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Effect of 1-methylcyclopropane in combination with Calcium chloride on postharvest storage and quality of green olives

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Fariba Amini[1]* and Ali Akbar Ramin[2]

1. Department of Biology, Faculty of Science, Arak University, Arak, Iran
2. Department of Horticulture, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

ABSTRACT

Green olive cultivars "Manzanila" and "Mission" were harvested at the mature green stage. They were either treated with 1-methylcyclopropane (1-MCP) at a concentration of 1.8 µL/L for 24 h at 20°C or kept untreated as a control. Both treated and untreated fruits were then immersed in water containing $CaCl_2$ of 0 (control), 50 and 100 mM for 2 h under 1.2 bar pressure. Fruits were then surface dried, put into plastic basket and stored at 6°C with relative humidity of 80% in a refrigerator for 12 weeks. The non-1-MCP treated fruits softened within 6 weeks after harvest. In contrast, the 1-MCP treatment inhibited fruit softening and color changes. Treatment with $CaCl_2$ delayed fruit softening, but had little effect on fruit color. The rate of ethylene production and respiration were also significantly ($P<0.05$) lower in fruits treated separately by 1-MCP and $CaCl_2$, compared to the control. However, the effect of 1-MCP in combination with $CaCl_2$ was more effective in the case of color change and softening during cold storage. It could be concluded that the fruits treated with a combination of 1-MCP and $CaCl_2$, were superior in preventing fruit softening and green color loss, and suffered minimum damage for 12 weeks at 6°C.

Keywords: calcium chloride, *Olea europea,* postharvest physiology, 1-Methylcyclopropane.

* **Corresponding author**: F-Amini@araku.ac.ir

Introduction

Research on olive cultivars indicate that detached olives show no climacteric respiratory rise. Green olives produce only traces of ethylene, while black fruit produce significantly higher quantities but still very low compared to climacteric fruits. Ethylene at 150-250 µL/L only slightly increases the respiration rate of green olives at 20°C, while it considerably increases respiration rates at 25 or 30°C with a climacteric-type rise depending on cultivar (1).

Non-climacteric fruits do not show a dramatic respiration or ethylene burst, nor do they continue to develop after harvest. Instead, they undergo a senescence process which is parallel to some of the same processes occurring in ripening fruit (2). However, slowing the process of ripening and senescence extends the storage and the shelf-life of fresh fruits and vegetables. It has been shown that inhibition of ethylene action delays ripening and senescence in several species of fruits and vegetables (3). Recently a new tool, 1-methylcyclopropene (1-MCP), has been added to the methods used for extending storage life and quality of plant tissue. A previous experiment with four cultivars of olive indicated that 1-MCP treatment effectively reduced loss of color changes and firmness for fruits stored at 5°C for 15 weeks (4). The success of the 1-MCP treatment depends on the method of application, duration and concentration as well as commodity factors such as cultivar.

Pre-and postharvest calcium applications have been used to delay senescence, to reduce postharvest decay, and to control the development of many physiological disorders in fruits and vegetables (5). In fruits, vacuum infiltration of calcium chloride ($CaCl_2$) has been used commercially to enhance storage potential (6). However, it is difficult to infiltrate enough calcium into some fruits to eliminate decay, without causing damage (5). In anna apple, using $CaCl_2$ in postharvest dips combined with heat treatment produced better fruit quality than with either treatment separately (7). In apple fruits, application of $CaCl_2$ coating significantly maintained the sensory quality of apple fruit by slowing down the metabolic changes. The 6% $CaCl_2$ treated fruit had the most intense color and better texture (8). Karemera and Habimana (9) evaluated the effect of pre-harvest calcium chloride on post harvest behavior of mango fruits and concluded that 1.5% $CaCl_2$ significantly increased the number of days taken for ripening of fruits, the shelf-life of fruits, physico-chemical parameters and organoleptic evaluation of mango fruits compared to the control. Postharvest dips in concentrated solutions of $CaCl_2$ have been used to improve firmness in fresh-cut strawberries (10). However, it is not clear if 1-MCP in combination with $CaCl_2$ will be of benefit for mature green olive cultivars. Therefore, in this paper the effects of dipping the two cultivars of olive in $CaCl_2$ solution and 1-MCP separately or in combination, on their quality and storage life are presented.

Materials and methods

Plant materials

Green olive (*Olea europea*) cultivars "Manzalina" and "Mission" were hand harvested on the 18th and 21st of September 2009 and 2010, respectively, from trees in the same orchard that received the same cultural practices at the mature green stage from the Isfahan University of Technology, Horticultural Department Experimental Orchard, and transported to the Postharvest Laboratory within 1 h. Sampling was done from three adjacent trees and from different

parts of each tree, so as to minimize the effect of watering, sun exposure and differences related to different maturation stage. Olives were sorted to obtain fruit uniform size and color and distributed randomly in 0.5 kg lots placed in 2L glass jars as one replication.

MCP and Ca treatments

Olives were treated with 1- methylcyclopropan (1-MCP) at a concentration of 1.8 µL/L (4) for 24 h at 20°C or kept untreated as a control. Both treated and non-treated fruits were then immersed in water containing $CaCl_2$ of 0 (control), 50 and 100 mM for 2 h under 1.2 bar pressure. Fruits were then surface dried and put into plastic basket and stored at 6°C with relative humidity of 80% in the refrigerators for 12 weeks. Sample of 20 olives were randomly removed from each treatment: 10 olives were used for firmness and 10 olives were used for color measurement. Observations of decay, firmness, and color value were made over 12 weeks, at an average interval of 2 weeks, during the course of the experiment.

Measurement of firmness

Flesh firmness was measured with a Fruit Hardness Tester (Model 10576, OSK, Japan) equipped with a modified 5 mm conic diameter and 2 mm-long tip plunger. Flesh firmness measurements were taken after careful removal of skin and penetration of the flesh to about 2 mm (flesh width ranged from 3-4 mm). Two firmness measurements were taken from opposite sides of each fruit.

Color measurement

External skin color (opposite sides) was periodically measured with a Minolta Chromameter (Model CR-200, Minolta Camera, Co, Japan), calibrated with a white standard (y=94.3; x=0.3142; z=0.3211). Values of *a* and *b* readings were taken and color was reported as a (green-red) and b (blue-yellow). Change in the H angle (H°) was calculated as h=arctan [b/a], which can be used effectively for visualizing the color of fruits (1).

Chlorophyll fluorescence measurement (Fv/Fm)

Chlorophyll fluorescence of olive fruits was measured using a plant efficiency analyzer (PEA, Hansatech, LTd, UK). When using a PEA, the fruit sample is darkened with a light weight plastic leaf clip clipped 6 min before the measurement. During measurement, the PEA sensor unit is held over the clip and the shutter remains open. A single button press activates the high intensity of the LED array of the sensor head which provides a maximum light intensity of 3000 µmol $m^{-2}s^{-1}$. Maximal PSII photochemical efficiency (Fv/Fm), the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm), was calculated automatically. Measurement was made from opposite sides of each olive. This technique has recently been used for postharvest horticultural communities for better indication of storage environments (11).

Polyphenols measurement

To understand color changes better, total polyphenols of fruits were analyzed. Measurements were carried out according to a previously published protocol (12), employing the Folin-Ciocalteu methodology. Gallic acid was used as the reference standard, and results were expressed as mg gallic acid equivalents per 100 g of fresh tissue.

Respiration

Respiration and production rates were measured by placing each treatment and replication in 4L glass jars, hermitically

sealed with a rubber stopper for 1 h at 20ºC, following 6 weeks of storage at 6ºC. CO_2 evaluation in samples was made from the exit flow from each jar, using infrared CO_2 detection.

Ethylene production

Ethylene production was determined by injecting 1 ml of the head-space gas extracted after 1 h and ethylene was quantified on a gas chromatograph (Varian GC-3400, series) fitted with FID and a stainless steel Porapak N column. The injector, oven and detector temperatures were 110, 90 and 250ºC, respectively. N_2 was used as the carrier gas at a flow rate of 0.37 ml s^{-1}.

Statistical analysis

The experiment was set up as a completely randomized design with three replications of each treatment. Data were subjected to ANOVA, and least significant (LSD) differences were determined at $P<0.05$ to compare the means.

Results

Color, a* and Hº value

Difference in color changes (a* value) for two cultivars of olives (Manzalina and Mission) appeared after 12 weeks of storage at 6°C (Fig. 1A). Both cultivars showed significant difference in a* value when fruits were treated with 1-MCP and 50 and 100 mM $CaCl_2$ in comparison to the control (0 1-MCP and $CaCl_2$) treatments. The appearance of 1-MCP treated fruit was better than that of $CaCl_2$ fruit due to the smaller changes in a* value following storage at 6ºC. However, the quality of fruits (skin color) was improved after treatment with a combination of 1-MCP and $CaCl_2$. Increase in *a** value indicated loss of greenness as could be seen in non-treated fruits (Fig. 1A).

Changes in Hº angle are another indication of ripening in many fruits. Regardless of cultivars, the fruits treated with 1-MCP and $CaCl_2$ from 50 to 100 mM resulted in better quality after 12 weeks of storage, compared to non-treated (control) fruits (Fig. 1B). The H° values confirmed that 1-MCP alone or 1-MCP in combination with $CaCl_2$ was chromatically greener than control fruits at the end of the treatment. The appearance of treated fruits was better than the control fruits due to smaller changes in H° (Hue angle) during storage. Hue angle of fruits treated with 1-MCP or 1-MCP in combination with $CaCl_2$ remained significantly ($P<0.05$) high at the end of storage, compared to the control fruits. There were no significant differences in Hº angle in fruits treated with $CaCl_2$ in both cultivars (Fig. 1B). However, there were no significant differences in Hº in fruits treated with 1-MCP alone or 1-MCP in combination with $CaCl_2$.

Fruit firmness

An olive fruit dip in $CaCl_2$ solution prior to storage significantly ($P<0.05$) improved fruit firmness in both cultivars following 12 weeks of storage at 6ºC, compared to the control (0 $CaCl_2$) treatment (Fig. 2A). The treatment with 100 mM $CaCl_2$ was the most effective. However, the fruits treated with 1-MCP showed significantly higher fruit firmness compared to those treated by $CaCl_2$ alone. Fruit firmness after 12 weeks of storage ranged from 1.5 to 1.7 kg in controlled fruits, which is lower than the firmness of fruits treated with 1-MCP and $CaCl_2$. Fruits treated with 1-MCP, especially in combination with 100 mM $CaCl_2$ treatment, became significantly ($P<0.05$) harder than those treated with either $CaCl_2$ or 1-MCP alone (Fig. 2A).

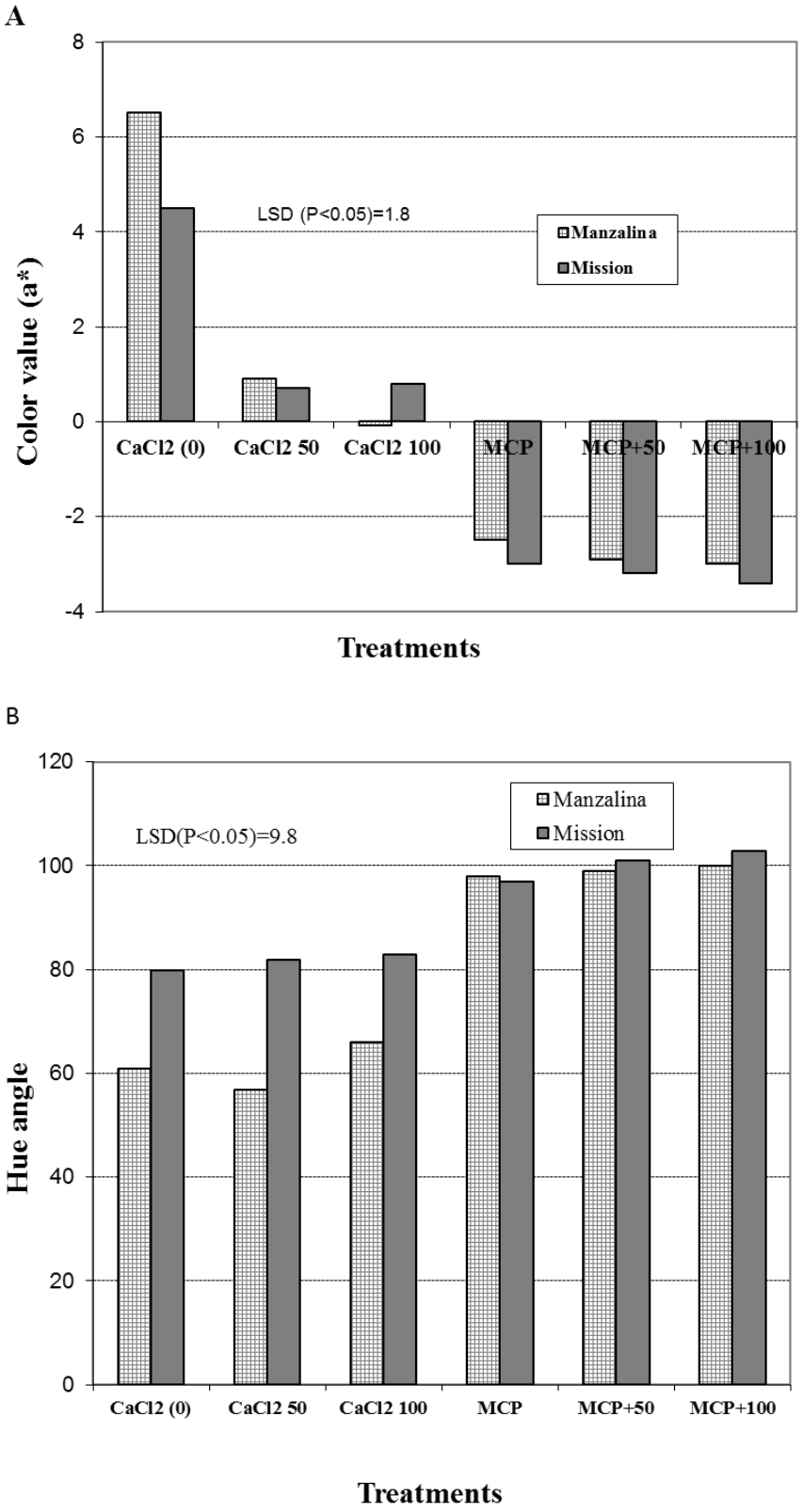

Figure 1. Effect of 1-MCP (1.8µL/L) and $CaCl_2$ (mM) on color value (a^*) (A) and (H^o angle) (B) of olives after 12 weeks of storage at 6°C

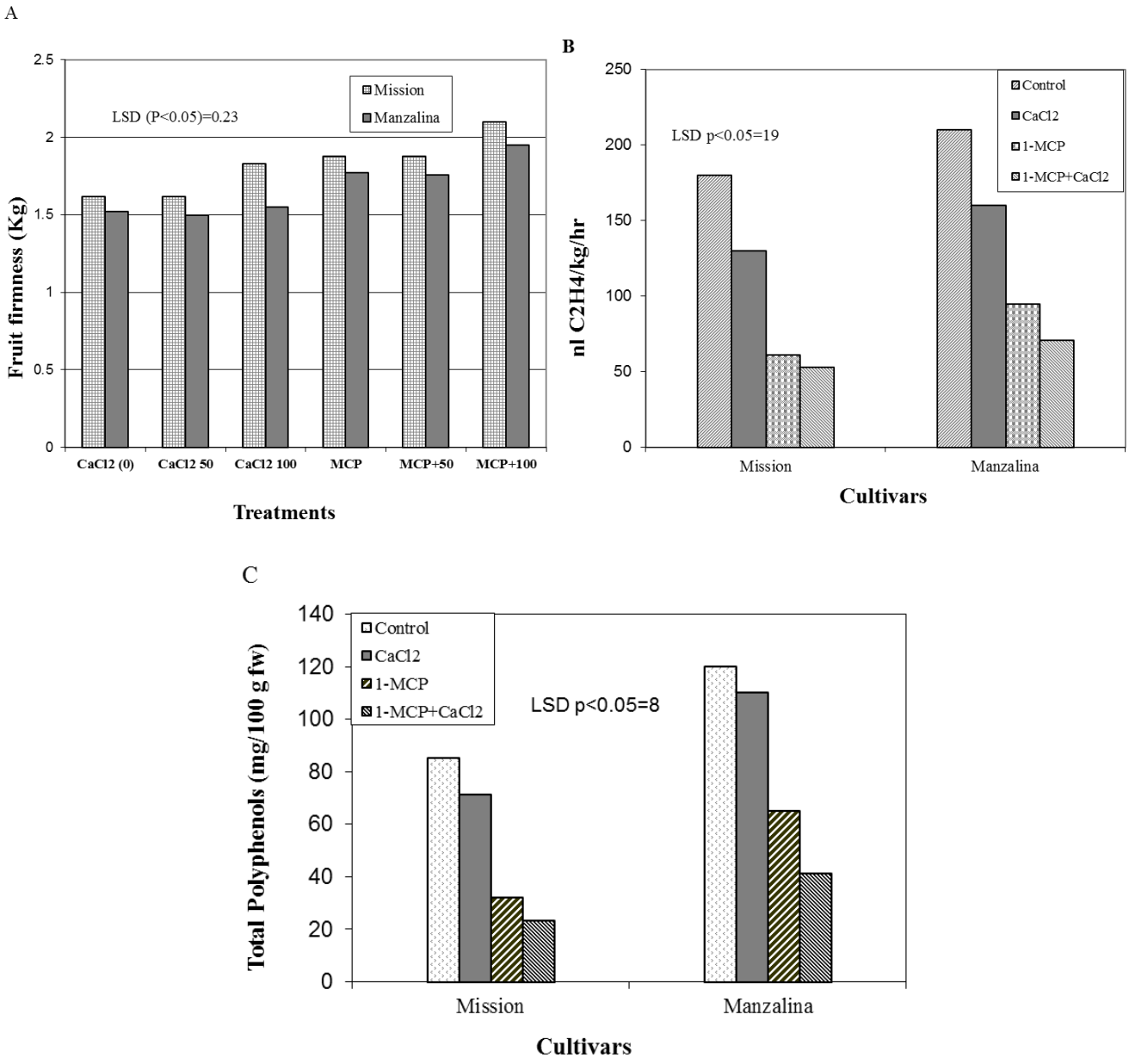

Figure 2. Effect of 1-MCP (1.8 µL/L) and $CaCl_2$ (mM) on fruit firmness (A), rate of ethylene production (B) and total polyphenols (C) of olives after 12 weeks of storage at 6°C

Ethylene production rate

Olive fruits in the control, 1-MCP, and $CaCl_2$ treatments showed similar ethylene production rates in both cultivars with slightly higher rates in cv. "Manzalina", but the combination of 1-MCP and $CaCl_2$ dip slowed down ethylene production (Fig. 2B).

Total Polyphenol

The level of total polyphenol compounds in the olive is shown in Figure 2C. The content of total polyphenols in olives after 12 weeks of storage at 6ºC following 1 day at 20ºC were *ca*. 80 and 120 mg/100 g fresh weight in control for cvs "Mission" and "Manzalina", respectively. During 12 weeks of storage at 6ºC, phenolics increased in control fruit, while less polyphenol accumulation was detected in $CaCl_2$ and 1-MCP treated fruit. However, total polyphenol synthesis was significantly reduced when the fruit was treated with 1-MCP in combination with

$CaCl_2$, compared to the control. In general, the total polyphenol content in control fruits was approximately 4 and 3 times higher than that in 1-MCP+$CaCl_2$ treated fruit in cvs "Mission" and "Manzalina", respectively.

Chlorophyll fluorescence

Changes in chlorophyll fluorescence (Fv/Fm) were observed during olive storage in all treatments (Fig. 3A). Both cultivars improved and showed greater value of Fv/Fm compared to the control. Olive fruits treated with 1-MCP and $CaCl_2$ individually or in combination after 12 weeks of storage showed significantly ($P<0.05$) greater value in Fv/Fm than the control fruits.

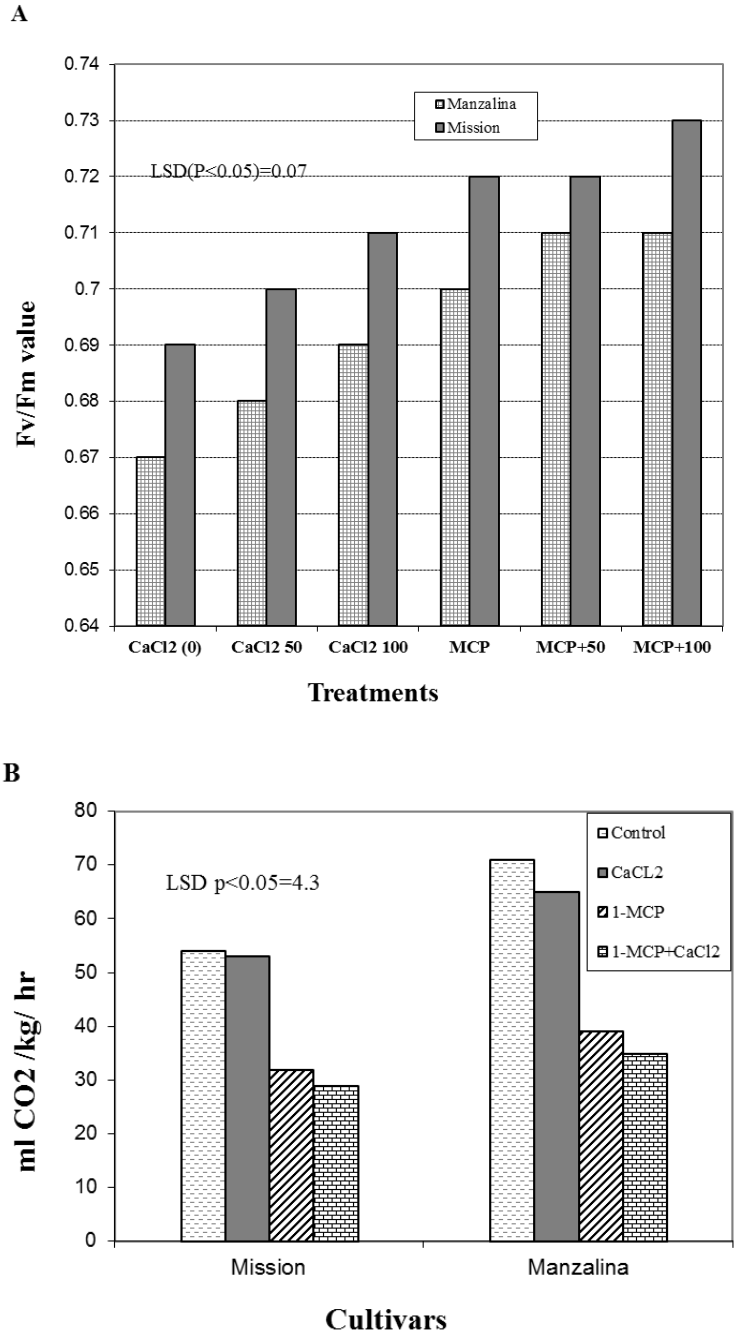

Figure 3. Effect of 1-MCP (1.8 µL/L) and $CaCl_2$ (mM) on F_v/F_m value (A) and respiration (B) of olive fruits after 12 weeks of storage at 6ºC

Respiration rate

Both cultivars showed a high initial respiration rate, which declined with either 1-MCP or $CaCl_2$ treatments (Fig. 3B). However, no significant differences were found in fruits' respiration between control and in calcium-treated fruits alone after 12 weeks of storage at 6ºC but, significant differences ($P<0.05$) were observed in respiration rate when fruits were treated with 1-MCP, compared to un-treated fruits. The fruits treated with 1-MCP reduced respiration rate to *ca*. half of the control. Subsequent reductions in respiration rate were observed when 1-MCP was combined with $CaCl_2$, but the differences were not statistically significant.

Discussion

The results of this study regarding the effect of 1-MCP on quality of olives are in agreement with previous reports by Ramin (4) in four cultivars of olives. Generally, skin color changed from green at the beginning of the treatment to green-red at the end of storage. Red color development is detrimental to these fruits as only green fruits can be processed. The non-treated fruits showed more changes in green color (a* value) in comparison with those in 1-MCP treatment. 1-MCP treated fruits in combination with $CaCl_2$ were more effective in maintaining the green color of skin than other treatments. Use of 1-MCP was able to maintain fruit color and this behavior seems to be a general effect of 1-MCP in most studied fruits (13, 14). In many fruits and vegetables, color development was accelerated by ethylene. Moreover, ethylene is involved in de-greening of fruits and many horticultural crops. 1-Methylcyclopropene inhibits ethylene action by blocking its receptor for extended periods and delays maturity and ripening (2).

In olives, postharvest treatment, 1-MCP, and calcium applied by dips may act synergistically to maintain or even enhance the initial firmness values of the fruits during storage. The results of this study are in agreement with previous reports by Ramin (4) about olives and Blankenship and Dole (2) concerning other fruits. Wang *et al*. (15) reported that 1-MCP treatment significantly delayed the decrease of firmness, total soluble solids, titratable acidity, inhibited increase in weight loss, suppressed the increase in respiration rate and ethylene production. Similar effects have been found in apple by different authors (16). The effect of calcium might be direct, by promoting the formation of calcium bridges among pectin molecules, which might increase the rigidity of the wall (17) or indirect by reducing wall porosity and limiting the mobility of cell wall polysaccharide-degrading protein (18, 19). Mackvandi *et al*. (20) evaluated the effects of postharvest application of 1-MCP and $CaCl_2$ dip or their combination on storage quality of mature-green olives and concluded that the combination of 1-MCP and $CaCl_2$ had synergistic effect on preventing fruit softening.

The chlorophyll fluorescence technique has been found useful for predicting the quality of a wide range of fruits and vegetables (21). Early measurement showed a sustained decrease in dark-adapted (Fv/Fm) and an increase in (Fv/Fm), indicating the occurrence of environmental stress in fruits and vegetables (22, 11).

Both cultivars (Mission and Manzalina) showed a high initial respiration rate, which declined with either 1-MCP or $CaCl_2$ treatments. Fruit respiration might have increased in response to fruit damage, and the reduced production of CO_2 by calcium or 1-MCP-treated olives could be associated with reduced tissue disruption (23).

In this study, the control and $CaCl_2$ treated fruits had higher C_2H_4 production rates compared to fruits exposed to 1-MCP, with or without a $CaCl_2$ dip. This may be partly due to possible microbial growth and general deterioration of the tissue as a result of senescence in these treatments (10). Also, Bower *et al*. (24) observed that 1-MCP treatment (0.01, 0.1 or 1.0 µL/L) reduced C_2H_4 production and the increased emission of CO_2 by 1-MCP treated strawberry was associated with the earlier onset of rot.

During a 12-week storage of Mission and Manzalina cultivars at 6ºC, phenolics increased in control fruit, while less polyphenol accumulation was detected in $CaCl_2$ and 1-MCP treated fruits. It is known that free phenolics are present mainly in the vacuole of cells. However, they are synthesized in the cytoplasm and may also be deposited in cell walls.Low-temperature storage treatments induce membrane damage in cell organelles, such as vacuoles, the vacuolar phenolics might possibly make contact with PPO, thus causing browning reaction in plant materials (25).

Conclusion

Olive cultivars' "Manzalina" and "Mission" can be stored for several weeks when treated with 1-MCP, and this could be extended when fruits are treated with (50-100 mM) $CaCl_2$ before storage.

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