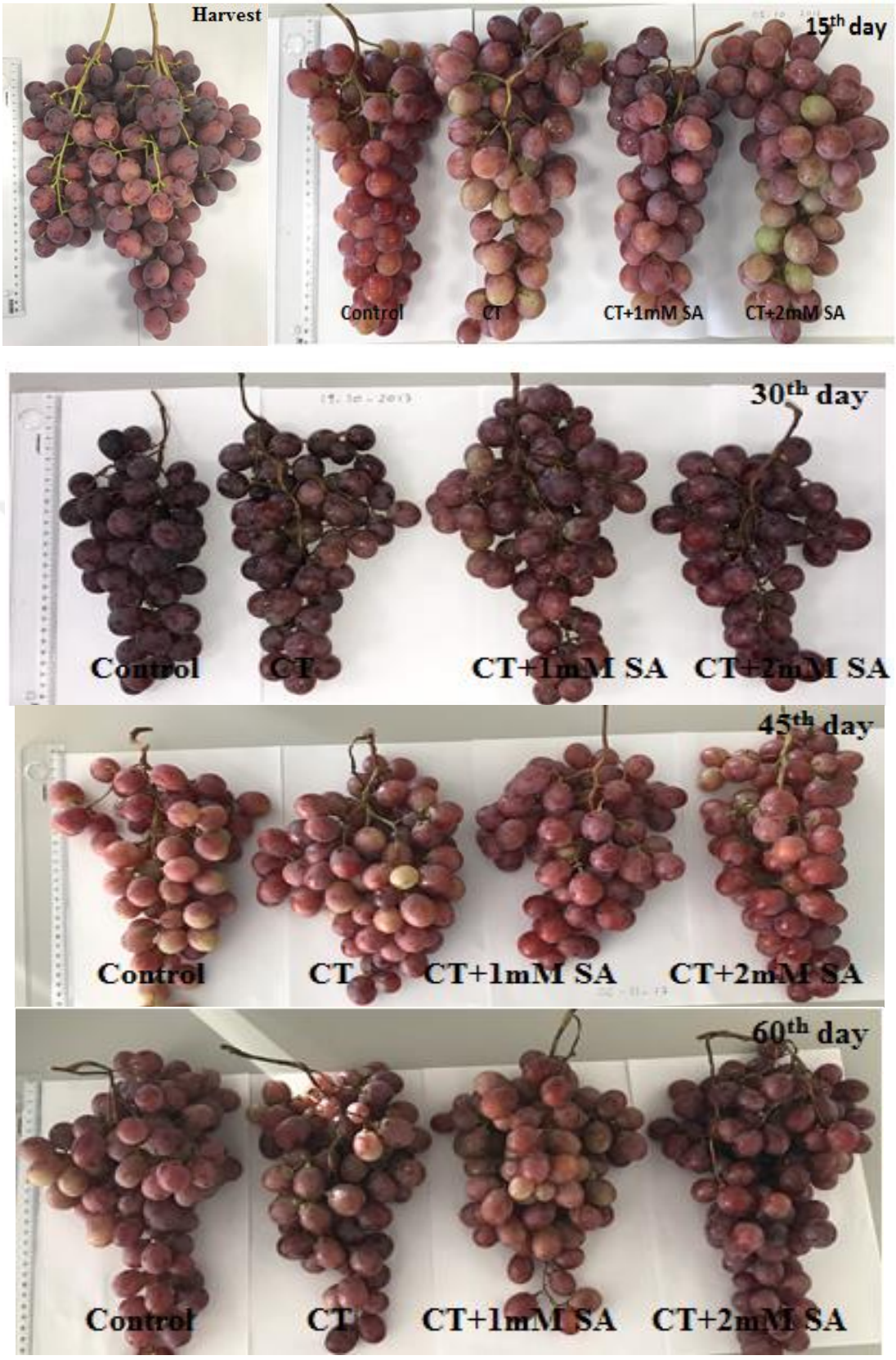


Photo 3.5. Samples taken out from the storage once after 15 days for 60 days

3.2.2 Fruit decay

Fruit decay of table grapes in each treatment during storage period was determined by counting the decay berries at 15 ,30, 45, 60 day at ±0.5℃, respectively and following 1 day shelf life intervals at room temperature. Fruit decay was calculated as percentage of decay berries from total berries.

3.2.3 Weight loss (%)

Weight loss of fresh table grapes in each treatment during storage were measured by monitoring weight changes of the fruit at 15, 30, 45, 60 d at ±0.5℃, respectively and following 1 d shelf life intervals at room temperature. Weight loss was calculated as percentage loss of initial weight like following formula.

Weight Loss (%) = {[Initial weight (g) -Weight (g)] / Initial Weight)}*100

3.2.4 Flesh firmness and fruit color

Flesh firmness was measured by fruit texture analyzer. It was read in g and then converted to Newton (N). Fruit color was measured with a Minolta CR-200 Chromo Meter (Minolta Camera Co., Osaka, Japan) in L* a* b* color space coordinates and then converted to Chroma values (C*) (Mcguire 1992).

3.2.5 Total soluble solids, pH, and titratable acidity

Total soluble solids (Brix) was measured by using a digital refractometer. Subsequently, the pH values of the fruit juices obtained by manually pressing 50 fruit juices and were measured using a pH meter.

10 g fruit tissues from ten grape berries were suspended in 100 mL of distilled water, mixed in a blinder. 0.1 N sodium hydroxide (NaOH) solution was added to the homogenized berry juice until to the endpoint of pH 8.2. The amount of NaOH used for titration was determined, and the amount of titratable acidity was calculated as % of tartaric acid.

3.2.6 Sampling and extraction

For sample extraction, berries were homogenized in an ice cold blender after removal of seeds and a 25 g of the homogenate were macerated in 100 mL of ethanol containing 0.1% HCl and set aside overnight in darkness. Then the extracts were filtered and the filtered extracts were centrifuged at 6000 rpm at 4 °C for 15 min. One part of the extracts was separated for determining anthocyanin content and the other remaining portions concentrated by a rotary evaporator at 50°C and were used for determining total phenolic, flavonoid contents, and antioxidant potential of samples.

3.2.7. Determination of total phenolic concentrations

Total phenolic concentrations of the extracts were determined by using a modified Folin-Ciocalteu colorimetric method (Slinkard and Singleton, 1977). Due to this method 0.02mL of extract was added to a 15mL measuring cylinder then 2.480 mL of (DDW) added. After that 0.2 mL folin was added and waited for eight minutes. There after 0.3 mL Na_2CO_3 was added to the mixture and waited for 60 minutes in room temperature and then the absorbance values of the samples were read at 750 nm by a spectrometer. Total phenolic content of samples was obtained from the calibration curve prepared with gallic acid (GAE) (20-250 mg/l) and expressed as mg GAE / kg of fresh weight.

3.2.8 Total flavonoid content

Total flavonoid concentration of the extracts were determined by the modified colorimetric method (Zhishen *et al.* 1999). According to this method, 0.25 mL of grape fruit extract or standard catechin solution (20, 50, 80, 100, 250 mg / L) was added to a 15 ml measuring cylinder and then 4.75 mL of DDW, 0.3 mL of 5% $NaNO_2$, the mixture was added 0.3 mL of 10% $AlCl_3$. Then, at the first minute, the mixture was added with 2 mL of 1 M NaOH, and the total volume was completed with 10 ml of DDW. The mixtures were thoroughly mixed and after 40 minutes, the absorbance of the samples was read at a wavelength of 510 nm in the spectrophotometer. Total flavonoid content was expressed as mg of catechin equivalents (CE) per kg g fresh weight.

3.2.9 Determination of total anthocyanin content

Total anthocyanin content of samples were determined by using the pH differential method (Giusti and Wrolstad 2005). Anthocyanin content of the fruit extracts were measured in a spectrophotometer (UV-visible spectrophotometer, Shimadzu 1601) at 510 and 700 nm with buffers at pH 1.0 and 4.5 (Wrolstad 1976; Giusti and Wrolstad 2005). All values were calculated as malvidin-3-O-glucoside using a molar extinction coefficient of 28.000.

3.2.10 Determination of antioxidant potential

Total antioxidant potential of the samples comprises simply antioxidant activity and antioxidant capacity. Total antioxidant activity was detected whereby DPPH radical scavenging assay and expressed as percentage of inhibition. However, total antioxidant capacity of samples were determined by the ferric reducing power assay (FRAP).
The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of different berry extracts were measured according to DPPH standard method (Blois 1958). IC_{50} values of the extracts i.e. concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

The total antioxidant capacity of samples were determined by the ferric reducing antioxidant power (FRAP) assay (Oyaizu 1986).FRAP assay is a measurement in reduction of Fe^{3+} $(CN-)_6$ to $Fe^{2+}(CN-)_6$, resulting in formation of the Perl's Prussian Blue complex following the addition of excess ferric ions (Fe^{3+}). One mL of grape extract (20 µg/mL) was mixed with 2.5 mL of 0.2 M (pH 6.6) phosphate buffer and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN_6)$]. The mixture was incubated at 50°C for 20 min, then rapidly cooled and mixed with 2.5 mL of 10% trichloroacetic acid then centrifuged at 1500 rpm for 15 min. An aliquot of the supernatant (2.5 mL) will be diluted with distilled water (2.5 mL) and then freshly prepared 0.5 mL of 0.1% $FeCl_3$ was added and allowed to stand for 10 min. The absorbance was measured at 700 nm. Butylhydroxytolune (BHT) (20-250 mg/mL) was used as standard for construction of the calibration curve and reducing power was reported as BHT equivalent per mg/mL of extract.

3.2.11 Statistical analysis

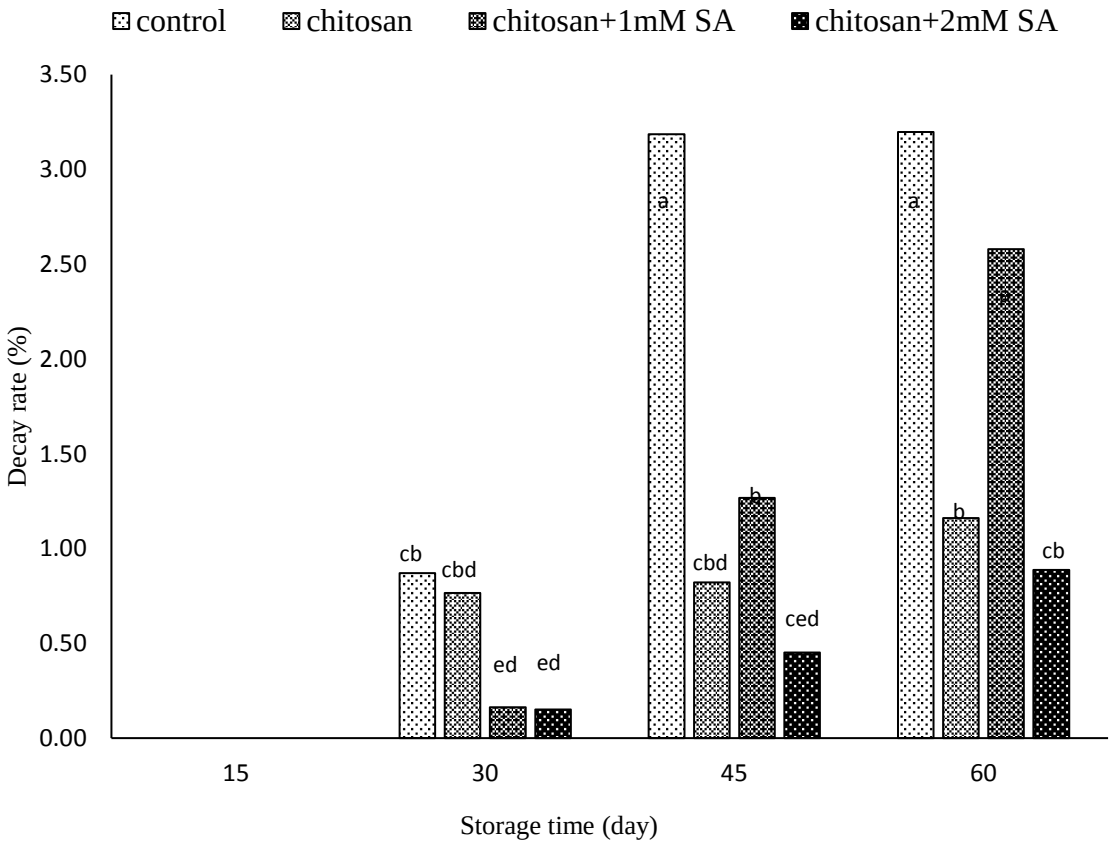
All data were analyzed using SAS V.9 statistical package program at $P \leq 0.05$ significance level with variance analysis followed by LSD multiple comparison test.

CHAPTER IV

RESULTS

4.1 Decay Rate

Fruit decay rate of grape fruits are shown in figure 4.1. Decay rate was increased by increasing storage time. Chitosan treatment significantly decreased the fruit decay rate. Furthermore, the interaction between chitosan treated berries and storage time was also significant ($P \leq 0.05$) as shown in table 4.1. Fruit decay rate was 0% in first 15days (Figure 4.1), in second 15 days fruit decay has started and after 60days the was more than 3.196% fruit decay in control group which in treatment groups were more less decay by 0.88% in CT+2mM SA.



The differences between fruit decay values were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4.1. Fruit decay (%) during storage at ±0.5°C

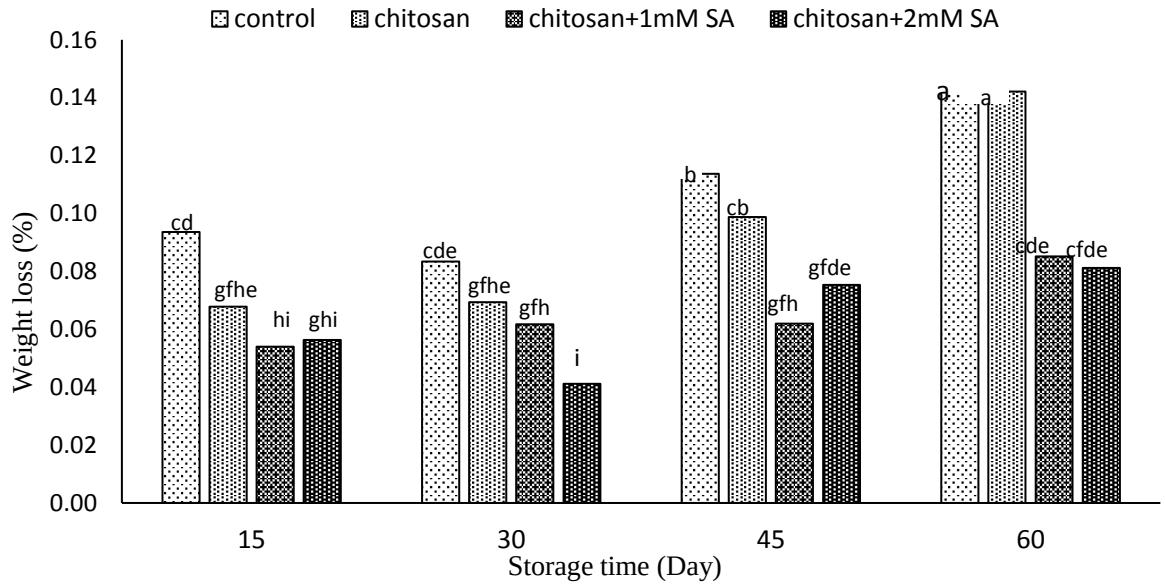
Table 4.1. Statistical analysis of the effects of storage period and applications on fruit decay

Source	DF	Fruit decay (%)
Application (A)	3	< 0.0001*
Storage period (T)	4	< 0.0001*
A*T	12	< 0.0001*

*: Express statistically significant at 5% level

4.2 Weight Loss

Weight loss of grape berries during the storage is shown in Figure 4.2. The statistical analysis of the effectiveness of applications on cluster weight loss are shown in table 4.2. While chitosan coating treatment significantly decreased weight loss in grape berries, the interaction between applications and cold storage time was also significant ($P \leq 0.05$). CT+2mM SA has shown more effective on decreasing weight loss during storage compare with the other treatments. The maximum weight loss after 60 days in storage was in control group with 0.145% and CT+2mM SA had the least weight loss with 0.081%.



The differences between the weight loss values were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4.2. Variations in weight loss (%) during storage at ±0.5°C

Table 4.2. Statistical analysis of the effects of storage period and applications on fruit weight loss

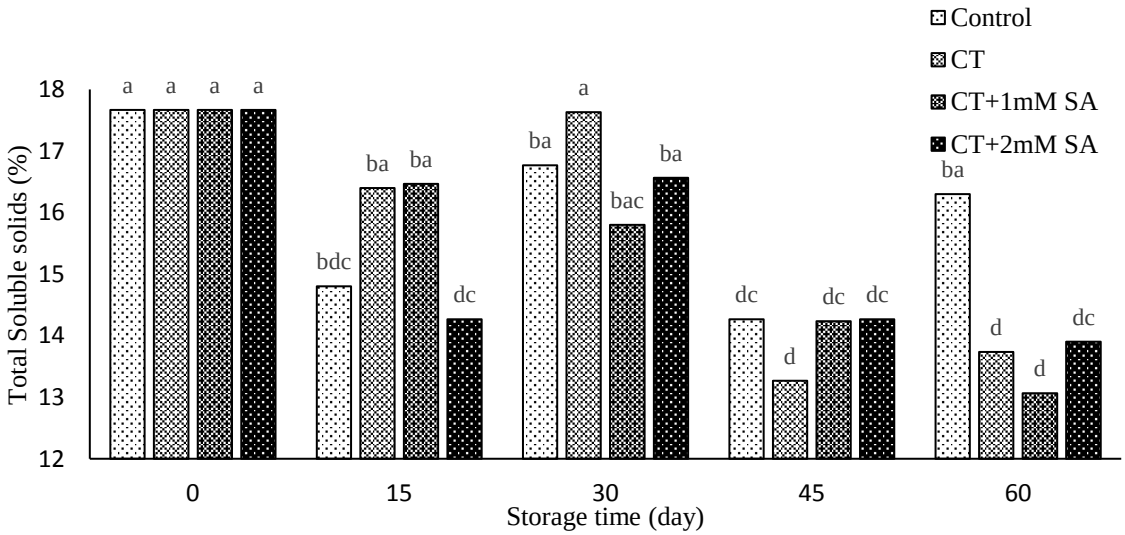
Source	DF	Weight loss (%)
Application (A)	3	< 0.0001*
Storage period (T)	4	< 0.0001*
A*T	12	< 0.0004*

*: Indicates statistically significant at 5% level

4.3 Total Soluble Solids (TSS, %)

The graph of changes in the amounts of TSS obtained during storage at ±0.5°C for control, CT, CT+1mM SA, CT+2mM SA coatings are presented in figure 4.3, and statistical analysis of these changes are shown in table 4.3.

During storage period TSS was decreased (figure 4.3), which at harvest time the amount of TSS was 17.66% and after storage of 60th days in CT+1mM SA treatment group was found the least amounts of TSS by 13.066%. The effect of chitosan coating treatment was not significant ($P \geq 0.05$). While the duration time during storage was found significant at $P \leq 0.05$ level. In addition in this study the interaction between chitosan treatment and time was found not significant ($P \geq 0.05$).



The differences between the total soluble solids values were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4.3. Variations in TSS (%) during storage period at ±0.5°C

Table 4.3. Statistical analysis of the effects of storage period and applications factors on total soluble solids

Source	DF	Total soluble solids (%)
Application (A)	3	0.4971
Time (T)	4	0.0001*
A*T	12	0.0761

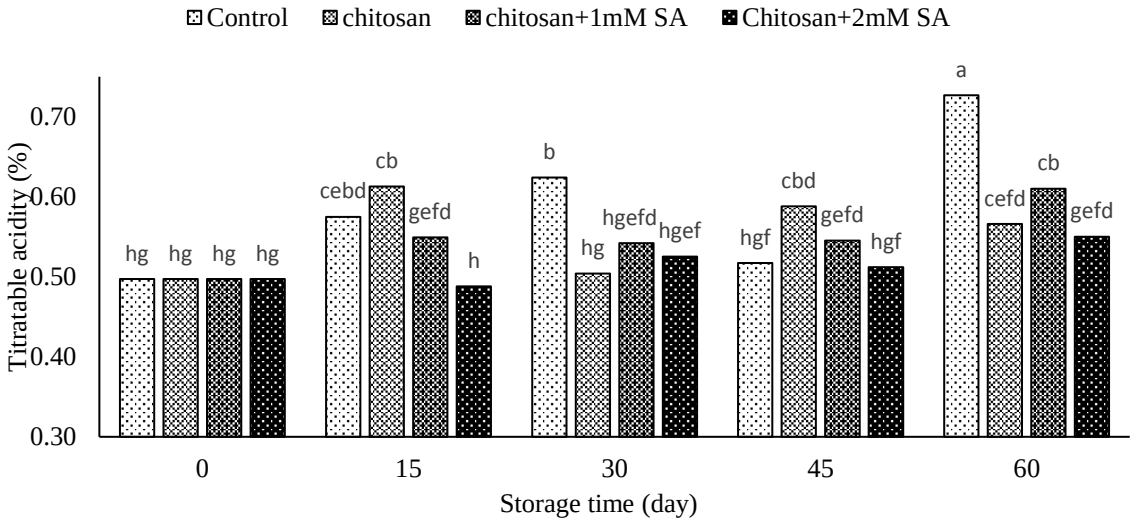
*: Express statistically significant at 5% level

4.4. Titratable Acidity Rate (TA, %)

TA content in samples changed in control group and CT, CT+1mM SA, CT+2mM SA treatment groups which comes after 60 days storage at ±0.5ºC are shown in figure 4.4. Statistical analysis of these changes are shown in table 4.4.

As a result of the study, it was observed that there is fluctuation in the amount of acidity of the uncoated and coated treatments during the cold storage. The highest rate of TA after 60 days found in control group by 0.727% (Figure 4.4), which significantly controlled the increasing TA rate in treatment groups ($P \leq 0.05$).

In addition, the effect of storage period on this parameter and was found the interactions between treatments and storage period in this study were also significant at $P \leq 0.05$ level.



The differences between the titratable acidity values were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4.4. Variations in TA (%) values during storage period at ±0.5ºC

Table 4.4. Statistical analysis of the effects of storage period and applications on titratable acidity

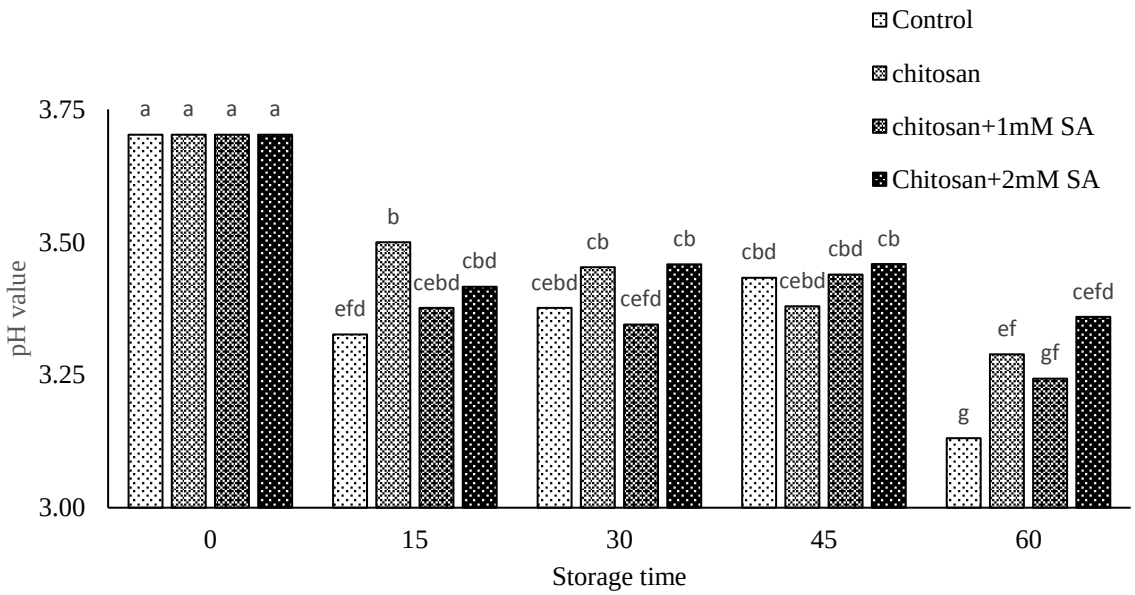
Source	DF	Titratable acidity (%)
Applications (A)	3	< 0.0001*
Storage period (T)	4	< 0.0001*
A*T	12	< 0.0001*

*: Express statistically significant at 5% level

4.5 pH

pH values of control, CT, CT+1mM SA and CT+2mM SA coating application groups are calculated and the graph which shows these changes are presented in figure 4.5. Statistical analysis of these changes are shown in table 4.4.

The pH values of the control and coated grape berries decreased from 3.702 to 3.131 (Figure 4.5). While the effect of coating applications on the pH values was significant, the effect of storage period was also significant ($P \leq 0.05$). Furthermore, on the pH values, the interaction between treatment and time was not significant in this study at $P \leq 0.05$ level.



The differences between the pH values were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4.5. Variations in pH values during storage period at ±0.5ºC

Table 4.5. Statistical analysis of the effects of storage period and applications on pH value

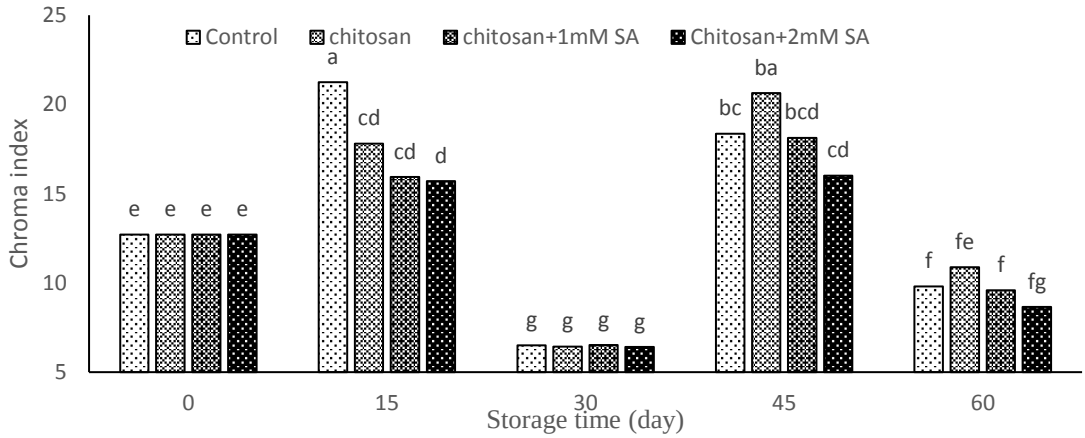
Source	DF	pH
Application (A)	3	0.0144*
Storage period (T)	4	< 0.0001*
A*T	12	0.1857*

*: Express statistically significant at 5% level

4.6 Fruit Color

The figure 4.6 shows fruit skin color (Chroma index, C*) of the control samples, CT, CT +1mM SA and CT+2mM SA coatings which stored for 60 days at ±0.5°C and the table 4.6 present the statistical analysis of these changes.

During storage period, C* values of coated and uncoated berries were increased and decreased (Figure 4.6). At the end of storage the highest C* value which was detected in fruits (10.877) was from CT group, and the least Chroma value which detected (8.653) was in CT+2mM SA. During storage period, the highest C* value was observed in the first 15th day of storage in control group, and the lowest C* value was observed after 30 days storage from CT+2mM SA (Figure 4.6). While the differences in the C* values of the applications were significant, the effect of the storage period during storage on the C*values was also significant, and the interaction between time and applications were also found significant at $P \leq 0.05$ level in this study (Table 4.6).



The differences between the Chroma index values were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4.6. Variations in Chroma index during storage period at ±0.5°C

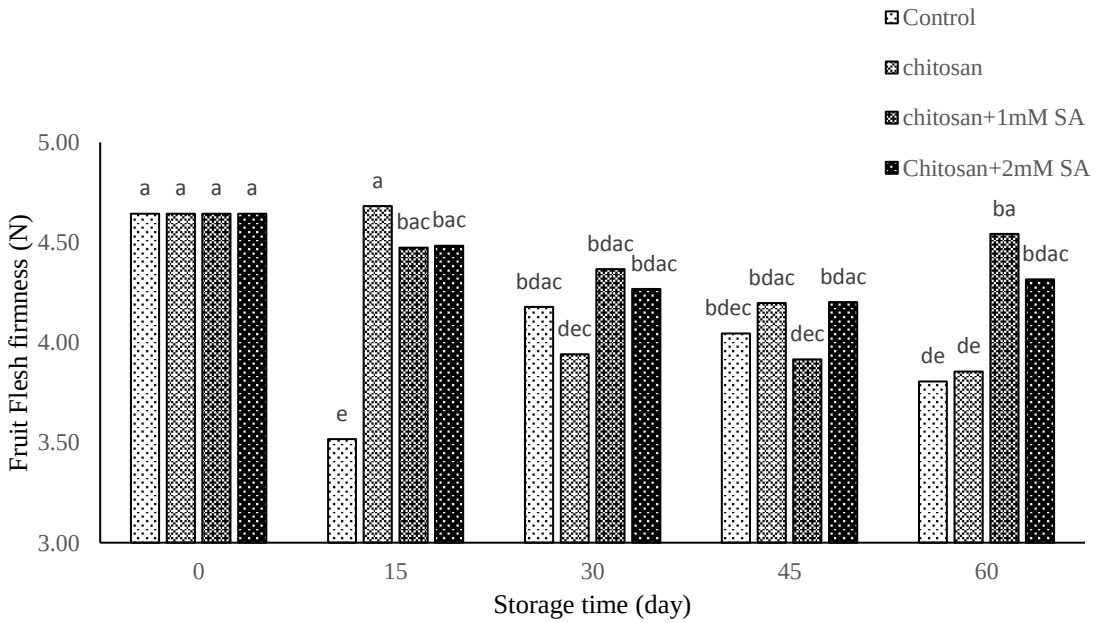
Table 4.6. Statistical analysis of the effects of storage period and applications on Chroma

Source	DF	Chroma
Application (A)	3	0.0035*
Storage period (T)	4	< 0.0001*
A*T	12	0.0216*

*: Express statistically significant at 5% level

4.7 Flesh Firmness

The flesh firmness of the control samples, CT, CT +1mM SA and CT+2mM SA coatings which stored for 60 days at ±0.5°C are calculated, and the graph of the variation of these findings were shown in Figure 4.7. Statistical analysis of these changes were shown in table 4.7.As a result of the study, it is observed that there is fluctuation in the flesh firmness value (N) of the uncoated and coated samples during storage (Figure 4.6). As you see the figure 4.6, control and CT group have lost faster their firmness than CT+1mM SA and CT+2mM SA. The highest firmness value after 60 days found in CT+1mM SA group by 4.543N (Figure 4.6).In addition the effect of application and storage period were found significant in this study ($P \leq 0.05$), the interaction between time and applications were not significant (Table 4.7).



The differences between the fruit flesh firmness were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4.7. Variations in flesh firmness (N) during storage period at ±0.5°C

Table 4.7. Statistical analysis of the effects of storage period and applications on flesh firmness

Source	DF	Fruit firmness (N)
Application (A)	3	0.0372*
Storage period (T)	4	0.0038*
A*T	12	0.0725

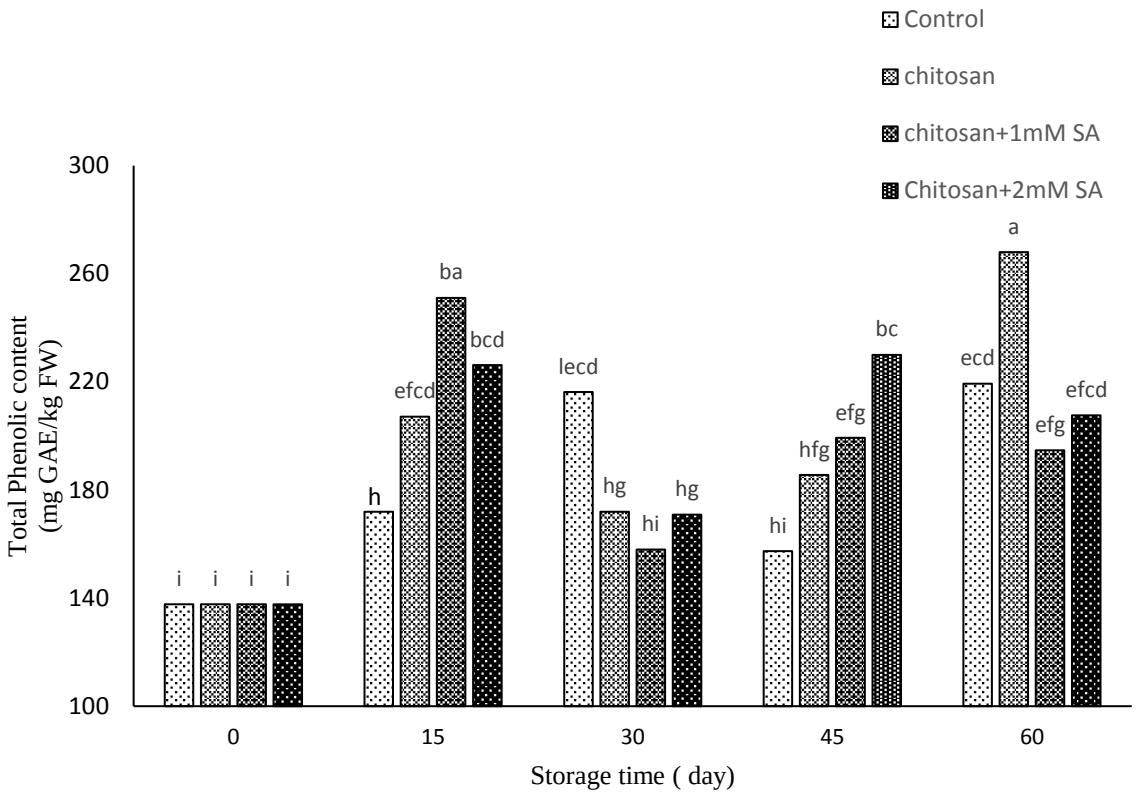
*: Express statistically significant at 5% level

4.8 Total Phenolic Content

The total phenolic content of the controls, CT, CT +1mM SA and CT+2mM SA coatings which stored for 60 days at ±0.5^{o}C are calculated, and the graph showing the changes of these findings are shown in figure 4.8. Statistical analysis of these changes are shown in table 4.8.

According to the beginning of storage the total phenolic contents in all treatments generally tended to increase during storage period. At the early stage of the storage period total phenolic contents in all treatments were increased then in the middle stage total phenolic contents decreased and at the end of stage total phenolic contents increased again. Effect of storage period during storage was found Signiant ($P \leq 0.05$) in the study (Table 4.8).

The highest phenolic content was found with CT application (267,99mg GAE/kg FW) and the lowest was found in the CT+1mM SA (194.65 mg GAE/kg FW) after 60 days of storage (Figure 4.8). While the effect of applications was not significant ($P \geq 0.05$). In addition the interaction between time and application was found to be significant at $P \leq 0.05$ level (Table 4.8).



The differences between the total phenolic compounds values were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4. 8 The variations in total phenolic compounds during storage period at ±0.5ºC

Table 4.8. Statistical analysis of the effects of storage period and applications on total phenolic contents

Source	DF	Total Phenolic content
Application (A)	3	0.1065
Storage period (T)	4	< 0.0001*
A*T	12	< 0.0001*

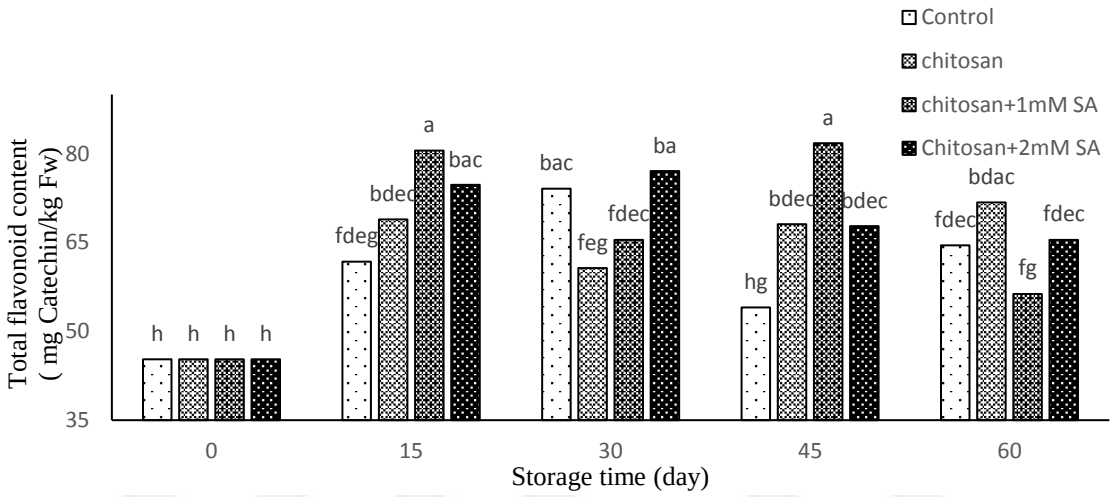
*: Express statistically significant at 5% level

4.9 Total Flavonoid Content

The changes of total flavonoid content of the control samples, CT, CT +1mM SA and CT+2mM SA coatings which stored for 60 days at ±0.5ºC showed in figure 4.9, and statistical analysis of these changes were shown in table 4.9.

This study reveals that the total flavonoid concentration of all treated and untreated grapes during storage increased in the early stage then decreased at the end of storage period

(Figure 4.9). The highest flavonoid content was found with CT+1mM SA (80.541mg C/kg FW) after 15 days storage and at the end of storage period the highest amount was observed in CT (71.769 mg C/kg FW) and the lowest was found with control (64.518 mg C/kg FW) (Figure 4.9). While the effect of applications during storage period was found significant ($P \leq 0.05$). In addition the effect of storage period was found significant ($P \leq 0.05$), furthermore the interplay between period and applications was found to be significant at $P \leq 0.05$ level (Table 4.9).



The differences between the total flavonoid contents values were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4.9. The variations in total flavonoid content (mg CE / kg FW) during storage period at ±0.5°C.

Table 4.9. Statistical analysis of the effects of storage period and applications on total flavonoid contents

Source	DF	Total flavonoid content
Applications (A)	3	0.0306*
Storage period (T)	4	< 0.0001*
A*T	12	< 0.0001*

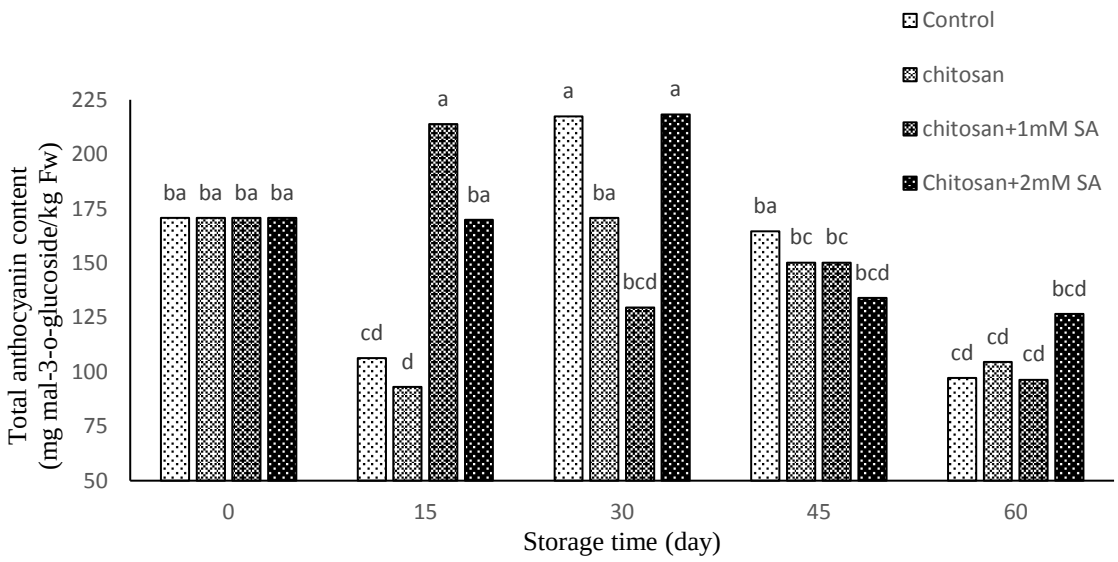
*: Express statistically significant at 5% level

4.10 Total Anthocyanin Content

The graph of changes in the total anthocyanin content in the controls, CT, CT +1mM SA and CT+2mM SA coatings which stored for 60 days at ±0.5ºC are shows in figure 4.10, and statistical analysis of these changes are shown in table 4.10.

The total anthocyanin content in grape samples before storage was determined as 170.67 mg malvidin-3-O-glucoside/kg FW. During storage, amount of anthocyanins changed soporifically. At early stage of storage the total anthocyanin decreased and after storage period of 30 days again they increased then at the last stage again decreased. At the end of storage, total anthocyanin values were 126.61 mg malvidin-3-O-glucoside / kg FW (CT+2mM SA), 104.58 mg malvidin-3-O-glucoside / kg FW (CT), 97.23 mg malvidin-3-O-glucoside/kg FW (Control) and 96.35 mg malvidin-3-O-glucoside/kg FW (CT+1mM SA) (Figure 4.10).

Applications were significantly controlled the decreasing in total anthocyanins during storage period ($P \leq 0.05$), while effect of storage duration was also significant ($P \leq 0.05$). In addition, the interaction between storage period and application were also found significant in this study. ($P \leq 0.05$) (Table 4.10).



The differences between the total anthocyanin content values were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4.10. The variations in total anthocyanins (mg malvidin-3-O-glucoside / kg FW) during storage period at ±0.5ºC

Table 4.10. Statistical analysis of the effects of storage period and applications on total anthocyanin content

Source	DF	Total Anthocyanin content
Application (A)	3	0.2107
Storage period (T)	4	< 0.0001*
A*T	12	0.0004*

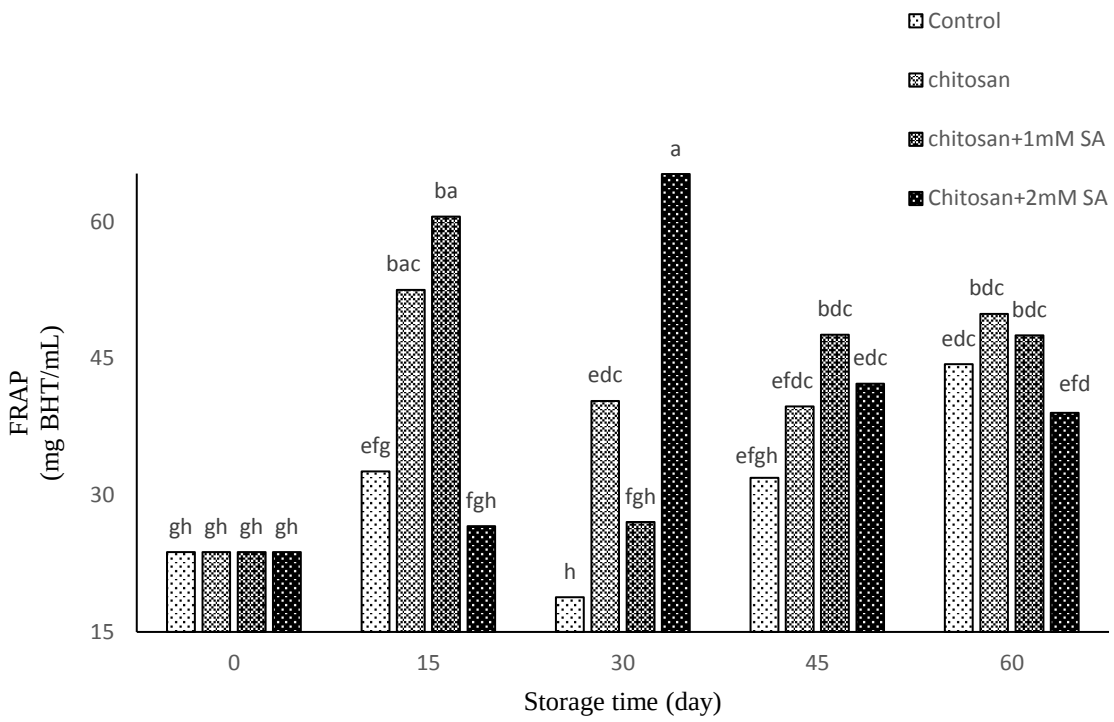
*: Express statistically significant at 5% level

4.11 Total Antioxidant Potential

Total antioxidant potential of the grapes with different coating applications during 60 days of storages determined as antioxidant activity and antioxidant capacity. Total antioxidant activity was detected whereby DPPH radical scavenging assay. However, total antioxidant capacity of samples were determined by the (FRAP). Findings obtained as a result of these methods are given under subheadings.

FRAP (mg BHT/mL extract): Figure 4.11 shows the findings of total antioxidant capacity of control, CT, CT+1mM SA and CT+2mM SA grape fruits obtained by FRAP method during storage at ±0.5^{o}C for 60 days. Table 4.11 shows statistical analysis of these changes.

Total antioxidant capacity of grapes was determined as 23.76 mg BHT/mL before storage, and total antioxidant capacity of coated and uncoated grapes increased during storage. The highest antioxidant capacity after a storage period of 60 days at ±0.5^{o}C were found in CT by 49.89 mg BHT/mL and the lowest antioxidant capacity were found by CT+2mM SA by 39.05mg BHT/mL. The effect of applications were found significant ($P \leq 0.05$). In addition the effect of storage period were also found significant in this study. Furthermore, the interactions between storage period and application were also found significant at $P \leq 0.05$ level (Table 4.11).



The differences between the FRAP values were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4. 11 Changes during the storage period at ±0.5ºC in the total antioxidant capacity (mg BHT / mL) resulting by FRAP method

Table 4.11. Statistical analysis of the effects of storage period and applications on FRAP

Source	DF	FRAP
Application (A)	3	0.0005*
Storage period (T)	4	< 0.0001*
A*T	12	< 0.0001*

*: Express statistically significant at 5% level

DPPH:- The antioxidant capacities of the four different groups (control, CT, CT+1mM SA and CT+2mM SA) for each 15th days storage period were revealed in Figure 4.12-Figure 4.15. The ability to inhibit the DPPH free radical of extracts at different concentrations (0.1 and 1.0 mg / mL) for each species was expressed as%. The highest DPPH inhibition (%) after 15 days of storage was detected in CT by 70.792 % in 1mg/mL (Figure 4.12), after 30 days of the storage the highest DPPH inhibition was found in CT+1mM SA by 76.404% in 1mg/mL (Figure 4.13), 73.724% in 1 mg/mL was observed in CT after 45 days of the storage period (Figure 4.14) which was the highest DPPH inhibition and at the end of the storage the highest DPPH inhibition was found in CT+1mM SA by 66.244% in 1mg/mL (Figure 4.15).

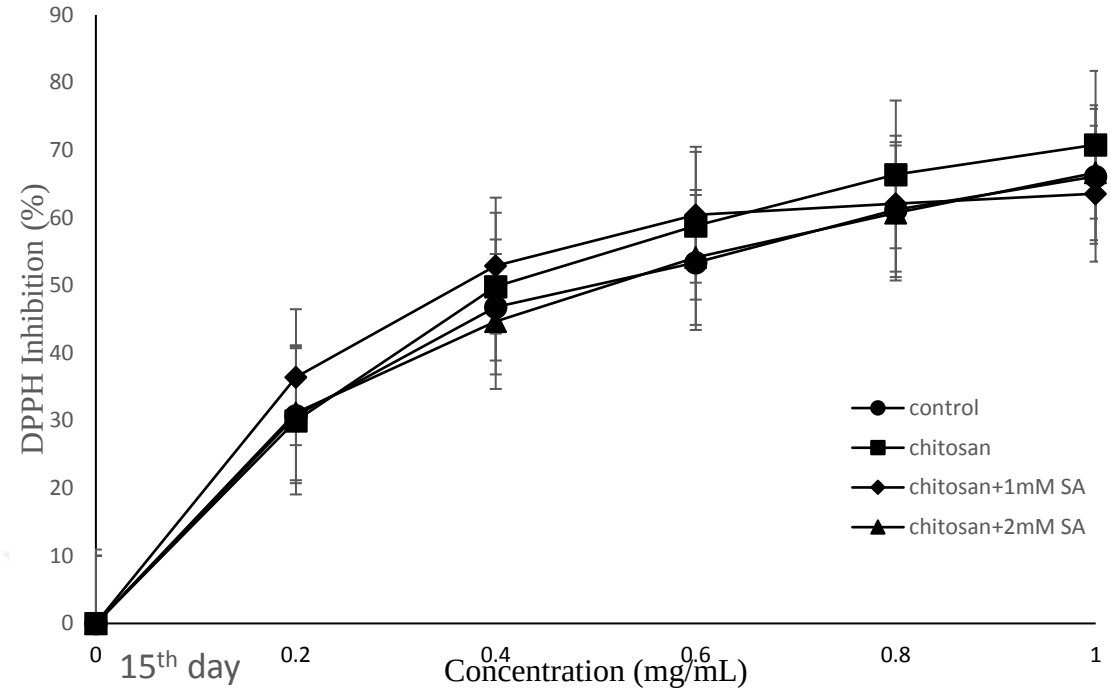


Figure 4.12. DPPH inhibition (%) of applications after 15 days of storage

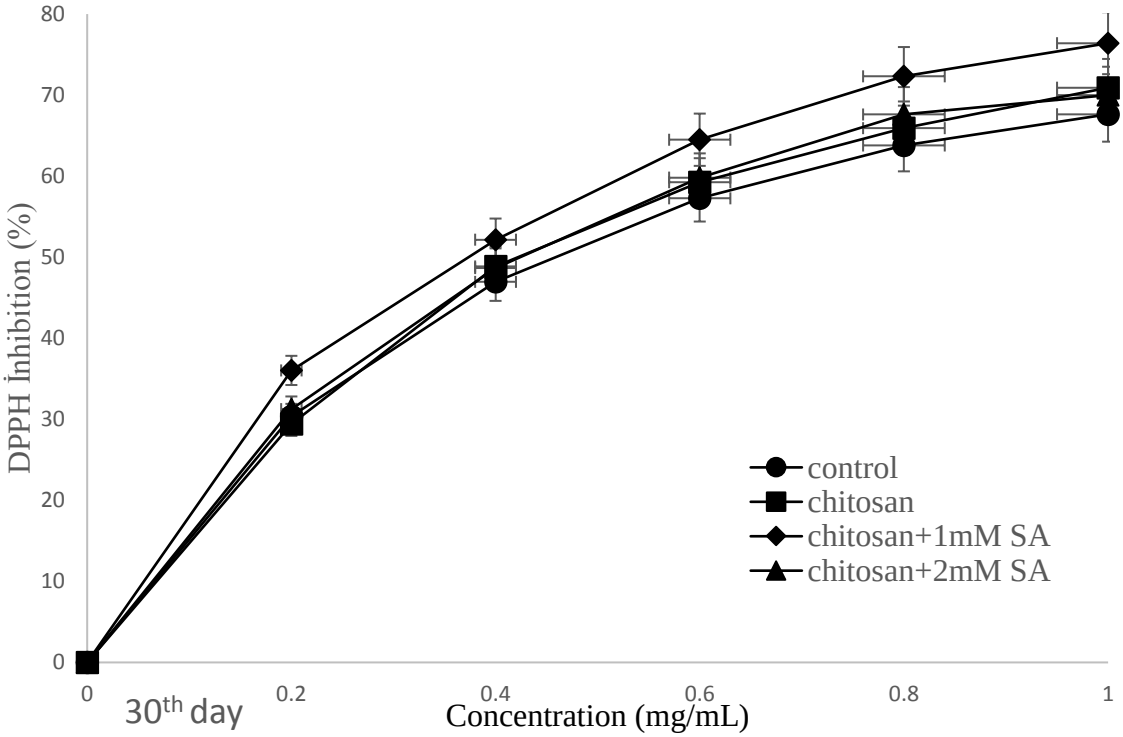


Figure 4.13. DPPH inhibition (%) of applications after 30 days of storage

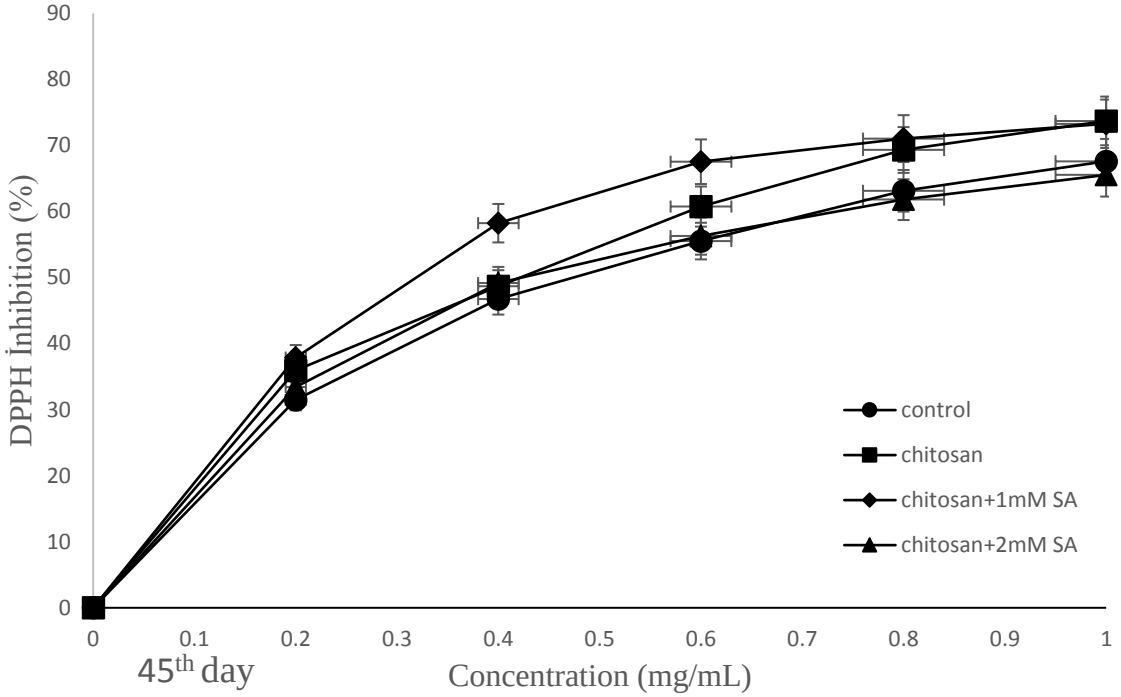


Figure 4.14. DPPH inhibition (%) of applications after 45 days of storage

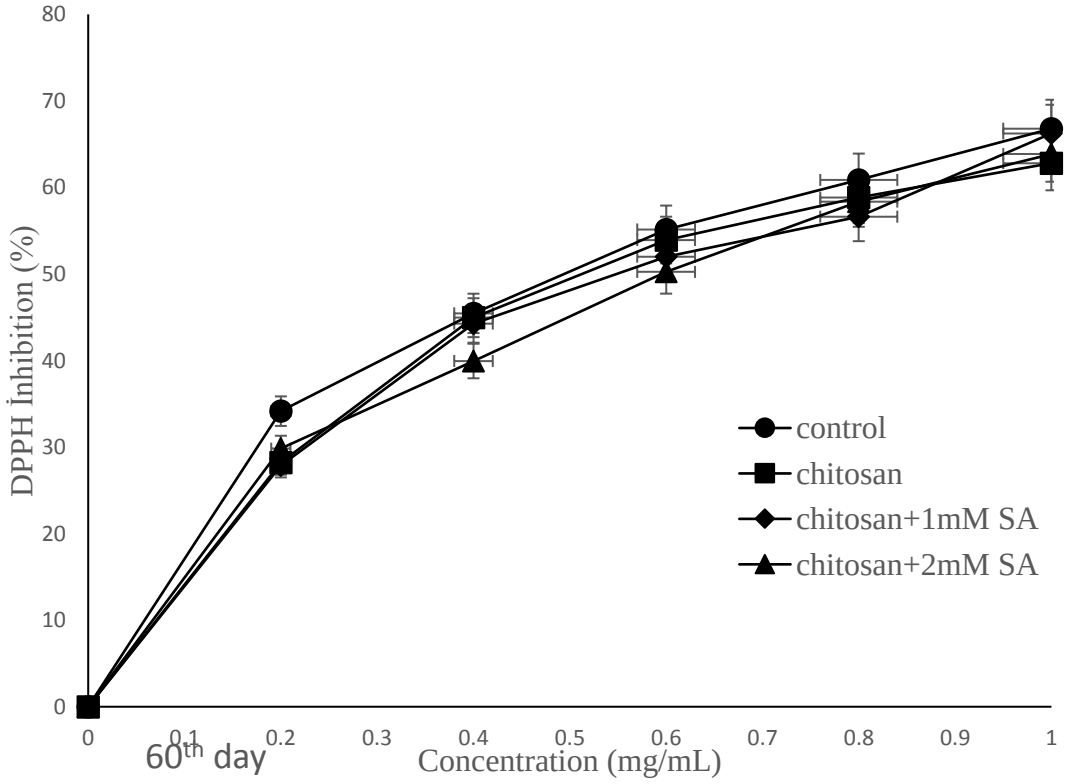
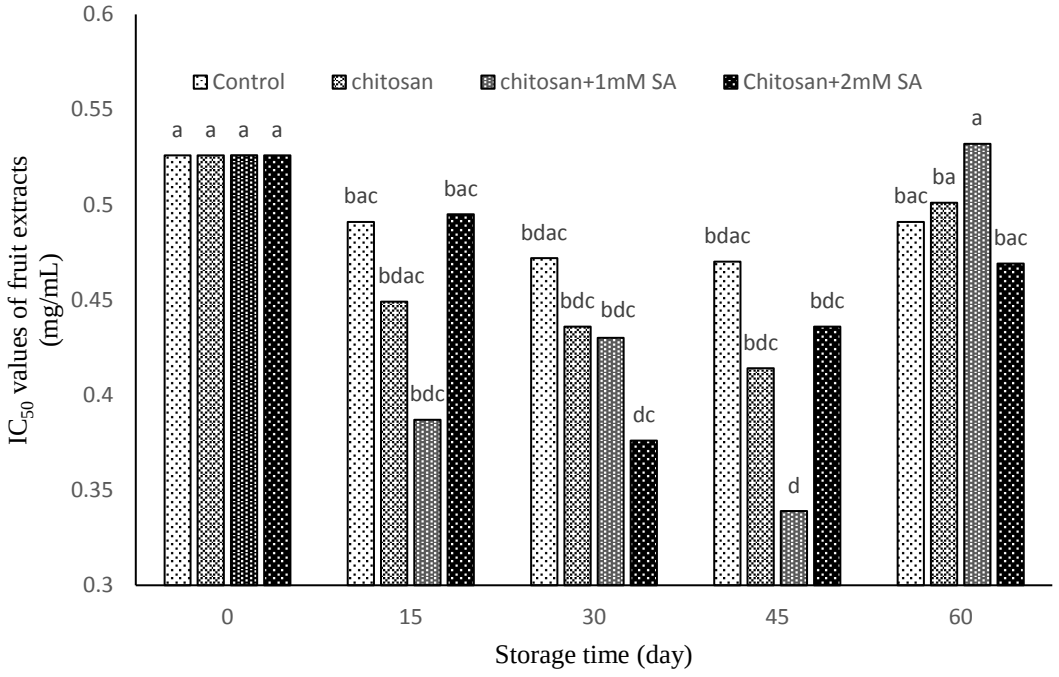


Figure 4.15. DPPH inhibition (%) of applications after 60 days of storage

Using these graphs, antioxidant activities (IC_{50} mg / mL) values of the extracts of control, CT, CT+1mM SA and CT+2mM SA during storage for 60 days at ±0.5°C is presented in figure 4.16. The IC_{50} refers to the amount of extract (mL) required to sweep 50% of the free radical of DPPH free from the medium per unit time, and statistical analysis of these changes are shown in the table 4.12.

During storage, IC_{50} (mg / mL) values of all treatment samples decreased. However, the lowest total antioxidant activity (%) was determined after 45 days storage period in CT+1mM SA samples by 0.339 mg/mL (Figure 4.16), and at the end of storage total antioxidant activity were started to increase again (Figure 4.16). The pre-storage IC_{50} value of the grape berries was determined 0.526 mg / mL, and after storage the IC_{50} value of control, CT, CT+1mM SA and CT+2mM SA were determined 0.491mg/mL,0.501mg/mL, 0.532mg/mL and 0.469mg/mL respectively. The effect of applications on antioxidant activity in this study weren't significant ($P \leq 0.05$), while the effect of storage period was found significant ($P \leq 0.05$). In addition the interaction between storage period and application were also not significant at $P \leq 0.05$ level (Table 4.12).



The differences between the DPPH values were determined according to the LSD test and expressed in letters. The different letters in the same column indicate a difference of $P \leq 0.05$.

Figure 4.16. Changes during the storage period at ±0.5°C in the amounts of IC50 (mg / mL) as a result of DPPH analysis

Table 4.12. Statistical analysis of the effects of storage period and applications on DPPH

Source	DF	DPPH
Application (A)	3	0.3933
Storage period (T)	4	0.0032*
A*T	12	0.6512

*: Express statistically significant at 5% level

CHAPTER V

DISCUSSION

The results showed that the application of chitosan alone or combined with SA, both significantly ($P \leq 0.05$) reduced the decay rate in grape berries compared with control during storage at ± 0.05 °C for 60 days (Figure 4.1). Similarly reported in previous studies (Romanazzi et al., 2012; Gao et al., 2013; Al-Qurashi and awad, 2015; Shen and Yang, 2017). Fruit decay in grapes mainly causes by *Botryits cinerea pers..*, decrease by CT coating (Gao et al., 2013). Liu et al., (2007) found that CT significantly prevent spore germination and mycelial growth of *B.cinerea* by damaging plasma membrane of the spores *in vitro*. In another study conducted by Meng et al. (2008) reported that CT pre-harvest spray and post-harvest coating have a good control effect on decay of table grapes.

In this study, the results supported the ideas of the CT coating were effective to decrease decay of grapes. Also, the results in the experiment showed that CT+2mM SA coating had a better function to reduce the natural decay incidence of grape berries compared to CT alone. Similarly result in the study of Shen and Yang (2017) and reported that CT with SA was more effective than CT alone in inducing grape berries resistance to fungal diseases, because SA also form a film on the surface of the grape berries that act as a physical barriers against infection. Overall, CT+2mM SA performed best among the control and chitosan treatment tasted during storage on ± 0.5 °C for 60 days (Figure 4.1), as for the shelf life of the CT+2mM SA treated berries it depends on the time and temperature in the application.

Weight loss in fresh fruit and vegetables is mainly because of water which causes by transpiration and respiration process (Zhu et al., 2008). Weight loss in all treatments showed an increasing pattern over storage (Figure 4.2). After 60 days storage period at ± 0.5 °C, weight loss in controls reached up to 0.145% whereas CT, CT+1mM SA, CT+ 2mM SA reached values of 0.142%, 0.085% and 0.081%, respectively. Numerous researchers demonstrated that weight loss was associated with respiration process and evaporation of water from the fruits (Amarante et al., 2001). Previous studies admitted that the CT coating functioned as self-control atmosphere and selectively percolated C_2H_4, CO_2 and O_2 inside and out of the fruit, therefore reducing fruit respiration metabolism (El

Ghaouth et al., 1991; Hagenmaier, 2005). In this study, it has been found that all CT treatments, showed less weight loss at ± 0.5 ^{o}C than control fruits. Similar performances of deferring weight loss has been reported in CT coated table grapes (Gao et al., 2013) and also reported for CT coated sweet cherry, strawberry, litchi fruit, peach, mango, banana and longan fruit (Petericcione et al., 2014, Hernández-Muñoz et al., 2008; Dong et al., 2003; Li and Yu, 2001; Kittur et al.,2001; Jiang and Li, 2001). However, CT+1mM SA and CT+2mM SA, showed more effective on decreasing weight loss than other treatments due to its sustained release of SA, in the agreement with the results of Zhang et al., (2015) and Shen and Yang (2017).

The quality parameters of the grapes (TSS, TA, pH, color, firmness), is also important index for evaluating the storage effect. In this study found that during storage at ± 0.05 ^{o} C for 60 days decreased the rate the TSS content and of the grape fruits, and the effect of CT coating on TSS was not significant but a significant effect of storage time was detected in this study. Similar result was found in the study of (Sanchez-Gonzalez et al., (2010) in grapes and reported that after 8 days TSS in samples subsequent, a sharp decreased and said that it was depended on the samples type. Meng et al. (2008) also found same result in grapes and reported that it may related to the inhibition of fruit respiration. Al- Eryani et al., (2008) studied on effect of different concentration of CT coating on storage life and quality of papaya and reported that high concentration of CT (0.5% and 1%) decrease TSS (Al-Eryani et al., 2008). Lower TSS were also reported on bananas and mangoes when coated with CT (Kittur et al., 2001).

pH values of the grapes also decreased during storage in this study which the initial value was 3.702 and after 60 days of the storage the pH value of control, CT, CT+1mM SA, CT+2mM SA were 3.131, 3.289, 3.242, 3.359, respectively. Similarly reported with (Sanchez-Gonzalez et al., (2010) and reported pH values decrease in grapes fruit and attributed to the natural variability of the product (Sanchez-Gonzalez et al., 2010). Vargas et al. (2006) studied on quality of cold- stored strawberries as effected by chitosan-oleic acid edible coating and reported that pH values decrease due to nature of the fruit organic acids, the usual decrease in fruit acidity (Vargas et al., 2006).

TA rate of grapes in this study increased during storage (Figure 4.4). Which the initial value was 0.497% and after storage TA rate of control, CT, CT+1mM SA and CT+2mM

SA were 0.727%, 0.566%, 0.610% and 0.550%, respectively, which CT coating significantly controlled the increasing of TA in grapes during storage and CT+2mM SA showed more effective than other treatments. This result is consistent with similar studies in which the application of CT coating and SA after harvest are examined on strawberry, guava fruit and banana (Asghari, 2006; Hong et al., 2012; Maqbool et al., 2011; Shafiee et al., 2010).

Fruit color is the most important parameter determining the quality of the fruit (Esti et al., 2002). In this study C* values studied as a color parameter of the grapes. During storage period, C* values changed, although, there were difference between samples. Similarly results were reported on grapes (Sanchez-Gonzalez et al., 2010). The highest saturated value found in CT and the least C* value found in CT+2mM SA. Perdones et al., (2012) reported that vivid color decreasing during storage and found highest value in pure CT coating after storage of strawberry, which similarly result found in this study. Asghari, M. and Aghdam, M.S. (2010) studied on impact of SA on postharvest physiology of horticulture crops and reported that SA prevented red color.

Flesh firmness of all berries decreased with prolonging storage period (Figure 4.7). During the cold storage period, significantly decline of firmness was in control samples compared to treated samples (Figure 4.7). Firmness changes of fruit flesh during storage attributed to degrading of primary cell wall and middle lamella structures (Yang et al., 2011). In addition, it has been also reported that fruit softening were influenced by degradation enzymes such as polygalacturonas, pectinesterase and cellulose (Gao et al., 2013). Deng et al., (2005) reported that during storage, pectinases catalyzes the protopectin to water soluble pectin. When pectin enters to the cell sap under osmotic pressure, it forms a gel and causing fruit softening. In this study, the results showed that CT, CT+1mM SA and CT+2mM SA might be to delay berry softening by inhibiting cell wall-degrading enzymes activities. Previous studies have reported a similar performance of delaying softening by CT coating in grapes (Gao et al., 2013; Guerra et al., 2016) in strawberries (Perdones et al., 2012) and by SA coating in peach (Wang et al., 2006) and in banana (Srivastava and Dwivedi, 2000). Firmness of the fruits were related to turgor pressures loss in the cells reduced by water loss.

Total phenolic contents in all treatments were increased and decreased during storage (Figure 4.8). Generally, total phenolic contents may either increase or decrease in fruit and vegetables, and it depends on the storage conditions (Kalt, 2005). Phenylalanine ammonia lyase (PAL) is involve in the phenylpropanoid pathway, a secondary plant metabolic pathway which produce a variety of phenolics with structural and defense related functions (Wallace and Fry, 1999). Therefore, phenolic contents increased due to PAL activity. The highest phenolic content was found in CT application at the end of the storage period and similar result was reported in grapes (Shen and Yang, 2017) and in kiwifruit (Tavarini et al., 2007). In this study, the lowest phenolic content was found in CT+SA treatments. In previous researches it was reported that SA inhibit the PAL activity during storage in pomegranate and loquat (Cai et al., 2006; Sayyari et al., 2009).

Total flavonoids in all treatment were increased at early stage then decreased at last the stage during storage (Figure 4.9). The initial amount of flavonoids were 45.307mg CE / kg FW. After 30 days, amount of flavonoids increased in all treatments. The phenylpropanoid pathway induces biosynthesis of flavonoids and pigment as well as lignin and phenolic compounds (Xu et al., 2007). This result is thought to be that flavonoid synthesis, one of the secondary metabolites, may be increased under low temperature stress (Quan et al., 2008). Similar reports have been reported in cherries (Gimenez et al., 2014), pomegranate (Sayyari et al., 2016) and in kiwi fruits (Tavarini et al., 2007). Khan et al., (2003) reported that CT oligomers may elevate phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase activities in soybean leaves. This may lead to the induction of other phenolic compounds, including phenolic acids and flavonoids such as catechins, which produced by the phenylpropanoid and flavonoids pathway where phenylalanine ammonia lyase (PAL) is a leading enzyme (Shiri et al., 2013). At the end of storage period amount of flavonoids decreased again (Figure 4.9). Flavonoids are phenolic derivatives, and in grapes are found in substantial amount (Shiri et al., 2013). This explain the increase and decrease in flavonoids associated with the total phenolic content during storage.

It is also believed that these results are due to the fact that CT may have a limiting effect on the polyphenol oxidase enzyme activity responsible for the degradation of phenolic compounds (Jiang and Li, 2001).

Anthocyanins which are effective on the coloring of the fruit are synthesized in the fruit skin and the fruit flesh (Kim et al., 2005). Therefore, it is an important parameter for fruit quality because of its effect on fruit appearance and its antioxidant properties. The total amount of anthocyanin in grape samples before storage was determined as 170.67 mg cy-3-rut / kg FW. During storage, amount of anthocyanins changed. At early stage of storage the total amount of anthocyanins were decreased and after 30 days storage again amount of anthocyanins increased then at the last stage again decreased. At the end of storage period, the values for total anthocyanin were 126.61 mg malvidin-3-O-glucoside/ kg FW (CT+2mM SA), 104.58 mg malvidin-3-O-glucoside/kg FW (CT), 97.23 mg malvidin-3-O-glucoside / kg FW (Control) and 96.35 mg malvidin-3-O-glucoside / kg FW (CT+1mM SA) (Table 4.10). CT films form an effective gas barrier, probably due to dense structure of the film (Wong et al., 1992). So, a possible modification of the internal in coated samples due to CT coating may explain this behavior. CO_2 and O_2 content affect anthocyanin synthesis or degradation rates. In same way Perz and Sanz, (2001) studied on strawberries and reported that a lower anthocyanin concentration in strawberries stored in controlled atmospheres.

Total antioxidant potential was determined by antioxidant activity and antioxidant capacity. Total antioxidant activity was detected by DPPH radical scavenging assay and total antioxidant capacity of the samples were determined by FRAP assay.

Total antioxidant capacity of the grapes in all treatments increased during storage (Figure 4.11). Tavarini et al., (2007) found the increasing total antioxidant capacity by FRAP method in kiwifruit and reported that antioxidant capacity increasing during storage (Tavarini et al., 2007). Connor et al., (2002) determined a reduction of the antioxidant capacity in 9 cultivars of blue berry fruits during storage. These authors found an increase in the antioxidant capacity and linked the increase in antioxidant capacity to the phenolic contents. Shivashankara et al., (2004) pointed that an increase in antioxidant capacity during low temperature storage maybe possible on in fruits which the contribution of total phenolic contents is greater than ascorbic acid.

CT solution have antioxidant capacity and it can be used as an antioxidant and anti-browning agent in the food industry (Devlieghere et al., 2004). In this study the highest FRAP value was detected in coated samples. In parallel with the findings Badway et al.,

(2017) found the highest total antioxidant capacity in strawberries coated with chitosan, Nair et al., (2017) found in guava fruit was coated with 1% CT by FRAP method.
The antioxidant activities of grapes, measured by DPPH assay. During storage, in this study observed a decrease in the antioxidant activities in grapes with higher values in coated compared with uncoated fruits (Figure 4.12). Similar results in grapes (Al-Qurashi et al., 2015), strawberries (Badway et al., 2017) and sweet cherries (Petericcione et al., 2014) coated with chitosan. Antioxidant activity can be attributed to synergistic and additive effects between different phytochemicals (Liu 2003; Kim et al., 2005).

The decline in antioxidant activity in untreated grape fruit at the end of storage might be because of senescence and decay. This indicated that the CT treatment is not only extend the shelf life, but also it can also enhance the health promoting natural antioxidant activity in grapes (Al-Qurashi et al., 2015).

CHAPTER VI

CONCLUSION

Grapes are one of the most popular among the commonly featuring table fruit. They have been widely regarded as "fruit queens" since ancient times. These small European and Mediterranean origin berries are the source of many nutritional health boosters such as polyphenolic antioxidants, vitamins, and minerals. No wonder why most of us often include them in our diet. Table grapes are highly perishable and non-climacteric. Quality of table grapes is mainly based on their chemical contents (sugar contents, taste, flavor, nutritional value and etc.) or physical characteristics (texture, appearance) or composition of these two factors. The processes developed for extending storage and shelf life and new technologies are great importance.

For this reason, the effects of CT, with SA on increasing storage life and quality of Red globe grape cultivar during storage at ± 0.5^{o}C with at ± 90–95% humidity for 60 days was evaluated. The recommendations based on the results of the examined features and some results are presented below.

- At the end of storage period fruit decay was 3.196% in control group where the decay rate in CT was 1.16%, 2.578% in CT+1mM SA and 0.887% in CT+2mM SA was found.
- The application of CT alone or combined with SA, both significantly ($P \leq 0.05$) reduced the decay rate in grape berries compared to control during storage.
- The results in the experiment showed that CT+2mM SA coating had a better function to reduce the natural decay incidence of grape fruits compared to chitosan alone. Because SA also form a film on the surface of the grape berries that act as a physical barriers against infection (Shen and Yang, 2017).
- At the end of storage weight loss in control, CT, CT+1mM SA and CT+2mM SA were 0.145%, 0.142%, 0.085% and 0.081%, respectively.
- Application of CT alone or with SA significantly ($P \leq 0.05$) controlled weight loss than control groups during storage.
- CT+1mM SA and CT+2mM SA, showed more effective on decreasing weight loss than other treatments due to its sustained release of salicylic acid.

- The quality of the grapes (TSS, TA, pH, color, firmness), is also important index evaluating the storage effect.
- During storage TSS was decreasing, which at harvest time the TSS was 17.66% and after storage at 60^{th} day in CT+1mM SA treatment group was found the least TSS by 13.066%.
- The effect of CT coating treatment was not significant ($P \geq 0.05$) in the TSS
- pH values of the grapes also decreased during storage, in this study which the initial value was 3.702 and after 60 days of the storage the pH value of control, CT, CT+1mM SA, CT+2mM SA were 3.131, 3.289, 3.242, 3.359, respectively.
- TA rate of grapes in this study increased during storage. Which the initial value was 0.497% and after storage TA rate of control, CT, CT+1mM SA and CT+2mM SA were 0.727%, 0.566%, 0.610% and 0.550%, respectively, which CT coating significantly controlled the increasing of TA in grapes during storage and CT+2mM SA showed more effective than other treatments.
- In this study C* values studied as a color parameter of the grapes. During storage Chroma values changed, although, there were difference between samples.
- The highest saturated value found in CT and the least C* value found in CT+2mM SA.
- In this study the results shows that CT, CT+1mM SA and CT+2mM SA might be to delay berry softening by inhibiting cell wall-degrading enzymes activities.
- In the results of the above findings, it was concluded that the addition of surface-active substances such as Tween-80 or plasticizers such as Tween-80 to coating solutions for future work would increase the success of the work because the coating solutions would effectively increase the film making capacity and can effectively limit the moisture and gas passages.
- The highest phenolic content was found with CT application (267,99mg GAE/kg FW) and the lowest was found in the CT+1mM SA (194.65 mg GAE/kg FW) after 60 days of storage. While the effect of application was not significant ($P \geq 0.05$).
- The total amount of flavonoids in the grape samples used in the experiment was determined as 45.307 mg CE / kg FW. At the end of storage, the amounts of flavonoids were determined as 64.518 mg CE / kg FW (Control), 71.769 mg CE / kg FW (CT), 56.330 CE / kg FW (CT + 1 mM SA), 65.453 mg CE / kg FW.

- At the end of storage, the values for total anthocyanin were 126.61 mg malvidin-3-O-glucoside/kg FW (CT+2mM SA), 104.58 mg malvidin-3-O-glucoside/kg FW (CT), 97.23 mg malvidin-3-O-glucoside/kg FW (Control) and 96.35 mg malvidin-3-O-glucoside/kg FW (CT+1mM SA).
- Total antioxidant potential of the grapes with different coating applications during 60 days of storages determined by antioxidant activity and antioxidant capacity. Total antioxidant activity was detected whereby DPPH radical scavenging assay. However, total antioxidant capacity of samples were determined by the ferric reducing power assay (FRAP).
- Total antioxidant capacity of grapes determined 23.76 mg BHT/mL extract before storage, and total antioxidant capacity of coated and uncoated grapes were increased during storage. The highest antioxidant capacity after 60 days storage at ±0.5^{o}C were found in CT by 49.89 mg BHT/mL extract and the lowest antioxidant capacity were found by CT+2mM SA by 39.05mg BHT/mL extract. The effect of application were found significant ($P \leq 0.05$).
- The pre-storage IC50 value of the grape fruits was 0.526 mg / mL. At the end of storage period, the IC50 values obtained were 0.491 mg / mL in control, 0.501 mg / mL in CT, 0.532 mg / mL in CT+1mM SA and 0.469 mg / mL in CT+2mM SA.
- These results indicated that chitosan+ SA treatment in combination with low temperature was really a useful non-chemical, non-toxic strategy for maintaining table grapes quality and extending the postharvest life.

REFERENCES

Aider, M., "Chitosan application for active bio-based films production and potential in the food industry: review", ***LWT–Food Science and Technology,*** 43, 837–842 2010.

Al Eryani, A.R., Mahmud, M.T., Omar, S.S.R., Zaki, M.A.R., Ali, H.I., "Effect of different concentrations of chitosan coating in storage life and quality characteristics of papaya (*Carica papaya* L.)", ***Asian Journal of Microbiology, Biotechnology and Environmental Sciences,*** Sc. Vol. 10 2008.

Ali, Asgar, Muhammad, M.T.M., Sijam, K., Siddiqui, Y., "Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (Carica papaya L.) fruit during cold storage", ***Food Chemistry***, 124, 620-626 2011.

Al-Qurashi D. A., "Postharvest chitosan treatment affects quality, antioxidant capacity,antioxidant compounds and enzymes activities of 'El-Bayadi' tablegrapes after storage", ***Scientia Horticulturae***, 197 (2015) 392–398 2015.

Amarante, C., Banks, N. H., Ganesh, S., "Relationship between character of skin cover of coatetd pears and permeance to water vapour and gases", ***Postharvest Biology and Technology***, 51,110-117 2001.

Arvanitoyannis, IS., "Totally and partially biodegradable polymer blends based on natural and synthetic macromolecules: preparation, physical properties, and potential as food packingmaterials" **Journal of Macromolecular Science,** ***Part C—Reviews in Macromolecular Chemistry and Physics***, 39, 205–271 1999.

Arvanitoyannis, IS, Nakayama, A., and Aiba, S., "Chitosan and gelatin based edible films: state diagrams, mechanical and permeation properties", ***Carbohydrate Polymers***, 37(4), 371–382 1998.

Asghari, M and Aghdam, M.S., "Impact of salicylic acid on postharvest physiology of horticulture crops", ***Trends in Food Science and Technology***, 21 (2010), 502-509 2010.

Asghari M, Ahadi L and Riaie S., "Effect of salicylic acid and edible coating based *Aloe vera* gel treatment on storage life and postharvest quality of grape (*Vitis vinifera* L. cv. Gizel Uzum)", ***International Journal Agriculture Crop Science.*** Vol., 5 (23), 2890-2898 2009.

Asghari, M., "Effects of salicylic acid on Selva strawberry fruit, antioxidant activity, ethylene production and senescence, fungal contamination and some other quality attributes", **PhD Thesis, Institue of Science, Tehran, Iran**, 2006.

Badway, M.E.I., Rabea,E.I., EL-Nouby, M.A.M., Ismail, R.I.A., Taktak, N.E.M., "Strawberry Shelf Life, Composition, and Enzymes Activity in Response to edible Chitosan Coating". ***International Journal of Fruit Science***, 17:2,117-136 2017.

Baez-Sanudo, M., Siller-Cepeda, J., Muy-Rangel, D., Heredia, J.B., "Extending the shelf life of bananas with 1-methylcyclopropene and a chitosan-based edible coating". ***Journal Recommendation service***, 89, 14, 2343-2349 2009.

Baldwin, E. A., Nisperos-Carriedo, M. O., Chen, X., and Hagenmaier, R. D., "Improving storage life of cut apple and potato with edible coating", ***Postharvest Biology and Technology***, 9, 151–163 2011.

Blois, M. S., "Antioxidant determinations by the use of a stable free radical", ***Nature*** 181(4617), 1199-1200 1958.

Bourliew, C., Guillard, V., Vall_es-Pami_es, B., Guilbert, S. and Gontard, N., "Edible moisture barrier: how to assess of their potential and limits in food products shelf-life extension? Crit. Rev", ***Food Science Nutrition***, 49:474–499 2009.

Cai, C., Li, X., and Chen, K. S., "Acetylsalicylic acid alleviates chilling injury of postharvest loquat (Eriobotrya japonica Lindl) fruit", ***European Food Research and Technology***, 223, 533-539 2006.

Cha, D. S., and Chinnan, M. S., "Biopolymer-based antimicrobial packaging: A review", ***Critical Review in Food Science and Nutrition***, 44, 223−237 2004.

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

Crisosto, C. H., Palou, L., Garner, D., and Armson, D. A., "Concentration by time product and gas penetration after marine container fumigation of table grapes with reduced doses of sulfur dioxide", ***Horticulture Technology***, 12, 241–245 2002.

Connor, A.M., Luby, J.J., Hancock, J.F., Berkheimer, S and Hanson, E.J., "Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage". ***Journal of Agricultural and Food Chemistry***, 50, 893-898 2002.

Dang, Q.F., Yan, J.Q., Li, Y., Cheng, X.J., Liu, S.C and Chen, X.G., "Chitosan acetate as an active coating material and its effect on the storing of *Prunus avium* L.''. ***Journal of Food Science.*** 75, 125–13 2010.

Debeaufort, F., Quezada-Gallo, J.A and Voilley, A., "Edible films and coatings: tomorrow's packaging: a review", ***Critical Reviews in Food Science and Nutritions***, 38: 299–313 1998.

Deng, Y., Wu, Y., and Li, Y.F., "Physiological responses and quality attributes of kyoho grapes to controlled atmosphere storage". ***Food science and Technology***, 39,584-590 2005.

Dhall, R. K., "Advances in edible coatings for fresh fruits and vegetables: *a review",* ***Critical Reviews in Food Science and Nutrition***, 53, 5, 435-450 2013.

Diaz-Mula, H. M., Serrano, M., and Valero, D., "Alginate coatings preserve fruit quality and bioactive compounds during storage sweet cherry fruit", ***Food Bioprocess Technology,*** 5, 2990–2997 2012.

Ding, C. K., and Wang, C. Y., "The dual effects of methyl salicylate on ripening and expression of ethylene biosynthetic genes in tomato fruit", ***Plant Science***, 164, 589-596 2003.

Ding, C. K., Wang, C. Y., Gross, K. C., and Smith, D. L., "Reduction of chilling injury and transcript accumulation of heat shock protein genes in tomatoes by methyl jasmonate and methyl salicylate", ***Plant Science***, 161, 1153-1159 2001.

Dong, H., Cheng, L., Tan, J., Zheng, K and Jiang, Y., "Effects of chitosan coating on quality and shelf life of peeled litchi fruit", ***Journal of Food Engineering***, 64, 355-258 2003.

Dutta, P. K., Tripathi, S., Mehrotra, G. K. and Dutta, J., "Perspectives for chitosan based antimicrobial films in food applications", ***Food Chemistry,*** 114(4), 1173-1182 2009.

Devlieghere, F., Vermeulen, A and Debevere, J., "Chitosan: Antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables", ***Food Microbial***. 21:703-714 2004.

El Ghaouth, A., Arul, J., Ponnampalam, R., Boulet, M., "Chitosan coating effect on storability and quality of fresh strawberries". ***Journal of Food Science***, 56, 6, 1618-1620 1991.

El Ghaouth,A., Smilanick, J. L and Wilson, C. L., "Enhancement of the performance of Candida saitoana by the addition of glycolchitosan for the control of postharvest decay of apple and citrus fruit", ***Postharvest Biology and Technology***, 19, 103–110 2000.

Esti, M., Cinquanta, L., Sinesio, F., Moneta, E. and Di Matteo, M., "Physicochemical and sensory fruit characteristics of two sweet cherry cultivars after cool storage", ***Food Chemistry.*** 76(4), 399-405 2002.

FAO, "Global food losses and food waste", **In: International Congress "SAVE FOOD!" Dusseldorf, Germany**, 2011.

FAO, 2016. **Table and dried grapes**. http://www.fao.org/3/a-i7042e.pdf

Gao, P.S., Zhu, Z.Q and Zhang, P., "Effects of chitosan-glucose complex coating on postharvest quality and shelf life of table grapes", ***Carbohydrate Polymers*** 95,371–378 2013.

Giusti, M., and Wrolstad, R. E., "Characterization and measurement of anthocyanins by UV-visible spectroscopy". ***Handbook of Food Analytical Chemistry***, pp. 19–31.New York: John Wiley and Sons, 2005.

Guerra, I.C.D., de Oliverira, P.D.L., Santos, M.M.F., Lucio, A.S.S.C., Tavres, J.F., Barbosa-Filho, J.M., Madruga, M.S and Leite de Souza, E., "The effects of composite coating chitosan and Mentha (*Pierita* L. or *x villosa* huds) essential oil on postharvest mold occurrence and quality of table grapes cv. Isabella", ***Innovative Food Science and Emerging Technologies***, 66, 96-103 2016.

Giménez, M. J., Valverde, J. M., Valero, D., Guillén, F., Martínez-Romero, D., Serrano, M. and Castillo, S., "Quality and antioxidant properties on sweet cherries as affected by preharvest salicylic and acetylsalicylic acids treatments", ***Food Chemistry***, 160, 226-232 2014.

Hagenmaier, R.D., "A comparison of ethane, ethylene and CO_2 peel permeance for fruit with different coatings". ***Postharvest Biology and Technology***, 37, 1, 56-64 2005.

Han, J. H. and Gennadios, A.,"Edible Films and Coatings: A Review; Innovations in Food Packaging", ***Elsevier Academic Press Amsterdam***, 15, 239-262 2005.

Han C, Lederer C, McDaniel M and Zhao Y., "Sensory evaluation of fresh strawberries (*Fragaria ananassa*) coated with chitosan-based edible coatings", ***Journal Food Science*** 70(3):172–8 2005.

Harsler, C.M., "Functional Foods: Their role in disease prevention and health promotion". ***Food Technology***, 52, 2, 57-62 1998.

Hernandez-Mu˜noz P, Almenar E, Ocio MJ and Gavara R., "Effect of calcium dips and chitosan coatingsonpostharvest life of strawberries (*Fragaria×ananassa*)", ***Postharvest Biology Technology.*** 39:247–53 2006.

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

Hirano, S., Ohe, Y and Ono, H., "Selective N-acylation of chitosan", ***Carbohydrate Research***, 47, 315–320 1976.

Hong, K., Xie, J., Zhang, L., Sun, D. and Gong, D., "Effects of chitosan coating on postharvest life and quality of guava (*Psidium guajava* L.) Fruit during cold storage", ***Scientia Horticulturae*** 144, 172-178 2012.

Hubbard, N. L., Pharr, D. M., & Huber, S. C., "Sucrose phosphate synthase and other sucrose metabolizing enzymes in fruits of various species", ***Plant Physiology***, 82, 191-196 1994.

International Organisation of Vine and Wine (OIV), "http://www.oiv.org/en/technical-standards-and-documents/statisticalanalysis/ statistical-data", ISBN 979-10-91799-56-0 2012.

Jayakumar, R., Prabaharan, M., Reis, R. L., and Mano, J. F., "Graft copolymerized chitosan-present status and applications", ***Carbohydrate Polymers***, 62, 142–158 2005.

Jiang, Y.M., Li, J and Jiang, W.B., "Effects of chitosan coating on shelf life of cold stored litchi fruit at ambient temperature", ***LWT Food Science Technology***, 38, 757-761 2005.

Jiang, Y. M and Li, Y. B., "Effects of chitosan coating on postharvest life and quality of longan fruit", ***Food Chemistry***, 73, 139-143 2001.

Jiang, Y. M., Yao, L. H., Lichter, A., and Li, J. R., "Postharvest biology and technology of litchi fruit", ***International Journal of Food, Agriculture and Environment***, 1(2), 76–81 2003.

Jianglian, D., and Zhang, S., "Application of chitosan based coating in fruit and vegetable preservation: A Review" ***Food Processing and Technology***, 4:5 2013.

Kader, A. A., "Increasing food availability by reducing postharvest losses of fresh produce", ***Acta Horticulture***. 682:2169–2175 2005.

Kalt, W., "Effect of production and processing factors on major fruit and vegetable antioxidants", ***Journal of Science***. 70, 11-19 2005.

Khan, W., Prithiviraj, B., Simith, D.L., "Chitosan and chitin oligomers increase phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities in soybean leaves". ***Journal of Plant Physiology***, 160, 859-863 2003.

Khwaldia, K., Perez, C., Banon, S., Desobry, S., and Hardy, J., "Milk proteins for edible films and coatings", ***Critical Review in Food Science and Nutrition***, 44, 239−251 2004.
Kerch, G., "The potential of chitosan and its derivatives in prevention and treatment of age-related diseases", ***Marine Drugs***, 13(4), 2158-2182 2015.

Kim, D.-O., Heo, H. J., Kim, Y. J., Yang, H. S. and Lee, C. Y., "Sweet and sour cherry phenolics and their protective effects on neuronal cells*",* ***Journal of Agricultural and Food Chemistry***, 53(26), 9921-9927 2005.

Kim, S.Y., Lim, J.H., Park, M.R., Kim, Y.J., Park, Y and Won Seo, Y., "Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under saline stress", ***Journal of Biochemistry and Molecular Biology***, 38, 218-224 2005.

Kittur, F. S., Saroja, N. S and Habibunnis – Tharanathan, R. N., "Polysaccharide based composite coating formulations for shelf-life extension of fresh banana and mango", ***European Food Research and Technology***, 213,316-311 2001.

Klessig, D. F., and Malamy, J., "The salicylic acid signal in plants", ***Plant Molecular Biology***, 26, 1439-1458 1994.

Koçak, H., Bal, E and Hasat Sonrası "UV-C ve Yenilebilir Yüzey Kaplama Uygulamalarının Kiraz Meyve Kalitesi ile Muhafaza Süresi Üzerine Etkiler", ***Turkish Journal of Agriculture and Forestry,*** 4(1), 79-88 2017.

Koç, B. E and Özkan, M., "Gıda endüstrisinde kitosanın kullanımı", ***Gıda Dergisi ,***36(3), 161-168 2011.

Langenkemper, G., McHale, R., Gardner, R. C., and MacRae, E., "Sucrose phosphate synthase steady-state mRNA increases in ripening kiwifruit. ***Plant Molecular Biology***", 36, 857—869 1998.

Le Tien, C., Lacroix, M., Ispas-Szabo, P., and Mateescu, M. A., "N-acylated chitosan: Hydrophobicmatrices for controlled drug release", ***Journal of Controlled Release***, 93, 1−13 2003.

Lin, D., and Zhao, Y., "Innovations in the development and application of edible coatings for fresh and minimally processed fruits and vegetables", **Comprehensive Reviews in *Food Science and Food Safety***. 6, 60-75 2007.

Liu, J., Tian, S, P., Meng, X and Xu, Y., "Effects of chitosan on control postharvest diseases and physiological responses of tomato fruit", ***Postharvest Biology and Technology***. 44, 300-306 2007.

Li H, Yu T., "Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit", ***Journal Science Food Agriculture.*** 81:269–74 2000.

Li, H., Yu, T., "Effect of chitosan on incidence of brown rot quality and physiological attributes of postharvest peach fruit", ***Journal Agricultural and Food Chemistry***. 81,269-274 2001.

Lin, L., Wang, B., Wang, M., Cao, J., Zhang, J., Wu, Y. and Jiang, W., "Effects of a chitosan-based coating with ascorbic acid on postharvest quality and core browning of "Yali" pear (*Pyrus bertschneideri* Rehd.)", ***Journal Science Food Agriculture***. 88:877–884 2008.

Liu, R.H., "Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals", ***American Journal of Clinical Nutrition***, 78, 517-520 2003.

Maqbool, M., Ali, A., Alderson, P. G., Zahid, N. and Siddiqui, Y., "Effect of a novel edible composite coating based on gum arabic and chitosan on biochemical and physiological responses of banana fruits during cold storage", ***Journal of Agricultural and Food Chemistry.*** 59(10), 5474-5482 2011.

McGuire, R. G., "Reporting of objective color measurements", ***HortScience.*** 27(12), 1254-1255 1992.

Meng, X. Li, B., Liu, J and Tian, S., "Physiological responses and quality attributes of table grape fruit to chitosan pre-harvest spray and postharvest coating during storage", ***Food Chemistry***. 106, 501-508 2008.

Mohammed, A., "Chitosan application for active bio-based films production and potential in the food industry: Review", **Food Science Technology.** 43: 837-842 2010.

Nair, M. S., Saxena, A. and Kaur, C., "Effect of chitosan and alginate based coatings enriched with pomegranate peel extract to extend the postharvest quality of guava (*Psidium guajava* L.)", ***Food Chemistry***. 240, 245-252 2017.

No, H., S. Meyers, W. Prinyawiwatkul, and Z. Xu, "Applications of chitosan for improvement of quality and shelf life of foods: *A review",* ***Journal Food Science***. 72:87–100 2007.

Oyaizu, M., "Studies on products of browning reaction". ***The Japanese Journal of Nutrition***, 44, 6 1986.

Perdones, Á. I., Escriche, A. Chiralt, and M. Vargas., "Effect of chitosan–lemonessential oil coatings on volatile profile of strawberries during storage", ***Food Chemestry.***197:979–986 2016.

Perdones, A., Sanchez-Gonzalez, L., Chiralt, A and Vargas, M., "Effect of chitosan-lemon essential oil coatings on storage-keeping quality of strawberry", ***Postharvest Biology and Technology***, 70, 32-41 2012.

Perez, A. G and Sanz, C., "Effect of high oxygen and high carbon dioxide atmospheres on strawberry flavor and other quality traits", ***Journal Agriculture Food Chemistry***. 49, 2370-2375 2001.

Petriccione, M., "Influence of postharvest chitosan treatment on enzymatic browning and antioxidant enzyme activity in sweet cherry fruit", ***Postharvest Biology and Technology,*** 109, 45-56 2015.

Petriccione, M., Sanctis, D. F., Pasquariello, S.M., Mastrobuoni, F., Rega, P., Scortichini, M and Mencarelli, F., "The effect of chitosan coating on the Quality and Nutraceutical traits of sweet cherry during postharvest life", ***Food Bioprocess***, 8,394-408 2014.

Quan, L. J., Zhang, B., Shi, W. W. and Li, H. Y., "Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network", ***Journal of Integrative Plant Biology,*** 50(1), 2-18 2008.

Rinaudo, M., "Chitin and chitosan: Properties and applications", ***Progress in Polymer Science*** 31, 603-32 2006.

Romanazzi, G., Lichter, A., Gabler, F.M and Smilanick, J.L., "Recent advances on the use of natural and safe alternatives to conventional methods to control postharvest gray mold of table grapes", ***Postharvest Biology, Technology***, 63, 141–147 2012.

Romanazzi, G., Feliziani, E., Bano, B. S and Sivakumar, D., "Shelf life extension of fresh fruit and vegetables by chitosan treatment"***, Critical Reviews in Food Science and Nutrition***, 57:3, 579-601 2017.

Sanchez-Gonzalez, L., Pastor, C., Vargas, M., Chiralt, A., Gonzalez-Martinez, C and Chafer, M., "Effect of hydroxyl propyl methyl cellulose and chitosan coatings with and without bergamot essential oil on quality and safety of cooled stored grapes", ***Postharvest Biology and Technology***, 60, 57-63 2011.

Sanchez-Gonzalez, L., Chafer, M., Chiralt, A., Gonzalez-Martinez, C., "Physical properties of edible chitosan films containing bergamot essential oil and their inhibitory action on *Penicillium italicum*". ***Carbohydrate Polymers***, 82, 2,277-283 2010.

Sanz, C., Perez, A. G., Olias, R and Olias, J. M., "Quality of strawberries packed with perforated polypropylene", ***Journal Food Science,*** 64, 748-752 1999.

Sayyari, M., Babalar, M., Kalantari, S., Serrano, M., and Valero, D., "Effect of salicylic acid treatment on reducing chilling injury in stored pomegranates", ***Postharvest Biology and Technology***, 53, 152-154 2009.

Sayyari, M., Aghdam, M.S., Salehi, F., Ghanbari, F., "Salicyloyl chitosan alleviates chilling injury and maintains antioxidant capacity of pomegranate fruits during cold storage". ***Scientia Horticulturae***, 211,110-117 2016.

Shahidi, F., "Chitin and chitosan from marine by-products. In F. Shahidi (Ed.), maximizing the value of marine by-products .Abington", ***Wood Head Publishing Limited***. 340–373 2007.

Shafiee, M., Taghavi, T. S. and Babalar, M., "Addition of salicylic acid to nutrient solution combined with postharvest treatments (hot water, salicylic acid, and calcium dipping) improved postharvest fruit quality of strawberry", ***Scientia Horticulturae,*** 124(1), 40-45 2010.

Shao, X. F., Tu, K., Tu, S. and Tu, J., A combination of heat treatment and chitosan coating delays ripening and reduces decay in "Gala" apple fruit**.** ***Journal Food Quality***, 35:83–92 2012.

Shen, Y. and Yang, H., Effect of pre-harvest chitosan-g-salicylic acid treatment on postharvest table grape quality, shelf life, and resistance to *Bothryis cinerea*-induced spoilage. ***Scientia Horticulturae***, 224, 367-373 2017.

Shiekh, R. A., Malik, M. A., Al-Thabaiti, S. A., and Shiekh, M. A. Chitosan as a novel edible coating for fresh fruits**.** ***Food Science and Technology Research***, 19, 139-155 2014.

Shiri, M. A., Ghasemnezhad, M., Bakhshi, D. and Sarikhani, H. Effect of postharvest putrescine application and chitosan coating on maintaining quality of table grape cv. "Shahroudi" during long-term storage. ***Journal Food Process***, 37:999–1007 2012.

Shiri, M.A., Bakhshi, D., Ghasemnezhad Dadi, M., Papachatzis, A., Kalorizou, H. "Chitosan coating improves the shelf life and postharvest quality of table grape (*Vitis vinifera*) cultivar Shahroudi". ***Turkish Journal of Agriculture and Forestry***, 37, 148–156 2013.

Shivashankara, K.S., Isobe, S., Al-Haq, M.I., Takenaka, M., Shina, T., "Fruit antioxidant activity, ascorbic acid, total phenol, quercetin and carotene of Irwin mango fruits stored at low- temperature after high electric field treatment". ***Journal of Agricultural of Food Chemistry***, 52, 1281-1286 2004.

Silva, N.D.L.F., Schmidt, M.E., Messias, L.C, Eberlin, N.M, Sawaya, F.H.C.A., "Quantitation of organic acids in wine and grapes by direct infusion electrospray ionization mass spectrometry". ***Analytical Methods***, 7, 53-62 2014.

Slinkard, K. and Singleton, V. L., "Total phenol analysis: Automation and comparison with manual methods". ***American Journal of Enology and Viticulture*** 28(1), 49-55 1977.

Smilanick, J. L., Harvey, J. M., Harstell, P. L., Henson, D. J., Harris, C. M., Fouse, D. C., "Influence of sulfur dioxide fumigant dose on residues and control of postharvest decay of grapes". ***Plant Disease***, 74, 418–421 1990.

Sousa, L.L., Andrade, S.C.A., Athayde, A.J.A.A., Oliveira, C.E.V., Sales, C.V., Madruga, M.S., Souza, E.L., "Efficacy of *Origanum vulgare* L. and Rosmarinus officinalis L. essential oils in combination to control postharvest pathogenic Aspergilli and autochthonous mycoflora in *Vitis labrusca* L. (table grapes)". ***International Journal Food Microbiology***. 165(3), 312–318 2013.

Srivastava, M. K., Dwivedi, U. N., "Delayed ripening of banana fruit by salicylic acid"**.** ***Plant Science***, 158, 87-96 2000.

Tavarini, S., Degl'Innocenti, E., Remorini, D., Massai, R., Guidi, L.," Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of Hayward kiwifruit". ***Food Chemistry***, 107, 282-288 2007.

USFDA, 2013. GRAS notice inventory. GRN No. 397. www.fda.gov. Accessed 9 January 2013.

Valle, D.V., Hernández-Muñoz, P., Guarda, A and Galotto, M.J., "Development of a cactus-mucilage edible coating (*Opuntia ficus* indica) and its application to extend strawberry (*Fragaria ananassa*) shelf-life", ***Food Chemistry***, 91, 751-756 2005.

Vargas M, Albors A, Chiralt A,Gonz´alez-Martinez C., Quality of cold-stored strawberries as affected by chitosan-oleic acid edible coatings. ***Postharvest Biology Technology***, 41:164–171 2006.

Velickova, E., Winkelhausen, E., Kuzmanova, S., Alves, V. D. and Moldão-Martins, M., "Impact of chitosan-beeswax edible coatings on the quality of fresh strawberries (*Fragaria ananassa* cv Camarosa) under commercial storage conditions". ***LWT-Food Science and Technology,*** 52(2), 80-92 2013.

Vu, K.D, Hollingsworth, R.G., Leroux, E., Salmieri, S., Lacroix, M., "Development of edible bioactive coating based on modified chitosan for increasing the shelf life of strawberries" ***Food Research International***, 44, 198-203 2010.

Wang, S. Y., Gao, H., "Effect of chitosan-based edible coating on antioxidants, antioxidant enzyme system and postharvest fruit quality of strawberries (*Fragaria×aranassa* Duch)". ***Food Science and Technology***, 52, 71–79 2013.

Wallace, G., Fry, S. C., "Action of diverse peroxidases and Laccases on six cell wall-related phenolic compounds". ***Phytochemistry,*** 52, 769-773 1999.

Wills, R., McGlasson, B., Graham, D., & Joyce, D., "Postharvest, an introduction to the physiology and handling of fruit & vegetables and ornamentals, (Fourth ed.)." Sydney 2052, Australia: University of New South Wales Press Ltd.\University of New South Wales. 1998.

Wang, L., Chen, S., Kong, W., Li, S., & Archbold, D. D., "Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage". ***Postharvest Biology and Technology***, 41, 244-251 2006.

Wong, D., Gastineau, F., Gregorski, K. S., Tillin, S.J., Paclath, A. E., "Chitosan-lipid films microstructure and surface energy". ***Journal Agriculture Food Chemistry***, 40, 540-544 1992.

Wrolstad, R. E., "Color and Pigment Analysis in Fruit Products". *S Bulletin 624*, Agricultural Experiment Station, Oregon State University, Corvallis, 1976.

Xu, C., Zhao, B., Wang, X., Yuan, X and Wang, Y., "Elicitor-enhanced syringing production in suspension cultures of saussureamedusa", ***World Journal microbial Biotechnology***, 23, 965-970 2007.

Xu,W. T., Qu,W., Huang, K. L., Guo, F., Yang, J. J., Zhao, H.,. "Antibacterial effect of grapefruit seed extract on food-borne pathogens and its application in the preservation of minimally processed vegetables". ***Postharvest Biology and Technology***, 45, 126–133 2007.

Yang, Y., Cui, J., Zheng, M., Hu, C., Tan, S., Xiao, Y, "One step synthesis of amino-functionalized fluorescent carbon nanoparticles by hydrothermal carbonization of chitosan" ***Chemical Communications***, 48, 380–382 2012.

Yang, Z., Cao, S., Cai, Y. and Zheng, Y., "Combination of salicylic acid and ultrasound to control postharvest blue mold caused by Penicillium expansum in peach fruit", ***Innovative Food Science & Emerging Technologies*** 12(3), 310-314 2011.

Zhang, Y. Z., Zhang, M. I., Yang, H. Q., "Postharvest chitosan-g-salicylic acid application alleviates chilling injury and preserves cucumber fruit quality during cold storage". ***Food Chemistry***. 174(5), 558-563 2015.

Zhang, Y., Chen, K., Zhang, S., & Ferguson, I., "The role of salicylic acid in postharvest ripening of kiwifruit"**.** ***Postharvest Biology and Technology***, 28, 67-74 2003.

Zeng, K. F., Cao, J. K., & Jiang, W. B., "Enhancing disease resistance in harvested mango (*Mangifera indica* L. cv. Matisu) fruit by salicylic acid". ***Journal of the Science of Food and Agriculture***, 86, 694-698 2006.

Zhishen, J.,Mengcheng, T.,&Jianming,W., "The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals". ***Food Chemistry***, 64, 555–559 1999.

Zhou, R., Mo, Y., Li, Y., Zhao, Y., Zhang, G., Hu, Y., "Quality and internal characteristics of Huanghua pears (*Pyrus pyrifolia* Nakai, cv. Huanghua) treated with different kinds of coatings during storage". ***Postharvest Biology and Technology***, 49, 171-179 2008.

Zhu, X., Wang, Q.M., Cao, J.K., Jiang, W.B., "Effects of chitosan coating on postharvest quality of mango (*Mangifera indica* L.CV. Tainong) fruits". ***Journal Food Process. Preservation,*** 32, 770-784. 2008.

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