EFFECTS OF PREHARVEST AND POSTHARVEST TREATMENTS ON QUALITY CHARACTERISTICS OF GRAPES

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ABSTRACT

EFFECTS OF PREHARVEST AND POSTHARVEST TREATMENTS ON QUALITY CHARACTERISTICS OF GRAPES

Gray mold, caused by *Botrytis cinerea*, is the most important disease which causes major quality and quantity losses in preharvest period and postharvest storage of grapes. Grapes are commonly treated with sulfurdioxide (SO_2) to control decay caused by *Botrytis cinerea* both in vineyard and during storage. However, there is need for natural alternatives to replace SO_2 because of many considerations related with sulfite residues, emergence of *B.cinerea* resistant strains, hazards for human and environment, negative effects on quality characteristics and sustainability, and certification for organic grapes. In this study, the effects of alginate solution with or without (w/wo) vanillin as preharvest spray and postharvest edible coating on quality, safety and biochemical properties of grapes from Semillon Blanc, Alphonse Lavalleé and Razaki cultivars were investigated. Soluble solid content, pH, titratable acidity, total phenolic content, antioxidant activity, yeast-mold counts, lactic acid content, color, and firmness were determined at the day of preharvest treatment, at harvest and during 35 days of storage at 4±2°C. In addition, sensory evaluation of grapes coated w/wo vanillin were also performed using hedonic scale test. Alginate treatments w/wo vanillin were effective in preventing weight, soluble solids, acidity, and firmness losses. Incorporation of vanillin into alginate coating provided significant reduction in yeast-mold growth. Moreover, phenolic content and antioxidant activity of grapes treated with alginate coating incorporating vanillin were higher than others during postharvest storage. In sensory evaluation, appearance was ranked as the highest for alginate coating wo vanillin. As a conclusion, alginate solution enriched with vanillin could be a natural alternative of synthetic fungicides to prevent deteriorations and enhance quality of grapes.

ÖZET

HASAT ÖNCESİ VE HASAT SONRASI UYGULAMALARIN ÜZÜMLERİN KALİTE ÖZELLİKLERİ ÜZERİNE ETKİSİ

Üzümlerde hasat öncesi dönemde ve hasat sonrası depolamada büyük ölçüde kalite ve miktar kayıplarına neden olan en önemli etmen *Botrytis cinerea*'nın neden olduğu gri küf hastalığıdır. *Botrytis cinerea*'nın neden olduğu bozulmanın kontrol edilmesi amacıyla üzümler genellikle bağda ve depolama boyunca kükürt dioksit (SO_2) ile muamele edilmektedir. Ancak, üzümlerde görülen sülfit kalıntıları, dirençli *Botrytis cinerea* türlerinin ortaya çıkması, insan sağlığı ve çevreye zararları, üzümlerin kalite özellikleri ve sürdürülebilirlik üzerine negatif etkileri, organik üzüm üretimi gibi pek çok unsur nedeniyle kükürt dioksit kullanımının yerine geçebilecek doğal alternatiflere ihtiyaç duyulmaktadır. Bu çalışmada, vanilin içeren ve içermeyen aljinat çözeltilerinin hasat öncesi sprey ve hasat sonrası yenilebilir kaplama olarak uygulamasının Semillon Blanc, Alphonse Lavalleé ve Razaki üzüm çeşitlerinde üzümlerin kalitesi, güvenliği ve biyokimyasal özellikleri üzerine etkisi incelenmiştir. Üzümlerde suda çözünen kuru madde miktarı, pH, titreedilebilir asitlik, toplam fenolik madde, antioksidant aktivitesi, küf-maya sayımı, laktik asit miktarı, renk değerleri ve meyve sertliği hasat öncesinde, hasatta ve hasat sonrası 35 gün boyunca 4±2 °C'de depolama sırasında belirlenmiştir. Ayrıca, vanilin içeren ve içermeyen yenilebilir kaplama ile kaplanan üzümlerin duyusal olarak değerlendirilmesi hedonik skala testi kullanılarak yapılmıştır. Vanilin içeren ve içermeyen aljinat bazlı yenilebilir kaplama ağırlık, suda çözünen kuru madde ve sertlik kaybını önlemede etkili olmuştur. Vanillin içeren aljinat bazlı yenilebilir kaplama maya ve küf gelişiminde önemli derecede azalma sağlamıştır. Bununla birlikte, vanilin içeren yenilebilir kaplamalarla kaplanan üzümlerin fenolik madde miktarının ve antioksidant aktivitesinin depolama boyunca diğer örneklerden daha yüksek olduğu görülmüştür. Duyusal değerlendirme sonucu, vanilin içermeyen yenilebilir kaplamalarla kaplanan üzümlerin görünüşü en yüksek derecelendirilmiştir. Sonuç olarak, üzümlerde bozulmaları önlemek ve kalitelerini arttırmak için vanilin ile zenginleştirilmiş aljinat çözeltisinin sentetik fungisitler yerine doğal bir alternatif olarak kullanılabileceği belirlenmiştir.

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CHAPTER 1

INTRODUCTION

As the healthy diet trend increasing, demand for fresh and processed fruit and vegetables has been increasing. Fresh fruit and vegetables offer high nutrition fact with delicious taste for consumers. However, quality and quantity losses in fresh fruit and vegetables appear from harvesting until they reach to consumers. These require different technologies to maintain quality and safety of fresh fruit and vegetables within the supply chain. In addition, industry products of fresh fruit and vegetables such as fresh-cut produce, fruit or vegetable juice, frozen or dried fruit and vegetables need high quality of the raw material to obtain desired quality and ensure safety of the final product. Enviromentally friendly packaging technologies can provide quality and safety of fresh fruit and vegetables during transportation and storage. Edible coatings are type of bio-based food packaging which maintain the quality and safety of food as well as provide functionality while reducing packaging wastes. Major postharvest quality losses of fresh fruit and vegetables such as color, texture, flavor and nutritional value can be prevented by edible coatings. Beside these, edible coatings may increase functionality and extend the shelf-life of food by being carriers of active ingredients such as aroma compounds, antimicrobial and antioxidant agents. Another benefit of edible coating or biodegredable food packaging is the reduction of plastic packages produced from polymers and reduction of food waste, especially fresh produce by slow down ripening.

Preharvest treatments are also applied to maintain quality characteristics of fresh fruit and vegetables from maturation until harvesting. Because insects and microorganisms such as bacteria, yeast and fungi cause the deteriorations during growning in the farm. Preharvest and postharvest deteriorations are mainly caused by fungal decay in fresh fruit and vegetables. Thus, chemical fungicides are commonly used by the farmers, but the main trend driven by consumers is natural organic fresh fruit and vegetables. Organic agriculture deals with preharvest losses due to the restriction of synthetic chemicals to preserve the human health and environment. Therefore, there is also a need for alternative strategies to replace with synthetic fungicides in order to prevent preharvest and postharvest losses.

Grapes are one of the non-climacteric fruits widely produced worldwide and considered as a valuable source of phenolic compounds, anthocyanins, flavonoids, and antioxidants. Gray mold, caused by *Botrytis cinerea*, is the most important disease of grapes in both preharvest period and postharvest storage. Preharvest spray applications of sulfurdioxide as synthetic fungicide are commonly carried out during growning period of grapes in vineyard. As the grapes are harvested, they are also periodically treated with sulfurdioxide to prevent postharvest diseases. However, fumigation with sulfurdioxide is limited due to residues, toxicity, human health and environment concern, emergence of resistant strains and food quality. Thus, incorporation of biocontrol agents such as antimicrobial agents into edible coatings can be natural alternatives which is applied as preharvest treatment and postharvest treatment in order to prevent fungal decay, maintain quality characteristics and extend shelf-life of grapes.

The objectives of this study are to develop alginate based bioactive edible films and coatings enriched with vanillin and to investigate the effects of preharvest alginate spray application and postharvest alginate coating with or without(w/wo) vanillin on quality, safety and biochemical properties of grapes from different cultivars in order to prevent deteriorations in vineyard (preharvest) and during storage (postharvest). Soluble solid content, pH, titratable acidity, total phenolic content, antioxidant activity, yeast-mold counts, lactic acid content, color and firmness values, and sensory properties were determined by means of quality characteristics of grapes. Furthermore, characterisation of alginate based edible film was studied by water solubility, color and mechanical properties, oxygen and carbondioxide transmission rate and antifungal activity against *Botrytis cinerea.*

CHAPTER 2

LITERATURE REVIEW

2.1. Edible Films and Coatings

Consumer demand for natural foods having high quality and safety has been increased dramatically. Companies and researchers lead to develop different ways to maintain quality, freshness, and safety of foods. Packaging plays an important role for maintaining food quality and safety during storage. Edible films and coatings are kind of food packaging which maintain the quality and safety of food while reducing packaging wastes due to their biodegradability (Espitia, Du, Avena-Bustillos, Soares, & McHugh, 2014). Edible films and coatings are defined as thin layers produced from edible, biodegradable materials including polysaccharides, proteins, and lipids. The layers applied on surface of food product have an ability to protect the product from physical damages, chemical and microbiological deteriorations. In addition, incorporation of edible films and coatings into standard packaging technologies are the way to improve quality characteristics of foods (Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011).

Edible films and coatings have been studied extensively for quality enhancement and/or shelf-life extention of food products. They act as a semi-permeable barrier on the food surface to control moisture and gas transmission e.g. oxygen and carbondioxide gas exchange (Sharma & Rao, 2015). Use of edible coatings on highly perishable foods plays an important role with providing a modified atmosphere that reduce moisture and aroma losses as well as respiration and oxidative reaction rates, delay color and texture changes, and improve the general appearance of the product through storage (Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008). In addition, they can serve as carriers for active ingredients such as aroma compounds, antimicrobial and antioxidant agents which improve the quality and functionality of foods. They also have capacity to maintain desirable concentrations of color, flavor, spiciness, sweetness, saltiness, etc. that enhance organoleptic properties of food products. Incorporation of these bioactive compounds as antimicrobials, antioxidants, aroma compounds, nutraceuticals,

probiotics represents an innovative concept for the edible films and coatings (Huber & Embuscado, 2009).

Applications of edible coatings are rapidly increasing due to their simpleness and eco-friendly characteristics (Sharma & Rao, 2015). There are some considerations for edible coatings and films to be used in foods. These considerations are cost, accessability of coating material, effective barrier properties for water vapor, oxygen and carbondioxide, being GRAS (generally recognized as safe), having good optical and mechanical properties (Falguera et al., 2011; Olivas & Barbosa-Cánovas, 2005).

In the food industry, edible films and coatings have been used for many centuries. However, a new trend has been developed by incorporation of bioactive compounds into the formulation that brings some functionalities and it results in quality preservation and shelf-life extension of food products. Addition of antimicrobial agents, antibrowning agents, antioxidants and flavors into the structure of coating create an active packaging concept which is an innovative packaging technology that extend the shelf-life and maintain the quality of the food by changing the conditions (Vermeiren, Devlieghere, Van Beest, De Kruijf, & Debevere, 1999). Another advantage is that the additional nutrients can be consumed along with the food by application of edible film or coating (Guilbert, Gontard, & Gorris, 1996).

Edible films and coatings containing bioactive compounds play an essential role in food technology as active food packaging. Active packaging has been defined as "a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food" which was stated in the European FAIR-project CT 98-4170 (Vermeiren et al., 1999). As an active packaging, edible film or coating carrying antibrownings, antioxidants or antimicrobials considerably improve the quality and extend the shelf-life of food. Active properties of edible coating are associated with release of bioactive compound incorporated into coating to avoid deteriorations in food. Antioxidative and antibrowning agents such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ascorbic acid, citric acid, tocopherol have been used for preventing enzymatic browning, inhibiting oxidation and improving antioxidant properties of various foods such as fresh fruit and vegetables (Baldwin, Nisperos-Carriedo , & Baker, 1995). In addition, several natural antimicrobial compounds have been used in food applications; these are including essential oils derived from plants (e.g., basil, thyme, oregano, cinnamon, clove, and rosemary), enzymes obtained from

animal sources (e.g., lysozyme, lactoferrin), bacteriocins from microbial sources (nisin, natamycin), organic acids (e.g., sorbic, propionic, citric acid) and naturally occurring polymers (chitosan) (Lucera, Costa, Conte, & Nobile, 2012). Recently, edible coatings are commonly used as a structure to incorporate bioactive compounds and it offers biodegredable and active packaging in order to improve quality of food products.

2.2. Applications of Edible Coatings on Fresh Produce

Promoting healthy diet and increasing world population poses rising consumption of fresh fruit and vegetables. However, fresh fruit and vegetables require considerable time to reach consumer or enter processing from an orchard or field. Their quality characteristics and nutritional value must be prevented during the transportation and storage. In this point, alternative technologies are required to keep quality, safety and nutritional value of fruit and vegetables from the time of harvesting until the time of consumption. There are different methods to improve quality and keep safety of fresh fruit and vegetables. Among other methods, use of edible coatings are widely preferred for maintaining freshness of fruit and vegetables due to their easy application, cost-effective and enviromentally-friendly properties. Edible coatings are also applied on fresh produce as active packaging by incorporating active substances into polymer matrix. In addition, this eco-friendly active packaging can reveal functional fruit and vegetable products when it is incorporated with functional ingredients.

Main purposes of applying edible coating on fresh produces are to produce a modified atmosphere, delay color changes, improve appearance, reduce water and aroma loss and be carries of antimicrobials, antioxidants, texture exchangers and nutraceuticals (Olivas & Barbosa-Cánovas, 2005). Control of internal atmosphere by use of edible coating minimize quality losses and reduce quantity losses in fresh fruit and vegetables (Park, 1999). Edible coatings interact with the coated fruit and/or the surrounding environment in a desirable way through their function as a barrier and maintain fruit freshness by preventing water losses and aroma losses as well as reducing respiration rate. Industrial fruit coating, which has not been commonly applied yet, are performing mostly by keeping fruits in flow with vibration or rolling motion, so that the fruits are exposed to coating dispersion. Another technique is spray coating for application of coatings, including a batch tank and spraying nozzles to disperse the

coating solution on food, then drive them to a drying step (Henriette Monteiro Cordeiro de, 2012).

The effect of edible coating on quality of fruit and vegetables depends on the composition of coating solution. Thus, it is very important to determine the formulation of coating according to the properties of fruit and vegetables (M. A. Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2009). Due to the respiration process, fruit and vegetables need oxygen and produce carbondioxide. Therefore, the permeability of coating material is very significant to keep adequate oxygen and carbondioxide ratio. The coating of fruit and vegetables with edible film having good O_2 and CO_2 permeability is considered as another way to apply modified atmosphere (Cemeroğlu, 2011). Polysaccharide-based edible coatings are commonly used for fruit and vegetables because of their selective permeabilities to O_2 and CO_2 (Oms-Oliu et al., 2008). Polysaccharides such as starch, pectin, alginate, chitosan, cellulose, carrageenan and gums have good gas barriers for fruit and vegetables (Baldwin et al., 1995).

Starches are polymers of d-glucopyranosyl, consisting of a mixture of two polymers, amylose and amylopectin. Edible films or coatings produced from starch films are limited by two major disadvantages. These are very brittle and require the addition of plasticizers to improve the flexibility. Additionally, the high hydrophilicity of starch results poor barrier properties in high relative humidity. Thus, starch is not prefered when working with fruits (Henriette Monteiro Cordeiro de, 2012).

Pectin is commonly used polysaccharide, being the main component in apple and citrus by-products. Pectin is composed of β-1,4-linked d-galacturonic acid where carboxyl groups of uronic acid are either fully (DE>50%, HMP: high methoxyl pectin) or particually (DE<50%, LMP: low methoxyl pectin) methyl esterified. Low methoxyl pectin tend to form gel through divalent cations (Silva, Bierhalz, & Kieckbusch, 2009). In order to control enzymatic browning and microbial growth, pectin based edible coating incorporating antioxidants and antimicrobial agents were applied on fresh-cut persimmon fruits. In this study, 10 g/kg citric acid and 10 g/kg calcium chloride were incorporated as antioxidants into coating solution. Potassium sorbate at 2 or 4 g/kg, sodium benzoate at 4 g/kg, and nisin at 500 IU/ml were used as antimicrobial agents. Pectin based coatings with antioxidants and antimicrobials effectively reduced the growth of mesophilic bacteria and controlled enzymatic browning of fresh-cut persimmon during storage at 5°C. However, for sensory quality of fruit, judges reported that the incorporation of antioxidants and antimicrobials into the pectin coating resulted

in slight acidity in samples and did not represent the characteristic flavor of persimmon (Sanchís et al., 2016).

Chitosan is another valuable polysaccharide which exhibits antibacterial and antifungal properties for preservation of food products. The effect of chitosan coatings on postharvest quality of fresh fruit and vegetables were widely studied. Polyphenol content of fresh fruit and vegetables declines during storage due to the activity of specific enzymes such as polyphenol oxidase (PPO) and peroxidase. Applications of chitosan coating were found to be effective on enzyme activity to control browning in fruits during storage (Kerch, 2015). The study performed with plums investigated impact of chitosan coating incorporated with ascorbic acid on quality of plum. It was reported that chitosan coating with combination of ascorbic acid exhibited considerably lower PPO activity and lower respiration rate during storage. It also significantly delayed the decreases in firmness and changes in color of plums (Liu, Yuan, Chen, Li, & Liu, 2014). Biodegradable coatings produced from chitosan (CH), methylcellulose (MC), carboxymethyl cellulose (CMC) or hydroxypropyl methylcellulose (HPMC) were applied on mandarins which are prone to loss weight, firmness and flavor. Among these polysaccharides, CMC was determined as the best coating for mandarins with the good firmness, lower weight loss and favorable glossiness. In this study, selected two polysaccharides which were CMC and chitosan used in layer by layer coating of mandarin. All quality characteristics of fruits were developed with layer by layer coating consisting an internal layer from CMC and an external layer from chitosan (Arnon, Granit, Porat, & Poverenov, 2015). In another study, chitosan coatings incorporated with calcium gluconate were evaluated as active packaging for the development of nutritionally fortified strawberries. It exhibited a significant increase in calcium content of fruit compared to control. In addition, chitosan coating was effective on controlling fungal decay, maintaining firmness and delaying color changes of strawberries at 20°C during 4 days (Hernández-Muñoz, Almenar, Ocio, & Gavara, 2006).

Alginates are water-soluble natural biopolymers extracted from several species of brown algae such as *Laminaria hyperborea, Ascophyllum nodosum, and Macrocystis pyrifera*. On a structural bases, alginate contains two acid units, β-(1-4)-linked D-mannuronic acid (M) and α-(1-4) linked L-guluronic acid (G), conjugated in the form of homopolymeric (MM or GG) and heteropolymeric blocks (MG or GM). Its unique properties includes film forming, gel producing, emulsion stabilizing, biocompatibility

and biodegradability. Gel forming, the most important property of alginate, occurs through the interaction and cross-linking between the G units of alginate and the cation in solution, specially calcium ions to produce strong gels (Pereira, Tojeira, Vaz, Mendes, & Bártolo, 2011; Rhim, 2004).

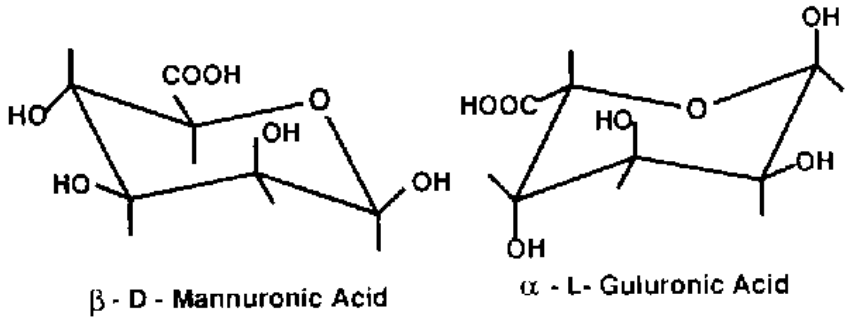


Figure 2.1. Monomers of alginate

Alginate is being an attractive polysaccharide because of its functionalities such as gel producing, film forming, emulsion stabilizing with non-toxicity and low price. Sodium alginate differs from the other kinds of alginates by producing water-soluble, tasteless, odorless, glossy and flexible edible films with low permeability to oxygen and oils (Tavassoli-Kafrani, Shekarchizadeh, & Masoudpour-Behabadi, 2016). Recently, sodium alginate is commonly used in developing biodegradable films and coatings for foods due to their good transparency and good resistance to gas exchange (Silva et al., 2014). Edible films produced from hydrocolloids like alginate show poor moisture barriers because of their hydrophilic structure. However, gel formation of alginate with calcium ions improve the water resistance of alginate films. It was shown that calcium treatment decreased significantly water vapor permeability (WVP) of alginate films (Rhim, 2004).

Alginate based edible coatings have been studied on different fruit and vegetables such as pineapple (Azarakhsh, Osman, Ghazali, Tan, & Mohd Adzahan, 2014), plum (Valero et al., 2013), apple (Olivas, Mattinson, & Barbosa-Cánovas, 2007), mushroom (Jiang, Feng, & Wang, 2013), melon (Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2008), strawberry (Fan et al., 2009) and arbutus berry (Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2015). The incorporation of lemongrass essential oil at different concentrations (0.1, 0.3 and 0.5% w/v) into alginate based edible coating was applied on fresh-cut pineapple. During 16 days of storage, alginate edible coatings enriched with lemongrass essential oil as a natural antimicrobial agent at 0.3 and 0.5% (w/v) concentrations were found more effective than the concentration at

0.1% (w/v) in reducing respiration rate, yeast and mold counts as well as total plate count of fresh-cut pineapple. Alginate coatings also maintained firmness, color values and sensory characteristics compared to uncoated samples. However, incorporation of 0.5% (w/v) lemongrass essential oil affected sensory attributes of fruit. Thus, alginate based edible coating including 0.3% (w/v) lemongrass essential oil was recommended to extend the shelf-life and maintain the quality of fresh-cut pine apple (Azarakhsh et al., 2014). In another study, postharvest quality characteristics of plum fruit such as weight loss, acidity decrease, softening, and color changes were delayed by application of 1% and 3% (w/v) alginate edible coating before storage (Valero et al., 2013). Moreover, incorporation of N-acetylcystine as an antibrowning agent into alginate and gellan edible coatings was succesfully applied on fresh-cut apples. It was demonstrated that alginate and gellan coatings prolonged the shelf life of fruit by reducing ethylene production, maintaining firmness and color as well as reducing the growth of microorganisms (Rojas-Graü, Tapia, & Martín-Belloso, 2008). Alginate coatings at 1%, 2% and 3% (w/v) concentrations maintained quality of button mushroom and extended its shelf-life to 16 days under high oxygen modified atmosphere. Particularly, alginate coating at 2% (w/v) concentration provided high level of firmness, reduced loss of ascorbic acid and delayed browning of button mushroom under high oxygen modified atmosphere (Jiang, 2013). Moreover, alginate coating incorporated with lemongrass oil (1.0 and 1.5% w/v), oregano oil (0.5% w/v) and vanillin (0.3% and 0.6% w/v) was found to be beneficial on quality and shelf life of fresh-cut apple during cold storage. These antimicrobial alginate coatings were effective in reducing growth of psychrophilic microorganisms and yeast and mold, also reducing ethylene production and preventing firmness and color changes in fresh-cut apples (Rojas-Graü et al., 2007).

The effect of different edible coatings incorporating selected bioactive compounds on different types of fruit and vegetables have been investigated. However, there is still need for further studies on some issues of edible coatings such as sensory attributes of coated foods, effectiveness of bioactive compound, characterisation of coating materials and application on different food matrices.

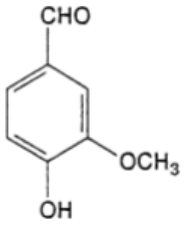
2.3. Bioactive Edible Coatings Incorporating Antimicrobial Agents

Edible coatings have been commonly applied as carriers of bioactive compounds which have a specific function to preserve the quality and safety of fresh fruit and vegetables. Edible coating incorporating antimicrobial agents is a form of antimicrobial packaging concept as an active food packaging. The use of antimicrobial agents into a food packaging system can control microbial growth by migration of the agents from the packaging to the surface of the product. Release of active substance incorporated into the film or coating generate the required concentration that inhibit the growth of releated microorganisms. The mass transfer of active substance is one of the most important point to provide antimicrobial activity. The other factors are consisting of chemical composition of film or coating, storage temperature, and characteristics of antimicrobial substance and food (Quintavalla & Vicini, 2002). In addition, the efficiency against the targeted microorganisms must be considered primarily in the selection of an antimicrobial compound and then, any interactions between the antimicrobial compound, coating material and food should be investigated (Kurek, Moundanga, Favier, Galić, & Debeaufort, 2013).

Antimicrobial edible coatings have been developed with several antimicrobial agents in order to preserve fresh produce from microbial contamination. As far as health issues, environmental problems and consumer awareness regarding synthetic chemicals concerned, researchers has focused on natural antimicrobial compounds to incorporate into edible coatings. Thus, there is an increasing demand for natural antimicrobial compounds from natural sources. The compounds with phenolic groups as oils of clove, oregano, rosemary, thyme, and vanillin are the most effective compounds because their antimicrobial activity are derived from chemical structure, in particular to the presence of hydrophilic functional groups, such as hydroxylgroups of phenolic components (Lucera et al., 2012).

Vanillin (4-hydroxy-3-methoxybenzaldehyde; Figure 2.2), which is the main component of vanilla, is widely used in food, beverage and pharmaceutical industries due to its functional properties (Walton, Mayer, & Narbad, 2003). Vanillin becomes one of the most attractive natural aroma compounds. Besides that, it exhibits bioactive properties e.g. antioxidant and antimicrobial properties against yeasts, molds and bacteria through many phenolic compounds in its structure. Studies also reported that it

can be considered as nutraceutical molecule by means of antimutagenic and antitumor characteristic (Mourtzinos, Konteles, Kalogeropoulos, & Karathanos, 2009). Therefore, vanilin has a potential to preserve foods beside its characteristic vanilla flavour. The antimicrobial activity of vanillin was investigated in fruit purées and it was found effective against yeast, molds, and bacteria at a concentration of 3000ppm (Cerrutti, Alzamora, & Vidales, 1997). Its effect in combination with other factors was tested and combination of vanillin with pH reduction showed synergistic effect on mold growth of *Aspergillus* species (Lopez-Malo, Alzamora, & Argaiz, 1998). Combined effect of vanillin and potassium sorbate also demonstrated synergistic effect on mold growth of *Penicillium* species (Matamoros-Leon, Argaiz, & Lopez-Malo, 1999). In addition, the inhibitory effects of vanillin (3-7mM) on the growth of *Aspergillus flavus, A. niger, A. ochraceus* and *A. parasiticus* in five fruit-based agar systems (apple, banana, mango, papaya and pineapple) were investigated and it was found to be significantly effective on growth rate. The minimum inhibitory concentration of vanillin on the mold growth was in the range of 1000-2000ppm and the most resistant mold was *A.niger* (López-Malo, Alzamora, & Argaiz, 1995).



Vanillin

Figure 2.2. Vanillin structure

Recently, many essential oils and natural volatile compounds such as eugenol, thymol, linalool, and carvacrol were commonly used to develop an antimicrobial packaging for the inhibition of microbial growth. Beside microbial inhibition, volatile compounds contribute to the flavor and odor of fruits (Ramos et al., 2013). Selecting the compound is very important because the most important quality attributes for fresh fruits are flavor and apperance for consumers (Velickova, Winkelhausen, Kuzmanova, Alves, & Moldão-Martins, 2013). Impact of edible film or coatings with antimicrobial agents on sensory quality of fresh produce should be considered. Edible film or coating generally have very little taste or smell because many proteins and polysaccharides do not have any taste or smell. However, volatile components especially from essential oils

which have intensive odor may expose an undesirable sensory characteristics for fresh produce (Liu, 2009). Consequently, adaptibility with sensory attributes of fresh produce and antimicrobial activity against targeted microorganisms are major considerations when making a decision about choice of antimicrobial agent. Vanillin is a generally regarded as safe (GRAS) flavoring compound that is usually compatible with fruit characteristics. (Matamoros-Leon et al., 1999). Therefore, vanillin becomes an appealing compound for fresh fruits with its flavor, antioxidant activity and antimicrobial property. Beside the inhibition of microbial growth, it also brings antioxidative protection which is also significant for keeping nutritional quality of fruits. Thus, it can be used as natural preservative instead of synthetic preservatives for fruits.

The flavor of grapes is derived from aromatic compounds which are mainly terpenes, norisoprenoids, benzene compounds, and C_6 alcohols (Noguerol-Pato et al., 2012). During storage, fruit respiration results in undesirable chemicals which can cause undesirable odor and affect the flavor of fruit, vanillin can also contribute to odor and flavor of fruits. As a result, vanillin seems a novel multifunctional bioactive agent to improve quality characteristics of fruit by inhition of microbial growth, keeping nutritional quality and prevention of changes in the volatile profile.

Nowadays, vanillin is widely used as a flavoring agent in beverages, biscuits, chocolate, ice cream, confectionary, desserts, etc. (Rupasinghe, Boulter-Bitzer, Ahn, & Odumeru, 2006). There are few studies based on edible films and coatings incorporating vanillin. Biodegradable polyhydroxybutyrate (PHB) films incorporating vanillin as a natural antimicrobial agent were produced and the films were found to be effective against bacteria with the minimum inhibitory concentration MIC ≥80 μg/g and fungi with MIC ≥50 μg/g in PHB films. The migration of vanillin from the PHB films were also investigated in food stimulants and it was faster into a hydrophobic environment than that into a hydrophilic environment (Raj Xavier, 2015). In another study, polyvinyl alcohol and bacterial cellulose monolayer and multilayered films containing vanillin as a functional food packaging material were characterised and the release of vanillin was faster in monolayer films compared to multilayer films (Stroescu, Stoica-Guzun, & Jipa, 2013). Chitosan films with vanillin at different ratios indicated a good antimicrobial activity against *E.coli* and the release kinetics of vanilin highlighted that these films could be used as flavor release for food or cosmetic product packaging (Stroescu et al., 2015). Alginate coating incorporating vanillin (0.3 and 0.6% w/v) has been shown to

have antimicrobial effect on fresh-cut apple and maintain firmness and color of fruit during cold storage (Rojas-Graü et al., 2007).

2.4. Preharvest and Postharvest Applications on Grapes

Grapes are one of the main fruit crops produced in the world and they are commonly consumed as fresh fruit, dried fruit, fruit juice, and wine production (Torregrosa, Vialet, Adivèze, Iocco-Corena, & Thomas, 2015). Grape and its products are rich in phenolic compounds such as flavanols, flavonols and anthocyanins which come with health benefits for consumers beside their delicious taste (Lima et al., 2014). However, grapes are one of the non-climacteric fruits which can easily loss the quality chracteristics due to rapid fungal growth, especially in high relative humidity (Martínez-Romero et al., 2007). Gray mold, caused by *Botrytis cinerea*, is the most important disease in both preharvest period and postharvest storage. Growth of the fungus during berry development shows difference according to climate conditions as temperature and relative humidity. Deterioration by *Botrytis cinerea* starts to appear at the stage of ripening when sugar content of berries reaches 10-12%. The stage is also known as "veraison" in which firm berries begin to soften in a short period (Zahavi et al., 2000). It spreads rapidly among the berries and causes major losses of grapes by gray mold infection. Postharvest deteriorations by *B.cinerea* in grapes develop in three main action which consist of subtle infection in preharvest period, contamination by fungal spores in the air or on the surface of grapes and nesting infection from contaminated and rotten berries (Teles, Benedetti, Gubler, & Crisosto, 2014).

Figure 2.3. Result of *Botrytis cinerea* growth in preharvest period of Sultani grapes

Among the other rot-causing molds, *B.cinerea* is able to grow effectively even at very low temperatures. Thus, the fungus is also responsible for the damage in cold storage (Parafati, Vitale, Restuccia, & Cirvilleri, 2015). It is not enough to prevent the growth of mold in ripening, it is also required to control its development during cold storage. Since, preharvest and postharvest decay by *B.cinerea* in grapes results in major economic losses for producer, synthetic fungicide treatment has been applied for eliminating diseases both in vineyard and in stored grapes. In vineyards, grapes are treated with sulfurdioxide (SO_2) as spray solution or powder form to inhibit the growth of mold and to minimize the amount of rotten grapes. Fumigation with SO_2 is significant for an effective control of gray mold in table grapes, wine grapes, and raisin grapes. After harvesting, SO_2 treatment was also repeated weekly to control gray mold development during cold storage which is not sufficient alone. There is a need to discover potential replacements for SO_2 because of many considerations related with sulfite residues, emergence of *B.cinerea* resistant strains to fungicide treatment, hazards for human and environmental health, negative effects on quality characteristics of grape and sustainability (Gabler, Smilanick, Mansour, & Karaca, 2010; Parafati et al., 2015).

Certificate of organic grapes includes prohibition of SO_2 use in USA. In addition, discharge of SO_2 to the environment after fumigation is not permited by regulatory agencies (Gabler et al., 2010). In Turkish Food Codex (Anonim, 2013), limits for sulfurdioxide and sulfides, which are coded by E220-E228, is 10 mg/kg (or mg/l) for table grapes. The restrictions for synthetic fungicides by legistlations make the control of *B.cinerea* difficult during postharvest period (Nigro et al., 2006). Thus, biocontrol applications can provide resistance against fungal decay in grapes under specific conditions. There is an increasing interest in development of formulations for biocontrol which can replace with fungicidies and be adaptable to traditional application methods (Elmer & Reglinski, 2006).

Recently, there have been different studies based on biological control of *B.cinerea* in grapes by using natural antifungal alternatives. Influence of carvacrol, which is a natural antimicrobial compound, on control of *B.cinerea* in grapes has been investigated. The study observed the effect of carvacrol vapour at four concentrations (1, 0.5, 0.2 and 0.05 ml/L) on the inhibition of *B.cinerea* in inoculated grape berries as well as in potato dextrose agar (PDA) and it was demonstrated that carvacrol was very effective in inhibiting the growth of *B.cinerea.* Moreover, ethylene production and respiration rate of treated table grapes were highly reduced compared to control grapes

(Martínez-Romero et al., 2007). Carvacrol could be a natural biocontrol agent to control fungal decay during postharvest storage of grapes. However, it was pointed out that the effect of carvacrol on sensory properties of table grapes should be investigated for consumer acceptance. In another study, combination of some essential oils and modified atmosphere packaging (MAP) was performed against rapid deterioration of grapes caused by fungi. The study remarked that the addition of thymol, eugenol, or menthol essential oils inside the packages was effective in reducing yeast and molds as well as mesophilic aerobics. Among these essential oils, eugenol was found to be the most effective antimicrobial compound in reducing yeast and mold growth of grapes by 2.1 log cfu/g reduction (Valverde et al., 2005).

A number of studies are available on organic and inorganic salt solutions to control of *B.cinerea.* Activity of potassium and calcium based salts against *B.cinerea* was evaluated in table grapes. Depending on in vitro trials and under artificial inoculation test, three selected salt solutions; potassium sorbate, potassium bicarbonate and calcium chelate were applied in table grapes with three application strategies which were preharvest spraying, post-harvest immersion after harvest, and combination of pre- and post-harvest treatment. All salt solutions indicated prevention of gray mold growth compared to control. However, the most effective salt solution by completely inhibition of *B.cinerea* (100%) was potassium bicarbonate. No significant difference was found between the single treatment by preharvest spraying and double treatment by pre- and post-harvest application. The study suggested that application of salt solution spraying one week before harvest is effective for control of *B.cinerea* in 'Italia' table grapes (Youssef & Roberto, 2014). In another study, calcium chloride, potassium carbonate, sodyum bicarbonate, and sodium carbonate were found to be effective to reduce gray mold in table grapes through assesment by in vitro and in vivo tests. It was demonstrated that postharvest deterioration caused by gray mold was reduced from 63.8% in untreated grapes to 22.5, 31.2 and 29.5% in calcium chloride, sodium carbonate and sodyum bicarbonate, respectively by using two salt applications (30 and 90 days before harvest) (Nigro et al., 2006). In addition, calcium and magnesium salts promoted capability of antagonistic yeasts against deterioration caused by *B.cinerea* on table grapes (Elmer & Reglinski, 2006).

The use of antimicrobial agents in edible coating is an another good alternative to control mold growth and to extend the shelf-life during postharvest storage of grapes. Besides inhibiting mold growth, edible coating incorporating bioactive agent can also

protect the main quality characteristics such as firmness, color, total phenol content, and antioxidant activity in grapes. Effect of hydroxypropylmethylcellulose (HPMC) edible coating incorporating propolis extract on quality characteristics and safety of table grapes was investigated during storage at 1-2 °C and 85-90 %RH (Pastor et al., 2011). In the study, propolis which is an aromatic compound was used as a preservative due to its antimicrobial, antifungal, and antioxidant properties. The HPMC (5% by weight) coatings including propolis extract at 0, 0.5, 1 and 1.5% by weight were significantly reduced weight loss and browning of table grapes. Color of grapes was improved by coating while there was no trend in phenol content and antioxidant activiy of samples. For safety, a slightly lower microbial growth was observed in coated samples, especially in treatment with 1.5% propolis extract. However, the difference between samples with and without propolis was not found significant. The study concluded that propolis can be considered as a nutritional compound in edible coating due to its benefits for human health (Pastor et al., 2011).

Chitosan has been tested in preharvest and postharvest treatments of grapes due to its antimicrobial property against several fungi. Effect of chitosan spray (1 g/l) as preharvest treatment and/or chitosan coating (10 g/l) as postharvest treatment on quality characteristics of table grapes was investigated during storage at 0 or 20°C and 90-95% RH. Weight loss was significantly reduced by postharvest chitosan coating. Decay incidence was also reduced by preharvest and postharvest chitosan treatment. However, decay incidence increased when fruits were stored at 20°C. Reduction in total phenolic content of grapes was significantly inhibited by postharvest chitosan coating. So, chitosan was suggested to be used in preharvest and postharvest treatments with its contribution to quality of table grapes (Meng, Li, Liu, & Tian, 2008). Decay incidence by gray mold was also reduced significantly by incorporation of grape seed extract (0.1%) into chitosan coating (1% w/v) on red globe table grapes during 4 weeks of storage at 0-1°C (Xu et al., 2007).

Researchers have also reported the application of Aloe vera gel as preharvest treatment for controlling fungal decay and maintaining quality of table grapes. With respect to research, preharvest treatment by Aloe vera gel inhibited microbial spoilage, delayed postharvest ripening and maintained postharvest quality of table grapes (Castillo et al., 2010). Aloe vera gel has also been applied as edible coating on table grapes in order to improve quality attributes. It was demonstrated that microbial spoilage was significantly reduced and weight loss, color and texture changes of grapes

were delayed by Aloe-treated edible coating during 35 days of cold storage (Valverde et al., 2005). Furthermore, controlled atmosphere (CA) is an another storage technique which create low level of oxygen and high level of CO_2 for fresh fruit and vegetables. Carbon dioxide enriched controlled atmospheres were developed to control fungal growth caused by *B.cinerea* by keeping quality attributes of grapes. Although high CO_2 concentration prevented fungal decay, concentration above 10 kPa resulted in quickly browning and off-flavor development on 'Redglobe' table grapes during cold storage (Crisosto, Garner, & Crisosto, 2002).

The aims of this study were to determine the effect of alginate solution with or without vanillin as preharvest spray on physicochemical properties and microbial safety and to investigate effect of alginate coating incorporated with or without vanillin as postharvest edible coating on postharvest decay, biochemical properties, quality and sensory attributes of table grapes from three cultivars including Semillon Blanc, Alphonse Lavalleé, and Razaki. Effect of preharvest alginate spray treatment and postharvest alginate coating on the quality and safety of grapes was evaluated during growing in vineyard and postharvest storage. In order to understand the effect of preharvest and postharvest treatments, total soluble solid content, pH value, titratable acidity, total phenol content, antioxidant activity, yeast and mold counts, color values, fruit firmness, and lactic acid content were determined at the day of preharvest treatment, at harvest and during 35 days of storage at 4±2 °C. In addition, sensory evaluation of grapes coated with or without vanillin were performed.

CHAPTER 3

MATERIAL AND METHODS

3.1. Materials

3.1.1. Grape samples

Grape samples (*Vitis vinifera*) used in this study were harvested from two different vineyards in Cumaovası and Gödence regions of İzmir. Semillon Blanc was obtained from Cumaovası vineyard, and Alphonse Lavalleé and Razaki were obtained from Gödence vineyard. Clusters of uniform size, color, and shape were selected for treatments and grape clusters were immediately transported to laboratory after harvesting.

3.1.2. Chemical Reagents

All chemicals used in the experiments are shown in Table 3.1 with the brand and code.

Table 3.1. Chemicals used in the analyses

Chemical Name	Brand	Code
Alginic acid sodium salt from brown algae	Sigma	A2033
Vanillin	Merck	8.18718.0100
Glycerol	Merck	1.04092.2500
Methanol	Merck	1.06007.2500
Potato Dextrose Agar (PDA)	BD	213400
Peptone water (buffered)	Merck	1.07228.0500
Calcium chloride dihydrate	Riedel-de Haën	12022
Folin Ciocalteu's Phenol Reagent	Fluka	47641

Table 3.1 (cont.). Chemicals used in the analyses

Gallic Acid	Sigma	G7384
6-Hydroxy-2,5,7,8-Tetra-methylchromane-2-carboxylic acid (Trolox)	Fluka	56510
pH 4 Buffer	WTW	108800-TPL4
pH 7 Buffer	WTW	108802-TPL7
Sodium Hydroxide (NaOH)	Riedel-de Haën	06203
Fenol phthalein	Merck	1.07233.0100
L(+) Lactic acid, approx 98%	Sigma	L1750
Sulfuric acid	Sigma	435589

3.2. Methods

3.2.1. Films Characterization

3.2.1.1. Preparation of Edible Films

In order to determine film characteristics edible films based on alginate at %1 and %2(w/v) concentrations were prepared with or without vanillin. Alginate based film forming solutions were prepared by dissolving 1 g and 2 g of alginate into 100 ml of distilled water for alginate films at 1% and 2% (w/v) concentrations, respectively. Continuous mixing was done using a magnetic stirrer (IKA RCT Basic, Germany) at 500 rpm for 8 h. The solution was held one night at 100 rpm to obtain homogeneous solution and then 1 g of glycerol(1% w/v) was added to the solution which was allowed to mix for 60min. Finally 1 g of vanillin(1% w/v) was added to the solution and mixed at 500 rpm for 16 h. Alginate films were produced by pouring 15 ml of each coating solutions into petri dishes having 8 cm diameter and then dried for 36 h in a drying oven(Membert ULM 500, Germany) at 45°C and 50% RH.

3.2.1.2. Film Thickness

The thickness of films was measured at eight randomly selected points of the films using a digital micrometer(Comecta Electronic Digital Micrometer, Cod. 5900602, Spain) with a sensitivity of 0.001 mm. Measurements were taken on three film samples for each type of films. Mean thickness values were used to determine water vapor permeability and mechanical properties of films.

3.2.1.3. Water Solubility

Water solubility (WS) of films was determined according to previously described method by researchers (Gontard, Guilbert, & Cug, 1992). Film samples were dried at 105 °C for 24 h in a drying oven (Membert ULM 500, Germany). The dried film pieces having the initial dry weight (w_i), were immersed in 30 mL of distilled water and stirred at 100 rpm for 24 h at 25 °C. Then solutions were filtered by using pre-weighted filter paper. The filter paper containing undissolved film materials was dried at 105 °C for 24 h in the drying oven (Membert ULM 500, Germany). The weight of dried samples was recorded as the final dry weight (w_f). The water solubility (%) was calculated according to equation (3.1.) and three replications were done.

$$Water\ Solubility\ (\%) = \frac{W_i - W_f}{W_i} x100 \quad (3.1.)$$

3.2.1.4. Film Color

Color of films was determined with a colorimeter (Konica Minolta Sensing Inc., Japan) and CIE L*, a* and b* color values were measured. Total color difference between the films with or without vanillin was calculated according to equation (3.2.). Measurements were performed on three film samples for each type of films and five readings were taken at different points of the film.

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \quad (3.2.)$$

where $\Delta L = L_{wov} - L_{wv}$, $\Delta a = a_{wov} - a_{wv}$, $\Delta b = b_{wov} - b_{wv}$

3.2.1.5. Mechanical Properties

Mechanical properties of film samples were determined using a texture analyser (Stable Macro Systems, Texture Analyser TA-XT Plus, UK) according to the ASTM D882-95 method (ASTM, 1995). Samples were stored at 25 °C, 50 %RH for 24 h before testing. Film samples were analyzed with an initial grip seperation of 40 mm, a cross speed of 10 mm/s and a load cell of 5 kg. Tensile strength (TS), elongation at break (EB) and Young's modulus (YM) were determined from the stress-strain curve in eight replicates for each film formulation. TS was calculated by dividing the maximum load for breaking the film by cross-sectional area (thickness x width) of film. EB, also known as strain, was evaluated as percentage ratio between changed length and initial length after breakage. YM was calculated as the slope of the initial linear part of stress-strain curves (Figure 3.1).

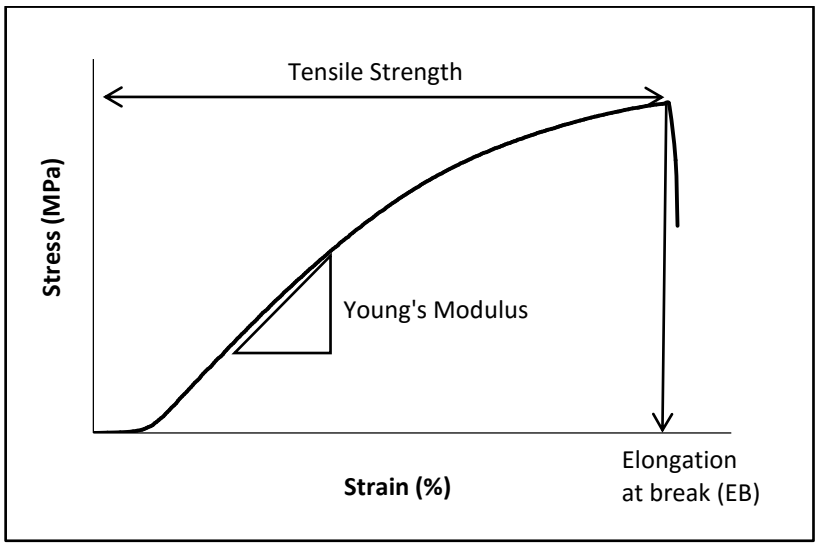


Figure 3.1. Stress-strain curve of films for mechanical properties

3.2.1.6. Oxygen and Carbon dioxide Transmission Rates

Oxygen and carbondioxide gas transmission of films were determined by isostatic method based on ASTM D3985 Standard Test Method by using a gas permeability tester instrument (L100-5000, Switzerland) with some modifications. Film sample is placed into a gas transmission cell where one chamber includes the test gas at spesific high pressure and other chamber comprises the permeating gas at low pressure

(Massey, 2003). Oxygen and carbondioxide transmission rate is obtained from instrument when the sample has reached to the steady-state rate of oxygen and carbondioxide transmission through film. Oxygen and carbondioxide gas transmission rates of films were expressed as ml of gas per unit area over 24 h (ml/m^2/24 h) at 25 ºC.

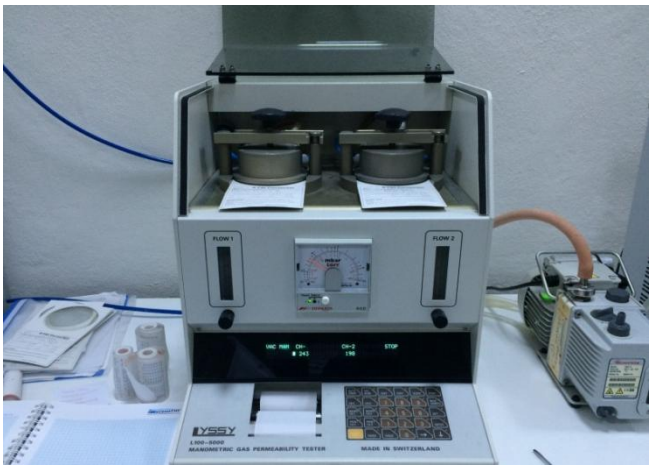


Figure 3.2. Gas Permeability Tester Instrument

3.2.1.7. Antifungal Activity against *Botrytis cinerea*

Antifungal activity of alginate films with or without vanillin was determined according to method described by Sánchez-González, Quintero Saavedra, and Chiralt (2014). Edible films incorporating vanillin at 0, 0.5, 1.0 and 1.5 % concentrations with the same diameter as Petri dishes were freshly prepared. Film samples were placed on potato dextrose agar (PDA) plate previously steriled and buffered at pH 4.5 and inoculated with 100µl of *Botrytis cinerea* suspension at concentration of 10^5 spore/ml. Inoculated plates which did not carry films were used as control. All plates were incubated at 25 ºC for 15 days. *B. cinerea* counts were carried out on PDA periodically at 0, 5, 10 and 15 days of the storage. The agar was removed aseptically from Petri dishes and transfered into sterile plastic bag. Then, the sample was homogenized with 0.1 % sterile peptone water using a stomacher (Bagmixer 400P, Interscience, France). Serial dilutions were prepared from homogenates and 0.1 ml from appropriate dilutions was inoculated on potato dextrose agar (PDA) and the plates were incubated at 25 °C for 5 days. All tests were performed in duplicate. Colonies were counted after incubation and results were expressed as log cfu/g.

3.2.2. Preharvest and Postharvest Treatment

For preharvest treatment, 1%(w/v) spray solution of alginate was prepared by dissolving sodium alginate in distilled water under continuous stirring at 500 rpm. 1%(w/v) vanilin was incorporated into alginate solution. Alginate spray w/wo vanillin were sprayed on whole grape clusters 2 weeks before harvesting. After spraying alginate solution, 0.3M $CaCl_2$ solution was also sprayed on the surface of fruits. Clusters without any treatment were used as control.

For postharvest treatment, 2% (w/v) alginate coating solution was prepared with same method as described above and 1% (w/v) vanillin was incorporated into coating solution. In addition, 1% (w/v) glycerol was added as a plasticizer. Grape clusters were dipped in coating solution for 30 s and allowed to remove excess solution. Then clusters were dipped in 0.3M $CaCl_2$ solution. Coated grape clusters were dried at 10 °C and %75 RH for 6 h and then stored in plastic containers at 4±2 °C for 35 days. Total soluble solid content, pH value, titratable acidity, total phenol content, antioxidant activity, yeast and mold counts, color values, fruit firmness, and lactic acid content were determined during storage. In addition, sensory evaluation of grapes were performed in seperately prepared samples.

3.2.3. Evaluation of Fruit Quality

3.2.3.1. Weight Loss

Weight losses were measured as a percentage loss of initial weight by recording weight changes of table grapes during storage. Measurements were done in three grape clusters selected from each treatment and weight losses (WL) were calculated by using following equation (3.3.) during storage.

$$Weight\ Loss\ (\%) = \frac{W_i - W_f}{W_i} x100 \qquad (3.3.)$$

where W_i is the initial weight of fruit (g) at the first day of storage and W_f is the final weight of fruit (g) at the end of storage.

3.2.3.2. Soluble Solid Content, Titratable Acidity and pH

Total soluble solid content (SSC) of grapes was determined in juices obtained from grapes by using a refractometer (Mettler Toledo, RE50 Refractometer, USA). Samples (10 g) were taken and homogenized with 100 ml water and pH was measured in suspensions by using a pH meter (Inolab Level 3, Weilheim, Germany). Titradable acidity represents the sum of all acids and it is commonly expressed in tartaric acid for grapes. Titratable acidity (TA) was determined by titration of diluted grape juice (1 ml of grape juice in 25 ml of distilled water) with 0.1N NaOH solution until the end of titration (pH =8.1) and expressed as equivalent concentration of tartaric acid by using equation (3.4.). Fenol phthalein was used as an indicator. All analyses were performed in triplicate.

$$Titradable\ Acidity\ (g/100ml) = \frac{VxNxEx\ 100}{m} \quad (3.4.)$$

where *V* is volume of NaOH (ml), *N* is normality of NaOH, *E* is milliequivalent factor (0.075 for tartaric acid) and *m* is amount of sample (ml).

3.2.3.3. Total Phenolic Content and Antioxidant Activity

Extraction method of phenolic compounds from grapes was determined using a method derived from total phenolic and flavonoid compounds extraction method with some modifications (Melgarejo-Flores et al., 2013). To assess total phenolic content and antioxidant activity, 10 g of grape was homogenized in 10 ml of methanol (% 80 v/v) by using homogenizer (IKA ULTRA-TURRAX T25, Germany) at 6000 rpm. Then, the extraction of phenolic compounds was carried out in ultrasonic bath for 60 min at room temperature. In order to eliminate solid particles, extract was centrifuged at 3000 rpm for 15 min at room temperature by using a centrifuge(Hettich Zentrifugen, Rotina 380R, Germany). The supernatant was used for analysis of total phenolic content and antioxidant activity. All experiments were performed in triplicate.

Total phenolic content of samples was determined using the Folin-Ciocalteu colorimetric method based on total phenol analysis (Slinkard & Singleton, 1977). Each extract (20 µl) was added into tubes, and 1.58 ml of distilled water was added. Then,

100 µl of Folin-Ciocalteau reagent (diluted 1:10 ratio with distilled water) was added and immediately mixed. After waiting for 8 min, 300 µl of sodium carbonate solution [7.5% (w/v) in distilled water] was added and mixed. Then, the solutions were incubated in a dark place for 2 h at room temperature. The absorbance of each solution was measured at 765 nm with a spectrophotometer (Shimadzu UV-2450, Japan). The total phenolic content of samples was calculated as mg gallic acid equivalent per kg fresh fruit by using a standard curve of gallic acid. For standard curve, gallic acid stock solutions at 100, 200, 300, 400, 500, 600, 700 800 and 1000 mg/ml concentrations were prepared and the absorbance of each solution was determined by using same method. The calibration curve was plotted by absorbance values against concentration.

Antioxidant activity of samples was determined according to the spectrophotometric analysis of the ABTS [2,20 -azinobis- (3-ethylbenzothiazoline-6-sulphonic acid)] radical scavenging activity (Re et al., 1999). ABTS solution at 7 mM and potassium persulfate solution at 2.45 mM were prepared by dissolving in distilled water. ABTS+ cation radical was produced by mixing equal amount of the ABTS and potassium persulfate solutions, then allowing the mixture to stand in the dark at room temperature for 16 h. The ABTS+ solution was diluted with phosphate buffer (0.1 M, pH :7.4) to an absorbance of 0.70±0.02 at 734 nm. After addition of 1.0 ml of diluted ABTS+ solution to 10 µl of sample, the reduction in absorbance at 734 nm was recorded 1 min after mixing up to 5 min. The percentage inhibition of absorbance at 734 nm was calculated by equation (3.5.). The antioxidant activity of samples was expresed as mg of Trolox equivalent(TEAC) per gram fresh fruit by using a standard curve of Trolox. In order to obtain standard curve, Trolox stock solutions at 25, 100, 175, 250, 325, 400, 475, 550, 625 µM concentrations were prepared and the percantage inhibiton of absorbance in each solution was determined by using the same method. The standard curve was plotted as a function of Trolox concentration for the percentage inhibition.

$$inhibition\ \% = \frac{(A_{ABTS\ +} - A_{sample}\ )}{A_{ABTS\ +}} x100 \quad (3.5.)$$

where A_{ABTS+} was the absorbance of $ABTS^{+}$ solution at 734 nm (0.70±0.02) and A_{sample} was the absorbance of $ABTS^{+}$ solution mixed with sample at 734 nm.

3.2.3.4. Color and Fruit Firmness Measurements

Color values (CIE L*, a* and b*) of grapes were determined by using a Minolta colorimeter (Konica Minolta Sensing Inc., Japan) during storage. Measurements were taken in same samples previously selected for each treatment. Fruit firmness were measured by using a texture analyser (Texture Analyser TA-XT2 Stable Macro Systems, Godalming, UK) with a 5 kg load cell and cylindrical probe having 2 mm diameter at a prespeed and postspeed of 2 mm/sec. Measurements were performed on 10 grape berries for each treatment and firmness was expressed as Newton (N).

3.2.3.5. Yeast and Mold Counts

In order to understand the effect of treatments on yeast and mold growth in grapes, microbiological analysis was carried out according to the method described by Sharma and Rao (2015). A representative sample of grapes (10 g) was transferred to a sterile stomacher bag and then homogenized for 1 min with 90 ml of 0.1 % sterile peptone water using a stomacher (Bagmixer 400P, Interscience, France). Serial dilutions were prepared from homogenates and 0.1 ml from each dilutions was inoculated on potato dextrose agar (PDA) and the plates were incubated at 25 °C for 5 days. All tests were performed in duplicate. Yeast and mold counts of grapes were determined periodically during storage and results were expressed as log CFU/g.

3.2.3.6. Lactic Acid Content

Lactic acid analysis was carried out at the Biotechnology and Bioengineering Research and Application Center (BIOMER) in İYTE. Lactic acid content of grapes was analyzed by high performance liquid chromatography (HPLC) equipped with a Bio Rad Aminex HOX-87H column according to previously reported method with some modifications (R. Liu, Wang, Qin, & Tian, 2016). As a mobile phase, 5 mM sulfuric acid was used at a flow rate of 0.6 ml/min. Lactic acid was detected at 210 nm with DAD detector at temperature of 65 °C and volume of injected sample 20 µl. Juices obtained from grape samples were diluted 1:4 ratio with ultrapure water. The standard solutions were prepared at different concentrations with ultrapure water. Standard

solutions and grape juice samples were filtered through a 0.45 µm millipore membrane filter (Sartorius Minisart Syringe Filter, Germany). Concentration of lactic acid was evaluated using peak areas of standard solutions and results were expressed in g lactic acid per liter juice (g/l).

3.2.3.7. Sensory Evaluation

Postharvest treatments, alginate coating w/wo vanillin, were carried out separately on Alphonse Lavalleé grapes for sensory analysis. A sensory panel was performed with 14 semi-trained panelists to evaluate the quality of grapes at 0, 7, 14, 21, 28 days of storage. Panelists, which consisted of Food Engineering Department students and staffs, were asked to rate given samples for appearance, color, odor, taste, texture and overall acceptability by using a nine-point hedonic scale (1=dislike extremely, 5=neither like nor dislike, 9=like extremely). Sensory test form is shown in Figure 3.3. Three grape berries from each treatment were presented in individual white cups for observation and one washed and prepared ready-to-eat grape sample from each treatment was presented only for tasting.

3.2.3.8. Statistical Analysis

All data were analysed by using one-way analysis of variance (ANOVA). Significant difference was considered at $p<0.05$ and Tukey's test was used to determine statistical significant difference between treatments with Minitab Software (Minitab Inc., State College, Pa., USA).

Panelist name:
Date:

Please evaluate three grape samples and write your score which best describes your feeling for each attribute. Thank you.

Scores

(9) Like extremely
(8) Like very much
(7) Like moderately
(6) Like slightly
(5) Neither like nor dislike
(4) Dislike slightly
(3) Dislike moderately
(2) Dislike very much
(1) Dislike extremely

ATTRIBUTE	SAMPLE CODES		
	413	691	279
Appearance			
Color			
Odor			
Taste			
Texture			
Overall acceptability			

Figure 3.3. Sensory test form

CHAPTER 4

RESULTS AND DISCUSSION

4.1.Film Properties

4.1.1. Water Solubility and Film Thickness

Water solubility of each type of films was measured to observe the effect of film composition and addition of vanillin on the water resistance of films. Table 4.1 shows water solubility (%) and thickness (mm) values of alginate films (AF) prepared at %1 and 2% (w/v) concentrations w/wo vanillin. The solubility of alginate films within the range of 26.76 to 35.18 %. Solubility increased with increasing alginate concentrations. The solubility of alginate films was not significantly influenced by addition of vanillin. The results indicated that the water solubility of alginate films incorporated with vanillin slightly increased from 26.76 to 30.17 % in alginate films at %1(w/v) concentration and slightly decreased from 35.18 to 33.78 % in alginate films at %2(w/v) concentration. Thickness values of alginate films were found between 0.030 and 0.080 mm. Increasing the concentration of alginate and addition of vanillin increased the thickness of films.

Table 4.1. Water solubility (WS) and thickness of alginate films (AF) at 1% and 2% (w/v) concentrations w/wo vanillin

Film	***Water solubility (%)***	***Thickness (mm)***
1% AF wo vanillin	26.76 ± 0.37	0.030 ± 0.002
1% AF w vanillin	30.17 ± 0.68	0.065 ± 0.004
2% AF wo vanillin	35.18 ± 1.25	0.049 ± 0.004
2% AF w vanillin	33.78 ± 0.31	0.080 ± 0.003

mean±std (n=3 for water solubility and n=8 for thickness)

4.1.2. Film Color

The color properties of edible films are one of the most important characteristics that can directly affect the color of food product. Alginate-based edible films are typically colorless film. Thus, application of alginate based edible coating do not change appearance of food product which is crucial for consumer acceptance. Table 4.2 shows color properties including *L** (lightness), *a** (green-red), *b** (blue-yellow), and *ΔE* (total color difference) of alginate films (AF) prepared at %1 and 2% (w/v) concentrations w/wo vanillin. Alginate films incorporated with vanillin has slightly different color parameters compared to alginate films without vanillin. Ligthness values (*L**) was a little higher in alginate films without vanillin than alginate films containing vanillin. This is probably due to the characteristic white color of vanillin. Positive a* value, which refers to red color, was lowest in 2% (w/v) alginate film incorporated with vanillin, whereas %1 alginate films w/wo vanillin and 2% (w/v) alginate films wo vanillin showed a similar values. Incorporation of vanillin into alginate films affected *b** (blue-yellow) color values. Negative b* value, which refers to blue color, was observed in alginate films at 1% and 2% (w/v) concentrations without vanillin while positive b* value, which refers to yellow color, was observed in alginate films at 1% and 2% (w/v) concentrations with vanillin. Total color difference (*ΔE*) between alginate films with or without vanillin was 6.09 for 1% (w/v) alginate film and 10.59 for 2% (w/v) alginate film. Total color difference (*ΔE*) of films increased with increasing alginate concentration.

Table 4.2. L*, a*, b* values and color difference (ΔE) of alginate films at 1% and 2% (w/v) concentrations w/wo vanillin

Film	***L****	***a****	***b****	***ΔE***
1% AF wo vanillin	90.76 ± 0.17	2.20 ± 0.36	-3.15 ± 0.22	-
1% AF w vanillin	88.69 ± 0.54	2.04 ± 0.08	2.57 ± 0.36	6.09
2% AF wo vanillin	91.01 ± 0.49	2.06 ± 0.03	-2.65 ± 0.32	-
2% AF w vanillin	88.15 ± 0.44	0.72 ± 0.19	7.46 ± 0.46	10.59

mean±std (n=3 for L*, a* and b*)

4.1.3. Mechanical Properties

Mechanical properties of edible films are generally explained by tensile strength (TS), elongation at break (EAB) and Young's modulus. Tensile strength (MPa), elongation at break (%) and Young's modulus of alginate films (AF) prepared at %1 and 2% (w/v) concentrations w/wo vanillin are given in Table 4.3. The tensile strength (TS) is response for the mechanical resistance of film due to the cohesion between the chains and the elongation at break (EAB) accounts plasticity of film that is explained as the capacity of the film to extend before breaking (Galus & Lenart, 2013). Tensile strength values of films ranged from 9.15 to 25.92 MPa with the lowest value for 1% (w/v) alginate film incorporated with vanillin and the highest value for 1% (w/v) alginate film without vanillin. Incorporation of vanillin into 1% (w/v) alginate film resulted in weakened film, but vanillin addition did not affect tensile strength of 2% (w/v) alginate film. This could be explained by increasing alginate concentration limited changes in tensile strength of film when vanillin was incorporated. Elongation at break changed from 12.44% to 38.35% with highest value for 2% (w/v) alginate film without vanillin and the lowest value for 2% (w/v) alginate film incorporated with vanillin. 1% (w/v) alginate films with and without vanillin have similar elasticity presented by elongation at break 36.58 and 27.04 %, respectively. 2% (w/v) alginate film incorporated with vanillin led to be stronger but less flexible edible films with elongation at break value of 12.44%. In literature, similar results for elongation at break values of alginate films as 15.9 and 14.9 % were given by Pereira et al. (2011). Young's modulus of alginate films at %1 and 2% (w/v) concentrations with and without vanillin were between 0.56 and 4.76.

Table 4.3. Tensile strength (TS), elongation at break (EAB) and Young's modulus of alginate films at 1% and 2% (w/v) concentrations w/wo vanillin

Film	***TS (MPa)***	***EAB(%)***	***Young's modulus***
1% AF wo vanillin	25.92 ± 3.71	27.04 ± 2.55	1.95 ± 0.42
1% AF w vanillin	9.15 ± 2.26	36.58 ± 4.38	0.59 ± 0.14
2% AF wo vanillin	14.44 ± 3.38	38.35 ± 2.38	0.56 ± 0.38
2% AF w vanillin	15.16 ± 2.30	12.44 ± 1.84	4.76 ± 0.15

mean±std (n=8 for TS, EAB and Young's modulus)

4.1.4. Oxygen and Carbon Dioxide Permeability

Oxygen and carbon dioxide transmission rate is one of the important properties of edible films. Because the balance between oxygen and carbon dioxide percentage on the environment impact the shelf-life of fresh produce. During respiration process, oxygen is consumed by fruit and carbondioxide is produced. As a result of transmission rate of gases, modified atmosphere can be formed on the surface of fresh produce upon coating (Maria A. Rojas-Graü et al., 2008). Oxgen and carbon dioxide transmission rates of alginate films (AF) at 1% and 2% (w/v) concentrations w/wo vanillin are given in Table 4.4. In the matter of oxygen transmission rates, it was observed that alginate films incorporating vanillin result in lower transmission rate compared to alginate films without vanillin. The oxygen transmission rate values range between 43.16 and 63.23 $ml/m^2/24$ h. Alginate edible films w/wo vanillin exhibited good oxygen barrier properties and addition of vanillin into alginate films slightly decreased oxgen transmission rates of films. Carbon dioxide transmission rates of alginate films increased when the concentration of alginate increase, but incorporation of vanillin into alginate films did not considerably change carbondioxide transmission rates of films. In literature, there is limited study on determination of oxygen and carbondioxide transmission of alginate films. Alginate (2% w/w)–apple puree (38º Brix) edible films presented oxygen barrier properties around 10.20 $cm^3.\mu m/m^2.d.kPa$ and incorporation of antimicrobial agents, lemongrass oil (0.5% w/w) and citral (0.5% w/w) also resulted in a decrease for oxygen permeability of alginate-apple puree edible films (Maria A. Rojas-Graü et al., 2007). Furthermore, the study that investigated impact of alginate and gellan edible coatings on the quality of fresh-cut apples showed that O_2 and CO_2 gas composition of headspace in tray where coated and uncoated apples placed did not show significant differences (Maria A. Rojas-Graü et al., 2008). Generally, edible films having low oxygen permeability is desired for coating applications. In this study, results showed that alginate films had appropriate barrier properties for coating applications on fresh produces.

Table 4.4. Oxygen and carbon dioxide transmission rates of alginate films at 1% and 2% (w/v) concentrations w/wo vanillin

Film	***Oxygen Transmission Rate (ml/m^2/24h)***	***Carbondioxide Transmission Rate (ml/m^2/24h)***
1% AF wo vanillin	50.82	136.31
1% AF w vanillin	43.16	131.88
2% AF wo vanillin	63.23	149.23
2% AF w vanillin	48.80	147.21

4.1.5. Antifungal Activity

Antifungal activity of alginate films incorporated with vanillin at different concentrations was evaluated during 15 days at 25ºC in order to determine appropriate concentration of vanillin which will inhibite the growth of *Botrytis cinerea*. Effects of vanillin at 0.5, 1.0 and 1.5% concentrations on *B.cinerea* growth are shown in Figure 4.1. Survival of *B.cinerea* in alginate films without vanillin as same as control sample which did not carry films during 15 days of incubation at 25°C. However, incorporation of vanillin into alginate films significantly ($p<0.05$) inhibited growth of *B.cinerea* compared to control samples. As the concentration of vanillin in alginate films was increased, considerable inhibition of *B.cinerea* growth was observed. Thus, the lowest and highest inhibition was achieved at 0.5% and 1.5% concentrations of vanillin, respectively, but the difference on *B.cinerea* growth between alginate films incorporating 1.0% and 1.5% vanillin is negligible. So, both 1.0% and 1.5% concentrations of vanillin was effective on reducing *B.cinerea* growth. We concluded that alginate films without vanillin did not show any antifungal activity while alginate films incorporated with vanillin at 0.5, 1.0 and 1.5% (w/v) concentrations reduced 47.94, 64.15 and 65.56% of fungal growth, respectively, after 15 days of incubation.

Incorporation of antimicrobial agents into edible coatings can accomplish the prevention and control of microbial spoilage in fresh produces. One of the most important selection criteria for antimicrobial agent is the efficacy of antimicrobial compound into edible coating. The antimicrobial agent has to reach sufficient

concentration on the fruit surface for inhibition of microbial growth because the decay by microorganisms appear mainly at the surface due to postharvest handling (S. X. Liu, 2009). As a result of the antifungal acitivity test, we continued with alginate edible films enriched with vanillin at 1%(w/v) concentration to extend the shelf-life of fresh produces. Vanillin concentration at 1.5%(w/v) is not considered because the higher amount of antimicrobial compound come with higher cost and it is also an aromatic compound, so that can results in intensive vanillin aroma.

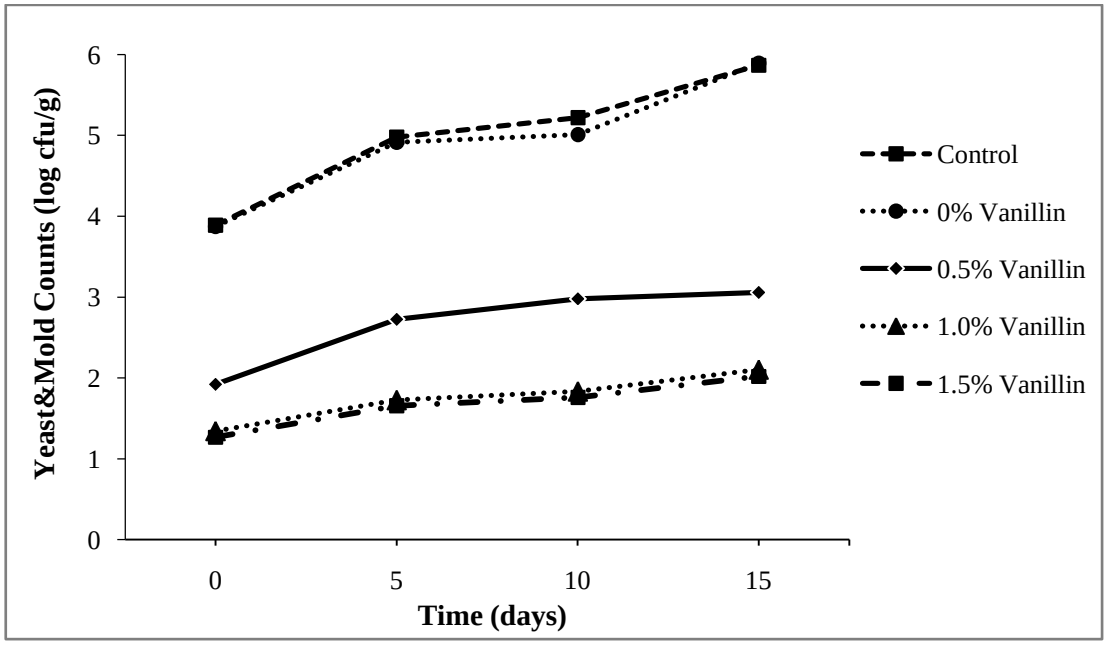


Figure 4.1. Effect of alginate films with and without vanillin on growth of *B.cinerea* during 15days of incubation at 25°C.

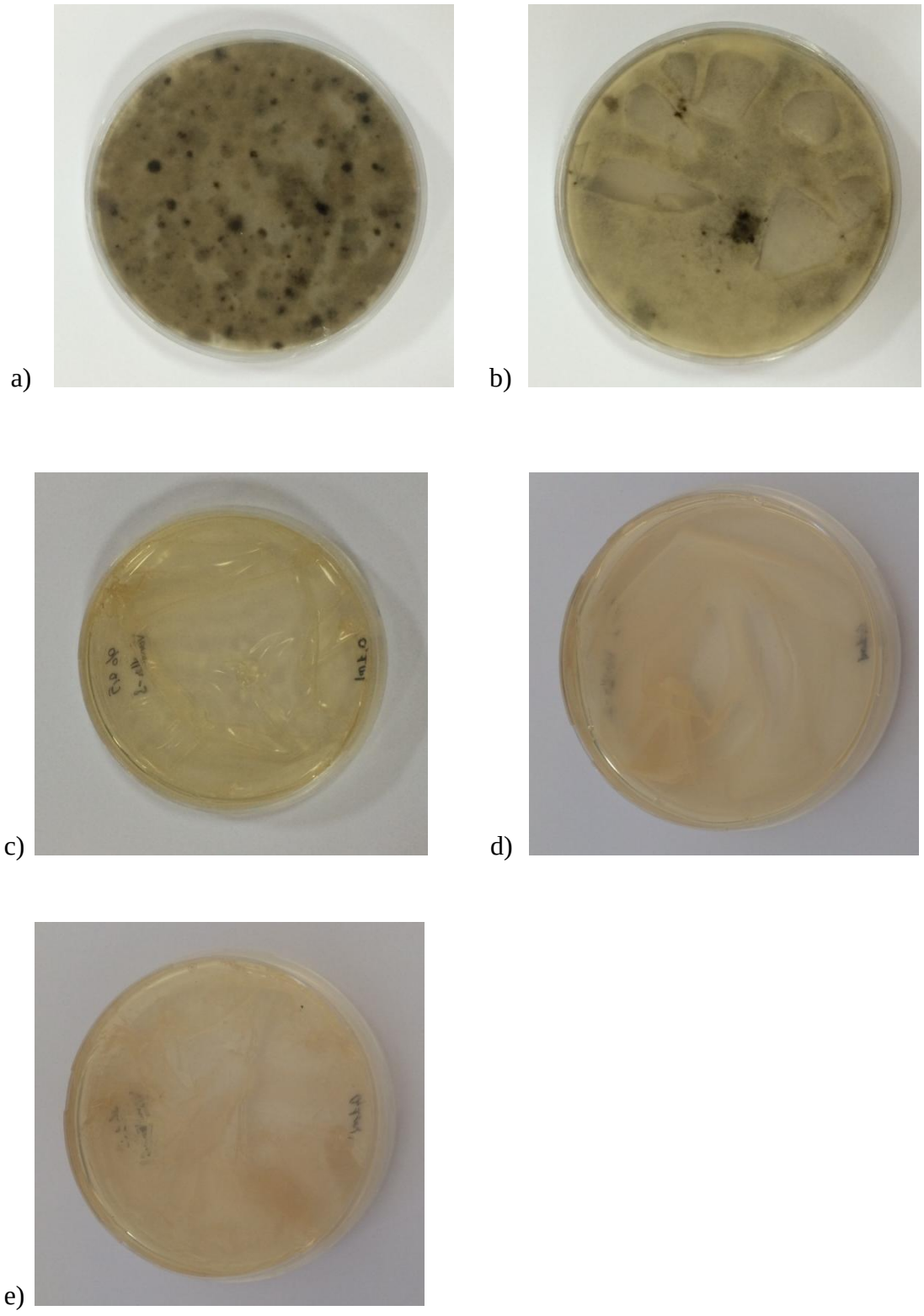


Figure 4.2. Potato dextrose agar plates inoculated with B.cinerea containing disc alginate films incorporating vanillin at different concentrations (b: 0%, c:0.5%, d:1.0%, e:1.5% w/v) and without film as control (a) after 15 days of incubation at 25 °C

4.2. Effect of Preharvest Treatments on Fruit Quality

Total soluble solid content (ºBrix), pH, titratable acidity, total phenolic content, antioxidant activity, yeast and mold counts and lactic acid content were determined at the day of spraying and at the day of harvesting in order to observe effect of preharvest treatments. All results are shown in Table 4.5 for Semillon Blanc, Alphonse Lavalleé and Razaki grapes.

Semillon Blanc grapes are generally used to produce sweet white wine while Alphonse Lavalleé and Razaki grapes are red and green table grapes which commonly sold in markets. Thus, these three different grapes differ from chemical composition such as total soluble solid content, titratable acidity, phenolic content, antioxidant activity and lactic acid content.

Soluble solid content (SSC) of grapes increased during maturation of grapes in vineyard. SSC of Razaki grapes increased from 11.30 ºBrix at spraying day to 24.95, 24.67 and 24.11 ºBrix at harvesting day for control, alginate spray and alginate spray incorporating vanillin groups, respectively. SSC of Alphonse grapes increased from 10.88 ºBrix at spraying day to 17.19, 17.51 and 16.08 ºBrix at harvesting day for control, alginate spray and alginate spray incorporating vanillin groups, respectively. Lastly, SSC of Semillon Blanc grapes increased from 14.61 ºBrix at spraying day to 19.63, 19.28 and 18.40 ºBrix at harvesting day for control, alginate spray and alginate spray incorporating vanillin groups, respectively. Meng et al. (2008) showed also an increase in SSC for *Vitis vinifera* cv. Jingxiu table grapes during 10 days of maturation period before harvesting. SSC, which determines the time of harvesting, changes with respect to the variety of grapes. Razaki grapes had the highest SSC following Semillon Blanc and Alphonse Lavalleé grapes at the day of harvesting. SSC of grapes treated with alginate spray including vanillin had slightly lower values than that of grapes treated with alginate spray. However, the difference between alginate spray treatment with or without vanillin was not found significant ($p>0.05$). In addition, there was no significant difference ($p>0.05$) in SSC between control and treatment groups for all of three cultivars.

Titratable acidity (TA) of grapes decreased with maturity development, so the high values of TA in unmature grapes at the day of spraying declined during growing in the vineyard. At the day of preharvest treatment, titratable acidity of Semillon Blanc,

Alphonse Lavalleé and Razaki grapes were 1.84, 1.69 and 3.34 g/100ml, respectively. When grapes were harvested, TA of Semillon Blanc grapes were 0.98, 1.03 and 0.99 g/100ml for control, alginate spray and alginate spray incorporating vanillin, respectively. For table grapes, TA of Alphonse Lavalleé and Razaki grapes were 0.71, 0.73 and 0.69 and 0.51, 0.51 and 0.45 g/100ml for control, alginate spray and alginate spray incorporating vanillin, respectively. There was no significant difference ($p>0.05$) in titratable acidity between control grapes and grapes sprayed with alginate solutions incorporating vanillin at 0 and 1% (w/v) concentrations for all of three cultivars. Similarly, there was a decrease in titradable acidity of grapes during maturation as previously reported in preharvest applications for grapes (Meng et al., 2008; Mirdehghan & Rahimi, 2016).

Briefly, control grapes, grapes treated with alginate spray and grapes treated with alginate spray incorporating vanillin did not show significant difference ($p>0.05$) on SSC and TA, so preharvest spray did not influence development of soluble solids and organic acids during maturation. Correlatively, alginate solutions sprayed on the fruit surface did not affect significantly ($p>0.05$) pH of grapes for all types. The pH values of control grapes, grapes treated with alginate spray and grapes treated with alginate spray incorporating vanillin were similar.

Preharvest treatment by alginate spray with or without vanillin was useful to provide microbial safety of grapes. There was a significant difference ($p<0.05$) in yeast and mold growth between untreated and treated Alphonse Lavalleé grapes. However, no significant difference ($p>0.05$) was observed in yeast and mold growth between untreated and treated Semillon Blanc and Razaki grapes. At the day of preharvest treatment, Semillon Blanc, Alphonse Lavalleé and Razaki grapes had 3.69, 3.04 and 2.64 log cfu/g for yeast and mold counts, respectively. At the day of harvesting, yeast and mold counts of untreated, treated with alginate spray and treated with alginate spray incorporating vanillin groups were respectively 3.95, 3.49 and 3.56 log cfu/g for Semillon Blanc grapes, 3.01, 2.54 and 2.59 log cfu/g for Alphonse Lavalleé grapes, 3.06, 2.89, 2.70 log cfu/g for Razaki grapes. Preharvest alginate spray treatments diminished slightly yeast and mold growth of grapes while yeast and mold counts of untreated grapes was increasing during maturation. Alginate spraying with orwithout vanillin indicated similar behaviour in diminishing yeast and mold growth. It is probably due to rapidly release of vanillin from grapes at high environmental temperature which were between 35 and 37°C on the vineyard. Environmental

conditions, especially temperature, can affect release of the aromatic compounds, so the high environment temperature may result rapid release. Degree of reduction in yeast and mold growth differ from type of grapes. The highest reduction in yeast and mold growth was observed in Alphonse Lavalleé grapes with 0.47 log cfu/g amount by preharvest treatment.

In the literature, there are number of researches based on preharvest treatments. Application of Aloe vera solution as preharvest spray 1 and 7 days before harvesting inhibited microbial spoilage, delayed postharvest ripening and reduced decay incidence of table grapes during cold storage. Preharvest application with Aloe vera gel indicated reduction (<1 log cfu/g) in yeast and mold counts of grapes and yeast and mold counts of table grapes after harvesting were between 3 and 4 log cfu/g. However, it was determined that the reduction in yeast and mold counts through preharvest spray was lower than that obtained by postharvest coating based on Aloe vera (Castillo et al., 2010). Similarly, in our work, preharvest spray treatment indicated lower reduction compared to postharvest coating treatment by alginate solution incorporating vanillin. The amount of vanillin in both preharvest and postharvest treatment was the same, but the environmental conditions were distinct and the high temperature during maturation of grapes in vineyards could result rapid release of vanillin. Regarding the release of vanillin, reduction in yeast and mold growth can be improved by increasing vanillin concentration in the preharvest spray.

All cultivars had similar total phenolic content which was between 652 and 655 mg GAE/kg at the day of preharvest spraying. Total phenolic contents of untreated grapes at harvest were respectively 694.19, 689.19 and 684.19 mg GAE/kg for Semillon Blanc grapes, Alphonse Lavalleé and Razaki grapes. At the day of harvesting, total phenolic content of control grapes, grapes treated with alginate spray without vanillin and treated grapes with alginate spray incorporating vanillin were respectively 694.19, 696.06 and 694.50 mg GAE/kg for Semillon Blanc grapes, 689.19, 702.94 and 701.06 mg GAE/kg for Alphonse Lavalleé grapes, 684.19, 690.44 and 686.06 mg GAE/kg for Razaki grapes. No significant difference ($p>0.05$) was observed in total phenolic content between control grapes and treated grapes by alginate spray with or without vanillin for all of three cultivars. Phenolic content of grapes from three cultivars increased during ripening in vineyard. Alphonse Lavalleé and Semillon Blanc grapes indicated higher phenolic content compared to Razaki grapes at harvest. Preharvest treatment had favourable effects on total phenolic contents of different grape cultivars.

Preharvest treatment by alginate spray with or without vanilllin was significantly effective on Alphonse Lavalleé in terms of increasing phenolic content. For other cultivars, the preharvest treatment by alginate spray did not considerably increase the phenolic content. However, the important point was that the application of preharvest treatment did not prevent synthesis of bioactive compounds in plant during growing.

When the fruits were treated with preharvest spray, antioxidant activity was 77.73, 77.46 and 79.42 μmol TEAC/L for Semillon Blanc, Alphonse Lavalleé and Razaki grapes, respectively. At the day of harvesting, antioxidant activity of control grapes, grapes treated with alginate spray without vanillin and treated grapes with alginate spray incorporating vanillin were respectively 82.45, 83.07 and 82.98 μmol TEAC/L for Semillon Blanc grapes, 80.88, 81.82 and 81.64 μmol TEAC/L for Alphonse Lavalleé grapes, 81.15, 81.60 and 81.51 μmol TEAC/L for Razaki grapes. There was no significant difference ($p>0.05$) in total phenolic content between control grapes and treated grapes by alginate spray with or without vanillin for all of three cultivars. Briefly, the level of antioxidants increased during ripening process in vineyard and the increase was not affected by preharvest treatment. Among three grape cultivars, Semillon blanc grapes had little higher values for antioxidant activity of phenolic compounds at harvest. It was mentioned that phenolic compounds those derived from cynnamic and benzoic acid (esterified or nonesterified form) are mainly founds in white grape cultivars. These phenolic acids are highly oxidative and produce brown compounds that also present antioxidant activity (Sánchez-González et al., 2011). The results for total phenolic content and antioxidant activity were compatible with literature such as total phenolic content and antioxidant activity of redglobe table grapes were found around 600 mg/kg GAE and in range of 40-60 trolox equivalents antioxidant capacitiy (TEAC) at the beginning of storage (Melgarejo-Flores et al., 2013).

Lactic acid bacteria (LAB) are responsible for lactic acid fermentation and they are naturally presented on the surface of grape and grape leaves (Bae, Fleet, & Heard, 2006). In anaerobic conditions, grapes can be affected by LAB which was identified by the formation of lactic acid from sugars such as glucose and fructose (König & Fröhlich, 2009). It was considered that preharvest alginate sprays remain as a very thin layer on the surface of grapes and the layer do not interfere with respiration process. Thus, changes in lactic acid content of grapes were determined as a precursor of anaerobic conditions. At the day of preharvest treatment, lactic acid content of Semillon Blanc, Alphonse Lavalleé and Razaki grapes was 0.24, 0.17 and 0.41 g/l, respectively.

At the day of harvesting, lactic acid content of grapes from untreated, treated with alginate spray and treated with alginate spray incorporating vanillin groups were respectively 0.21, 0.22 and 0.24 g/l for Semillon Blanc grapes, 0.19, 0.14 and 0.14 g/l for Alphonse Lavalleé grapes, 0.32, 0.34, 0.38 g/l for Razaki grapes. There was no significant difference ($p>0.05$) in lactic acid content between control grapes and treated grapes by alginate spray with and without vanillin for all of three cultivars. It was indicated that preharvest alginate spray did not limit oxygen around the berries. In literature, lactic acid content of grape juices obtained from fresh grapes(18-21º Brix) was found between 0.19 and 0.64 g/l while main organic acids were tartaric acid and malic acid (Lima et al., 2014). The results regarding the lactic acid content of grapes was consistent with previous reports.

To sum up, preharvest alginate spray with and without vanillin improved safety and maintained quality of grapes. Preharvest treatment did not affect significantly ($p>0.05$) maturation of fruit before harvesting.

Table 4.5. Effect of preharvest treatments on quality characteristics of Semillon Blanc, Alphonse Lavalleé and Razaki grapes

C	G	Brix (ºBx)	pH	TA (g/100ml)	TPC (mg GAE/kg)	AA (µMmol TEAC/l)	YMC (log cfu/g)	LAC (g/l)
Semillon Blanc	I	14.61 ± 0.60	3.51 ± 0.03	1.84 ± 0.21	655.75 ± 4.21	77.73 ± 0.61	3.69 ± 0.21	0.24 ± 0.03
	II	19.63 ± 0.57	3.98 ± 0.13	0.98 ± 0.07	694.19 ± 3.44	82.45 ± 0.67	3.95 ± 0.25	0.21 ± 0.02
	III	19.28 ± 0.47	3.72 ± 0.06	1.03 ± 0.04	696.06 ± 2.77	83.07 ± 0.75	3.49 ± 0.33	0.22 ± 0.03
	IV	18.40 ± 0.69	3.82 ± 0.04	0.99 ± 0.04	694.50 ± 3.68	82.98 ± 0.75	3.56 ± 0.17	0.24 ± 0.04
Alphonse Lavellee	I	10.88 ± 0.91	3.19 ± 0.01	1.69 ± 0.21	665.13 ± 5.25	77.46 ± 0.92	3.04 ± 0.09	0.17 ± 0.06
	II	17.19 ± 0.37	3.93 ± 0.06	0.71 ± 0.07	689.19 ± 3.73	80.88 ± 0.40	3.01 ± 0.03	0.19 ± 0.05
	III	17.51 ± 0.86	3.94 ± 0.02	0.73 ± 0.04	702.94 ± 3.40	81.82 ± 0.81	2.54 ± 0.16	0.14 ± 0.03
	IV	16.08 ± 0.32	3.81 ± 0.03	0.69 ± 0.11	701.06 ± 5.91	81.64 ± 0.43	2.59 ± 0.11	0.14 ± 0.05
Razaki	I	11.30 ± 0.23	3.08 ± 0.08	3.34 ± 0.07	652.94 ± 4.13	79.42 ± 1.34	2.64 ± 0.13	0.41 ± 0.07
	II	24.95 ± 0.18	4.37 ± 0.04	0.51 ± 0.04	684.19 ± 3.28	81.15 ± 0.91	3.06 ± 0.14	0.32 ± 0.03
	III	24.67 ± 0.49	4.28 ± 0.04	0.51 ± 0.04	690.44 ± 7.53	81.60 ± 0.95	2.89 ± 0.16	0.34 ± 0.02
	IV	24.11 ± 0.17	4.23 ± 0.0	0.45 ± 0.04	686.06 ± 4.89	81.51 ± 0.49	2.70 ± 0.28	0.38 ± 0.04

mean ±std (n=3 for TSS, pH, TA, LA; n=2 for TPC, AA, YMC)
I: Unmature grape
II: Control group
III: Group treated with alginate spray
IV: Group treated with alginate spray incorporating vanillin
C: Cultivars
G: Groups
TA: Titratable acidity
TPC: Total phenolic content
AA: Antioxidant activity
YMC: Yeast and mold counts
LAC: Lactic acid content

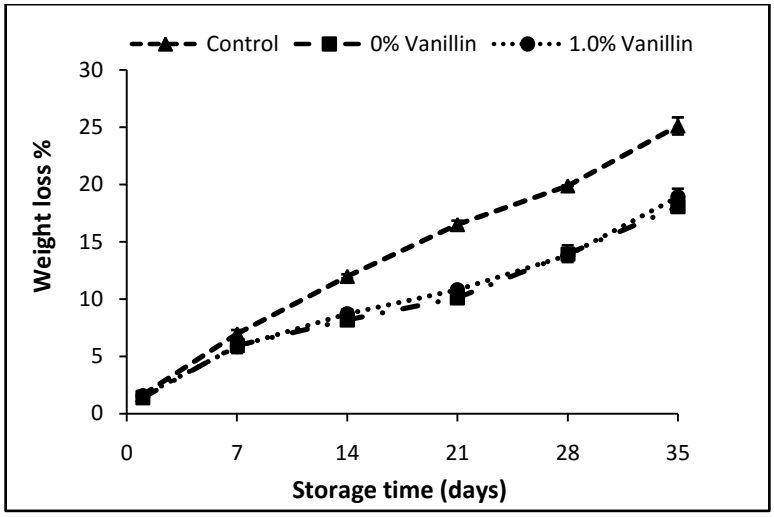
4.3. Effect of Preharvest and Postharvest Treatments on Fruit Quality

4.3.1. Weight Loss

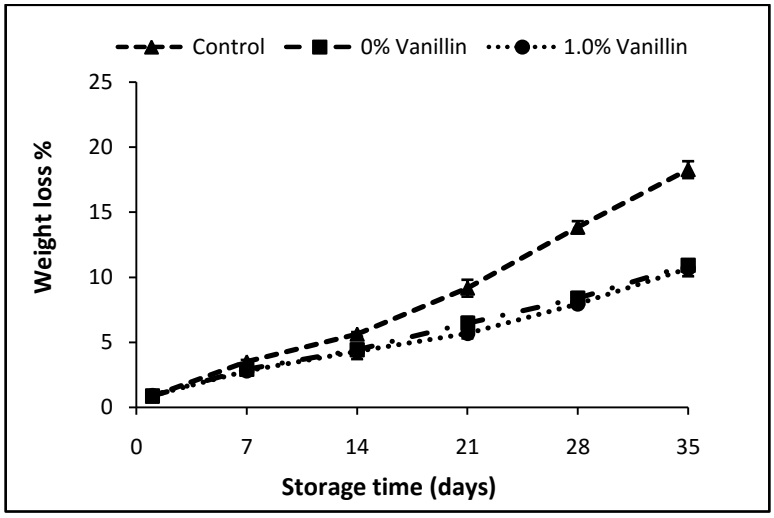
Coating application significantly ($P<0.05$) prevented weight loss in three types of grapes during storage compared to control (Figure 4.3). At the end of 35 days of storage, control grapes lost 25.11, 18.28, and 16.75% of their weight while weight loss of alginate coated grapes reached 18.07, 10.92, and 11.39% for Semillon Blanc (Figure 4.3.a), Alphonse Lavalleé (Figure 4.3.b), and Razaki grapes (Figure 4.3.c), respectively. Weight loss patterns for all three grape cultivars were similiar. No significant ($P>0.05$) difference was determined in weight loss among two types of coating which were alginate coating with or without vanillin.

Fruit and vegetables contain a relatively large amount of water. However, when fruit and vegetables are harvested, water loss occurred naturally and its rate depends on temperature and relative humidity of medium (Embuscado & Huber, 2009). Moreover, weight loss from fruits and vegetables is due to the gradient of water vapor pressure between fruit and atmosphere, resulting in transition of water vapor from surface of fruit to the air (Valero et al., 2013). Although alginate coatings are considered to be not very effective to reduce weigh loss, studies indicated that alginate-calcium coatings prevented water loss effectively through cross-linking alginate polymer with calcium ions. Thus, water vapor barrier properties of alginate coatings were improved by calcium cross-linking (Olivas et al., 2007). Reduction in weight loss by alginate coating has also been demonstrated in fruits and vegetables including plum (Valero et al., 2013), apple slices (Olivas et al., 2007), fresh-cut pineapple (Azarakhsh et al., 2014), mushroom (Jiang et al., 2013), and strawberry (Fan et al., 2009). In the present study, the results showed that alginate coatings with or without vanillin significantly ($p<0.05$) limited weight loss of grapes during 35 days of storage at 4±2 °C.

(a)



(b)



(c)

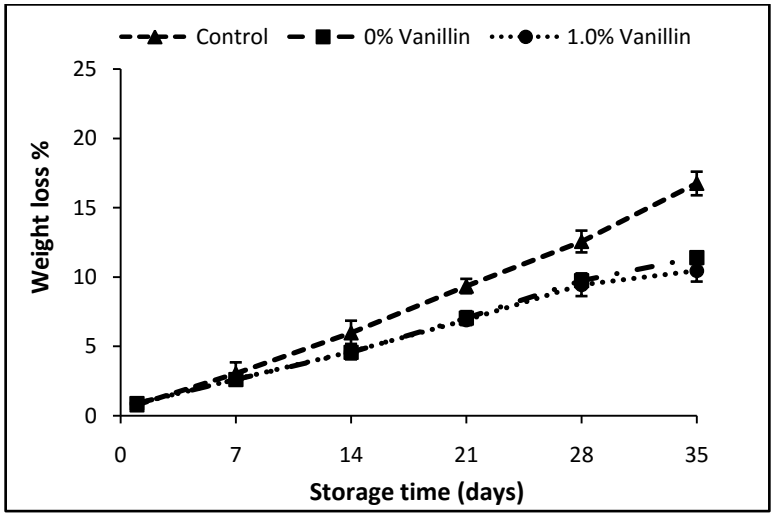


Figure 4.3. The effect of alginate based preharvest spray and postharvest coating on weight loss(%) of grapes from different varieties; (a) Semillon Blanc, (b) Alphonse Lavalleé and (c) Razaki during storage

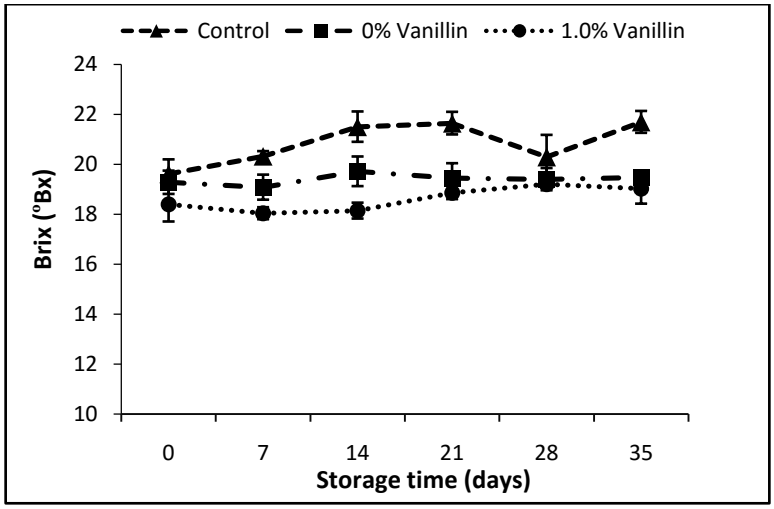
4.3.2. Soluble Solid Content, Titradable Acidity and pH

Changes in soluble solid content (SSC) during the storage in three different types of grapes are shown in Figure 4.4. For three grape cultivars, grapes coated with alginate coating incorporating vanillin showed lower SSC than samples coated with alginate coating during storage. It may be due to the addition of vanillin, but the difference in SSC of grapes coated alginate coating with or without vanillin was not significant($p>0.05$). As shown in Figure 4.4, SSC of coated grapes did not show considerable changes during storage, but SSC of uncoated grapes gradually increased in the initial period of the storage and then reflected a decrease similarly in Semillon Blanc (Figure 4.4.a), Alphonse Lavalleé (Figure 4.4.b), and Razaki grapes (Figure 4.4.c).

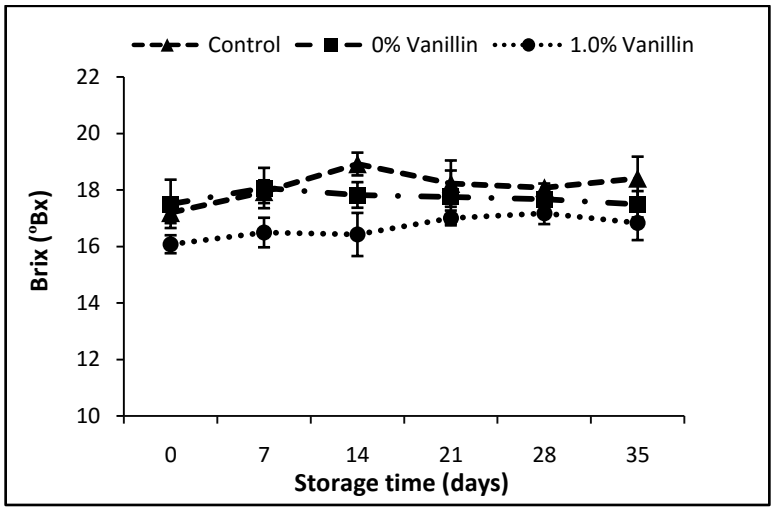
We concluded that alginate coating had positive effect on SSC of grapes during cold storage. Increase in SSC in the initial period of storage is probably due to continued fruit maturation. After gradual increase, there is a diminishing pattern for control samples. Consumption of soluble solids by respiration process results in a decline in soluble solid content for metabolic activities of fruit (Özden & Bayindirli, 2002). However, coated grapes showed regular pattern with slightly increases and decreases. Thus, the positive effect of alginate coating comes from the reduction of respiration process by forming gaseous environment in fruit surface.

Soluble solid content and titratable acidity are one of the most important quality characteristics of grapes to determine their quality during storage (Gao, Zhu, & Zhang, 2013). As soluble solids, organic acids in fruits are also exhausted during storage. Titratable acidity of uncoated and coated grapes during storage are shown in Figure 4.5 for three different types of grapes. Titratable acidity gradually decreased in control samples for all types of grapes. At the end of storage, grapes coated with alginate had lower decrease compared to uncoated grapes. In this study, results indicated that alginate coating provided lower loss in both soluble solid content and titratable acidity of grapes during storage. The decrease in titratable acidity is an index of increase in maturity and was also reported for grapes treated with preharvest chitosan spray and postharvest chitosan coating (Meng et al., 2008). The results for pH value of grapes are given in Figure 4.6 and it shows that there is no trend for pH value of samples. Briefly, postharvest alginate coating with or without vanillin was effective in maintaining soluble solid content and titratable acidity of grapes.

(a)



(b)



(c)

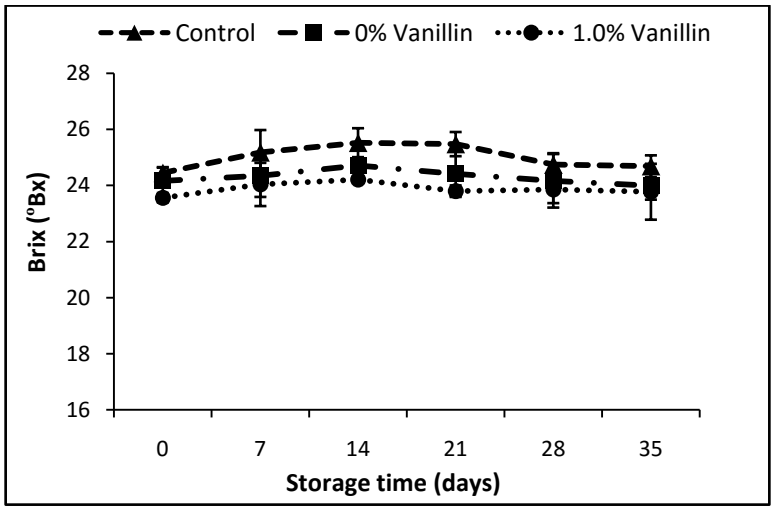
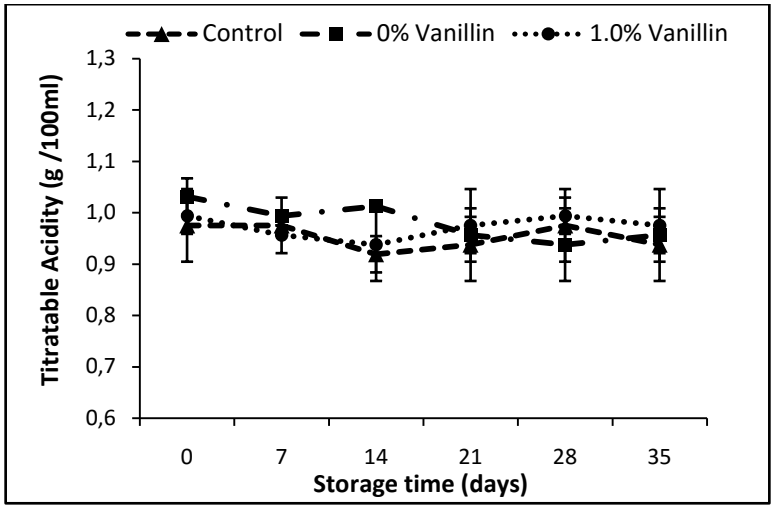


Figure 4.4. The effect of alginate based preharvest spray and postharvest coating on soluble solid content(°Brix) of grapes from different cultivars; (a) Semillon Blanc, (b) Alphonse Lavalleé and (c) Razaki during storage

(a)



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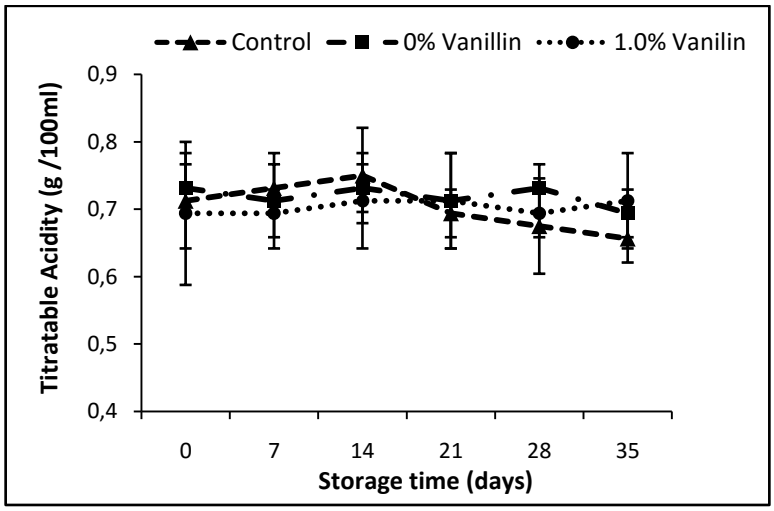

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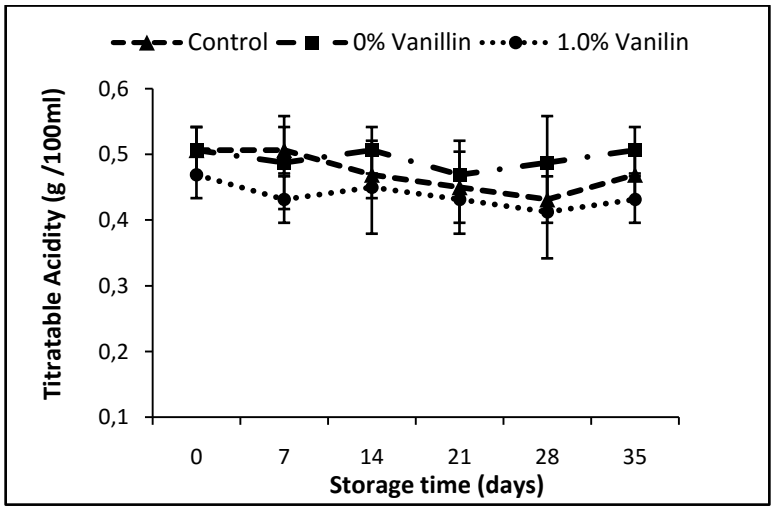
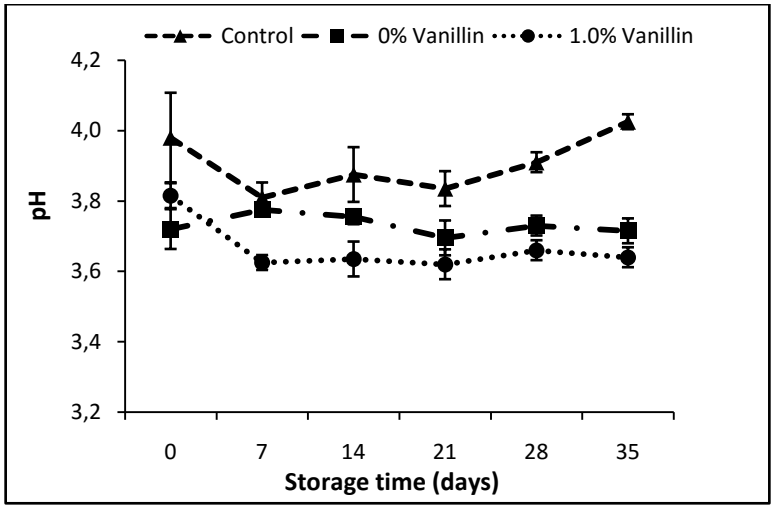
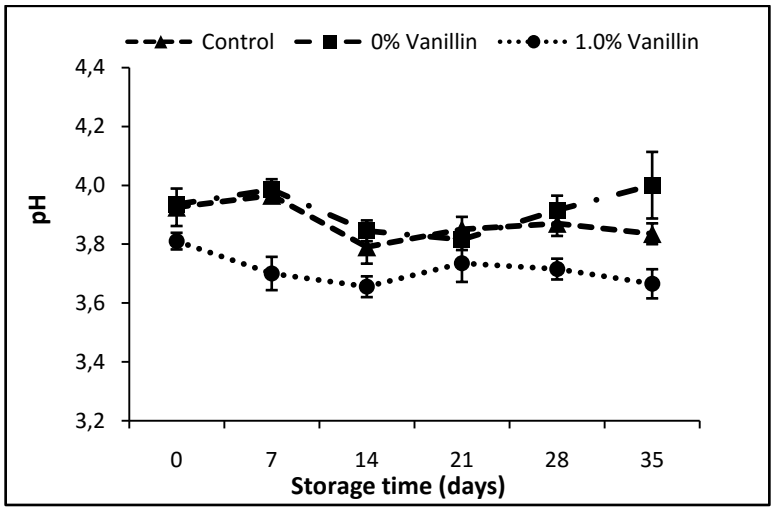


Figure 4.5. The effect of alginate based preharvest spray and postharvest coating on titratable acidity(g/100ml) of grapes from different cultivars;
(a) Semillon Blanc, (b) Alphonse Lavalleé and (c) Razaki during storage

(a)



(b)



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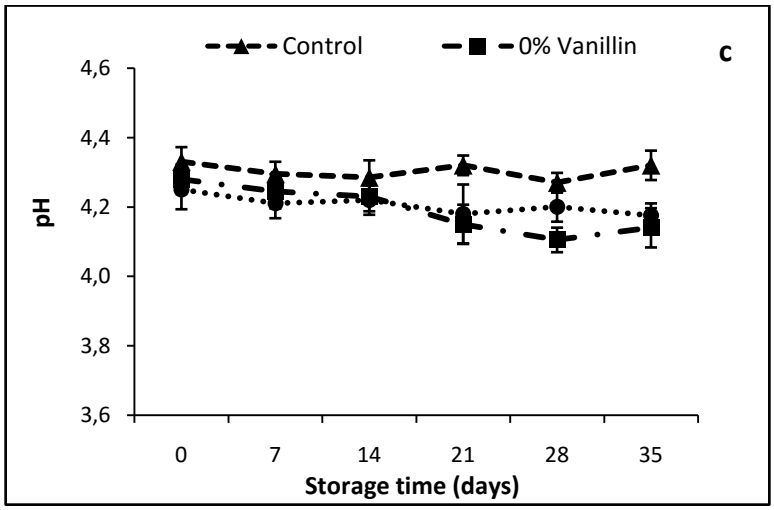


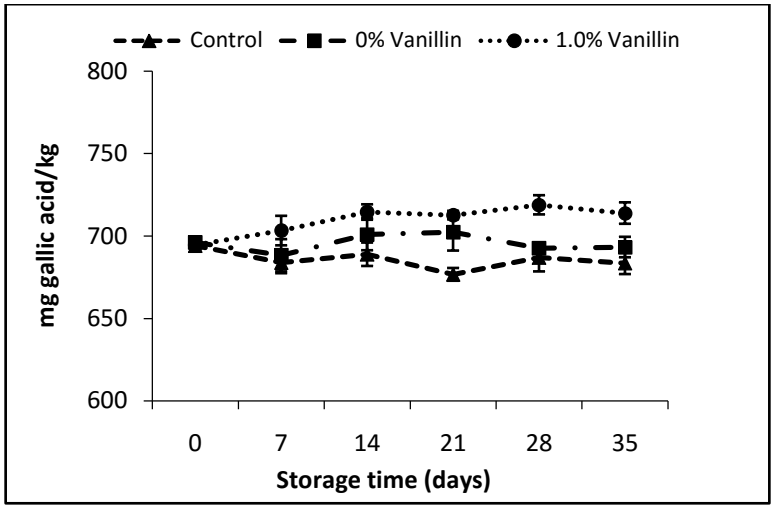
Figure 4.6. The effect of alginate based preharvest spray and postharvest coating on pH values of grapes from different cultivars; (a) Semillon Blanc, (b) Alphonse Lavalleé and (c) Razaki during storage

4.2.3. Total Phenolic Content and Antioxidant Activity

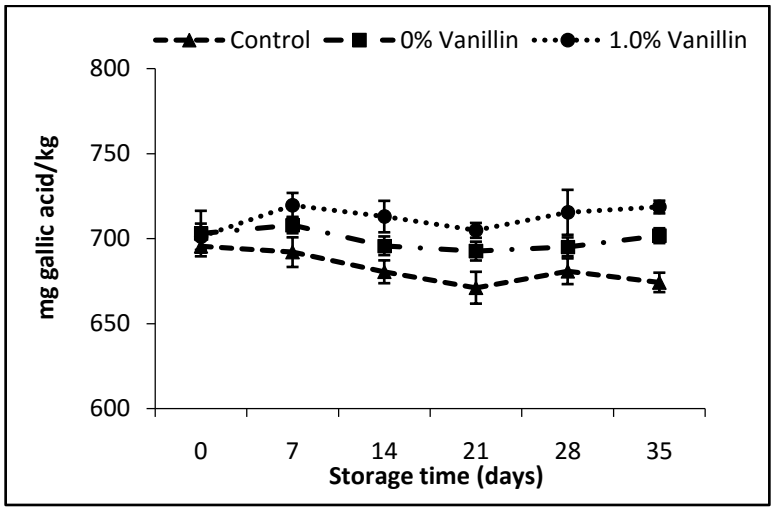
The changes in total phenolic content and antioxidant activity of grapes are shown in Figure 4.7 and Figure 4.8, respectively. Concentration of total phenolic compounds after storage was lower in control groups than initial while postharvest coatings have displayed similar values to initial concentration at the end of storage. Decline in total phenols concentration was prevented by incorporation of vanillin into the coating formulation. As a result, postharvest treatment by alginate coatings significantly ($p<0.05$) prevented the loss of phenolic compounds and addition of vanillin as a bioactive compound ensure higher total phenolic concentration than alone alginate coating throughout storage. Moreover, total phenolic content of Alphonse Lavalleé grapes was higher compared to other cultivars during storage. Total phenolic content of untreated(control) grapes slightly decreased during storage which was similar to total phenolic content changes reported for table grapes derived from *Vitis vinifera* cv. Muscatel cultivar (Pastor et al., 2011).

Antioxidant activity of phenolic compounds indicated progressively higher levels in both treated and untreated grapes during 2 weeks of storage. It was reported that formation of antioxidant compounds can occur as a result of enzymatic browning due to poylphenol oxidase (PPO) enzyme activity during postharvest storage of grapes (Meng et al., 2008). Therefore, the increase in antioxidant activity is probably due to formation of these compounds in the first period of postharvest storage. After 14 days of storage, decrease in antioxidant activity of grapes started, but antioxidant activity of grapes treated with alginate coating incorporating vanillin was always higher than other groups which were grapes treated with only alginate coating and control grapes. Alginate coating incorporating vanillin maintained initial antioxidant activity of grapes at the end of storage. The protective role of alginate coating incorporating vanillin on antioxidant activity was observed in all of three grape cultivars.

(a)



(b)



(c)

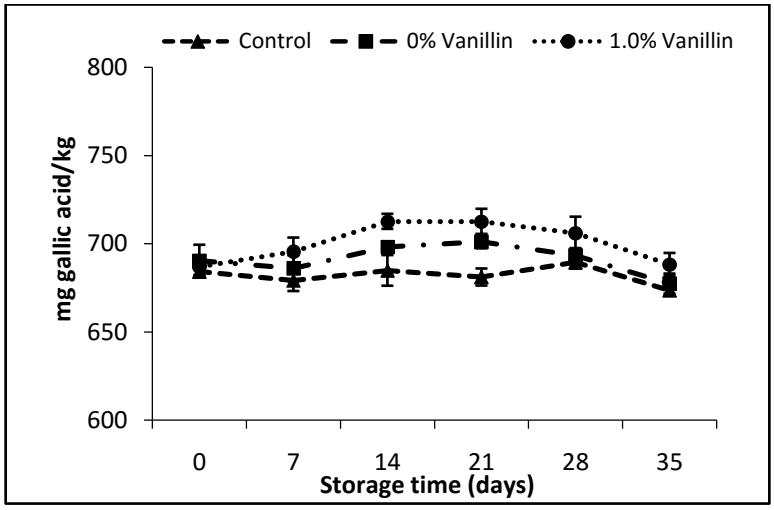
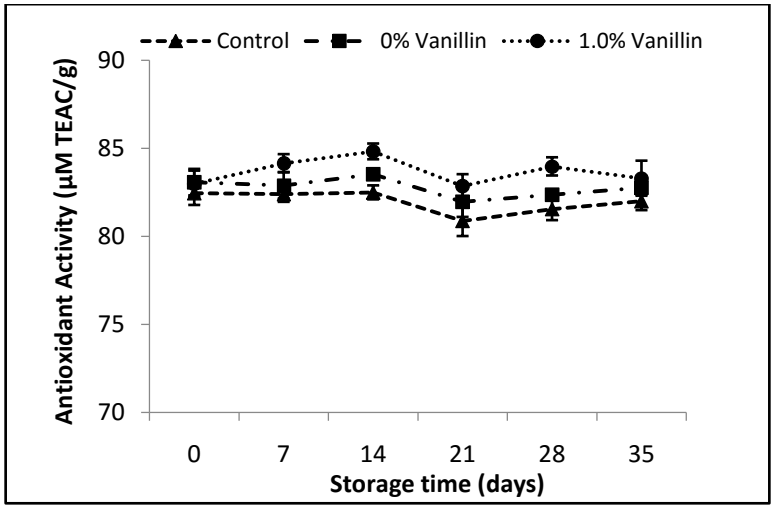
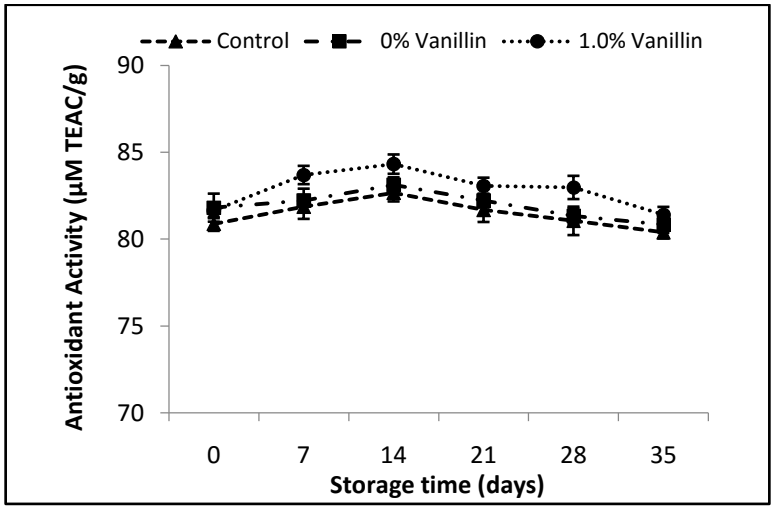


Figure 4.7. The effect of alginate based preharvest spray and postharvest coating on total phenolic content (mg gallic acid/kg) of grapes from different cultivars; (a) Semillon Blanc, (b) Alphonse Lavalleé and (c) Razaki during storage

(a)



(b)



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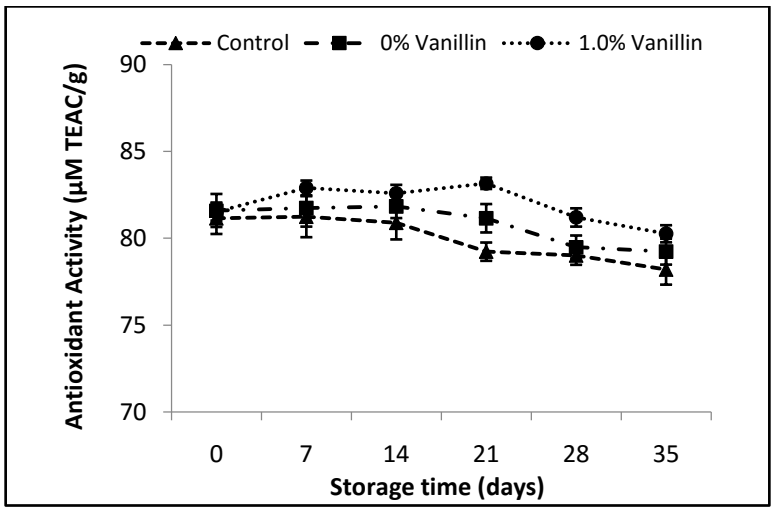


Figure 4.8. The effect of alginate based preharvest spray and postharvest coating on antioxidant activity(µmol TEAC/g) of grapes from different cultivars; (a) Semillon Blanc, (b) Alphonse Lavalleé and (c) Razaki during storage

4.2.4. Color and Fruit Firmness

Color changes and softening occurs during ripening and postharvest storage due to the degredation of components and enzyme activities (Serrano et al., 2008). Effects of alginate treatments on color values (CIE L*,a* and b*) of grapes are given in Table4.6 for three grape cultivars. Coating application was effective in maintaining color changes of grapes during storage. The main effect of alginate coating incorporating vanillin was observed in the lightness of skin color. Lightness (L*) values were higher in grapes coated with alginate coating incorporating vanillin than only alginate coating for all three cultivars. This higher lightness values of grapes having alginate coating incorporating vanillin can be explained by a characteristic white color of vanillin. There was no significant ($p>0.05$) difference between alginate coating without vanillin and control groups at the day of harvesting. However, lightness decreased with storage time and the decrease was significantly greater in control group. This is probably due to the enzymatic browning and coating play an important role in retarding enzymatic reactions which was reported in previous studies (Pastor et al., 2011).

Chroma values of samples showed differences depending on the grape varieties during storage. Incorporation of vanillin into alginate coating decreased the chroma values of grapes having green color (a*:-5.62 and -4,90) from Semillon Blanc and Razaki grape cultivars whereas that increased the chroma values of grapes having red color (a*:1.40) from Alphonse Lavalleé cultivar. Alginate coating incorporating vanillin revealed slightly changes in a* value for Semillon Blanc (a*:-4.59), Alphonse Lavalleé (a*:2.47), and Razaki (a*:-3.16) grapes. Lastly, hue value of grapes slightly decreased for Alphonse Lavalleé grapes and increased for Semillon Blanc and Razaki grapes by incorporation of vanillin into alginate coating. No significant ($p>0.05$) difference was observed in chroma and hue values of grapes coated with alginate coating and control group.

Table 4.6. The effect of alginate based preharvest spray and postharvest coating on color values of Semillon Blanc, Alphonse Lavalleé and Razaki grapes during storage

	Color Parameters	Treatments/Days	0	7	14	21	28	35
Semillon Blanc	**L**	Control	38.72	38.20	35.87	36.68	37.02	37.86
		0% Vanillin	39.41	37.29	38.40	37.93	40.24	38.61
		1.0% Vanillin	45.17	45.49	46.57	47.19	43.76	45.06
	a	Control	-5.62	-6.18	-6.11	-6.04	-5.93	-7.21
		0% Vanillin	-5.80	-5.50	-5.33	-5.26	-4.16	-5.84
		1.0% Vanillin	-4.59	-4.64	-4.54	-4.21	-4.68	-4.99
	b	Control	11.16	15.04	13.67	14.47	15.70	18.21
		0% Vanillin	14.60	14.54	13.74	14.10	11.49	13.71
		1.0% Vanillin	9.06	8.55	8.94	8.09	9.16	11.75
Alfonse	**L**	Control	23.54	23.51	21.63	22.05	21.09	21.74
		0% Vanillin	25.55	25.26	24.96	24.81	25.24	24.82
		1.0% Vanillin	32.91	32.28	33.13	33.37	32.64	32.12
	a	Control	1.40	1.48	1.91	1.65	1.71	1.76
		0% Vanillin	1.69	1.45	1.43	1.82	1.59	1.63
		1.0% Vanillin	2.47	2.45	2.20	2.06	2.09	2.21
	b	Control	-1.06	-0.73	-0.45	-0.64	-0.53	-0.49
		0% Vanillin	-0.29	-0.81	-0.81	-0.51	-0.61	-0.53
		1.0% Vanillin	-2.31	-2.14	-1.51	-1.92	-1.67	-1.49
Razaki	**L**	Control	46.48	48.47	47.11	46.61	46.12	45.91
		0% Vanillin	46.77	48.30	48.42	47.78	45.91	45.00
		1.0% Vanillin	49.44	49.74	49.25	48.94	50.73	47.64
	a	Control	-4.90	-5.07	-5.11	-4.44	-4.65	-3.98
		0% Vanillin	-6.20	-6.02	-5.97	-5.66	-5.83	-4.90
		1.0% Vanillin	-3.16	-3.69	-3.05	-3.42	-3.20	-3.25
	b	Control	18.68	19.15	20.60	21.40	20.02	20.96
		0% Vanillin	17.47	17.41	17.41	17.78	16.89	16.47
		1.0% Vanillin	9.77	10.49	10.86	10.23	10.72	11.79

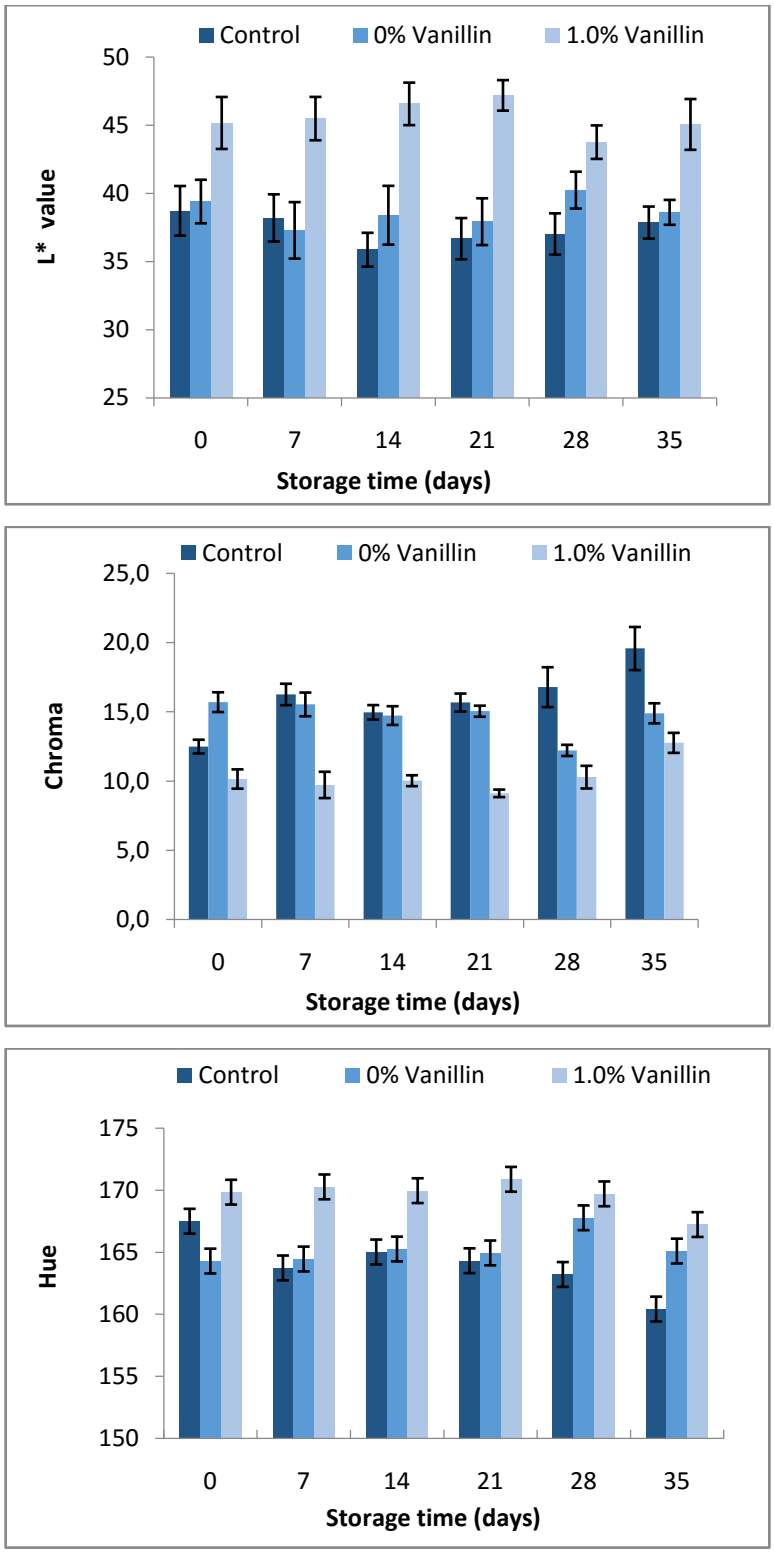


Figure 4.9. The effect of alginate based preharvest spray and postharvest coating on color values of Semillon Blanc grapes during storage

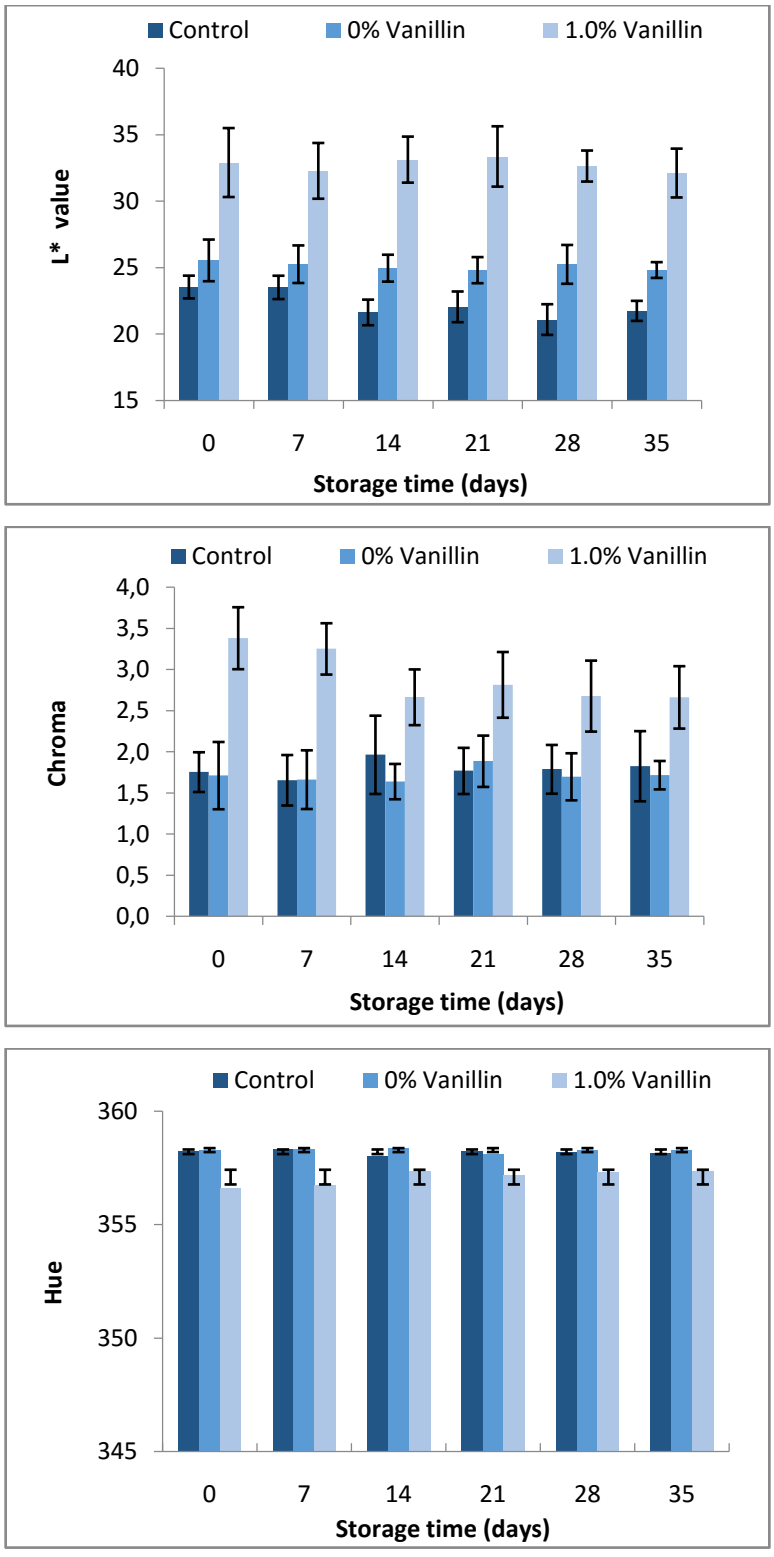


Figure 4.10. The effect of alginate based preharvest spray and postharvest coating on color values of Alphonse Lavalleé grapes during storage

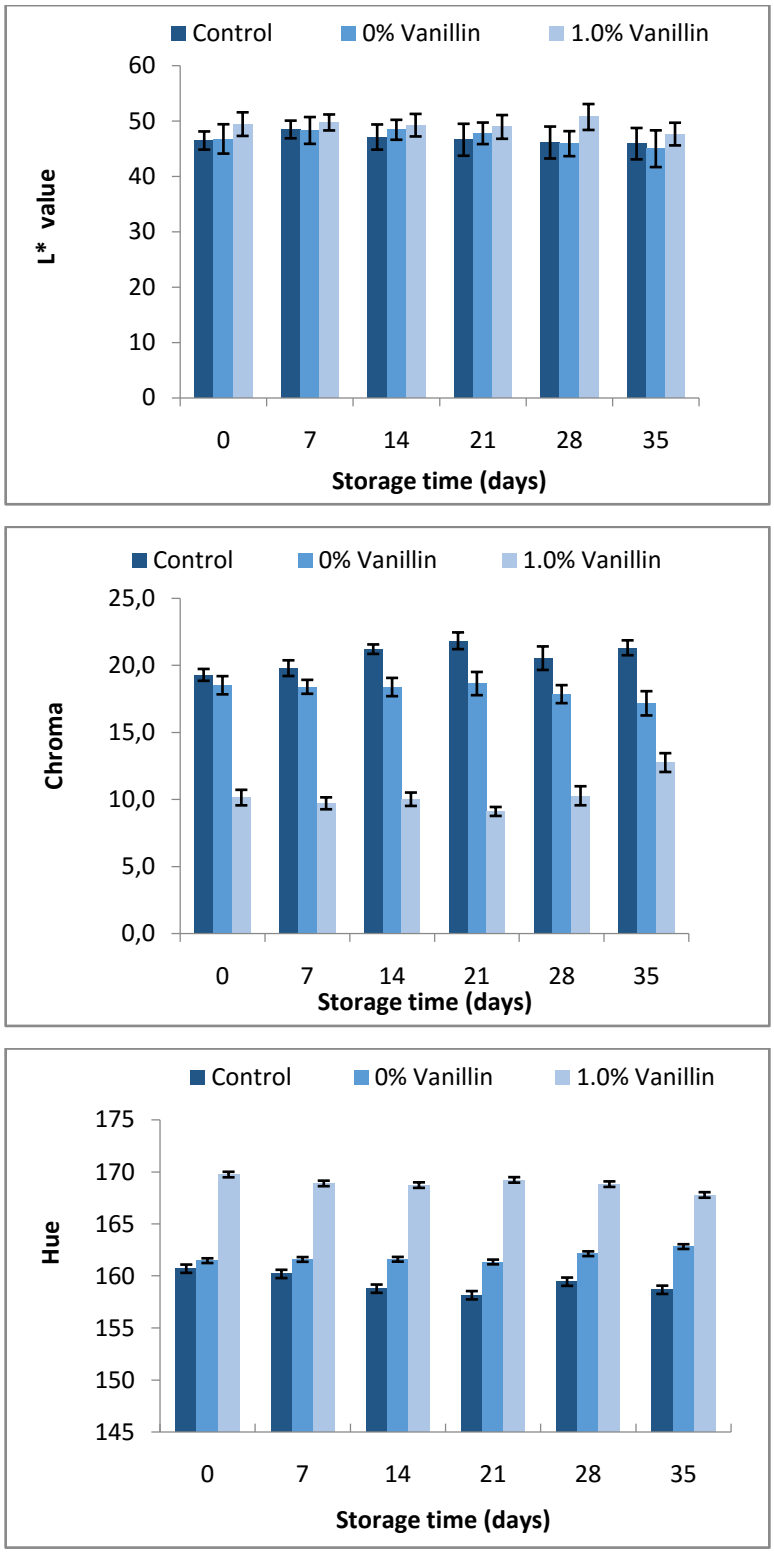
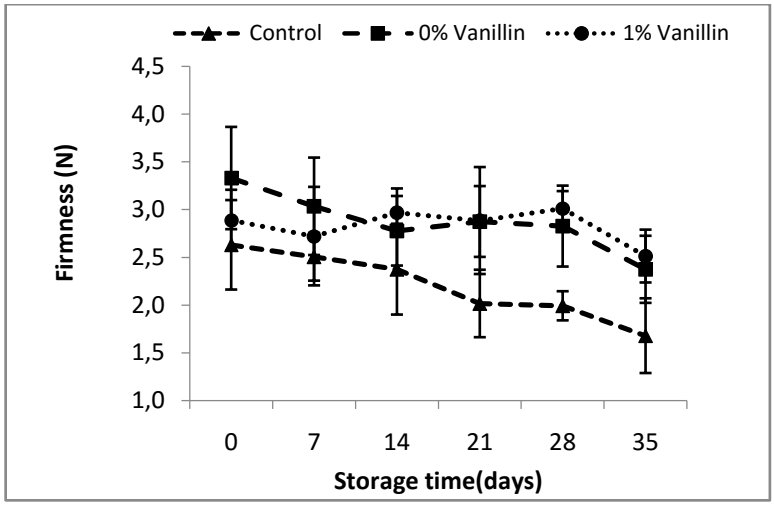


Figure 4.11. The effect of alginate based preharvest spray and postharvest coating on color values of Razaki grapes during storage

Fruit firmness values as function of storage time are presented in Fig. 4.12. It is remarkable that grapes treated with preharvest spray showed greater firmness values compared to untreated grapes at the beginning of storage. Firmness of control grapes sharply decreased with increasing storage time in all cultivars. Meanwhile, coating application maintained firmness of grapes during storage. ANOVA results also demonstrated that fruit deformation force (N) explained as fruit firmness of grapes was significantly ($p<0.05$) affected by alginate coating with or without vanillin. Addition of vanillin into coating did not significantly ($p>0.05$) influence the firmness of grapes in all cultivars. The highest reduction in firmness loss was observed in Alphonse Lavalleé grapes followed by Semillon Blanc and Razaki grapes. Control grapes from Alphonse Lavalleé cultivar lost approximately 50.47% of their firmness at the end of storage. However, firmness loss for Alphonse Lavalleé grapes treated with alginate coating with or without vanillin were about 19.56% and 18.28% at the end of storage. The reduction in firmness loss by alginate coating can be explained by minimizing weight losses through coating application that has been reported in table grapes (Sánchez-González et al., 2011), plum (K. Liu et al., 2014) and apples (Olivas et al., 2007) by different edible coatings. In addition, $CaCl_2$ treatment had good contribution to the fruit firmness. The positive effect of calcium in reduction of firmness loss during storage was reported in strawberries (Atress, El-Mogy, Aboul-Anean, & Alsanius, 2010).

Texture is an important quality characteristics that affects consumer preference of fresh fruits. Softening or firmness loss naturally occurs during postharvest period due to fruit ripening and water loss. Coating application maintains the texture attributes of fresh fruit by reducing the rate of methabolism and decreasing water loss (Zhou et al., 2008). Firmness of grapes directly influence the market value of fruit and the studies by different coating applications on table grapes have been explained that the firmness losses was declined with chitosan coating (Xu et al., 2007), Aloe vera coating (Valverde et al., 2005), and hydroxypropylmethylcellulose coating (Sánchez-González et al., 2011) during postharvest storage. In the study, grapes treated with alginate coating showed greater firmness than control grapes at the end of cold storage and values of fruit firmness are in accordance with the literature.

(a)



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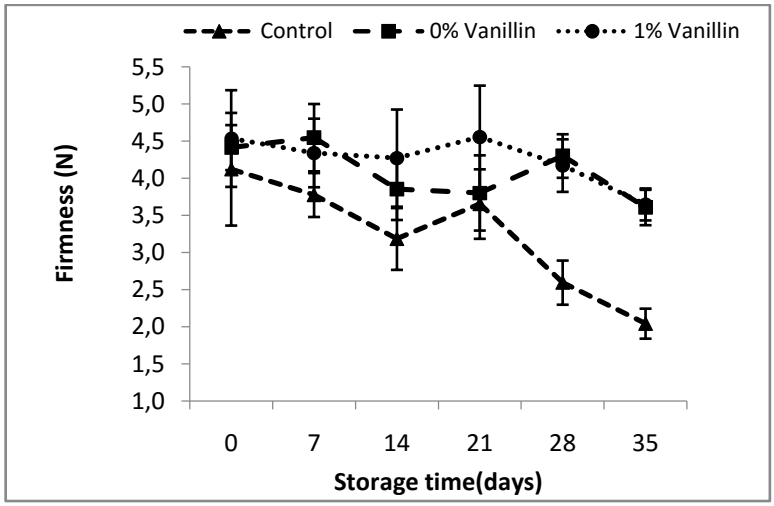

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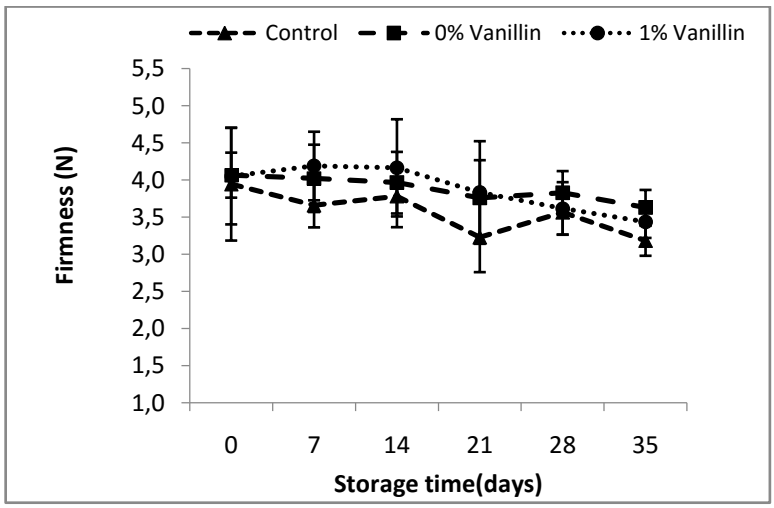


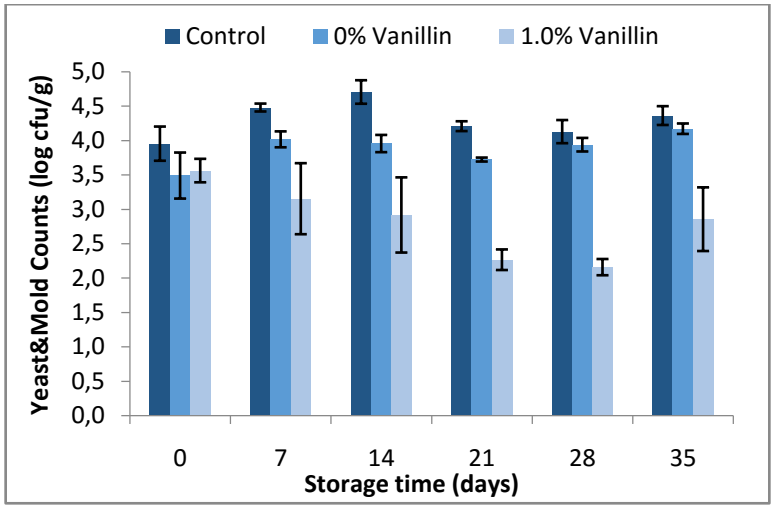
Figure 4.12. The effect of alginate based preharvest spray and postharvest coating on firmness value(N) of grapes from different varieties; (a) Semillon Blanc, (b) Alphonse Lavalleé and (c) Razaki during storage

4.2.5. Yeast and Mold Counts

Deterioration in fresh fruit and vegetables by microorganisms caused high quality and quantity losses. Microbial spoilage of grapes is caused by fungi and it is the main reason for preharvest and postharvest deteriorations. Changes in yeast and mold counts of grapes treated with preharvest spray and postharvest coating during storage are presented in Figure 4.13. It was remarkable that alginate coatings incorporating vanillin were considerably effective in reducing yeast and mold growth. Both uncoated grapes and only alginate coated grapes showed increasing pattern in yeast and mold counts during storage while grapes coated with alginate coatings incorporating vanillin having diminishing pattern in yeast and mold counts until 35 days of storage. Compared with control grapes, the addition of vanillin into alginate coating provided significant ($p<0.05$) reduction in yeast and mold growth by 1.51, 1.73 and 0.82 log cfu/g for Semillon Blanc, Alphonse Lavalleé and Razaki, respectively at the end of storage. Application of alginate coating incorporated with vanillin prolonged the shelf life of grapes by lowering yeast and mold counts significantly ($p<0.05$) during storage. Thus, postharvest edible alginate coating incorporated with vanillin could be used as alternative to prevent fungal decay and extend shelf life of grapes during cold storage.

A few studies in the literature have already demonstrated that vanilin has beneficial effect on reduction of yeast and mold growth in fresh fruits. (María A. Rojas-Graü et al., 2007) found that an alginate coating without incorporation of antimicrobial agents was not effective in reducing yeast and mold growth on fresh-cut apple. On the other hand, alginate coating incorporating lemongrass oil (1.0 and 1.5% w/v), oregano oil (0.5%w/v) and vanillin (0.3% and 0.6% w/v) inhibited the growth of yeast and mold. In addition, the growth rate of yeast and mold in fresh-cut apple coated with alginate coating incorporating vanillin did not exceed 3 log cfu/g at the end of 21 days storage at 4 °C. Our results were compatible with literature and indicated that yeast and mold growth significantly reduced by alginate coating when vanillin was added as a natural antimicrobial agent.

(a)



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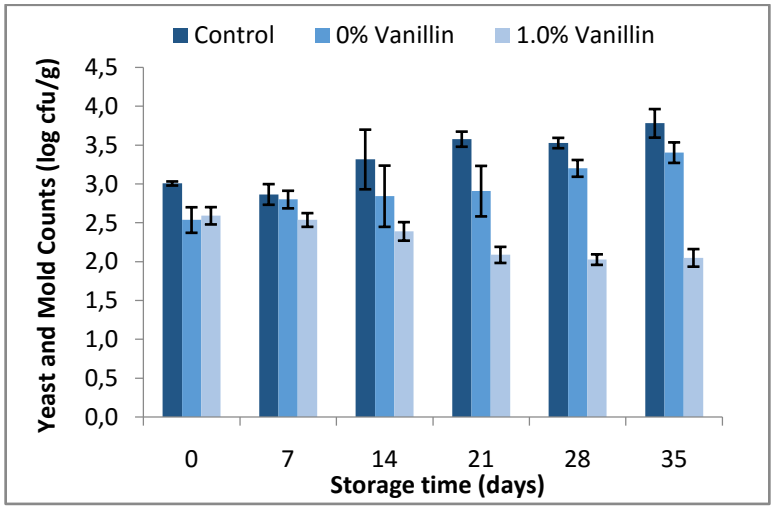

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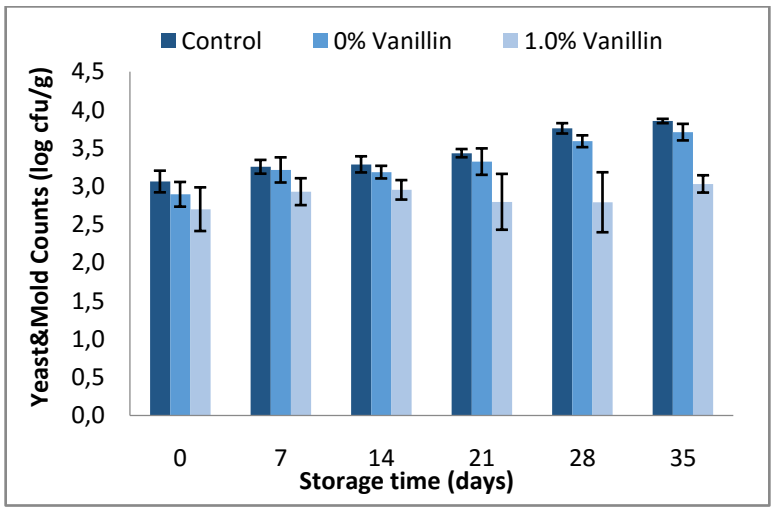
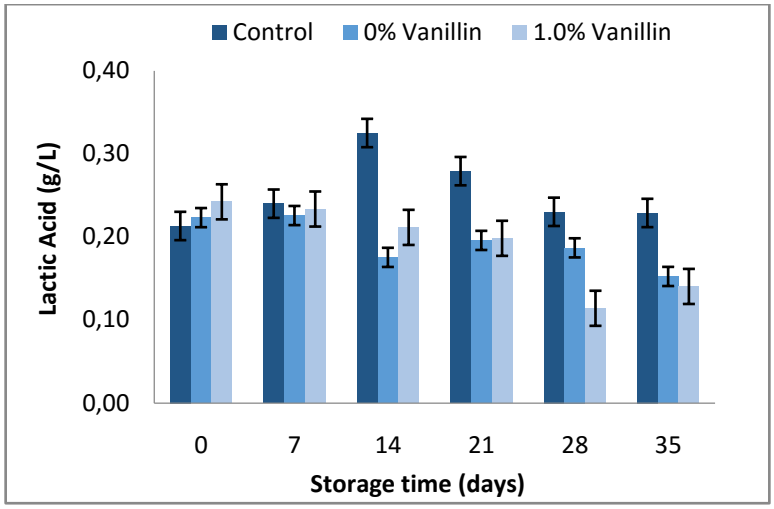


Figure 4.13. The effect of alginate based preharvest spray and postharvest coating on yeast and mold counts(log cfu/g) of grapes from different varieties; (a) Semillon Blanc, (b) Alphonse Lavalleé and (c) Razaki during storage

4.2.6. Lactic Acid Content

Grapes could be spoiled by lactic acid bacteria which is naturally found on the surface of grape and grape leaves and produce lactic acid from sugars in anaerobic conditions (Bae et al., 2006). In that respect, the balance between O_2 and CO_2 provided by coating play an important role. High lactic acid content can be an indicator of lactic acid fermentation due to anaerobic conditions. However, lactic acid is also one of the organic acids naturally presented in grapes. Lactic acid concentrations ranged from 0.11 to 0.32 g/l for Semillon Blanc, 0.10 to 0.29 g/l for Alphonse Lavalleé and from 0.19 to 0.46 mg/l for Razaki grapes. As we mentioned in result part of preharvest treatment, lactic acid content of grape juices from different varieties of *Vitis labrusca* grapes varied from 0.19 to 0.64 g/l (Lima et al., 2014). Lactic acid content of grapes were compatible with literature and did not show significant ($p>0.05$) changes in coated grapes during storage. It could be pointed out that alginate coating w/wo vanillin was efficient for providing internal atmosphere with O_2 and CO_2 during storage.

(a)



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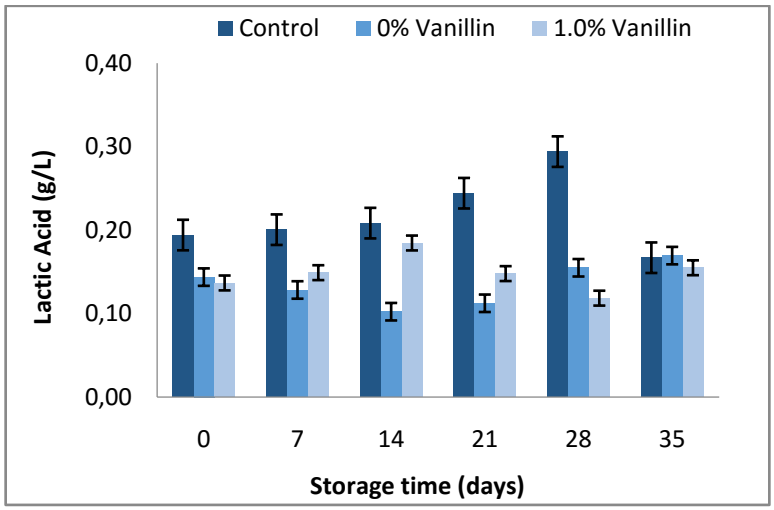

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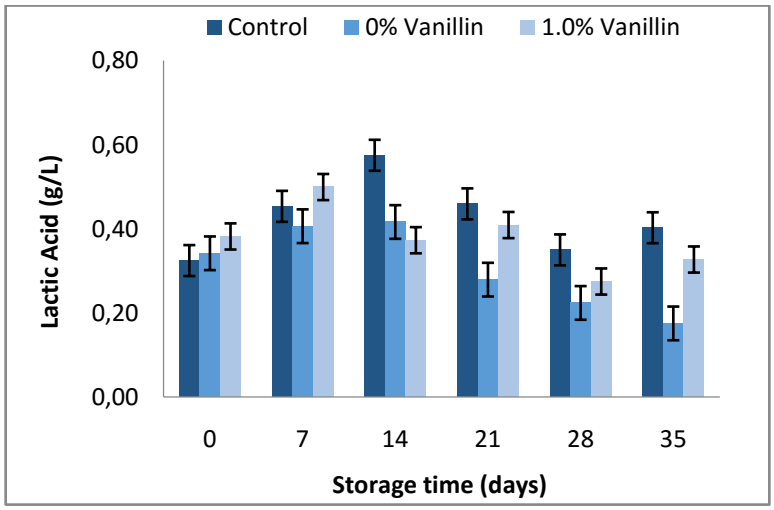

Figure 4.14. The effect of alginate based preharvest spray and postharvest coating on lactic acid content (g/l) of grapes from different cultivars; (a) Semillon Blanc, (b) Alphonse Lavalleé and (c) Razaki during storage

4.2.7. Sensory Evaluation

Impact of edible film or coatings with active agents on sensory quality of fresh produce should be considered. Adaptibility with sensory attributes of fresh produce and antimicrobial activity against targeted microorganisms are major considerations when making a decision about the selection of active agent. Vanillin is a generally regarded as safe (GRAS) flavoring compound that is usually compatible with fruit characteristics (Matamoros-Leon et al., 1999). The results for sensory attributes of Alphonse Lavalleé grapes are summarized in Table 4.7. Taste of grapes were performed until 21 days of storage due to the microbial spoilage consideration especially in control group. All sensory attributes decreased during 28 days of storage in both control and coated grapes. However, judges gave higher points for coated grapes than control groups. Appearance and color attributes of grapes were ranked as the highest in grapes coated with alginate coating without vanilin. Odor of grapes coated with alginate coating incorporating vanillin had the highest scores during storage. Our results also showed that grapes coated with alginate coating incorporating vanilin had higher scores for taste than control groups and grapes coated with alginate coating. High scores in odor and taste were most probably related with desired taste and flavor of vanillin. Briefly, coating of grapes by alginate protected the sensory attributes and incorporation of vanillin into coating provided desirable taste and flavor.

Table 4.7. Sensory attributes of Alphonse Lavalleé grapes with respect to alginate coating treatment with/without vanillin during storage.

Sensory Attribute	Treatments/Days	0	7	14	21	28
Appearance	Control	6.11	6.50	5.75	5.25	4.55
	0% Vanillin	8.33	7.50	6.00	5.88	5.15
	1.0% Vanillin	5.11	5.25	5.34	4.98	4.63
Color	Control	6.89	7.38	6.88	5.13	4.75
	0% Vanillin	8.44	8.25	7.50	6.23	5.69
	1.0% Vanillin	5.44	5.50	6.00	4.63	4.50
Odor	Control	6.00	6.38	6.38	5.01	4.28
	0% Vanillin	6.33	7.25	6.25	5.25	4.63
	1.0% Vanillin	6.67	7.38	6.75	6.00	5.38
Taste	Control	7.89	7.00	6.50	n.d.	n.d.
	0% Vanillin	7.89	7.63	6.88	n.d.	n.d.
	1.0% Vanillin	7.11	8.00	7.50	n.d.	n.d.
Texture in mouth	Control	7.22	7.63	6.88	n.d.	n.d.
	0% Vanillin	7.67	7.75	7.38	n.d.	n.d.
	1.0% Vanillin	8.22	8.00	7.13	n.d.	n.d.
Overall Acceptability	Control	7.33	7.13	6.25	5.13	3.75
	0% Vanillin	8.00	7.00	7.00	5.38	4.25
	1.0% Vanillin	6.56	8.00	6.63	4.63	3.65

n.d. : not determined

CHAPTER 5

CONCLUSION

Antimicrobial edible coating produced by incorporation of vanillin into alginate solution was effective in inhibition of fungal growth. Characterization of alginate based edible films with or without vanillin demonstrated that it could be a natural, simple, and economical way to extend the shelf-life of fresh produce. Preharvest spray treatment of alginate solution enriched with vanillin put forward an alternative method instead of sulfurdioxide treatment in order to control fungal decay and maintain quality of grapes during maturation. Based on the results obtained in postharvest coating of grapes, alginate coating incorporating vanillin protect the quality of grapes by slowing down respiration, which provide retention of soluble solids, titratable acidity, firmness, color, and also extended the shelf life of grapes by release of vanilin from coating. Significant difference ($p < 0.05$) was found in weight loss, firmness, yeast and mold counts, total phenolic content, and antioxidant activity; however, there was no significant difference in color values, lactic acid content, and soluble solid content between the grapes coated with alginate coating and uncoated grapes. Overall, our work suggested that bioactive coating developed by addition of vanillin into alginate coating could be an alternative of synthetic fungicides to prevent postharvest deteriorations and improve postharvest quality of grapes by avoiding harmful impact of chemicals on environment and human health.

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APPENDIX A

CALIBRATION CURVES

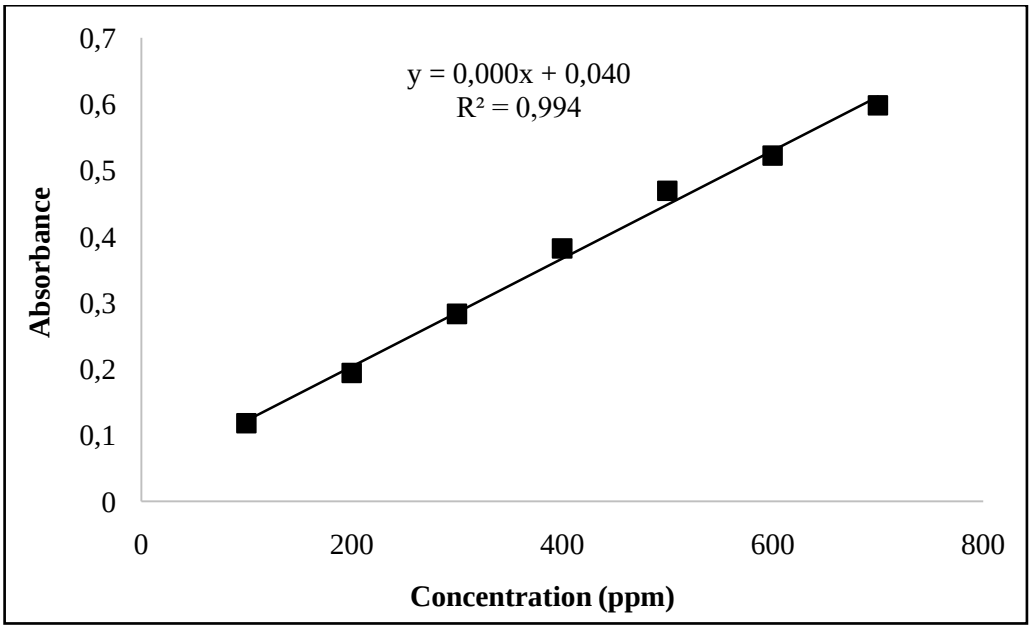

Figure A.1. Calibration curve of Gallic acid

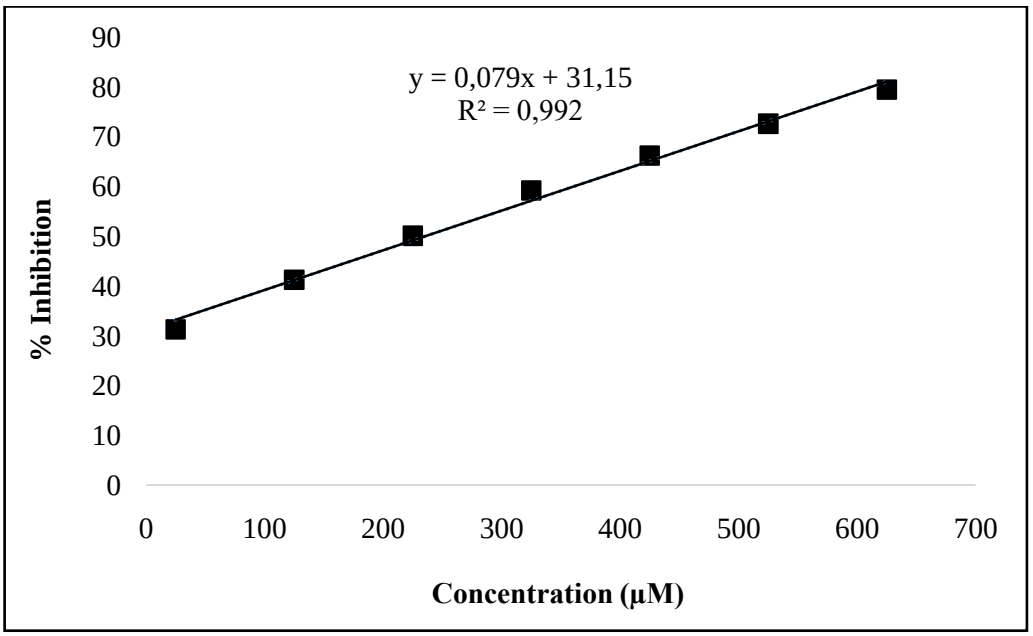

Figure A.2. Calibration curve of Trolox (6-Hydroxy-2,5,7,8-Tetra-methylchromane-2-carboxylic acid)

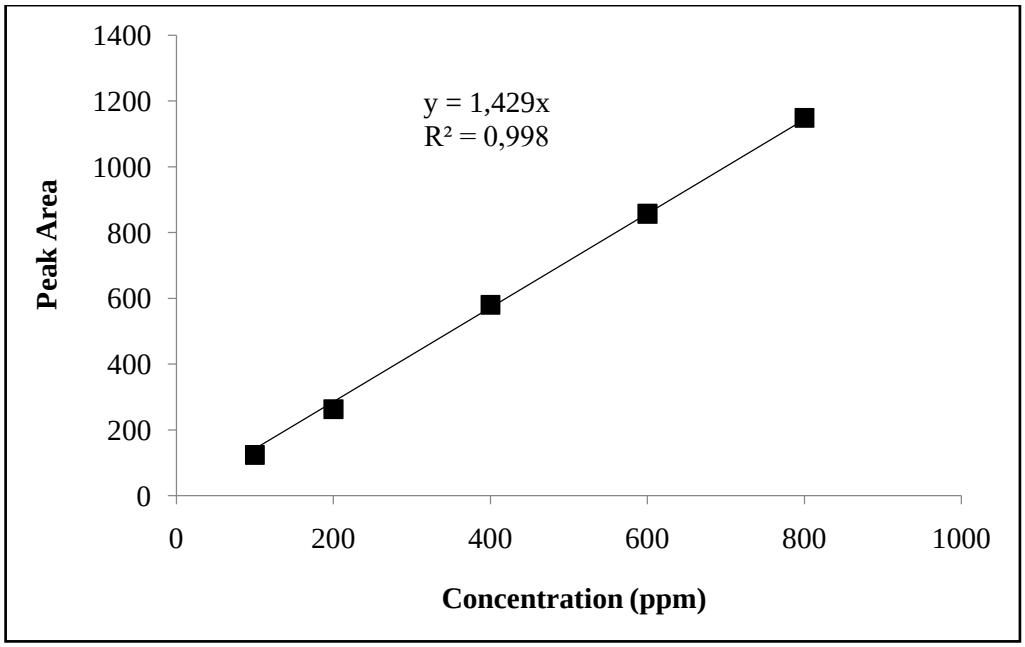

Figure A.3. Calibration curve of Lactic acid