

The role of some elicitors in inducing chilling stress resistance in apricot fruit

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SUMMARY

This study aimed to study the role of 2 mM salicylic acid and 0.2 mM methyl jasmonic acid in reducing the chilling injuries on two different apricot varieties fruits (Bergaraugé and Flavor cot). These treatments were applied for apricot fruit directly after harvest after measuring the zero time data. Then the fruit were stored at 1 °C for three weeks and were examined 7 day periodically, other group of fruit was stored at 1 °C for 15 days then transport to room temperature and examined after 4 and 8 days. The fruit samples were collected at each examination time, and the measurements containing the fruit firmness (Kg cm⁻²), chilling and decay index, membrane electrolyte leakage, total phenol content and phenylalanine ammonia lyase mechanism (PAL) activity. The results showed that there are no obvious differences between the tested varieties and the differences may back to genetic variations and they took the same trend for most of measurement after treated with SA and MJ. SA and/or MJ treated fruit achieved low percentage of fruit softening in comparison to water treated fruit. At the same time the chilling and decay index showed that the SA and/or MJ reduced the decay or breakdown symptoms in either tested varieties. The treated fruit with those elicitor help the fruit to keep about stable phenol content while water treated fruit lose the phenol content sharply at early storage and this stable level of phenol my because of the ability of those chemical to raise PAL activity in treated fruit.

Keywords: apricot fruit, chilling injuries, salicylic acid, methyl jasmonic acid, PAL

ÖSSZEFOGLALÁS

A tanulmány célja 2 mM szalicilsav és 0.2 mM metil-jázmonsav hatásának vizsgálata két sárgabarack fajta (Bergaraugé és Flavor cot) fagyási sérüléseinek enyhítésében. A kezeléseket közvetlenül szedés után végeztük. Ezután a gyümölcsöt három hétig 1 °C-on tartottuk és 7 napig vizsgáltuk. A sárgabarack másik vizsgált csoportját 15 napig tartottuk 1 °C-on, majd szobahőmérsékleten tárolva a 4. és a 8. napon vizsgáltuk. Mintát vettünk a gyümölcsökből minden egyes vizsgálat során és a következő tényezőket elemeztük: keménység (Kg/cm²), fagyási és romlás-index, elektrolitzivárgás a sejtmembránon, összes fenoltartalom, illetve fenilalanin ammónia-liáz (PAL) aktivitás. Az eredmények rámutattak, hogy nincs lényegi különbség a vizsgált fajták közt, illetve az esetleges különbségek genetikai gyökerek lehetnek és hasonló tendenciát mutattak a szalicilsav és a metil-jázmonsav kezeléseket követően. A szalicilsavval és/vagy metil-jázmonsavval kezelt gyümölcs alacsony szintű puhulást mutatott a vízzel kezelt gyümölcshöz képest. Ugyanakkor a fagyási és romlásindex alapján megállapítottuk, hogy a szalicilsavas és/vagy metil-jázmonsavas kezelés csökkentette a romlást és a bomlási folyamatokat mindkét vizsgált fajtában. Az elicitorok hatására a gyümölcs képes volt stabil szinten tartani a fenoltartalmat, míg a vízzel kezelt gyümölcs már a tárolási időszak elején rohamosan veszített fenoltartalmából. Ennek oka az lehet, hogy a kezeléshez használt anyagok hatására a gyümölcs növelni tudja PAL aktivitását.

Kulcsszavak: sárgabarack, fagyási sérülések, szalicilsav, metil-jázmonsav, PAL

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is classified under the *Prunus* species of *Prunoidae* sub-family of the *Rosaceae* family of the *Rosales* group. Apricot has an important place in human nutrition, and can be used as fresh, dried or processed fruit. Fruit quality is essential for the acceptance of apricot cultivars by consumers, especially due to the current situation of high competition in the markets with the presence of numerous new cultivars and other fruits and other foods. Low temperature has been widely used to store fruits and vegetables as its beneficial effects for delaying senescence and maintaining quality. Chilling injury (CI) is a physiological disorder induced by low, but not freezing temperatures, which affects fruit quality seriously. The increasing demand for consumption of fruits and vegetables, along with restriction on the use of synthetic chemicals, has encouraged scientific research to develop new technologies based on natural product such as salicylic acid (SA), acetyl salicylic acid (ASA), methyl salicylate (MeSA) and methyl jasmonate

(MJ). Although there are many methods to reduce CI in fruits and vegetables, salicylates and jasmonates treatments are inexpensive, easy to apply and can be used on fruits and vegetables (Asghari and Aghdam, 2010). Salicylic acid SA has been extensively used for quality improvement in a number of crops (Peng and Jiang, 2006). Literature has impressively argued the role of phenolic compounds such as salicylic acid about their influence on physiological or biochemical processes including ion uptake, membrane permeability, enzymes activity, heat production, growth development (Arberg, 1981). SA significantly reduced the quality loss in peaches (Wang et al., 2006), tomato (Ding et al., 2001), sweet peppers (Fung et al., 2004), and loquat fruits (Cai et al., 2005). SA and its derivatives are widely in use to enhance fruits postharvest life by controlling their firmness. SA has been documented to enhance flesh firmness of harvested peaches during storage (Li and Han, 1999; Wang et al., 2006), and banana fruits during ripening (Srivastava and Dwivedi, 2000). Thus, salicylic acid has remarkable ability to maintain the fruits quality during storage life of fruits. Several

natural volatile compounds have been reported to possess antimicrobial activity and maintaining fruit quality. The methyl jasmonate (MJ) has been studied for their effectiveness in maintaining the quality of fresh fruits and vegetables. It has been reported that MJ treatment can reduce the development of chilling injury symptoms in zucchini (Wang and Buta, 1994) and mango (Gonzalez-Aguilar et al., 2000). Treatment of tomato fruit with low concentrations of (0.01 mM) methyl jasmonate (MJ) or methyl salicylate (MeSA) substantially enhanced their resistance to chilling temperature and decreased the incidence of decay during low-temperature storage. (Ding et al., 2002). The aim of this work was to study the mechanism which SA and MJ can reduce the chilling injuries and prolong the shelf life for apricot fruits.

MATERIAL AND METHODS

Plant material and treatments

Fruits from different two apricot varieties (Bergaraugé and Falvor Cot) were hand harvested from the Gyümölcsért Kft. located in Boldogkőváralja, Hungary at ripe stage and selected for uniform size, colour and examined to exclude all visual defects. Each variety fruits were divided into three groups as shown in *Table 1*, for chemical treatment the Fruit were dipped into solution of 0.2 Mm MeJA and 2 Mm SA for about 15 minutes as inducing treatments and water treated fruit were control. Then each chemical treatments fruits were divided into 2 groups for storage treatment. The first group was for shelf life at 25 °C and the fruit were examined at 4 days and 8 days after treatment. The other group of storage treatment was for storage at 1 °C and the examinations were 7, 14 and 21 day after treatment. Each treatment has 3 replicats and the experiment repeated twice.

Effect of SA and MJ on fruit firmness

Fruit firmness measurements were performed on about 30 fruits immediately after harvest for each variety as zero time. Other fruits were undergone for the experimental treatments and examined subsequently in order to assess the effect of chemical treatment in

reducing the stress of cold storage. The fruit firmness (kg cm⁻²) was measured by Magness Tazlor penetrometer.

Evalaution of chilling injury and decay

Chilling injury (CI) and fruit decay were investigated at the end of shelf life and cold storage, by using 30 fruit per replicate. The degree of CI was visually investigated on the fruit surface following a double cut parallel to the axial diameter. The extent of flesh browning was divided into five classes: 0, no browning; 1, extensive browning covering <25% of the cut surface; 2, extensive browning covering ≥25% but <50% of cut surface; 3, extensive browning covering ≥50% but <75% of cut surface; 4, extensive browning covering ≥75% of cut surface. From this, a CI index was expressed as:

$$\text{CI index} = \frac{[(\text{CI level}) \times (\text{number of fruit at the CI level})]}{4 \times \text{total number of fruit in the treatment.}}$$

Symptoms of apricot fruit decay were superficial browning. The severity of the symptoms was assessed visually according to the following five-stage scale: 0, no browning; 1, browning covering ≥25% of the fruit surface; 2, browning covering ≥25% but <50% of the fruit surface; 3, browning covering ≥50% but <75% of the fruit surface; 4, browning covering ≥75% of the fruit surface. From this, a decay index (DI) was expressed as:

$$\text{DI index} = \frac{[(\text{DI level}) \times (\text{number of fruit at the DI level})]}{4 \times \text{total number of fruit in the treatment.}}$$

Determiration of cell membrane electrolyte leakage

Electrolyte leakage was determined using 30 disks (8 mm diameter, 3–4 mm thick) from flesh tissue of 5 fruits. Disks were washed with double-distilled water and then immersed in 40 mL double distilled water for 3 h. Ion leakage was measured as the amount of conductivity of the solution (HANNA, Italy). Disks were boiled for 30 min at 95 °C, cooled in room temperature, and then the total conductivity was measured. Electrolyte leakage was expressed as relative conductivity (100*the conductivity of tissue solution/the total conductivity).

Table 1.

The design of experiment		
Chemical treatment	Storage treatment	Time of examination
Control	Storage at 1 °C for 15 days then at Shelf at 25 °C	4 days
		8 days
	Storage at 1 °C	7 days
		14 days
		21 days
0.2 mM MeJA	Storage at 1 °C for 15 days then at Shelf at 25 °C	4 days
		8 days
	Storage at 1 °C	7 days
		14 days
		21 days
2 mM SA	Storage at 1 °C for 15 days then at Shelf at 25 °C	4 days
		8 days
	Storage at 1 °C	7 days
		14 days
		21 days

Measurement of total phenol content

Total phenol contents were determined using Folin-Ciocalteu's reagent according to the method of Singleton and Rossi (1965). Phenol content was calculated from a standard curve obtained by different concentrations of gallic acid.

Measurement the changes in PAL activity

Fruit samples

The samples were collected at 0, 7, 14 and 21 days at 1 °C and at for shelf life after storage for 15 days at 1 °C the samples were collected after 4 and 8 days at 25 °C, each treatment was replicated three times.

Enzyme extract

Flesh (10 g) from 10 fruits with 0.5 g PVPP was ground with 25 ml of 50 mmol l⁻¹ sodium borate buffer (pH 8.8, containing 5 Mm β-mercaptoethanol) for phenylalanine ammonia-lyase (PAL). The extracts were then homogenized and centrifuged at 10 000 × g for 20 min at 4 °C the supernatant were used for enzyme assay.

PAL activity was assayed according to the method of Assis et al. (2001), with slight modifications. Enzyme extract (1 ml) was incubated with 2 ml of borate buffer (50 mmol l⁻¹, pH 8.8) and 1 ml of L-phenylalanine (20 mmol l⁻¹) for 60 min at 37 °C. The reaction was stopped with 1 ml HCl (1 mol l⁻¹). PAL activity was determined by the production of cinnamate, which was measured by absorbance at 290 nm. The blank was the crude enzyme preparation mixed with L-phenylalanine with zero time incubation. PAL activity was defined as nmol cinnamic acid h mg⁻¹ protein.

Data analysis

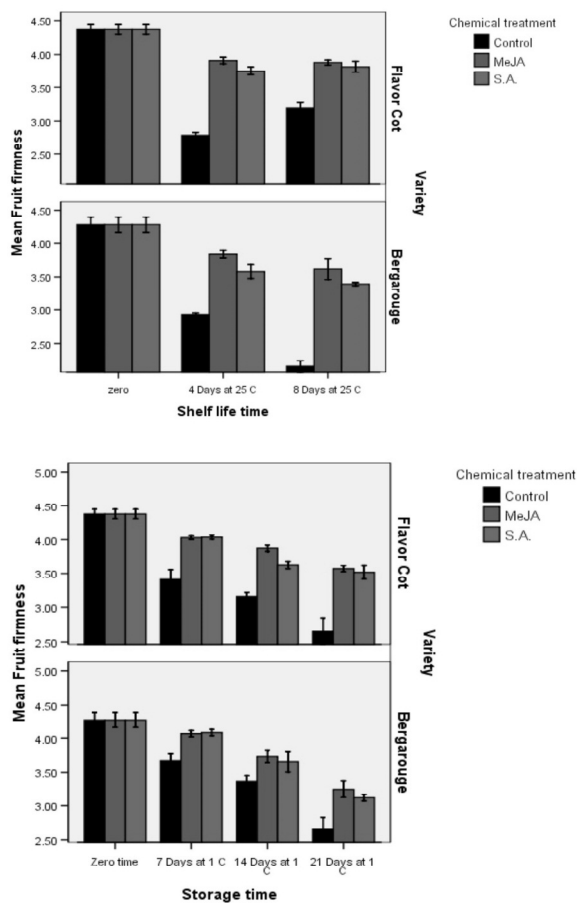
Experiments were performed using a completely randomized design. All analyses were performed with SPSS program (SPSS Inc., Chicago, IL, USA). The data were analyzed by one-way analysis of variance (ANOVA). Means separation was performed by Duncan's multiple range tests. Differences at p<0.05 were considered as significant.

RESULTS

Effect of SA and MJ in keeping fruit firmness of apricot fruits

Data in *Figure 1* shows that the fruit firmness was reduced in all treated fruit and this reduction was obvious in water treated fruit. The SA and MJ treated fruit showed lower reduction in firmness. The differences between chemical treated fruit either SA or MJ and the control fruit was significant (p<0.05). Also it is clear that MJ achieved lower softening in comparison to SA treated fruit without significant. For differences between varieties, the data shows that Flavor Cot has appearance to keep it firmness during storage better than Bergarauge. These results showed that the SA and MJ prolong the shelf life of apricot fruit at 25 °C either for Flavor Cot and Bergarauge with highly differences with water treated fruit (p<0.05).

Figure 1: The effect of 2 mM salicylic acid and 0.2 mM methyl Jasmonic acid on fruit firmness (Kg cm⁻²)



Effect of SA and MJ in chilling injury and decay index

The degree of chilling injury CI of control, MJ and SA treatments increased with storage time as observed by the CI index (*Table 2*). Meanwhile, pre-treated fruit with 0.2 Mm MJ and 2 Mm SA achieved low CI index than control (p<0.05) in all storage times and no differences was noticed for the tested varieties. The data in *Table 2* showed that Bergarauge has higher resistance to chilling than flavor cot and these differences were significant at 14 and 21 days of storage at 1 °C. The shelf life data showed that the CI increase dramatically after fruit expose to room temperature and SA and MJ treated fruit showed good resistance to these symptoms than control in tested two varieties with significant differences.

As can be seen in *Table 3*, non treated apricot fruit (control) showed dramatically increase in decay % at 1 °C as reached to 96.25±2.3% and 98.23±3.21a% after 21 days for Bergarauge and Flavor cot respectively. Whereas 0.2 Mm MJ and 2 Mm SA treatments depressed the development of fruit decay with significant different with control (p<0.05). MJ and SA showed obvious effect in keeping the apricot fruit away from fruit decay in shelf life treatments. Flavour cot fruit showed highly resistance to decay symptoms than Bergarauge with significant differences (p<0.05).

Table 2.

The effect of MJ and SA treatments on resistance of apricot fruits to chilling stress

Treatment	Varieties	CI index (%)				
		7 days at 1 °C	14 days at 1 °C	21 days at 1 °C	15 days at 1 °C then 4 days at 25 °C	15 days at 1 °C then 8 days at 25 °C
Control	Bergarouge	3.12±0.87a	16.68±1.45a	37.65±4.56a	18.67±2.35a	30.24±3.65a
	Flavor Cot	3.21±0.541a	19.23±0.351a	44.21±2.351a	20.12±2.65a	30.12±4.02a
0.2 Mm MeJA	Bergarouge	1.35±0.21a	4.52±1.98c	9.68±3.65d	8.65±1.09b	14.35±1.35c
	Flavor Cot	2.32±1.01a	6.21±1.68b	11.21±1.02b	10.21±2.32b	17.21±2.31b
2 Mm SA	Bergarouge	1.64±0.34a	5.58±0.68c	20.91±5.09c	7.68±2.34b	19.26±2.65b
	Flavor Cot	1.54±0.21a	5.21±0.98b	21.24±2.032c	9.21±1.32b	19.24±1.23b

Note: values within a column followed by different letters are significantly different at $p<0.05$ according to Duncan's multiple range tests. The results represent the means±SD of triplicate assay.

Table 3.

The effect of MJ and SA treatments on resistance of apricot fruits to decay

Treatment	Varieties	Fruit decay (%)				
		7 days at 1 °C	14 days at 1 °C	21 days at 1 °C	12 days at 1 °C then 4 days at 25 °C	12 days at 1 °C then 8 days at 25 °C
Control	Bergarouge	16.05±1.07a	56.24±5.35a	96.25±2.3a	66.36±2.65a	100±3.05a
	Flavor Cot	13.25±1.32a	40.21±3.25a	98.23±3.21a	52.12±4.21b	96.23±2.32a
0.2 Mm MeJA	Bergarouge	2.11±0.98b	6.57±1.08b	16.98±6.65b	16.35±3.65c	36.65±2.03b
	Flavor Cot	2.01±0.32b	6.21±1.22b	14.21±1.23b	12.35±1.21c	31.23±3.01b
2 Mm SA	Bergarouge	2.33±1.03b	7.58±2.08b	15.61±4.19b	20.36±4.09c	35.36±3.65b
	Flavor Cot	2.12±1.021b	6.35±1.32b	13.21±2.01b	15.12±2.31d	35.26±2.01b

Note: values within a column followed by different letters are significantly different at $p<0.05$ according to Duncan's multiple range tests. The results represent the means±SD of triplicate assay.

Cell membrane electrolyte leakage

The cell membrane electrolyte leakage exceeded to about 100% in water treated fruit while SA and MJ could reduce cell membrane electrolyte leakage in apricot fruits stored for 14 and 21 days. But when fruits were transported from low temperature to room temperature for shelf life, membrane electrolyte leakage increased and there was no significant difference between MJ treated and SA fruits while control fruit showed dramatically increasing (Figure 2). The same trend was noticed for the tested varieties.

Effect of SA and MJ on total phenol content

Total phenol content reduced sharply in water treated fruit after storage at 7 days meanwhile, MJ treated fruit showed slight increase in fruit phenol content with significant differences with control fruit but after 14 days of storage at 1 °C the phenols content took to decrease but still higher than control fruit ($p<0.05$) for Bergarouge fruits (Figure 3). At the same time, SA treated fruit achieved semi stable phenol content during first 2 weeks then decreased in either Bergarouge or Falvor cot. The differences between the varieties were not significant but phenol content of flavour cot fruit was higher than Bergarouge.

Effect of SA and MJ on PAL activity

Treated fruit with SA and MJ showed increasing in PAL activity during 7, 14 and 21 days at 1 °C (Figure 4). At the same time water treated fruit showed sharply

decreasing in PAL activity. MJ treated fruit showed priority than those treated with SA with significant in case of Bergarouge fruit. For the differences between varieties, Bergarouge fruit showed high response to SA or MJ treatment than flavour cot with significant differences. Fruit at room temperature was affected by SA and MJ, as the PAL activity start to increase slightly at 4 days at 25 °C then the fruit treated with MJ increased significantly than SA treated fruits for Bergarouge fruit. Also SA and MJ enhanced the PAL activity of Flavor cot but it was lower response than Bergarouge fruit.

DISCUSSION

In this work, during cold storage and further shelf life at 25 °C of control apricot, the loss of firmness occurred gradually along storage. The reduction in softening process being retarded by the use of both MJ and SA treatments. It has been postulated that MJ reduces pectinmethylesterase (PME) activity, decreasing de-esterification of pectin (Meng et al., 2009), and thus maintaining fruit texture. SA has been documented to enhance flesh firmness of harvested peaches during storage (Li and Han, 1999; Wang et al., 2006), and banana fruits during ripening (Srivastava and Dwivedi, 2000). Thus, SA has remarkable ability to maintain the fruits quality during storage life of fruits and these results in the same way like our finding (Figure 2). Other face of MJ mode of action on affecting is decreasing membrane lipid peroxidation and maintaining high superoxide dismutase (SOD) activity in strawberry under water stress (Wang, 1999).

Figure 2: The effect of 2 mM salicylic acid and 0.2 mM methyl Jasmonic acid on membrane electrolyte leakage (%)

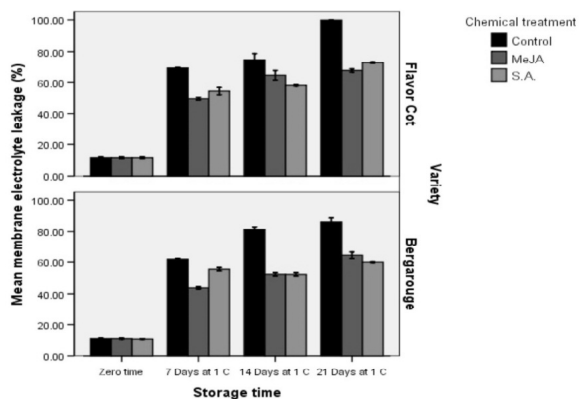


Figure 4: The effect of 2 mM salicylic acid and 0.2 mM methyl Jasmonic acid on PAL activity (nmol h⁻¹ mg protein⁻¹)

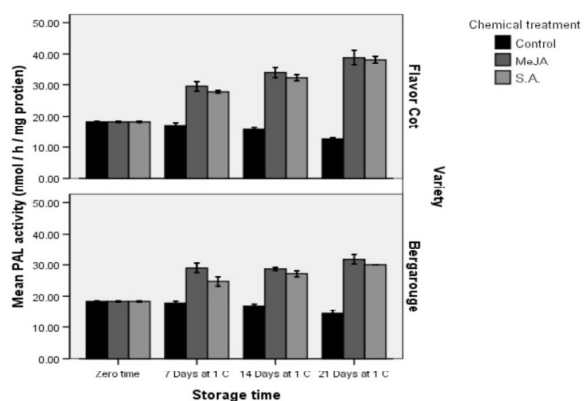
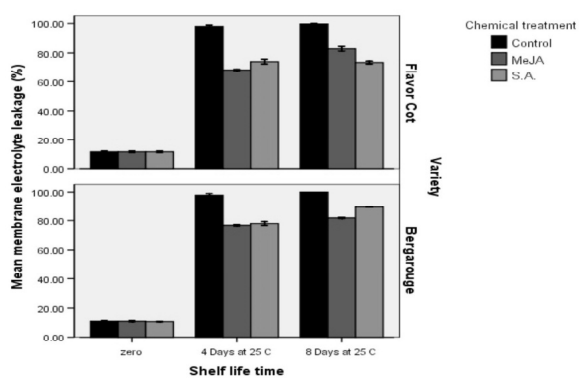
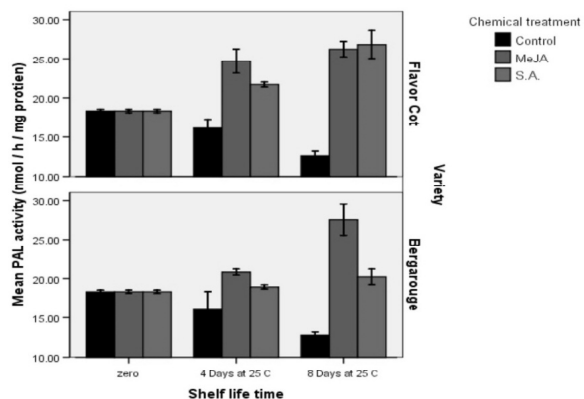
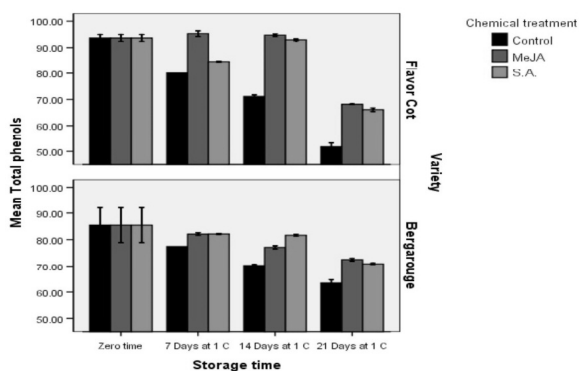
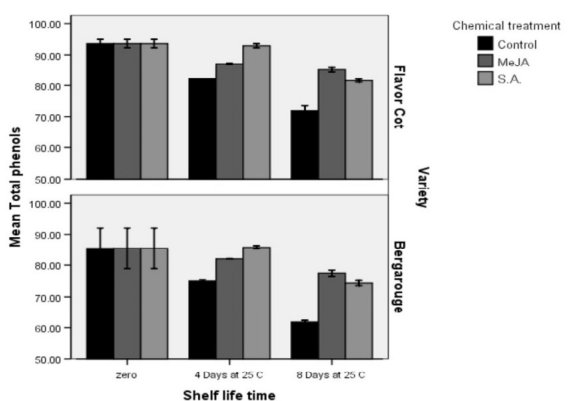


Figure 3: The effect of 2 mM salicylic acid and 0.2 mM methyl Jasmonic acid Total phenol (mg GAE 100 g FW⁻¹)



Our data demonstrated that the treatment of apricot fruit with methyl jasmonate (MJ) or salicylate (SA) substantially enhanced their resistance to chilling temperature and decreased the incidence of decay during low-temperature storage and shelf life.

Apricot fruit easily appear chilling injury when stored at low temperature. The CI symptoms were generally influenced and triggered by a combination of storage temperature and period (Li and Han, 1999). Our results state that using of MJ and SA play important role in alleviating CI symptoms. However MJ could keep the CI at lowest level in comparison with SA treatment after 21 days at 1 °C and after 8 days at 25 °C. It has been reported that the improvement of chilling tolerance in harvested horticultural crops is related to enhancement in activities of antioxidant enzymes. Sala (1998) found that the chilling-tolerant mandarins have a higher antioxidant enzyme activity than the chilling sensitive cultivars. This may interpret why MJ and SA is acting its role better in cold storage than shelf life (Table 2) may be because the chilling as kind of stress work as an inducer to express some important antioxidant genes as reported by Fung et al 2004 as they demonstrated that the expression patterns of alternative oxidase and seven other genes involved in defenses against oxidative stresses in pepper fruit treated with MeSA or MJ vapor increased transcript level of these enzymes even at room chamber of 25 °C, whereas no changes was observed with untreated and this was joined with preventing chilling injury.

MJ has been shown to induce the synthesis and expression of some stress proteins such as heat-shock proteins and pathogenesis-related proteins, which lead to the increased resistance of the stress and the decreased incidence of the decay (Ding et al., 2001; 2002). In this experiment, lower cell membrane electrolyte leakage was also found in MJ treated fruits (*Figure 1*), indicating the less disruption in plasma membrane under chilling stress. Thus, it is suggested that maintaining membrane integrity by SA and MJ treatment was beneficial for controlling flesh browning of peach fruits.

The activity of PAL was examined in this study to investigate the possible role of PAL in phenolic

metabolism of apricot fruit in response to MJ treatment. We found that fruit treated with MJ exhibited significantly higher levels of PAL activity, total phenolics, compared to the control fruit. These results suggest that MJ may improve the antioxidant status of apricot fruit by inducing PAL activity and thus positively affecting phenolic metabolism.

Some papers demonstrated that the SA play a vital role in induction of systematic acquired resistance in plant cells by its ability to induce defense and antioxidant enzymes such as polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL) and β -1, 3-glucanase (Qin et al 2003).

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