



Reserach Article

Preharvest Applications of Alginate, Salicylic Acid and Oxalic Acid Have a Synergistic Effect on Quality and Storability of Red Globe Grape Variety (*Vitis vinifera* L.)

Erdoğan Bal^{1*}  Demir Kök¹  Ali İzzet Torçuk²  Gamze Uysal Seçkin² 

¹Tekirdağ Namik Kemal University, Faculty of Agriculture, Department of Horticulture, 59300, Tekirdağ, Türkiye
²Growing Techniques Department, Tekirdağ Viticulture Research Institute, Republic of Turkey Ministry of Agriculture and Forestry, 59300, Tekirdağ, Türkiye
*Sorumlu yazar: ebal@nku.edu.tr

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Abstract

This study aimed to evaluate the synergistic effect of preharvest spraying with alginate and in combination with salicylic acid (SA) and oxalic acid (OA), on the quality parameters and storage life of 'Red Globe' grape variety at 0-1°C with 85-90% relative humidity for 60 days. Data of physical and chemical quality parameters were collected at 15-day intervals. The results have shown that alginate coatings delayed changes in weight loss, firmness and berry color compared to control. Alginate coating enriched with oxalic acid and salicylic acid proved to be the most effective applications in restricting stem browning and decay incidence in grapes. Moreover, these formulations contributed to the high antioxidant potential of coated grapes by enriching the phenolic compound content. These findings reveal that preharvest spraying of clusters with alginate combined with SA and OA applications may have high potential for improving storage quality of commercial grapes.

Keywords: Table grape, preharvest application, edible coating, quality.

Hasat Öncesi Uygulanan Alginat, Salisilik asit ve Okzalik Asit Uygulamalarının Red Globe Üzüm Çeşidinin (*Vitis vinifera* L.) Kalitesi ve Muhafazası Üzerine Sinerjik Etkisi

Öz

Bu çalışma, hasat öncesi dönemde alginatın teksel ve, salisilik asit ve okzalik asit ile birlikte uygulanmalarının Red Globe üzüm çeşidinin 0-1 °C ve % 85-90 oransal nemde 60 gün süre ile muhafaza edilebilme süresi ve kalite parametreleri üzerine olan sinerjik etkilerinin değerlendirilmesi amacıyla gerçekleştirilmiştir. Çeşide ait fiziksel ve kimyasal kalite parametre verileri 15 günlük aralıklar ile ölçülmüştür. Araştırma sonuçları kontrol ile karşılaştırıldığında alginat ile kaplama işleminin üzüm çeşidinde ağırlık kaybı, meyve sertliği ve tane kabuk rengi değişimlerinde gecikmelere neden olduğunu göstermiştir. Üzümüne uygulanacak alginatın okzalik asit ve salisilik asit ile zenginleştirilerek kaplanması, salkım iskeleti sararması ve çürüme durumunu sınırlamada en etkili uygulama olduğu ortaya konmuştur. Ayrıca bu kombinasyonlar ile kaplanmış üzümlerde daha yüksek antioksidan potansiyelinin oluşmasına katkı sağlayan fenolik bileşiklerin miktarının artmasına neden olmuştur. Bu bulgular, hasat öncesi dönemde salkımlara salisilik asit ve okzalik asit ile kombine edilmiş alginat uygulamalarının, ticari üzümün muhafaza kalitesini iyileştirmede yüksek potansiyele sahip olabileceğini ortaya koymaktadır.

Anahtar Kelimeler: Sofralık üzüm, hasat öncesi uygulama, yenilebilir kaplama, kalite

Introduction

Grapes are among the most widely produced fruits in the world, with approximately 73.5 million tons produced each year, and is one of the most diffused fruits. As of 2021, Turkey, one of the major countries producing grapes, was ranked sixth in the world with a 4.2 million tons grape production (Anonymus, 2021). The "Red Globe" cultivar, a premium, seeded, red table grape that can

be preserved in the cold for longer-term export and domestic market, is also widely grown throughout Turkey.

Table grape quality is attracting more and more attention from producers and exporters, driven by consumer demand and fruit market prices. Additionally, grapes have gained interest due to their high levels of phenolic compounds, anthocyanin and other naturally occurring antioxidants as well as certain nutritionally significant substances including sugars and minerals (Asgarian et al., 2021). Nevertheless, shelf life of grapes is often shortened because of loss of firmness, berry drop, stem discoloration, rachis dehydration and pathogen infection. *Botrytis cinerea* is also one of the most devastating and economically important diseases in grape all over the world and SO₂ fumigation is still used for controlling postharvest decay (Ni et al., 2016). However, there is a need for alternative applications to SO₂ due to problems with overexposure sulfide residues and its phytotoxic effects such as bleaching and hairline cracking.

During growth and development, the fruit is more sensitive to external stimuli due to rapid cell division and expansion. Fruits usually have a more active metabolism than stored fruit in cold room. For this reason, it is thought that the effects of preharvest elicitor sprays on delaying senescence and improving resistance may have more favorable effects compared to postharvest spraying (Gong et al., 2022).

Recently, many studies have been conducted on the application of various types of edible films and coatings, which are identified as a thin layer of biobased material covering the food surface (Krochta et al., 2011). The coatings are based on polysaccharides, proteins, and lipids to extend the postharvest life of various horticultural products. Among these materials, polysaccharide-based edible coatings such as chitosan and alginate have been gaining popularity recently. They act as barriers against atmospheric gases, water vapor and microbes, helping to reduce respiration and oxidation reaction rates (Singh and Packirisamy, 2022).

Postharvest coatings are often not advised for table grapes with a delicate pericarp and fleshy skin (Shen and Yang, 2017), despite the fact that alginate-based edible coatings are good at maintaining the postharvest quality of fruits (Valero et al., 2013; Rastegar et al., 2019). Recent studies showed that preharvest edible coating applications were effective to improve quality of grapes (Castillo et al., 2010; Nia et al., 2022). Furthermore, by adding biomolecules with antibacterial and antioxidant characteristics, these coatings can potentially become bioactive.

Salicylic Acid (SA) is a hormone produced by plants, that can function as a signaling molecule in their reactions to pathogenic or environmental stress. It is crucial for delaying the senescence, inhibiting ethylene production or action, and maintaining postharvest quality, all of which are advantageous characteristics for preserving postharvest quality of fruits and vegetables (Goni et al., 2017; Chen et al., 2023). Preharvest applications of salicylates have been also reported to improve grape quality characteristics and resistance to pathogen attacks in recent years (Champa et al., 2014; García-Pastor et al., 2020). Similarly, Oxalic Acid (OA) a ubiquitous organic acid in plants, has a variety of effects in delaying aging, controlling postharvest diseases, preventing enzymatic browning, and mitigating of chilling damage in fruits and vegetables (Zheng and Brecht, 2017). In previous reports, it has been found that preharvest applications with SA and OA have positive effects in increasing the content of bioactive compounds and antioxidant potential in commercial harvest (Shen and Yang, 2017; Kok and Bal, 2019; Gomes et al., 2021; García-Pastor et al., 2021).

Improvement in the properties of coatings has been reported in several studies by application of natural organic compounds. However, to our knowledge, there is no scientific literature about the effects of incorporation of SA and OA into alginate coating formulations on grapes quality at harvest or during storage. Therefore, this study was carried out to evaluate the possibility of alginate coating with SA or OA as a preharvest tool to improve the quality and shelf life of 'Red Globe' grape variety.

Materials and Methods

The experiment was carried out at Tekirdağ Viticulture Research Institute, located in Tekirdağ, using "Red Globe" grapes (*Vitis vinifera* L.) during 2020 growing season. The vineyard was a 7-years old planting of cv. Red Globe grafted onto 110R rootstock, bilateral guyot trained with a grapevine spacing 3.0 m × 1.5 m.

Alginate solution was prepared by dissolving 1% concentration (w/v) in hot water (45°C) and shaking continuously until the solution became clear. After cooling to 20°C, glycerol was added as a

plasticizer at 2% v/v (Zapata et al., 2008). For preparing Alg+SA and Alg+OA applications, 2 mM salicylic acid ($C_7H_6O_3$, $\geq 99\%$, Sigma-Aldrich) and 2 mM oxalic acid ($C_2H_2O_4$, $\geq 99\%$, Sigma-Aldrich) were incorporated into the alginate solution and stirred until achieving an emulsion by a complete dispersion. Then, tween 80 as penetrating agent at 0.1% was added to all solutions.

For pre-harvest applications in the vineyard, the vines are divided into four groups. Each coating applications were studied by sampling six clusters per vine from 16 vines. Five days before harvest, applications were carried out as follows: (1) control: grape clusters were sprayed deionized water; (2) Alginate application: 1% alginate was sprayed using a hand sprayer until the clusters were wetted by the flow; (3) Alg+SA application: clusters were sprayed with 1% alginate + 2 mM SA; (4) Alg+OA application: clusters were sprayed with 1% alginate + 2 mM OA.

Grape clusters were harvested at commercial maturity, wrapped in plastic boxes with a capacity of 15-20 kg and carried to the laboratory. Clusters were selected on the basis of uniform size, color and shape were randomly distributed into batches. For each experiment, packages were prepared by placing about 2 kg of table grapes inside the polyethylene plastic bags after pre-cooling grapes. These packages were stored at 0-1°C and 85-90% relative humidity for evaluation of postharvest quality. Measurements were repeated at 0, 15, 30 and 45 for up to 60 days during cold storage.

Clusters were weighed on day 0 and at each analysis period, and weight loss was expressed as a percentage of initial weight. Titratable acidity (TA) and soluble solid content (SSC) of grapes were evaluated in the juice obtained from 30 fruits in each replication. SSC content of grape was estimated by using hand refractometer (Atago Model) and expressed as °Brix. TA of grape was determined by titration method and it was expressed as g tartaric acid 100 ml⁻¹ juice. Ripening Index (RI) was determined as a ratio of SSC and TA. Berry firmness was measured by a PCE PTR-200 handheld digital penetrometer and results were given as g/cm². Berry color was assessed in CIE L*a*b* color space (CM-5 Konica-Minolta Camera Co., Osaka, Japan). Depending on these values, hue angles (arctan b/a) were calculated. Color values were obtained from 30 berries for each replicate. Measurements were taken from opposite sides of each fruit along equatorial axis of berry.

Total phenol content (TPC) of the grapes was determined by Folin Ciocalteu colorimetric method (Slinkard and Singleton, 1977) and expressed as mg gallic acid equivalent per kg fw (mg GAE kg⁻¹). Briefly, a portion of the extract and Folin–Ciocalteu reagent were mixed with sodium carbonate, shaken for 60 minutes, and then the absorbance was read at 765 nm by a spectrophotometer (Shimadzu UV-Mini 1240, Japan).

DPPH (2,2-diphenyl-1-picrylhydrazil) radical scavenging assay was used to determine the antioxidant activity, based on the methods of Brand-Williams et al. (1995). The free radical scavenging activity of the extracts was expressed as micromoles trolox equivalent (TE) per gram of fresh sample ($\mu\text{mol TE g}^{-1}$) from the triple extracts using Trolox's calibration curve.

Stem browning was performed visually using a 0-5 rating scale, according to Chervin et al. (2005): where 1= <10% stem browning, 2= 10-30% stem browning, 3= 30-50% stem browning, 4= 70-90% stem browning and 5= >90% stem browning.

Berries with visible rottenness caused by fungus or any other microorganisms' infection were regarded as decayed fruit. Decay incidence was calculated by the following formula: Decay incidence (%)=(number of decayed berries/total number of berries)×100.

A completely randomized design was utilized and each result was expressed as mean \pm SE of three replicates. All analyses were performed using the SPSS software package v. 18.0 (IBM, NY, USA). Means compared by using Fisher's Least Significant Difference (LSD) test ($p < 0.05$).

Results and Discussion

Weight loss

Weight loss of grapes is one of the most critical quality features of postharvest life and marketability (Bal et al., 2017). The results showed that weight loss increased with prolonged storage time (Figure 1). Although there were no statistically differences between preharvest alginate, Alg+SA and Alg+OA applications (2.1, 2.2 and 2.3%, respectively), these applications considerably reduced the weight loss in grapes compared to the control (3.3%). Alginate reduces external change and water loss by acting as a protective barrier between the fruit and its environment. These positive effects of the alginate coating on fruit are based on their hygroscopic properties (Kontominas, 2020). Moreover, the addition of glycerol as plasticizer to the coating, as applied in the study, gave good results in terms

of weight loss reduction, according to previous records (Valero et al., 2013). Reduction in weight loss by alginate coating has also been demonstrated in tomato (Zapata et al., 2008), plum (Bal, 2019), ber fruit (Ramana et al., 2015) and strawberry (Fan et al., 2009).

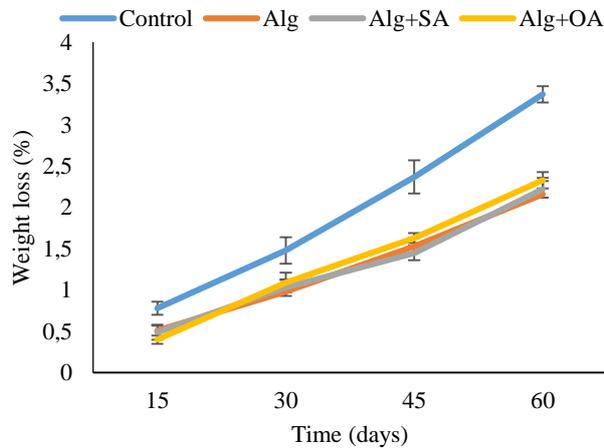


Figure 1. Alginate along with SA and OA affects weight loss of grapes

Berry firmness

In the study, the firmness of grapes declined both in treated and control fruit during storage (Figure 2). These changes in firmness were related to clusters lose their water content and physiological in their structure during storage period. Initially, the firmness values were similar with slight differences for both control and treated samples. As the storage period progressed, all samples gradually began to show loss of firmness, but preharvest Alg+SA and Alg+OA applications exhibited higher firmness in comparison to the control and Alginate samples. At the end of the 60-day storage period, berries treated with Alg+SA and Alg+OA maintained firmness levels of 462 and 429 g, respectively, while the control and Alginate had a firmness of 390 and 371 g. Higher retention of firmness applied with SA and OA enriched alginate coating may be due to lower activity of enzymes in the cell wall, resulting in a slower degradation rate. Similarly, delaying the softening process during storage have been also obtained after SA and OA applications in grape (Champa et al., 2014; Garcia-Pastor et al., 2021).

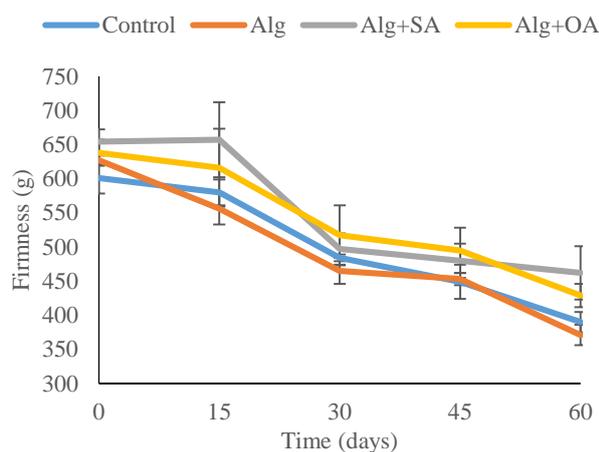


Figure 2. Alginate along with SA and OA affects firmness of grapes

SSC, TA and Ripening Index

SSC, TA and ripening index are basic indicators reflecting the organoleptic quality of table grapes. In current study, lower SSC content and higher TA were observed in grapes treated with Alg+SA, Alg+OA and Alginate compared to control at harvest (Figure 3), indicating a marked delay in ripening and maturation process, whereas RI index increased with advancing maturity and cold storage. However, data revealed that there was no significant difference in SSC, TA and SSC / TA between control and application groups during storage period.

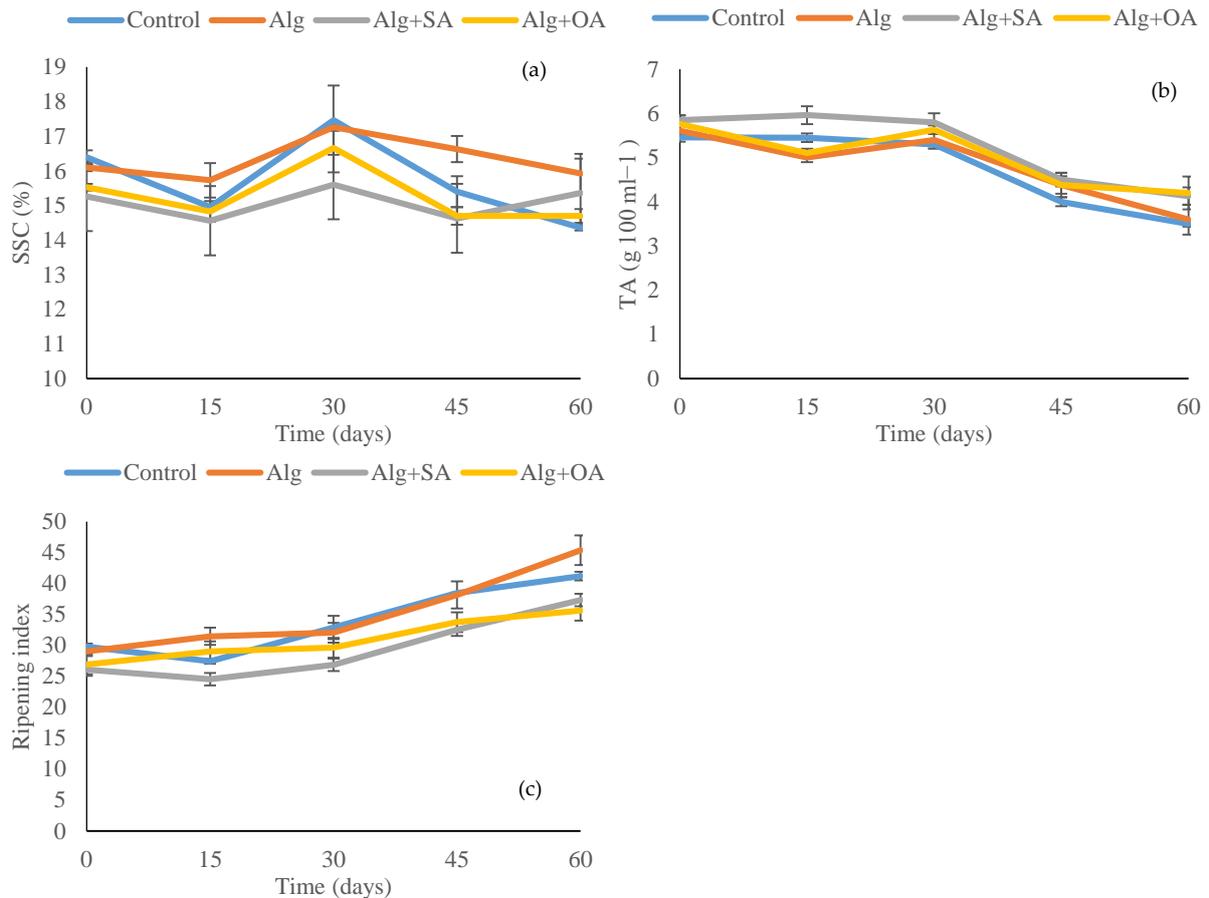


Figure 3. Alginate along with SA and OA affects SSC (a), TA (b) and Ripening index (c) of grapes

Berry color

Red Globe berries are large-round and range in color from light pink to deep red. In the study, L^* values of berry color slightly decreased and hue angle (h) values showed fluctuations in the form of increase-decrease during storage regardless of applications (Figure 4). This reduction has also been reported in other studies with coated grapes (Melo et al., 2018; Souza et al., 2021). h angle slightly changed without significant differences between control and treated fruit. These minor changes in skin color of ‘Red Globe’ grapes during storage follow a typical behavior for non-climacteric fruits (Cherian et al., 2014). During storage, berry lightness decreased continuously irrespective of applications. Clusters treated with Alg+SA, Alg+OA and Alginate maintained higher L^* values up to 60 days of cold storage on the contrary to control. The results are in line with Konuk Takma and Korel (2017) who reported that edible coatings showed a positive effect on maintaining the color brightness of grape berries.

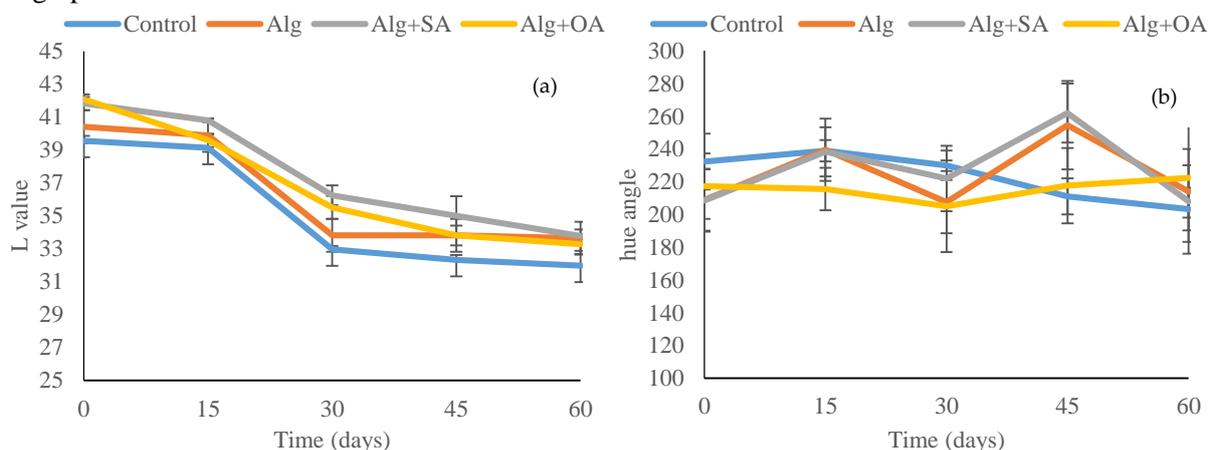
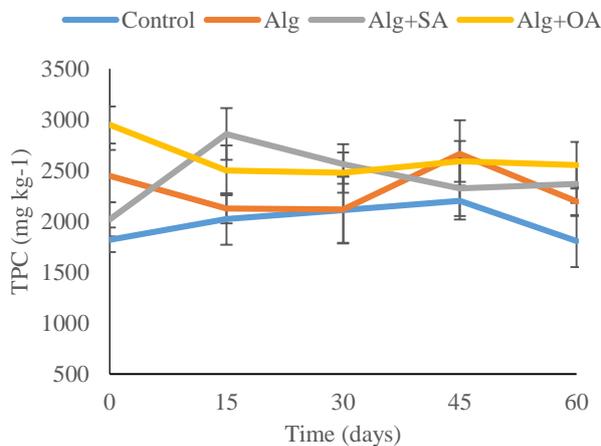


Figure 4. Alginate along with SA and OA affects L (a) and hue angle (b) values of grapes**Total Phenol Content**

The changes of TPC in grapes are presented in Figure 5. The TPC in grapes with Alg+OA was found to be the highest after harvest at 2948 mg kg⁻¹, and the lowest was in the control at 1820 mg kg⁻¹. During storage, fluctuations in the form of increase and decrease in TPC were observed. Similarly, Kalt (2005) reported that phenol content in fruits may increase or decrease depending on cold storage conditions. Although TPC value compared to initial values decreased in all applications except Alg+SA application according to the initial values at the end of storage, Alg+OA treated fruit had the highest TPC with 2553 mg kg⁻¹, followed by Alg+SA (2368 mg kg⁻¹) and Alginate (2196 mg kg⁻¹). On the other hand, the lowest TPC were obtained from control fruit (1807 mg kg⁻¹) on day 60. Since TPC was negatively correlated with weight loss and decay amount, TPC amount was found to be lower in control fruits due to increased weight loss and decay. It has been reported that the application of elicitors such as alginate, SA and OA improves the polyphenol content in fruits and thus increases their quality (Konuk Takma and Korel, 2017; Kok and Bal, 2019; Gomes et al., 2021; García-Pastor et al., 2021). These results indicate that SA and OA applications promoted TPC accumulation during storage due to activation of PAL, a key enzyme of the phenylpropanoid pathway (Shen and Yang, 2017; Martinez-Espla et al., 2019).

**Figure 5.** Alginate along with SA and OA affects total phenolic content of grapes**Antioxidants**

As shown in Figure 6, on the first day of the experiment, a difference in antioxidants was observed in fruits covered with SA and OA-enriched alginate coatings compared to control and alginate-coated fruits. Antioxidant activity in control grapes at harvest was 1.52 $\mu\text{mol TE g}^{-1}$ and significantly higher in Alg+SA and Alg+OA coated grapes, with increases of 17% and 13%, respectively. This difference might be due to the incorporation of SA and OA extract in the coating systems which had an antioxidant effect and triggered defense mechanisms (Kayashima and Katayama, 2002; Cisneros-Zevallos, 2003). During the experiment, the highest antioxidant activity was determined in grapes with Alg+OA application (1.90 $\mu\text{mol TE g}^{-1}$) on the 30th day. At the end of storage, the highest and lowest levels of antioxidant activity (1.81 and 1.50 $\mu\text{mol TE g}^{-1}$) were observed in Alg+OA coated grapes and control applications, respectively. Decrease in antioxidant content in untreated grapes during storage was likely a result of faster senescence. Likewise, changes in antioxidant activity were found similar to the trend observed in TPC. The positive effect of alginate coating with SA or OA, in minimizing loss of antioxidants, might be attributed to delayed oxidation of phenolics and enhanced the antioxidant enzymes. Enhanced of antioxidant in response to preharvest SA or OA application has also been reported in grapes (García-Pastor et al., 2021; Nia et al., 2022). Moreover, alginate coatings, restricted moisture loss and gas exchange, was reported effective in retaining higher antioxidants than control in sweet cherry and mango (Diaz-Mula et al., 2012; Rastegar et al., 2019).

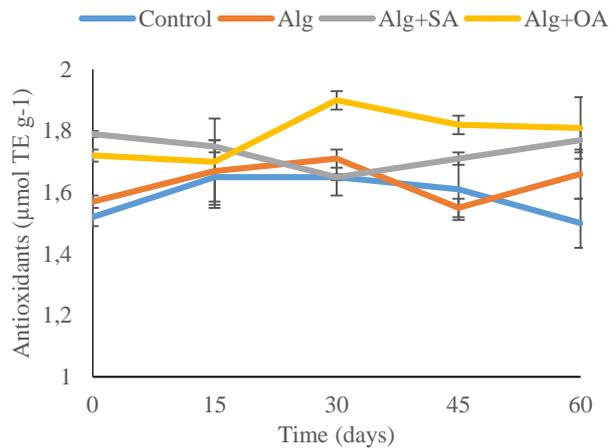


Figure 6. Alginate along with SA and OA affects antioxidant activity of grapes

Stem Browning

Stem browning is a sign of serious water loss in concomitance with oxidative processes that affects the quality of table grapes throughout the storage period (Hamie et al., 2022). In the study, it was observed that there was positive correlation between water loss and stem browning index. As seen in Figure 7 for stem browning, no browning symptoms on rachis were observed at day 15 of storage. The first symptoms were detected on the 30th day analysis and continued to increase depending on the applications. At the end of the storage, the least stem browning scores noticed in grapes treated with Alg+SA (1.63 point) followed by Alg+OA (1.73 point) and alginate (2.53 point) whereas the highest stem browning was observed in control (3.36 point). Stem browning and drying of ‘Red Globe’ was significantly lower on coated grapes than control. Especially in alginate coating combined with SA and OA, the laterals and rachis of the clusters were greener than alginate coating alone.

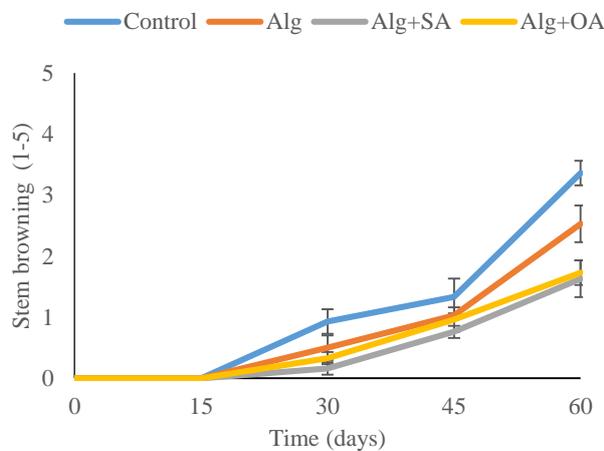


Figure 7. Alginate along with SA and OA affects stem browning of grapes

Decay Incidence

Fungal growth is an important factor that limits the storage life of grapes and causes economic losses. In the study, decay incidence increased as storage time progressed, but this increase was significantly higher in the control compared to the other applications (Fig. 8). As can be observed, alginate coating alone or with SA or OX prevented fruit from suffering any kind of surface deterioration until 15th day of storage. Similar behavior has been reported by many authors and has been attributed to that coatings creating an altered atmosphere on the fruit surface that can inhibit microbial growth, resulting in lower decay incidence (Singh and Packirisamy, 2022). After the 15th day of storage, the loss of fruit at all applications was due mostly to fungal infection by *Botrytis cinerea*. Alg+OA (4%) and Alg+SA (5.3%) applications were the most effective applications in suppressing decay incidence at the end of the storage, while decay incidence in control was 14.6%. In the study, alginate combined with SA or OA probably worked synergistically and markedly suppressed the decay incidence in grapes. In the same way, Konuk Takma and Korel (2017) reported

that postharvest alginate coating incorporating vanillin could be used as an alternative to reduce microbial growth during grape storage. Guerreiro, et al. (2016) also reported a reduced microbial growth of raspberries coated with alginate-based coating enriched with essential oils during storage period. Moreover, SA and OA have both been also reported to have antimicrobial properties (Champa et al., 2014; Zheng and Brecht, 2017; García-Pastor et al., 2020).

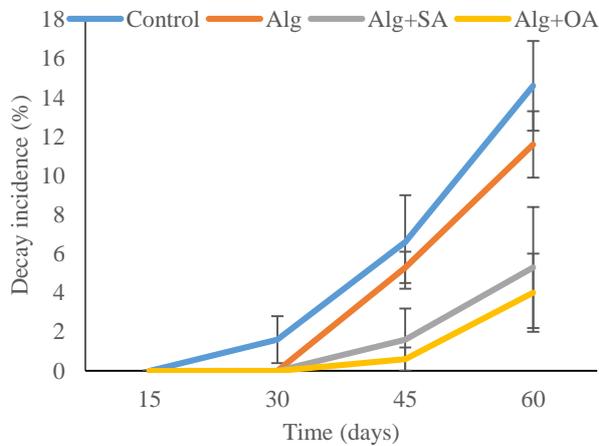


Figure 8. Alginate along with SA and OA affects decay incidence of grapes

Conclusions

Overall results showed that preharvest applications of grape clusters with Alg+OA and Alg+SA in Red Globe grape variety had positive influence on grape quality parameters at harvest and storage period. These applications led to stems with better freshness with lower browning symptoms, and lower incidence of decay after 60 days of storage. Moreover, the total phenol content and antioxidant activity were also found at higher levels in grapes from treated grapevines than in those from control ones, both at harvest and at the end of the storage. In conclusion, preharvest coating with elicitors points to the potential to modulate the postharvest quality and marketing life of fruits, and more research is needed in this regard.

Author Contributions

The authors declare that they have contributed equally to the article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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