

USE OF CARVACROL HELPS MAINTAIN POSTHARVEST QUALITY OF RED GLOBE TABLE GRAPE

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ABSTRACT

In this paper, role of carvacrol vapour on postharvest grape quality was studied. For this aim, 100, 250, 500 and 1000 µl carvacrol absorbed gauzes were placed near the clusters for treatments. Besides, pads generating sulfur dioxide (SO₂) and control grapes were also used for comparison. All clusters were packed with modified atmosphere packaging (MAP) and stored at 1 °C and conditions of 90±5% relative humidity (RH) in darkness for 120 days. Some quality characteristics were examined at 20 days intervals. As a result, all concentrations of carvacrol delayed the changes in titratable acidity (TA) and firmness, and reduced weight loss at the end of storage. In addition, the fungal decay also reduced dependent on the highest carvacrol concentration. No deterioration was observed during the 40th day of storage treated with all carvacrol treatments and SO₂. All the treated grapes sampled on 120th day of storage had lost marketability. Also in our study, carvacrol was found to be more successful in terms of taste than control and SO₂ application. The results showed in this study that carvacrol could be used as an innovative tool to maintain postharvest quality during table grape storage, as alternative to use of SO₂.

Key words: Essential oil, carvacrol, Red Globe, quality, postharvest treatment.

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INTRODUCTION

Grape is an important fruit crop cultivated on a large scale in Turkey as well as in the world. Grapes are highly perishable, non-climacteric fruits with a low rate of physiological activity which demonstrates serious problems during postharvest handling, storage and marketing. The postharvest life of table grapes is relatively shortened by firmness loss, skin browning, rachis dehydration and browning, berry shatter and also they are very susceptible to fungal decay due to their relatively low pH level (Crisosto *et al.* 2001; Martinez-Romero *et al.* 2007; Meng *et al.* 2008; Ranjbaran *et al.* 2011; de Sousa *et al.* 2013). The most common commercial method to control decay, maintain the fruit quality during long distance transportation and a long term storage is the use of sulfur dioxide (SO₂) during cold storage, either by fumigation or generators (Meng *et al.* 2008; Zoffoli *et al.* 2008). However, the use of this compound has been limited in most countries, in spite of its effectiveness in controlling fungal decay (Meng *et al.* 2008). This synthetic compound is harmful to bunches, human health, and difficult in using with colored grapes (Shi *et al.* 2013). Therefore, there is a need for safe, efficient and cost-effective alternative practices that reduce risks to people and at the same time maintain the quality of grapes (Badawy and Rabea 2011; Ciccarese *et al.* 2013). As has been reported previously, modified

atmosphere packaging (MAP) could be alternative to replace the use of SO₂ technique for keeping quality of grapes. (Meng *et al.* 2008). However, conventional MAP treatment is not enough to maintain product quality (Serrano *et al.* 2008). In years, essential oils have been gathered popularity due to possible application as natural antimicrobial and antioxidant agents that are safe for consumption. Moreover, consumers admit essential oils more easily (Tzortzakis 2007). The generally recognized as safe compounds status of essential oils promotes their application as biopesticides to control pests and disease to provide safe food (Sivakumar and Bautista-Banos 2014). Valero *et al.* (2006) stated that the combination of MAP and application of essential oils was reduced incidences of microbial spoilage also was preserved the quality of grapes. Efficacy of essential oils has been reported in several studies in grapes, suggesting their applications (Valverde *et al.* 2005; Valero *et al.* 2006; Martinez-Romero *et al.* 2007; de Sousa *et al.* 2013; Vitoratos *et al.* 2013). Among the various essential oil, carvacrol is one of the most extensively studied essential oil and can serve as a powerful tool to possess antimicrobial activity (Avila-Sosa *et al.* 2012; Peretto *et al.* 2014). There are insufficient studies available on the effect of carvacrol treatment with MAP on grapes during cold storage. This research aimed to determine the effect of carvacrol at the vapour phase on the storage life and maintaining quality of Red Globe grapes during storage in MAP.

MATERIALS AND METHODS

Plant materials and carvacrol treatment: Clusters (*Vitis vinifera* L. cv. Red Globe) were harvested at commercial ripening stage from a local vineyard in Büyükkabaca, Senirkent, Isparta and transferred to the laboratory. Clusters were selected for the experiment based on the uniformity in cluster size, color, the absence of mechanical injuries and disease, and the existence of healthy greenish rachis. The selected clusters were randomly divided into six groups thus each group contained about 1.5 kg grapes. Clusters were packed in MAP. Treatments with carvacrol (purity of 99.5%) was performed by placing 100, 250, 500 and 1000 µl carvacrol on sterile gauze inside the package, then sealing to minimize vaporization, immediately and avoiding the contact with berries. Control groups (without carvacrol treatment and treatment with SO₂ generator peds) were packed in the same conditions. All packages were stored at 1°C and 90±5% relative humidity (RH) in darkness for 120 days. Samples from the 0th, 20th, 40th, 60th, 80th, 100th and 120th days of the cold storage were analysed.

Respiration rate determination: To measure respiration rate, about 1 kg grapes from each group were sealed in airtight jars (3 L) at 20 °C for 24 hour, and 1 ml gas sample was withdrawn with a gas syringe and analyzed with Agilent GC-6890N model gas chromatography. Measurements were performed in split/splitless of inlet in split mode with gas sampling valve by using fused silica capilar column (GS-GASPRO, 30 m × 0.32 mm I.D., USA). Carrier gas flow and temperature of the oven were 1.7 ml/min and 250°C, respectively. The respiration rate was expressed as ml kg⁻¹ h⁻¹ and evaluated with the equation;

$$\text{Respiration rate} = \frac{[(\text{CO}_2_{\text{produced by fruit}} + \text{CO}_2_{\text{absorbed by fruit}})]}{(\text{time} \times \text{fruit weight})}$$

Weight loss, color and firmness determination: Weight losses were expressed as percent loss of weight with respect to the initial weight, and it was evaluated with the equation;

$$\text{Weight Loss} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100.$$

Color measurement was performed with a Minolta colorimeter. Standard white plate was used for calibration. The values were determined as CIE L*, a* and b*. Berry firmness was evaluated with a texture analyzer. The plunger (φ5 mm) was inserted into the mesocarp to a depth of 10 mm at the speed of 100 mm s⁻¹. The maximum penetration force was used as the firmness of the samples. Results were expressed in Newton (N). For berry firmness and color assessments, samples of 10 berries in each package for per replicate were selected.

Soluble solid content (SSC) and titratable acidity (TA) determination: Fruit juice samples were obtained

randomly from 10 berries in different part of clusters. Total soluble solid of berry juice was determined by using a digital refractometer (Atago Pocket PAL 1) and the results were expressed as °Brix. TA was determined by titration with NaOH (0.1 N) to end point of at pH 8.1 and the results were expressed as g tartaric acid l⁻¹ grape juice.

Berry appearance and rachis browning: Berry appearance was evaluated from visual inspection of berries and assignment of score, i.e. 1: extremely poor; 3: poor; 5: fair; limit of usability; 7: good; 9: excellent (Artes-Hernandez *et al.* 2004). Rachis browning was graded using the scoring system, i.e., 1: healthy; 2: slight; 3: moderate; 4: severe (Crisosto *et al.* 2002).

Taste: Taste analysis of grapes was evaluated according to a hedonic scale, i.e., 1: worst, 2: bad, 3: moderate, 4: good, 5: best (Koyuncu *et al.* 2012).

Fungal decay assessment: Berry decay was evaluated by scoring the number of contaminated berries by fungi per clusters. i.e., 1: normal (no decay on fruit surface); 2: trace (up to 5%); 3: slight (5-20%); 4: moderate (20-50%); 5: severe (>50% of fruit) (Babalar *et al.* 2007).

Statistical analysis: Descriptive statistics for the variables were presented as Mean and Standard deviation. One-way ANOVA was used to compare group (treatments) means. LSD multiple comparison test was also used to identify different group means followed by ANOVA. Statistical significance level was considered as 5% and JMP 8 (SAS Institute Inc., Cary, NC) statistical program was used for all statistical computations.

RESULTS AND DISCUSSION

Weight loss: Weight loss increased with prolonging storage period in overall treatments, but in treated grapes, it was less compared to the control grape (Keskin *et al.* 2014; Keskin *et al.* 2015). (Fig 1A). Weight loss was delayed more in 250 µl carvacrol treated grapes with 0.43% at the end of the storage. There was difference among the treatments after 80 days of storage (p<0.01). Weight loss occurs due to water loss and decrement of carbon accumulation via transpiration. The rate of transpiration depends on the vapor pressure gradient between the fruit tissue and the surrounding atmosphere (Sogvar *et al.* 2016). Water loss results in a reduction in appearance quality such as wilting, shriveling, less gloss, and limpness, which will reduce market value. Furthermore susceptibility to fungal decay of table grapes is influenced by weight loss (Valverde *et al.* 2005). MAP has been known to limit weight losses by reducing moisture loss from the package. In this study, carvacrol treatment became effective in inhibiting weight loss in MAP through retarding senescence process during

storage. These findings were similar to the results of Serrano *et al.* (2005), Valero *et al.* (2006), Guillén *et al.* (2007), Abdolahi *et al.* (2010); Sabır *et al.* (2010). They showed that usage of essential oils decreased the weight loss in fruit, but the detailed mechanism still unclear.

Firmness: The effects of different concentrations of carvacrol on the firmness are shown in Fig 1B. Fruit firmness decreased during storage and there was no difference among the treatments. SO₂ treated grapes softened more rapidly by 80th days of storage. Berry firmness is one the most important limiting quality factors for grapes evaluated. Grape berries soften considerably during storage. While softening has been supposed to result from changes in cell wall composition due to the activity of the some enzymes, berry firmness is strongly associated with the turgor pressure of mesocarp cells (Nunan *et al.* 1998; Serrano *et al.* 2008). Valero *et al.* (2006) stated that the presence of eugenol or thymol resulted in maintenance or slightly reduction of flesh firmness during storage.

Titrateable acidity (TA) and Soluble solids content (SSC): TA content of grape juice decreased in all treatments during storage time (Fig 1C) and TA was found to be significantly affected by treatments ($p < 0.01$) for each storage time. TA contents of untreated grape fruit at 120 days storage time were the lowest values with 3.60 g l⁻¹ tartaric acid. At 80 days storage time, TA contents of 250 µl carvacrol treated grapes found as 3.72 g l⁻¹ tartaric acid. All treatments almost significantly increased SSC. Compared with the other treatments, SSC contents of 100 µl carvacrol treated grapes at 60 days storage time were the highest, and untreated control grape at 100 days storage time were the lowest (Fig 1D). In our study SSC increase and TA decreases in grapes during storage. This finding is similar to previous reports in grapes and other fruits (Valverde *et al.* 2005; Abdolahi *et al.* 2010; Bal *et al.* 2011; Sogvar *et al.* 2016; Sabır *et al.* 2018). This could be explained as low consumption of sugar for respiration during postharvest life in grapes due to the fact that grapes are nonclimacteric fruit and show very low respiration rates (Ranjbaran *et al.* 2011).

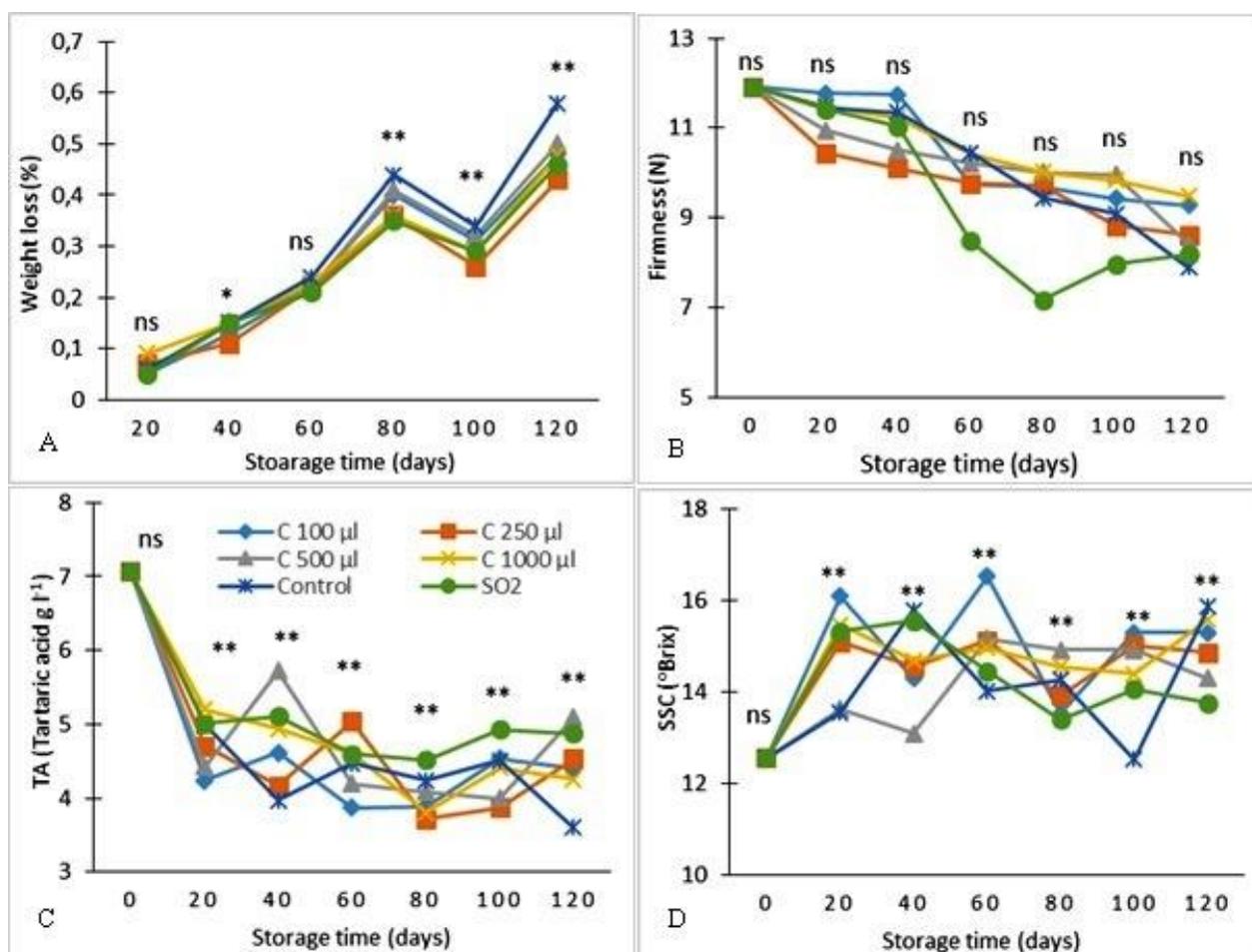


Figure 1. A) Weight loss (%), B) firmness (N), C) TA (g l⁻¹ tartaric acid), D) SSC (°Brix) values of Red Globe grapes during the storage. ns: non significant, *: significant as $p < 0.05$ and **: significant as $p < 0.01$ difference according to the LSD values for each storage time.

Respiration rate: Carvacrol had no significant effect on the respiration rate among the treatments at 0 and 40 days of storage time. At the other storage times, there was significant difference among the treatments. Untreated grape fruit at 100 days storage time was the highest respiration rate values and the lowest values were obtained 250 μl and 1000 μl carvacrol treated grapes. At 120 days storage time, respiration rate of 1000 μl carvacrol treated grapes was highest respiration rate

(Table 1). The lower the respiration rate during storage the higher the shelf life of fruits and vice versa (Misir *et al.* 2014). The reduction of fruit respiration is correlated with a delayed senescence and a reduced susceptibility to decay (Martinez Romero *et al.* 2007; Serrano *et al.* 2008; Castillo *et al.* 2010). In our study, the use of 1000 μl carvacrol reduced the respiration rate of grapes only at 100 days storage time. In other storage times, carvacrol treatments were not as effective as sulfur dioxide.

Table 1. Respiration rate ($\text{ml CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) in Red Globe either treated with carvacrol at different concentrations or untreated and untreated with SO_2 generator pads.

Treatments	Storage time (days)						
	0	20	40	60	80	100	120
Control	1.2 \pm 0.31	1.73 \pm 0.49ab	1.29 \pm 0.34	1.31 \pm 0.18b	1.37 \pm 0.24b	2.00 \pm 0.38a	1.49 \pm 0.17bc
SO_2		1.23 \pm 0.28c	1.28 \pm 0.18	1.23 \pm 0.12b	1.29 \pm 0.30b	1.44 \pm 0.35bc	1.71 \pm 0.15bc
C 100 μl		1.84 \pm 0.02ab	1.48 \pm 0.00	1.46 \pm 0.03ab	2.41 \pm 0.24a	1.78 \pm 0.05ab	1.46 \pm 0.22c
C 250 μl		1.93 \pm 0.05a	1.47 \pm 0.02	1.29 \pm 0.12b	2.07 \pm 0.29a	1.17 \pm 0.09c	1.63 \pm 0.01bc
C 500 μl		1.93 \pm 0.05a	1.54 \pm 0.02	1.39 \pm 0.06b	1.41 \pm 0.07b	1.44 \pm 0.21bc	4.54 \pm 2.50b
C 1000 μl		1.46 \pm 0.03bc	1.13 \pm 0.29	1.66 \pm 0.20a	2.06 \pm 0.11a	1.30 \pm 0.19c	7.95 \pm 3.37a
	ns	*	ns	*	**	*	**

Data were mean \pm standard deviation. The symbol showed ns: non significant, *: significant as $p < 0.05$ and **: significant as $p < 0.01$ difference according to the LSD values for each storage time.

Fruit color: The changes in grape fruit color which were examined in treated and nontreated grapes during the storage period are shown in the Table 2. After 80 days of storage, L^* values were statistically significant ($p < 0.05$). At the end of the storage, L^* values which shows fruit brightness of the treated with SO_2 generator pads and 250 μl carvacrol fruits (38.76), were higher than control fruits (35.60). For the a^* values which indicates redness were difference among the treatments only at 80 days of storage ($p < 0.01$). The highest a^* value was 6.01 with 100 μl carvacrol treated grape while the lowest a^* values were 3.03 with 250 μl carvacrol and 3.58 with 1000 μl carvacrol treatments. Significant changes were observed in berry b^* colour value (representing yellowness) only at 80 and 100 days of storage ($p < 0.05$). Colour is important grape consumer attributes and usually responsible for fruit acceptability. During ripening color changes are due to the degradation of chlorophyll and accumulation of anthocyanins in grapes (Wrolstad *et al.* 2005). Serrano *et al.* (2008) stated that, the combination of MAP with essential oils delayed discoloration more than MAP alone. In our study, colour values of grapes showed differences and efficiency depending on the carvacrol concentrations during storage. The results regarding the changes in the colour indices are in agreement with previous study (Guillen *et al.* 2007), related to changes in the skin colour of grape during storage. This study has shown that MAP significantly reduces color changes when used with essential oils.

Berry appearance: As shown in Fig. 2A, visual appearance of the grapes deteriorated during postharvest storage in control. After 40 days of storage there was difference among the treatments ($p < 0.01$). Especially, treated with SO_2 generator pads and 1000 μl carvacrol concentration had significant positive effect on visual appearance. Also Pastor *et al.* (2011) found that the application essential oils had a better effect on appearance (Pastor *et al.* 2011). Results of this study are similar findings with Abdolahi *et al.* (2010), who stated that thyme oil indicated the highest impact on visual appearance in compared to control. Visual appearance could be related to berry and rachis browning, which was correlated with the lower dehydration and darkening levels during storage. This is probably due to the enzymatic browning, caused by reduced polyphenol oxidase (PPO) enzyme and inhibition of this enzyme activity in fruits might generally reduced using essential oils (Vial *et al.* 2005).

Taste: Taste of grapes in all treatments decreased during storage time and taste was found to be significantly affected by treatments at 60 ($p < 0.05$), 80 and 100 days ($p < 0.01$) storage time. Highest taste score was recorded with 1000 μl carvacrol treatment. On the other hand, lowest score was seen with non treated control grapes (Fig 2B). Literature data indicate that the usage of MAP has protected organoleptic quality of grapes during storage (Martinez-Romero *et al.* 2003). Also, Khezzadeh *et al.* (2013) stated that SO_2 treatment causes sulfure taste in grapes but essential oils had not negative effect owing

to evaporated and did not have any residue on fruits. Similar results were also observed in this study. In our study, MAP, combined with essential oil such as

carvacrol, was found to be more successful in terms of taste than control and SO₂ application.

Table 2. Color parameter (L^* , a^* and b^*) values in Red Globe either treated with carvacrol at different concentrations or untreated and untreated with SO₂ generator peds.

Treatments	Storage time (days)							
	0	20	40	60	80	100	120	
L^*	Control	41.25±0.50	41.28±2.49	37.17±0.43	39.81±0.29	39.36±0.75ab	37.09±1.05a	35.60±0.18b
	SO ₂		40.82±1.24	37.77±2.94	38.63±2.23	38.58±1.21bc	34.79±0.88bc	38.76±1.65a
	C 100 µl		40.07±1.96	38.41±2.13	38.35±2.61	36.45±1.50c	33.99±0.09bc	37.42±0.97a
	C 250 µl		41.15±0.82	40.73±2.80	39.93±1.09	37.75±0.85bc	33.55±1.18c	38.76±0.90a
	C 500 µl		41.68±0.62	42.45±2.23	38.82±1.18	40.83±1.19a	35.59±1.09ab	38.70±0.48a
	C 1000 µl		40.75±4.19	39.14±0.46	37.81±2.70	37.17±1.88bc	34.59±1.09bc	37.84±1.15a
a^*	Control	7.07±0.80	7.92±2.09	6.42±1.22	5.93±1.27	4.06±1.09c	4.41±0.95	3.73±0.74
	SO ₂		7.30±0.57	5.95±0.70	5.68±0.33	4.35±0.07b	4.70±0.23	4.35±0.85
	C 100 µl		6.42±0.55	6.77±2.17	5.99±0.28	6.01±0.78a	5.06±1.56	3.95±0.25
	C 250 µl		6.83±0.31	6.51±0.92	5.15±1.44	3.03±0.86c	3.21±0.87	5.43±1.38
	C 500 µl		7.08±1.22	7.81±0.51	5.09±1.04	4.09±0.19bc	4.53±0.27	3.96±0.73
	C 1000 µl		7.70±1.26	6.40±1.18	5.13±0.07	3.58±0.46c	3.78±0.60	5.83±1.62
b^*	Control	0.86±1.14	0.90±0.38	-1.16±0.82	-0.04±1.57	-1.29±0.74b	-1.08±0.93ab	-0.88±0.63
	SO ₂		0.74±0.59	-1.09±0.78	-0.28±1.06	-1.82±0.70b	-2.01±0.71b	-2.14±1.00
	C 100 µl		0.62±0.84	-0.02±0.93	-1.11±0.47	0.07±0.97a	-0.28±1.10a	-0.99±1.29
	C 250 µl		0.15±2.18	-1.50±0.87	-1.30±1.01	-2.52±0.51b	-1.13±0.59ab	-1.95±0.24
	C 500 µl		0.49±1.44	0.13±0.39	-2.03±0.10	-1.26±0.95b	-2.11±0.24b	-2.65±0.45
	C 1000 µl		0.40±0.55	-1.52±0.50	-1.54±0.73	-2.35±0.15b	-2.32±0.77b	-0.95±0.82
L^*	ns	ns	ns	Ns	*	**	*	
a^*	ns	ns	ns	Ns	**	ns	ns	
b^*	ns	ns	ns	Ns	*	*	ns	

Data were mean ± standart deviation. The symbol showed ns: non significant, *: significant as $p < 0.05$ and **: significant as $p < 0.01$ difference according to the LSD values for each storage time.

Fungal decay: As illustrated in Fig 2C, there was significant difference among the treatment at 40, 60, 100 and 120 days storage time. No decay was determined during the 40 days of storage treated with all carvacrol treatments and SO₂ generator peds. All treated grapes sampled on 120th day of storage were discarded, as they had lost marketability. Fumigation the storage by SO₂ is the most common method to control postharvest decay of grape (Kou *et al.* 2009). However, the use of SO₂ is associated with many problems such as berries bleaching, hairline cracking, discoloration, bleaching and rachis browning (Salimi *et al.* 2013). Therefore, innovative alternatives are required. In this regard, it was determined that treatment of essential oil has been powerful on reducing postharvest deterioration, delaying the ripening process and maintaining fruit quality in several fruits, such as grapes, cherry, apple, strawberry, pear, apricot and plums (Liu *et al.* 2002; Shahi *et al.* 2003; Martinez-Romero *et al.* 2004; Serrano *et al.* 2005; Valero *et al.* 2006; Tzortzakakis 2007; Alikhani *et al.* 2009). Also, the effectiveness of essential oils such as thymol, eugenol, menthol and carvacrol in MAP packaging in reducing

fungal decay in table grapes and sweet cherries has been well documented (Guille'n *et al.* 2007; Serrano *et al.* 2005; Valero *et al.* 2006; Abdolahi *et al.* 2012; Zhang *et al.* 2019). According to previous reports it seems that damage to cell walls and membrane structure and function is a result of antimicrobial action of essential oils (Rattanapitigorn *et al.* 2006).

Rachis browning: Rachis browning of grapes in all treatments increased during storage and there was significant difference among the treatments for each storage time ($p < 0.05$ and $p < 0.01$). Rachis browning was greater in 250 µl carvacrol and control treatment at the end of 120 days. Red globe grapes, the use of low concentrations of carvacrol (100 and 250 µl) increased stem browning by during storage. It was lower treated with 1000 µl carvacrol grapes like SO₂ ones (Fig. 2D). Crisosto *et al.* (2001) stated that stem browning was associated with cluster water loss. Similarly in our study, due to limiting weight loss during storage, using high concentrations of carvacrol and SO₂ generating pads, have shown fewer cluster browning even after 120 days of storage.

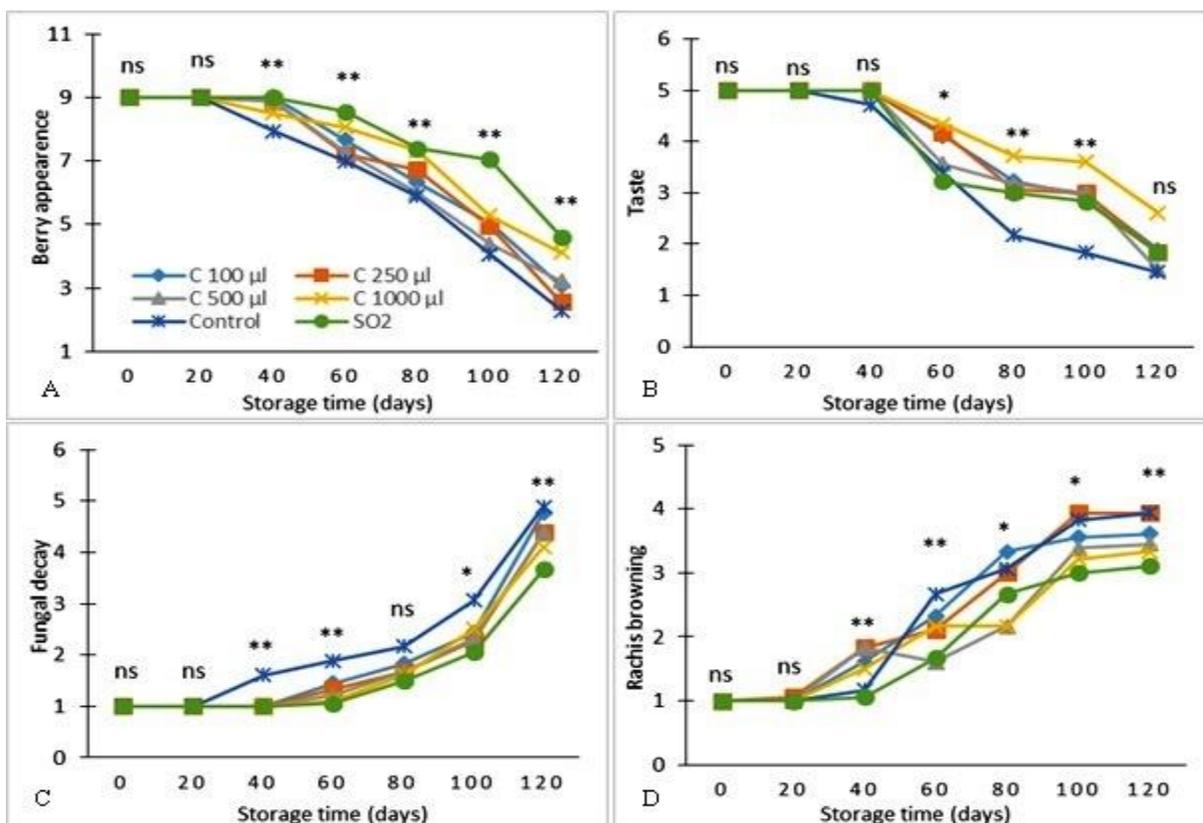


Figure 2. A) Berry appearance, B) taste, C) fungal decay, D) rachis browning values of Red Globe grapes during the storage. ns: non significant, *: significant as $p < 0.05$ and **: significant as $p < 0.01$ difference according to the LSD values for each storage time.

Conclusion: In this paper, it was found that postharvest treatment of carvacrol maintained the quality parameters, decreased the decay and increased storage life of grape cv. Red Globe, effectively. According to the results of this research and prior reviewed studies, it can be accomplished that the usage of essential oils such as carvacrol with MAP together is an innovative and utility tool as alternative to the use of SO₂ in grapes. Grapes treated with carvacrol and SO₂ could be stored for 80 days with marketable quality in MAP at 1°C and 90±5 % relative humidity. But control group lost its commercial properties after 60th days of cold storage. The SO₂ and 1000 µl carvacrol treatments gave the best result in terms of some quality attributes and sensory evaluation. However, more detailed research is needed to investigate the effect mechanism of carvacrol on postharvest quality and life of grapes. These findings may have considerable commercial significance.

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