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Combined Effects of MAP and Postharvest Salicylic Acid Treatment on Quality Attributes of Dill (*Anethum graveolens* L.) Bunches during Storage

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ABSTRACT

The effects of combinations of modified atmosphere packaging (MAP) with salicylic acid (SA) treatment on storage and shelf life quality of dill (*Anethum graveolens* L. cv. Asder) leaves were investigated. After harvest, dill leaves were dipped into an aqueous solution containing different concentrations of salicylic acid (1, 2 and 4 mM) for 2 minutes. The control group was immersed in distilled water only for 2 minutes. Treated samples were dried with blotting paper and placed in modified atmosphere package and stored at 0 ºC and 90±5% relative humidity (RH) conditions for 25 days. After cold storage, dill leaves were kept at 10 ºC and 55-60% RH for 2 days to simulate commercial practice (shelf life), and analyzed for same quality parameters performed during cold storage. Weight loss, color, respiration rate, gas composition in package, soluble solids content (SSC) and ascorbic acid content were determined initially and at 5 day-intervals. The dill bunches were also evaluated for visual quality during storage period. According to the results, SA treatment allowed dill leaves to stay green longer than those of control group. 1 mM concentration of SA was the best treatment for prolonging the storage life of dill leaves with keeping the quality.

Keywords: Dill; Cold storage; MAP; Salicylic acid

1. Introduction

Dill (*Anethum graveolens* L.), a member of celery family *Apiaceae,* is the only species of the genus *Anethum*. Dill is a valuable aromatic herb and has been used for enhancing flavor of some foods such as pickle, soups and salads (Sakaldaş et al 2010). In Europe and central Asia, people use fresh and dried leaves of dill in their food. Dill leaves are best while they are fresh because they lose their flavor rapidly when leaves are dried (Kılıç & Duyar 2016).

Leafy vegetables and herbs have a relatively short postharvest life because of the high respiration rate and rapid senescence process. Yellowing is a well-known senescence symptom of leafy vegetables and they lose their quality rapidly after harvest (Koukounaras et al 2006). Some of the reasons behind these losses are traditional approaches

to handling, lack of preservation technologies and marketing knowledge. Simple strategies for postharvest quality maintenance are required to reduce losses of economic crops (Ali et al 2013).

The modified atmosphere packaging (MAP) is widely used technology which reduces respiration rate, weight loss and decay, and prolongs the postharvest life of many horticultural products (Sakaldaş et al 2010). MAP reduces the respiration rate and other metabolic processes by modification of CO_2 and O_2 concentrations in the storage atmosphere surrounding the commodity (Kader et al 1989).

Nowadays, extend of the postharvest life by chemicals is limited because of their detrimental effect on the environment and human health. Therefore, we need to develop more safe and effective strategies (Mandal et al 2009). More attention is being paid to plant based metabolites as anti-ripening and anti-microbial agents for sustenance of maximum postharvest quality of products (Ali et al 2013). Salicylic acid (SA), a member of a group of phenolic compounds, is now considered as a hormonal substance (Awad 2013). SA has been reported to play an important role in regulating of many physiological processes (Zavala et al 2004) and in controlling quality losses of horticultural crops after harvest (Asghari & Aghdam 2010). In addition, the use of SA in pre- and post-harvest period has been reported to be effective in maintenance of quality and extension of storage life for some horticultural crops such as banana (Srivastava & Dwivedi 2000), tomato (Ding et al 2001), peach (Wang et al 2006), pomegranate (Sayyari et al 2009), and pineapple (Lu et al 2011).

There is no study available on the effect of SA treatment on dill leaves during cold storage. This research aimed to determine the role of SA on extending the storage life and maintaining quality of dill leaves during storage in MAP.

2. Materials and Methods

2.1. Plant material

The fully expanded green dill leaves (*Anethum graveolens* L.) of parsley cv. Asder were used as plant material. Dills were harvested in early morning and about 75-80 dill stems were grouped into bunches (~30 cm long, ~250 g). Foreign materials and withered yellow leaves were removed from research material. Bunches were homogenized and examined visually.

2.2. Salicylic acid treatment and storage conditions

Dill leaves were harvested from Isparta-Turkey, and transported to postharvest physiology laboratory of Fruit Research Institute, immediately. Dill bunches were pre-cooled (the external temperature of dills reduced to 4-5 °C within 24 hours) by forced air at 1 °C and 85-90% relative humidity (RH). After precooling, bunches were dipped in an aqueous solutions containing 1, 2 and 4 mM SA and Tween-20 for 2 minutes. Control group was immersed in distilled water and Tween-20 for 2 minutes. After treatments, bunches were drained and dried with blotting paper. Then bunches placed in modified atmosphere package (LDPE) were stored at 0 °C and 90±5% RH for 25 days. After cold storage, bunches were kept at 10 ºC and 55-60% RH for 2 days to simulate commercial practice (shelf life). Samples from the 0^{th}, 5^{th}, 15^{th}, 20^{th} and 25^{th} day of cold storage and shelf life were analyzed.

2.3. Quality analysis

Weight losses of dills were expressed as the percentage of loss of weight with respect to the initial weight. Weight loss was determined by the equation;

$$\text{Weight loss} = [(\text{First weight} - \text{Last weight}) / \text{First weight}] \times 100 \quad (1)$$

Respiration rate was measured in 3 bunches for each replicate. Weighed dill bunches were placed in airtight jars (1.3 L) at 20 °C for 1 hour. Then gas sample was taken from jars and injected into gas chromatographs (Agilent GC-6890N). Measurements were made in split/splitless (S/SL) of inlet in split mode with gas sampling valve with 1 ml gas sample by using fused silica capilar column (GS-GASPRO, 30 m × 0.32 mm I.D., U.S.A), with thermal conductivity detector (TCD) for respiration rate measurements by Agilent GC-6890N (U.S.A

and Canada) model gas chromatography (GC) and Chemstation A.09.03 [1417] software. Carrier gas flow was 1.7 mL min^{-1} in stable flow mode. Results were expressed as mL CO_2 kg^{-1} h^{-1}.

Soluble solids contents (SSC) of dills were measured with a digital refractometer (Atago Pocket PAL-1) and expressed as percentage (%).

Color measurement was performed at two parts of leaf surface with a colorimeter (Minolta CR-400, Japan) over 5 bunches in each replicate. Calibration was made by the standard white plate of manufacturer company. The values were expressed by the CIE L^* (brightness-darkness), a^* (+ a^*: red, - a^*: green) and b^* (+ b^*: yellow, - b^*: blue) system and the values were evaluated as L^*, a^* and b^*.

Gas concentration (O_2 and CO_2) of packages was determined by Gaspace 2 (Gas Headspace Analyzer, Systech Instruments).

Visual quality (external appearance) was determined using a scale of yellowing and freshness. Dill leaves were divided into four groups and graded as a scale of 1-4 (1 dark green-too fresh, 2 light green-fresh, 3 yellowish green-few fresh, 4 greenish yellow not fresh) (Sakaldaş et al 2010).

Vitamin C content of dills were determined by spectrophotometric method, using 2,6-dichlorophenolindophenol (Loeffler & Ponting 1942).

2.4. Statistical analysis

The experiment was set up according to the factorial randomized design with 3 replications. Main effects and interactions were analyzed and means were compared by LSD Tests at a significance level of 0.05. All analyses were performed with SPSS software package v.18.0 for Windows by General Linear Model (GLM) univariate test.

3. Results and Discussion

3.1. Weight loss

Weight loss of dills can lead to discoloration which reduces both market value and consumer acceptability. Evaporation of moisture from the surface of fresh commodities is responsible for reduction in weight (Ali et al 2013). The weight losses of dills are illustrated in Figure 1. Weight loss (%) increased with prolonging storage period and temperature. Generally, weight losses were minimized by cold storage. Weight losses of dills were at low values at the end of the cold storage but these losses reached the higher values when dills were transferred to 10 ºC. As expected in this research, the higher weight loss in shelf life condition is related to higher water vapor losses. The effects of treatments and storage periods on weight loss were significant. The weight loss of the dills treated with all concentrations of SA was relatively delayed compared to control group at the end of shelf life. MAP has been known to limit weight losses by reducing moisture loss from the package. SA application became effective in inhibiting weight loss in MAP through retarding senescence process during shelf life. The maintenance of cellular integrity by SA (Ali et al 2013) might be the reason in lowering weight loss of dill leaves in the present

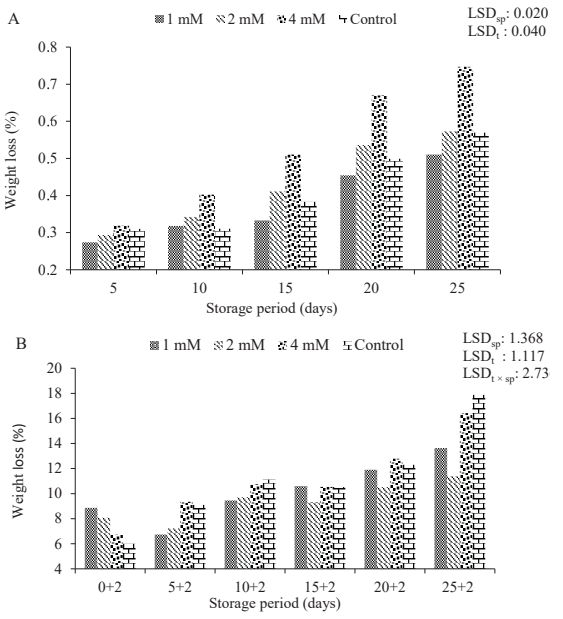

Figure 1- Effect of SA on weight loss of dill leaves stored at cold storage (A) and shelf life (B)

study. Likewise, Gill et al (2016) indicated that weight loss of crops was reduced by SA treatment.

3.2. Soluble solids content

The SSC of dills were given in Figure 2. SSC of dills was remittent during storage periods and there was no stable increase or decrease. However, SSC of dills stored at cold condition were less than those of shelf life condition. All treatment displayed a decreasing in SSC compared to initial values in cold storage condition. On the contrary of the cold storage, the SSC of dills increased with shelf life after 25+2 days. The breakdown of complex carbohydrates into more soluble sugars and reducing water content might be the reason in increased sugar concentration at shelf life condition. Likewise, according to Kluge et al (1996), sugar loss due to respiration could account for sugar increases with weight loss, and Rohani et al (1997) indicated that slower respiration rates resulted in slow degradation of complex carbohydrates into simple sugars.

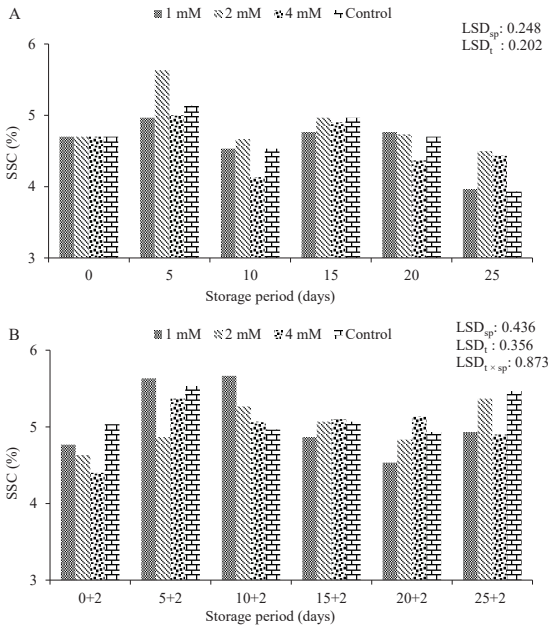

Figure 2- Effect of SA on SSC of dill leaves stored at cold storage (A) and shelf life (B)

3.3. Color

The color changes of SA-applied and control group dills during the storage were given on the Table 3. L* values, which represent brightness-darkness, of the dills were generally decreased during cold storage, except for 1 mM SA treatment. Dill leaves treated with 1 and 2 mM SA were slightly brighter than those of other treatments, especially after 15 days of cold storage. On the contrary of the cold storage, L* values increased compared to initial values after 25+2 day of storage. The increase of L* can be due to discoloration of leaves at shelf life condition (Figure 3).

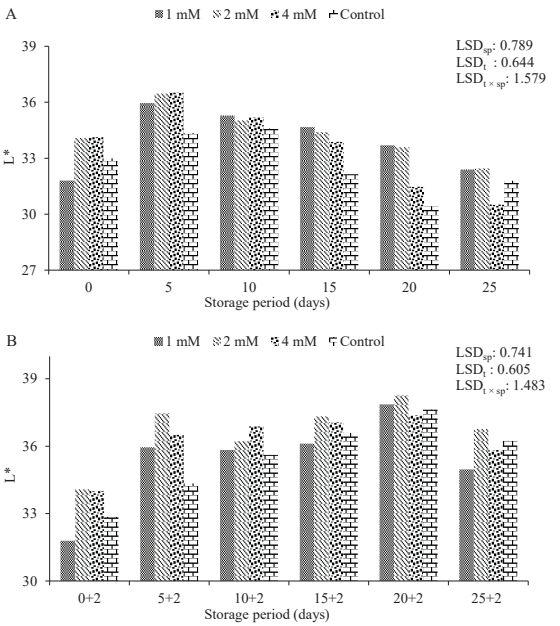

Figure 3- Effect of SA on L* values of dill leaves stored at cold storage (A) and shelf life (B)

Dill leaves were harvested at a stage when the leaves had fully green color. a* and b* values increased gradually in cold storage and shelf life. The increasing in a* and b* values were higher in the control group (Figure 4, 5). This means that the dill leaves in the control group had more yellow surface color than the others at the end of storage. The best results for maintaining color were obtained from dill

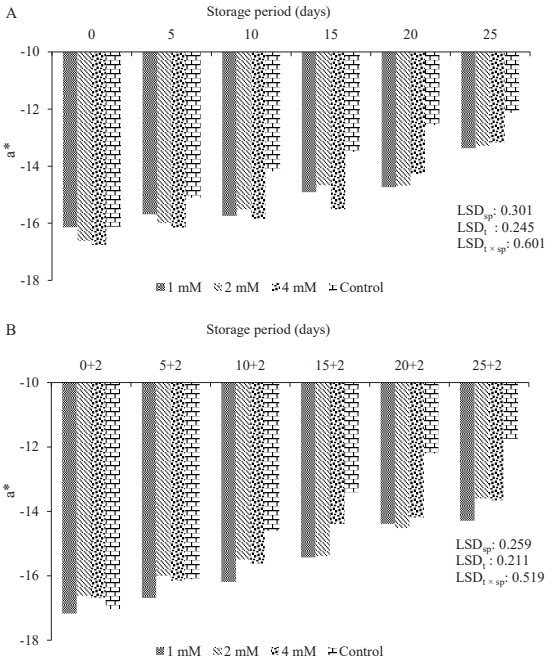

Figure 4- Effect of SA on a* values of dill leaves stored at cold storage (A) and shelf life (B)

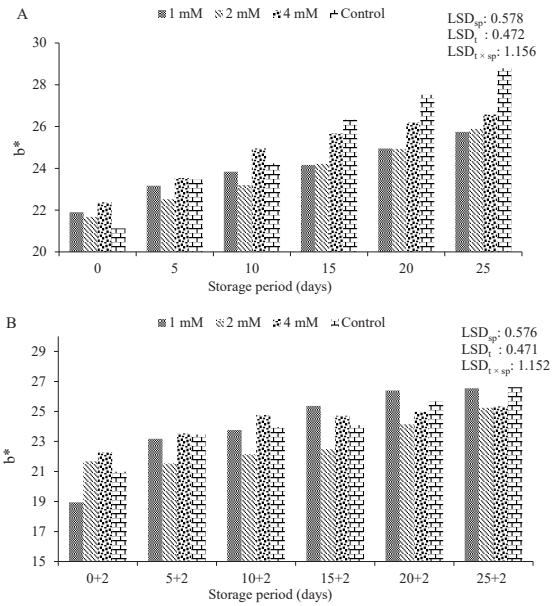

Figure 5- Effect of SA on b* values of dill leaves stored at cold storage (A) and shelf life (B)

leaves treated with 1 mM SA. These findings seem to be in accordance with the general effect of SA and MAP in delaying the senescence process during storage. Similarly, it was reported that SA treatment delayed discoloration (Peng & Jiang 2006) or prevent color change by decreasing senescence (Asghari & Aghdam 2010).

3.4. Gas composition in package

The gas composition in package is illustrated in Figure 6. The O_2 concentration of MAP fluctuated between 12.27% and 19.30% during the entire storage period. The O_2 levels on the 5th day were between 17.75% in the control group and 12.27% in the 4 mM SA treatment, while these values were found as 18.33% and 14.80%, respectively after 25 days of storage. As can be seen from Figure 6, the concentration of O_2 gas in the MAP went down to 14.93% (average value) within five days and remained fairly constant during the rest of storage period. The CO_2 level in MAP ranged from 1.20% to 3.13% throughout the storage. The lowest average

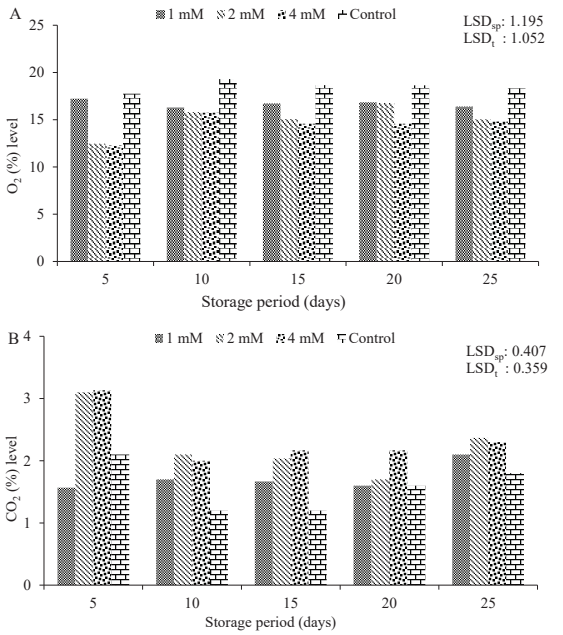

Figure 6- Effect of SA on O_2 (A) and CO_2 (B) composition in MAP

CO_2 level (1.58%) was found in the control group, while the highest one was obtained from the 4 mM SA treatment (2.35%). We observed a similar gas composition within the MAP during storage of dill leaves treated with different concentrations of SA. Although SA, in a concentration dependent manner, affected respiration rate of dill leaves, this trend could not be observed in the gas values in the package. This can be due to factors, which affect gas composition for MAP, such as weight of crops, homogeneous permeability of packaging materials, maturation stage of crops etc. These results were similar to the findings of Tano et al (2007).

3.5. Respiration rate

Respiration rate is a major factor affecting the postharvest quality loss of horticultural crops. Therefore, it is very important to maintain the respiration rate at minimum level to prolong the storage life of crops. The respiration rate of dill leaves began to increase and attained at maximum levels at the 15th days of the storage. However, on 20th and 25th day of cold storage, the respiration rate decreased. Similar results were obtained from shelf life storage. Control samples had the highest respiration rate (19.076 mL CO_2 kg^{-1} h^{-1}) followed by 4 mM (16.920 mL CO_2 kg^{-1} h^{-1}), 2 mM (14.451 mL CO_2 kg^{-1} h^{-1}) and 1 mM SA (10.130 mL CO_2 kg^{-1} h^{-1}) treatments, at the end of the cold storage. In storage for shelf life, these values were found as 15.940 mL CO_2 kg^{-1} h^{-1} (2 mM SA), 15.878 mL CO_2 kg^{-1} h^{-1} (control), 14.524 mL CO_2 kg^{-1} h^{-1} (4 mM SA) and 13.952 (1 mM SA). Lower concentrations of SA (1 and 2 mM) treatments successfully suppressed the respiration rate of dill leaves during cold storage (Figure 7). Similar trends were also observed by Norman et al (2004) in tobacco; Han et al (2003) in peach; Mo et al (2008) in sugar apple. The effect of SA may be due to retarded senescence of dill leaves during storage. Wills et al (1998) reported that SA treatment effectively reduced metabolic activity which delays crops senescence process. On the other hand, at the lower concentrations (0.1 mM or less), the effect of SA on respiration rate was transitory, but at higher concentrations, respiration rate was severely inhibited in tobacco (Norman et al 2004).

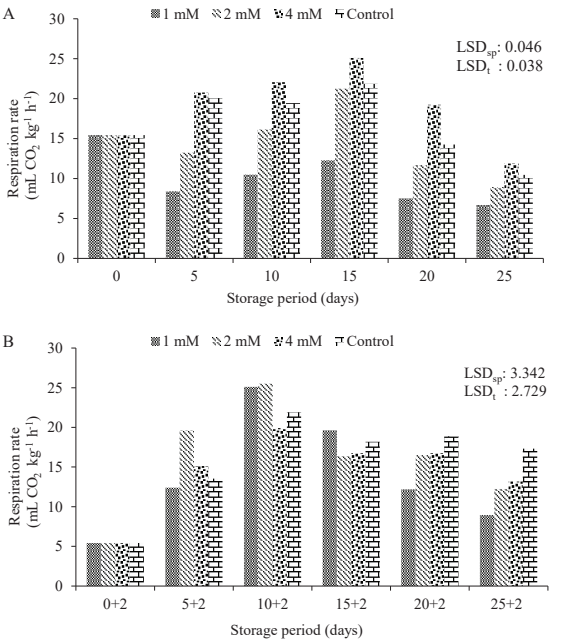

Figure 7- Effect of SA on respiration rate of dill leaves stored at cold storage (A) and shelf life (B)

3.6. Ascorbic acid

The ascorbic acid of dill significantly tended to decrease throughout the storage. Decline of ascorbic acid was strict after 15 day of storage. The best preservation of ascorbic acid was obtained from 2 mM SA treated dill bunches after 25 day of cold storage. However, no significant difference was found between SA concentrations and control group. Nevertheless, it was found that 1 mM and 2 mM SA concentrations gave the highest values of ascorbic acid compared to control after 25 days of storage (Table 1). Correspondingly, ascorbic acid was significantly decreased in control pomegranates but remained unchanged in fruit treated with the highest (2 mM) concentration (Sayyari et al 2009). In present study, the lowest ascorbic acid values (5.44-4.67 mg 100 mL^{-1}) were determined in dill leaves treated with 4 mM. Similarly, Maysoun (2016) reported that the highest SA treatments (5 mM SA) resulted in reduced ascorbic acid content compared with the control in tropical fruits. SA prevented ascorbic acid destruction in pineapple (Lu et al 2011), and it might

Table 1- Effect of SA on ascorbic acid content (mg 100 mL^{-1}) of dill leaves stored at cold storage and shelf life

Storage period (days)							
			Cold storage				
Treatments	*0*	*5*	*10*	*15*	*20*	*25*	*Means*
1 mM	11.762	10.202	8.074	1.804	3.424	1.322	6.098
2 mM	11.762	13.823	4.404	3.424	2.246	1.650	6.218
4 mM	11.762	11.298	5.749	1.730	1.585	1.138	5.544
Control	11.762	10.825	5.126	3.191	1.948	1.001	5.642
Means	11.762 A*	11.537 B	5.838 C	2.537 D	2.301 E	1.278 F	
			Shelf life				
Treatments	*0+2*	*5+2*	*10+2*	*15+2*	*20+2*	*25+2*	*Means*
1 mM	11.146	9.362	7.288	1.413	2.061	1.127	5.400
2 mM	11.146	9.803	7.419	1.588	1.287	1.027	5.378
4 mM	11.146	8.846	4.540	1.069	1.395	1.048	4.674
Control	11.146	9.275	4.397	1.370	1.090	0.984	4.710
Means	11.15 A	9.32 B	5.911 C	1.360 D	1.458 D	1.047 D	

*, means followed by different letters with in the same row are significantly different at P<0.01

be used to decrease deterioration of ascorbic acid in crops (Sayyari et al 2009).

3.7. Visual quality

The results pertaining to sensory tests of dill leaves are presented in Table 2. The visual quality of dill leaves decreased with prolonging storage period, and increasing storage temperature. Storage conditions and SA concentrations affected the visual quality of dill leaves. Dill leaves treated with 1 mM SA had the best quality compared to the other concentrations (2 mM and 4 mM). The quality loss of dill leaves was noticeable after 20 day cold storage. Similarly, Sakaldaş et al (2010) reported significant quality losses in dill leaves after 20 day cold storage. In present study, SA treated dill leaves

Table 2- Effect of SA on visual quality (1-4) of dill leaves stored at cold storage and shelf life

Storage period (days)							
			Cold storage				
Treatments	*0*	*5*	*10*	*15*	*20*	*25*	*Means*
1 mM	1.00	1.00	1.50	1.80	2.04	2.45	1.63
2 mM	1.00	1.33	1.62	1.98	1.96	3.11	1.83
4 mM	1.00	1.31	1.70	1.97	2.04	3.61	1.94
Control	1.00	1.33	1.97	2.00	2.33	3.73	2.06
Means	1.00 D*	1.24 D	1.70 C	1.94 BC	2.09 B	3.22 A	
			Shelf life				
Treatments	*0+2*	*5+2*	*10+2*	*15+2*	*20+2*	*25+2*	*Means*
1 mM	1.00	1.17	1.71	1.97	2.43	3.18	1.91
2 mM	1.00	1.39	1.93	2.15	2.63	3.72	2.14
4 mM	1.00	1.36	1.93	2.16	3.03	3.82	2.22
Control	1.07	1.40	2.15	2.27	3.39	3.85	2.36
Means	1.02 E	1.33 D	1.93 C	2.14 C	2.87 B	3.64 A	

*, means followed by different letters with in the same row are significantly different at P<0.01

almost maintained their quality until 20 day of cold storage. However these leaves reached to the non-marketable limit with 15 day cold storage plus 2 days shelf life except for 1 mM treatment (Table 3). The decreasing pattern in the score of visual quality in the present study was in agreement with previous studies by Catunescu et al (2012), who reported reductions of scores from 20.20 to 18.18 after 12 day storage of dill treated at 4 °C. In some previous researches conducted in fruits, SA treatments also decreased quality losses compared to control samples during storage (Sayyari et al 2009; Luo et al 2011; Ali et al 2013; Khademi & Ershadi 2013).

Table 3- ANOVA for dependent variables for treatments, storage period and their interactions for dill leaves

Parameters	*Cold storage*			*Shelf life condition*		
	SP	*T*	*T×SP*	*SP*	*T*	*T×SP*
Weight loss	**	**	ns	**	**	**
Respiration rate	**	**	ns	**	**	ns
Visual quality	**	ns	ns	**	ns	ns
SSC	**	*	ns	**	**	**
L*	**	**	**	**	**	**
a*	**	**	**	**	**	**
b*	**	**	**	**	**	**
Gas composition in MAP-O_2 level	*	**	ns	-	-	-
Gas composition in MAP-CO_2 level	**	**	ns	-	-	-
Ascorbic acid content	**	ns	ns	**	ns	ns

ns, represents non-significance at P<0.05; **, represents significance at the 0.01 level; *, represents significance at the 0.05 level; SP, storage period; T, treatments

4. Conclusions

According to the results of this research, SA treatment allowed dill leaves to stay green longer than those of control group. Dill leaves treated with lower concentrations of SA (1 and 2 mM) maintained their quality better than those of 4 mM SA and control treatment. The 1 mM SA was the best treatment for prolonging the cold storage and shelf life of dill leaves with keeping quality. Dill leaves treated with 1 mM SA could be stored for 15+2 days with marketable quality in MAP, but control leaves lost their commercial properties after 10+2 days. As a ubiquitous phenolic acid, a plant based metabolites and an endogenous hormone, SA could be a promising candidate for prolonging postharvest life and quality of dill leaves. However, further detailed research on this subject is needed to investigate.

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