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Effects of 1-methylcylopropene (1-MCP) and Modifed Atmopshere Packing (MAP) on postharvest browning and microbial growth of loquat fruit

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Summary

"Champagne de Grasse" loquat fruits were initially treated with 0 (control), 312.5 ppb and 625 ppb of 1-methylcylopropene (1-MCP) for 6 h at 20°C. After 6 h, treated fruits were divided into the two main groups: within the first main group, 1-MCP treated fruits were placed in Modifed Atmopshere Packing (MAP) and stored for 12 days at 5°C with 90% of relative humidity (RH). Control fruits without exposure to 1-MCP were placed in MAP and maintained under the identical storage conditions. Within the second main group, 1-MCP treated fruits were subjected to two different storage temperatures. The first were placed in trays and then kept for 12 days of storage at 5°C with 90% of RH and the second group was stored for 9 days at 20°C with 60% of RH. Similarly, the control not subjected to 1-MCP treatment was maintained under the same condition. Treatment groups and control were examined for the following parameters: the flesh fruit firmness, total soluble solid (TSS) content, titratable acidity (TA), color value (L and Chroma), browning index and microbial quality. Unpacked 1-MCP treatments (312.5 and 625 ppb) improved the storage quality of loquat fruits, but, the most efficient treatment was achieved with 625 ppb of 1-MCP. However, the combination of 1-MCP and MAP revealed a better effect on maintaining the fruit quality as indicated by the delay of softening, deterioration rates, TA, browning rate and the significant inhibition of total aerobic mesophiles, psychrotrophic aerobic bacteria, yeast and molds during the whole storage period. The results indicated that the most efficient application in all treatments was obtained using the combination of 1-MCP (625 ppb) with MAP at 5°C storage.

Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a member of the Rosaceae family that is widely cultivated in the subtropical regions of China, Japan, India, Israel and the Mediterranean area (Shaw, 1980). Turkey is one of the largest loquat producers known, dating back to over 30 years. Approximately 96% of the total production of loquats is primarily concentrated in the two provinces of Mediterranean region: Antalya and Mersin. A large proportion of fruits produced in Turkey are consumed mainly as fresh fruit and are also exported to Kuwait, Saudi Arabia, Russia, Jordan, Germany and other European countries (Karadeniz et al., 2009). Most fruits such as loquat fruits are highly perishable commodities, susceptible to decay, mechanical damage, moisture losses and microbial decay during the postharvest life. Consumer interest worldwide in the quality of fresh products has increased in recent years. Product quality is a complex issue, since it includes visual characteristics, physical properties such as texture, mineral and vitamin content, flavor, and other organoleptic characteristics. Once fruits are harvested, postharvest handling practices do not usually improve on the quality attained in the field; they only may slow the rate at which deterioration occurs (Fallik, 2008). Packaging is one of the most important processes to maintain the quality of food products for storage, transportation and end-use (Han, 2005). Modified Atmosphere Packaging extends the shelf life of food products and prevents (or at least retards) any undesirable changes in the wholesomeness, safety, sensory characteristics, and nutritive value of foods. 1-Methylcyclopropene (1-MCP) is an inhibitor of ethylene perception that binds irreversibly to the ethylene binding protein. It is currently known to have commercial applications for maintaining the storage life of horticultural products, with highly effective, with nontoxic and odorless properties (Sisler and Serek, 1997). Although ethylene-independence was observed during the ripening of some non-climacteric fruits, several studies revealed that ethylene has extended the storage life of some non climacteric fruits including cherries, citrus and strawberries (Shaw, 1980). The efficacy of 1-MCP treatment or MAP storage has been previously evaluated for their ability to maintain loquat fruit quality (Ding et al., 1998; 2002; Cai et al., 2000). However, literature surveys indicated that the combined effect of 1-MCP and MAP on quality attributes and shelf life of loquat fruits has not been investigated yet. Therefore, the purpose of this study was to determine the combined effect of 1-MCP and MAP on changes in fruit quality (firmness, skin color, TSS, pH, TA, browning rate), and shelf life of 'Champagne de Grasse' loquat fruit during refrigerator storage at 5°C and room temperature at 20°C.

Materials and methods

Fruit, treatment and storage

"Champagne de Grasse" loquat fruits were hand-harvested at ripe stage according to fruit skin color (fully yellow). The loquats were obtained from an orchard in district Erdemli (Mersin) and held in a cardboard box and alveolar trays during transport to the laboratory and analyzed within 3 h of harvest. Loquats were graded according to visual defects and uniformity of shape, size and color. Fruits were placed in 1 m^3 containers before the beginning of the 1-MCP treatment. Commercially available 1-MCP tablets were obtained from AgroFresh Inc./Rohm and Haas Company and the desired concentration of 1-MCP was prepared according to the manufacturer's instruction (EthylBloc, Bio-Technologies for Horticulture, Walterboro, SC). Briefly, each individual 1-MCP tablet including 312.5 ppb of 1-MCP was added into 15 mL of solution. The recommended dose of two tablets for upper concentration was also used in this investigation.

Afterwards, the tube containing 1-MCP tablet plus solvent was shaken thoroughly and immediately transferred to the polyethylene container. The ventilation process for each 1-MCP treatment in the polyethylene container including airtight lid were carried out for 6 h at 20°C and 60% relative humidity. Upon exposure to 1-MCP treatments, one set of fruits was divided and maintained under the following conditions: I) 312.5 ppb of 1-MCP+unpacked state; II) 625.5 ppb of 1-MCP+unpacked state; III) not treatment with 1-MCP (control)+unpacked state. After treatment, all unpacked loquats (treated and control) were stored for 12 days at 5°C with 90% RH and for 9 days at 20°C with 60% of RH. Unless otherwise stated, analyses of unpacked 1-MCP and control fruits were examined on

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the day of 0 and after 3, 6, and 9 days of storage at 20°C. In addition, all fruits stored at 5°C were examined on the day of 0 and after 4, 8, and 12 days of storage. The other set of loquats subjected to the same 1-MCP treatment as mentioned above were divided and maintained under the modified atmosphere packaging (also called polyethylene bulk liner, 30μ /p-plus antimist, 700X700 in bag size) : I) 312.5 ppb of 1-MCP+MAP; II) 625.5 ppb of 1-MCP+MAP; III) not treatment with 1-MCP (control) + MAP. 1-MCP+MAP and control fruits were stored for 12 days at 5°C with 90% RH. The fruits were taken and examined on the day of 0, 4, 8, and 12 in order to determine and compare the effects of MCP treatments+MAP and control stored at 5°C.

Fruit firmness and total soluble solids

Fruit firmness was determined by selecting 10 fruits per treatment at intervals during storage period. Fruit firmness was measured using a FHR-1 (1 kg) firmness tester (Nippon optical works CO. LTD.- Tokyo-Japan) equipped with a cylinder type probe (5-mm diameter). Measurements were made on two opposite sides of each fruit after removal of a small piece of peel. The total soluble solids (TSS) content (%) of loquat juice was measured with a hand held refractometer (Krüss-Germany). The results were expressed as % at 20°C.

Titratable acidity

Loquat pulp (40g) was homogenized in 50 ml of distilled water. Titratable acid (TA) content was determined by titration with 0.1 N NaOH to pH 8.1, and the results were expressed as percentage of malic acid (Mirdehghan, 2007).

Color value

Fruit skin color of loquat fruit was measured by two readings on the two different symmetrical faces of the fruit in each replicate, using a Konica Minolta (CR-400) chroma meter and colorimeter calibrated with a white standard tile. Results were expressed as a skin color as described Chroma value from $(a^{*2}/b^{*2})^{1/2}$ value.

Browning index

Browning index was observed every 2 day for 12 days for 4°C storage by measuring the extent of browning area as described by Akhtar et al. (2010). Using 30 fruits on the following scale: Using 30 fruits on the following scale: 0= no browning; 1 = less than ¼, 2 = ¼ to ½ browning; 3 = ½ to ¾ browning; 4 = more than ¾ browning; the browning index was calculated using the following formula:

Browning index= [1 x N1 + 2 x N2 + 3 x N3 + 4 x N4] x 100

Microbiological analyses

Total aerobic mesophiles, psychrotrophic aerobic bacteria, yeast and mold counts were enumerated to determine the combined effect of unpacked 1-MCP and 1-MCP+MAP treatments. For microbiological analysis, aseptically deseeded loquat fruit (25g) was homogenized for 2 min in 225 ml of sterile peptone water (0.1%, w/v) with a Stomacher (Interscience). The homogenized samples were subjected to ten fold dilution using the same diluent and pipetted into a sterile petri dish from each dilution, then appropriate melted agar poured in and mixed with the sample. The total aerobic mesophilic count was determined on Plate Count Agar (30°C for 3 days). The psychrotrophic count was enumerated on Plate Count Agar (5°C for 10 days). The yeast and mold count was enumerated on Potato Dextrose agar (25°C pH 3.5, for 5 days). Three samples per treatment were aseptically taken and analyzed during the storage at intervals. The results were then reported as log cfu/g.

Statistical analysis

Experiments were performed using a completely randomized design with 10 replicates per treatment. Three kg fruit was used in each MAP packed. All statistical analyses were performed with SPSS 13.0 software for Windows. The results were obtained using GLM multivariate procedures which provide analysis of variance by one or more factor variables. Means were compared by the least significant difference tested at significance levels of ($P < 0.05$).

Results and discussion

Fruit firmness

Application of 1-MCP (312.5 and 625 ppb) treatments delayed the softening of loquat fruits when they were kept both unpacked and in MAP storage for the first eight day of storage. Additionally, MAP without 1-MCP treatment had the same effect as the ones mentioned above. The effect of 1-MCP treatment in combination with MAP on the firmness of loquat fruit during 5°C storage was statistically different compared to the control. As shown in Fig. 1 and Fig. 2, the strongest effect on fruit firmness was obtained by 1-MCP (625 ppb)+MAP at 5°C storage when compared to unpacked 1-MCP (312.5 ppb) and control stored at 5°C and 20°C. Fruit firmness in the present work softened much more rapidly in unpacked control than in unpacked 1-MCP treated fruits at 5°C and 20°C, and 1-MCP+MAP treated fruits at 5°C storage.

The results in this work are in accordance with the results of Tian et al. (2000), Jiang et al. (2001) and Ding et al. (2002), who observed a significant delay of senescence associated with the inhibition of ripening rate and the loss of fruit flesh firmness when used with 1-MCP+MAP at low storage temperature.

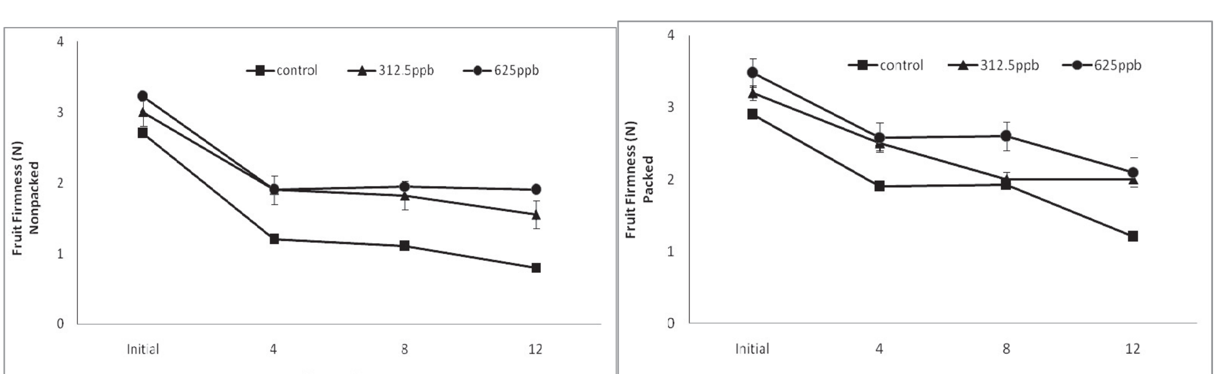

Fig. 1: Changes in fruit flesh firmness of loquat fruit stored at 5°C for 12 d after harvest. Bars indicate ± S.E.

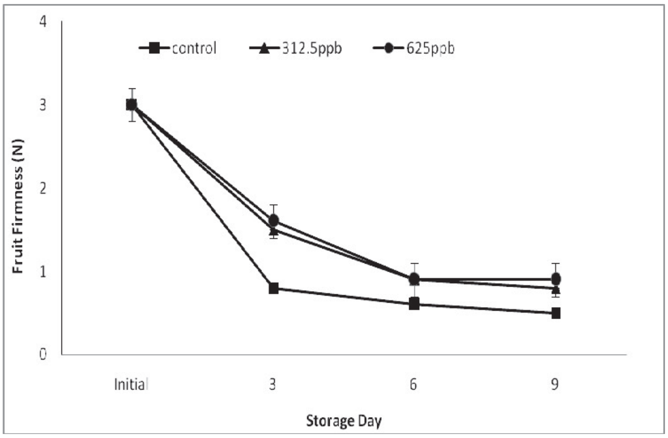

Fig. 2: Changes in fruit flesh firmness (N) of loquat fruit stored at 20°C for 9 d after harvest. Bars indicate ± S.E.

and other treatments applied. In contrast to the effects of 625 ppb of 1-MCP+MAP treatment at 5ºC, higher TSS content was obtained from 625 ppb of 1-MCP treatment at 20ºC (Fig. 4). TSS content of unpacked 312.5 and 625 ppb of 1-MCP treatments and control stored at 20ºC increased continuously up to 6 days of storage. However, this continuous increase declined slightly in unpacked 312.5 ppb of 1-MCP treatment and control. High TSS content in fruits is affected by several factors such as dehydration of fruits, treatment conditions, packaging material type and/or maturity stage at harvest, as suggested by Cai et al. (2006). In previous studies, it was also indicated that TSS value varied from fruit to fruit, treatment and/or storage conditions. For example, Bregoli et al. (2005) found the low TSS value of 1-MCP treated nectarine fruit stored at low temperature, while Watkins (2006) reported variable results of TSS content in relation to 1-MCP treated and untreated fresh fruits. In addition to those studies, Cai et al. (2006) also observed that TSS contents increased initially and then decreased in the control, 0.5 and 50 μL/L 1-MCP treated fruits stored at 20ºC for 8 days. The results of the present study did not differ from the findings of Bregoli et al. (2005) and Cai et al. (2006).

Soluble solids content

TSS content was determined using the control fruits, and after 1-MCP (312.5 and 625 ppb) treatment on fruits for 6 h at 20ºC. At the initial day, the lowest TSS content was obtained by 625 ppb of 1-MCP treatment among all applications (Fig. 3). Treatment of 312.5 ppb of 1-MCP+MAP, 312.5 and 625 ppb of 1-MCP+ unpacked and control fruits at 5ºC increased its TSS content for the first eight days but then decreased sharply. As shown in Fig. 3, 625 ppb of 1-MCP+MAP for 12 days at 5ºC significantly lowered the TSS content of loquats when compared to unpacked 625 ppb of 1-MCP

Titratable Acidity

The primary organic acid in loquat fruit is malic acid, which is the principal flavor component. Malic acid accounts for approximately 90% of the total acid contents in loquat fruit. At the initial day, 1-MCP (312.5 and 625 ppb) treated fruits after 6 h and the control were examined for titratable acidity (TA). TA of 625 ppb 1-MCP treated fruits was higher than 312.5 ppb 1-MCP treated fruits and control. Whereas, TA content of 312.5 ppb 1-MCP treated fruits + MAP and control at 5ºC increased up to the first fourth day of storage, then,

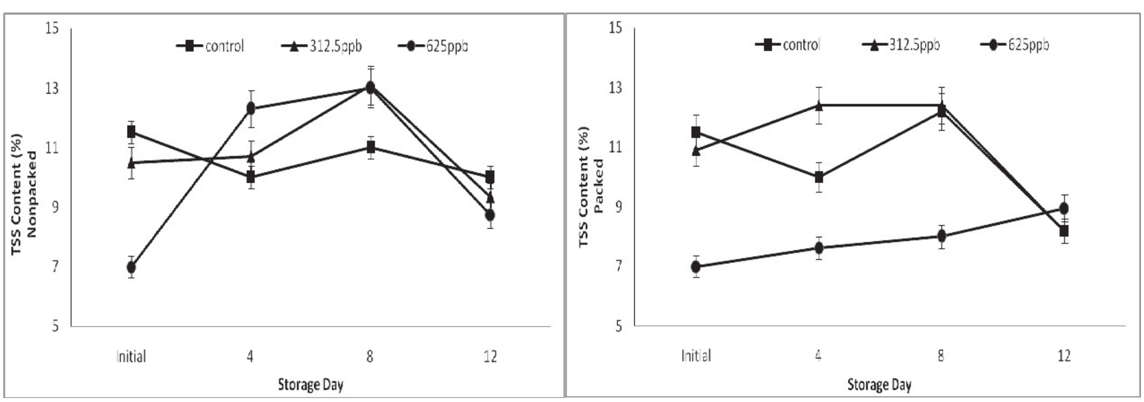

Fig. 3: Changes in fruit total soluble solids of loquat fruit stored at 5°C for 12 d d after harvest. Bars indicate ± S.E.

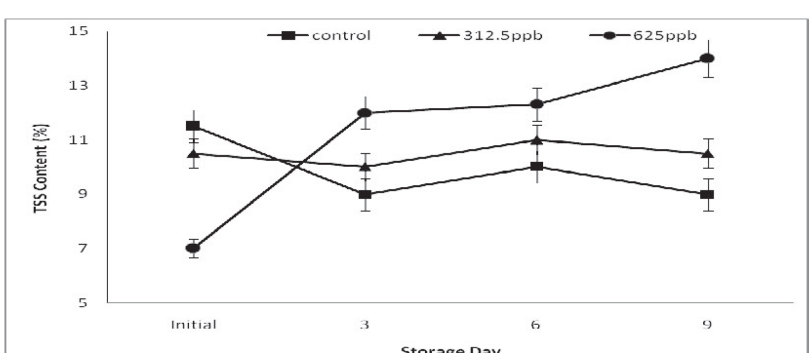

Fig. 4: Changes in fruit total soluble solids of loquat fruit stored at 20°C for 9 d after harvest. Bars indicate ± S.E.

this content decreased in control except 312.5 ppb 1-MCP treated fruits + MAP (Fig. 5). For the first six days of 20ºC storage, TA content of 1-MCP (312.5 and 625 ppb) treated fruits decreased significantly and was less than that of the control, but then increased with the storage time (Fig. 6). The effect of 1-MCP, MAP and storage temperature and the combination of those factors on TA value was statistically different. The present result indicated that 1-MCP (625 ppb)+MAP effectively reduces the loss of TA, as shown in Fig 5. It has been previously suggested that the decrease of TA value in some fruits treated with 1-MCP depends on the concentration of organic acids present in fruit, conversion of acids to sugars in fruits during respiration. The decrease in TA value in the present work may be due to these factors as stated by previous authors (DING et al., 1998; BREGOLI et al., 2005; WATKINS, 2006).

Fruit color changes

Changes in color intensity and quality are important indicators of maturity and quality for fresh loquat. Brightness (L) value, red intensity, and the yellow intensity were measured using a Minolta chromameter (*L*, *a, b*) and these values were used to calculate the chromaticity parameters: fruit lightness (L), chroma values. Fruit L* and Chroma values increased in 1-MCP (312.5 and 625 ppb)+MAP and control+MAP at 5°C up to 8 days and results were expressed in Fig. 7 and Fig. 8. In contrast, those values in all treatments decreased significantly with the ripening at 5ºC after 8 days of storage. 1-MCP (625 ppb)+MAP kept fruit chroma values lower than 1-MCP (312.5 ppb) and control at 5°C. At 20°C, L value of unpacked 1-MCP (312.5 ppb) and control decreased until 3 days of storage. While this value started to increase towards the end of storage. Unpacked 1-MCP (625 ppb) treatment in all applications and storage temperatures made the fruit lightness color value higher and/or longer. Unpacked 1-MCP (625 ppb) treatment on chroma value up to 6 days at 20°C appeared to be higher than the results obtained by unpacked 1-MCP (312.5 ppb) treatment and control (Fig. 9). Towards the end of storage, treatments of 1-MCP (312.5 and 625 ppb) and control groups showed approximate values as shown in Fig. 9. DING et al. (2002) reported that MAP storage inhibited fruit color changes slightly. But the result of 1-MCP treatment with or without MAP showed no change on fruit skin color. Towards the end of storage, it appeared that all results revealed nearly approximate values. In a similar study by AGUAYO et al. (2006), it was reported that skin luminosity (*L**) increased in correlation with the time of storage. 1-MCP treatment during storage was found to be significant for both the *a*/*b* ratio and *L** color parameters. Similar results were also reported in other fruits such as apple, pineapple. (BUDU and JOYCE, 2003).

Browning Index

The effects of 1-MCP treatment and MAP storage on browning index at 5°C and 20°C storage are presented in Fig. 11 and Fig. 12. There were significant differences between the combination of 1-MCP (625 ppb) with MAP and other treatments. The present study showed that value of browning of unpacked loquat fruit increased during the storage at 5°C for 12 d and 20°C for 9 d (Fig. 11 and Fig. 12). But, browning index seemed to be higher at 20°C. The best result for browning index in the present study was obtained in 625 ppb of 1-MCP treated fruits at 5°C for 12 d compared to other treatments. The browning index value was statistically significant

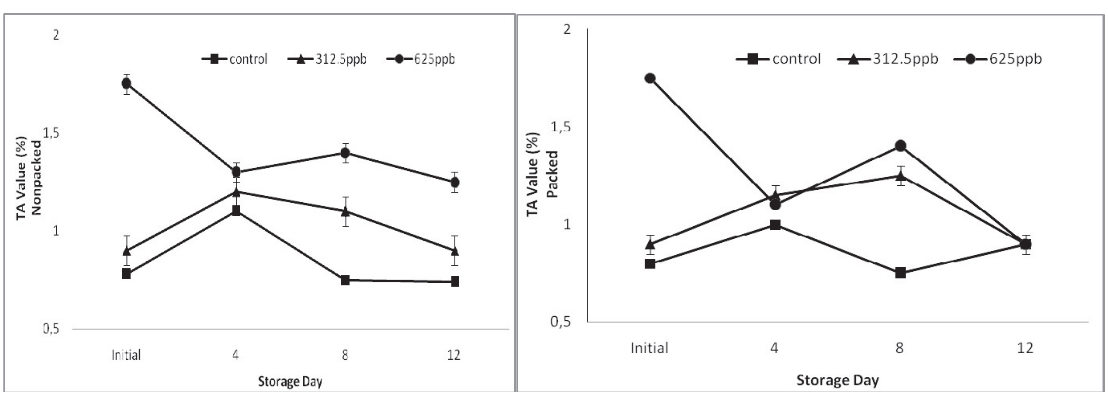

Fig. 5: Changes in fruit TA value of loquat fruit stored at 5°C for 12 d after harvest. Bars indicate ± S.E.

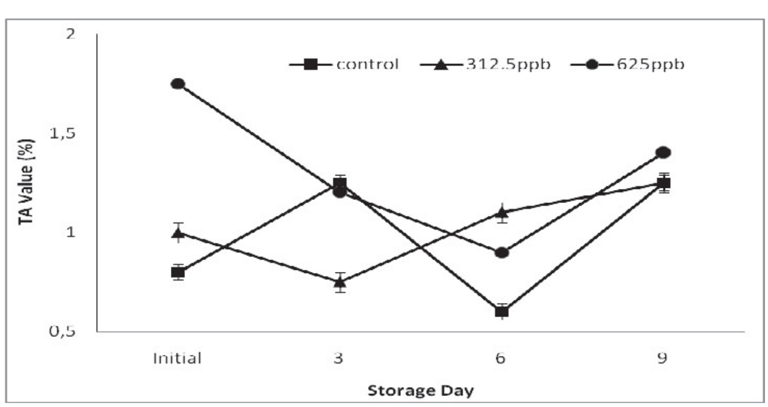

Fig. 6: Changes in fruit TA value of loquat fruit stored at 20°C for 9 d after harvest. Bars indicate ± S.E.

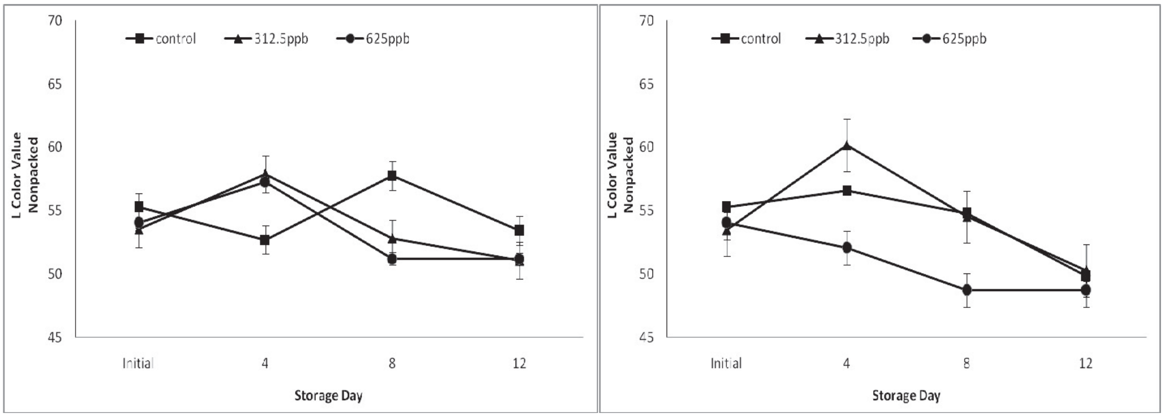

Fig. 7: Changes in fruit L value of loquat fruit stored at 5°C for 12 d after harvest. Bars indicate ± S.E.

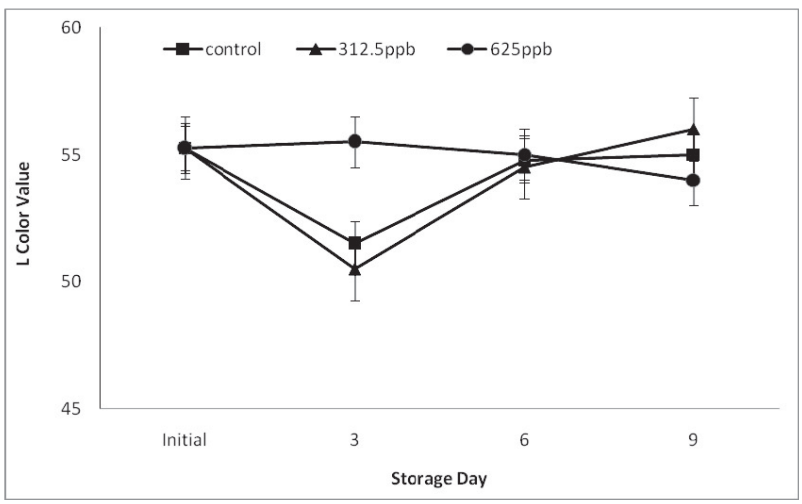

Fig. 8: Changes in fruit L color value of loquat fruit stored at 20°C for 9d after harvest. Bars indicate ± S.E.

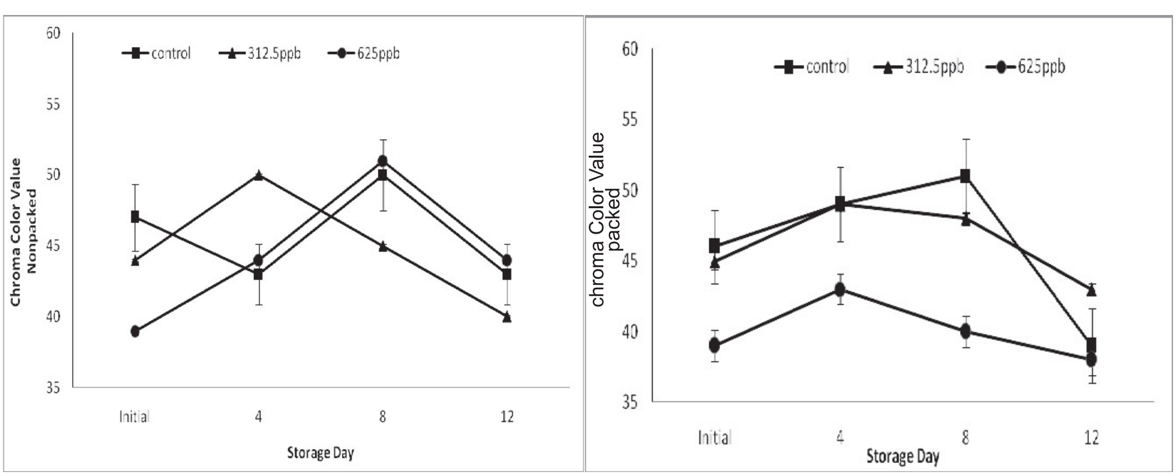

Fig. 9: Changes in fruit chroma color value of loquat fruit stored at 5°C for 12 d. Bars indicate ± S.E

at 1-MCP and/or MAP storage and the interaction between factors during storage at 5°C and 20°C. Two concentrations of 1-MCP and MAP reduced the browning index and additionally improved the visual quality maintenance of loquat during storage. This findings showed an accordance with the study of CAI et al. (2006), who also reported that 0.5 and 5 µl /L 1-MCP treatment had a reducing effect on fruit browning index. In an another study by DING et al. (1998), it was reported that loquat fruit retained its initial quality and chemical

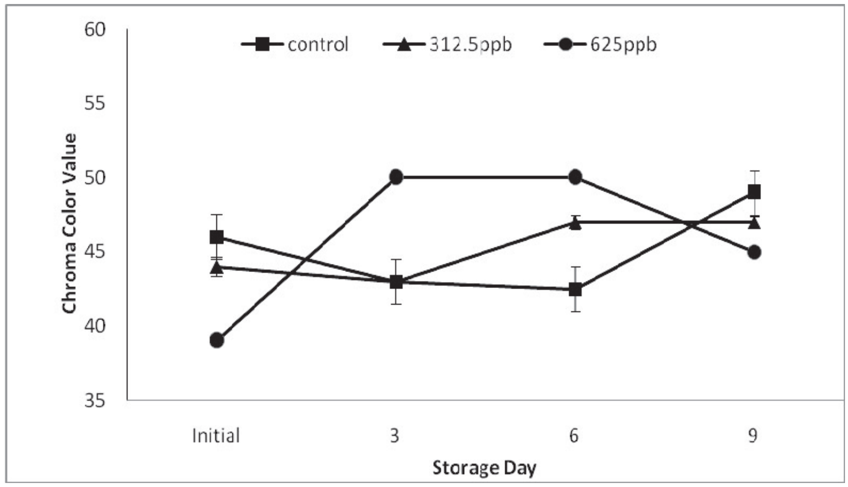

Fig. 10: Changes in fruit chroma color value of loquat fruit stored at 20ºC for 9d. Bars indicate ± S.E

components in 0.15% perforated polyethylene film packaging at 1°C and 5°C, which showed an accordance with finding of the present study. On the other hand, GUELFAT-REICH (1970) indicated that polyethylene wraps increased internal browning and postharvest rotting of loquat fruit.

Previous studies suggested that browning index value showed some kind of changes according to fruit variety, modified atmosphere packing material thickness, treatment and/or storage conditions. For instance, the effect of MAP on the browning tendency of loquat during 5ºC storage was observed by examining the Hunter L value in the report of DING et al. (2002). And the results showed that there was a rapid decrease when Hunter L value was measured. The result of the present study was similar to the findings of DING et al. (2002). Besides that, in the present study, L values revealed some differences between treatments during storage, but, it gave very close values for all treatments towards the end of storage.

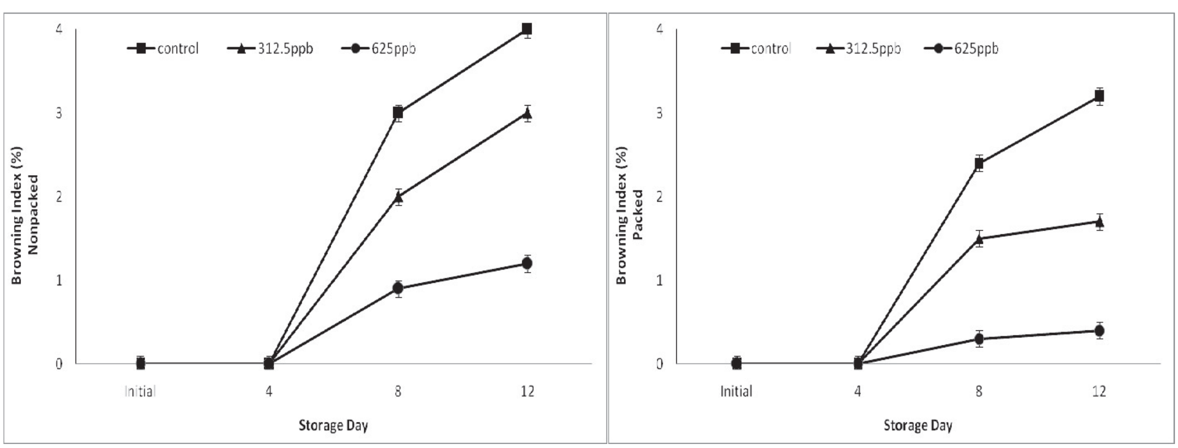

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Fig.11: Changes in fruit browning index of loquat fruit stored at 5°C for 12 d after harvest. Bars indicate ± S.E.

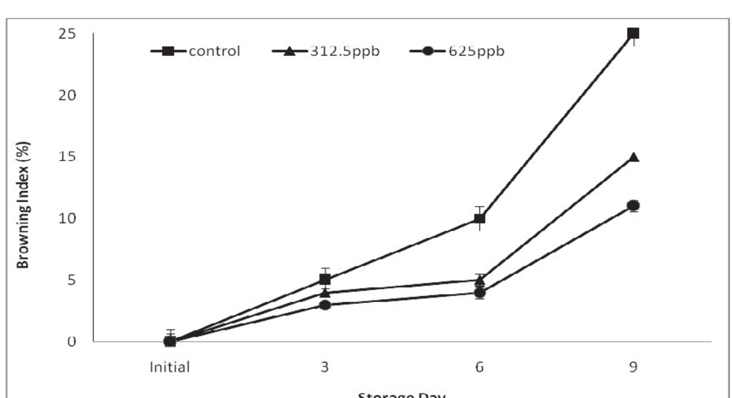

Fig. 12: Changes in fruit browning index of loquat fruit stored at 20°C for 9 d after harvest. Bars indicate ± S.E.

Microbial Count

Total aerobic mesophilic count (TMC) for the MAP and unpacked loquat stored at 5°C for 12 days and 20°C for 9 days, respectively were shown in Fig. 13 and Fig. 14. TMC (log CFU/g) of unpacked loquat fruits at 5 and 20°C significantly increased during the storage (Fig. 13 and Fig. 14). Combination of 1-MCP (625 ppb) + MAP suppressed the total aerobic mesophiles up to the four days of storage at 5°C in comparison to 1-MCP (312.5 ppb) treatment and the control. MAP significantly suppressed the growth of total aerobic mesophiles than unpacked one at 5°C

Total yeast and mold count (log CFU/g) of unpacked loquat stored at 5°C for 12 days and 20°C for 9 days increased significantly as shown in Fig. 15 and Fig.16. However, total yeast and mold count of 1-MCP (625 ppb) + MAP loquat fruit stored at 5°C for 12 days remained negligible compared to 1-MCP (312.5) + MAP and control + MAP (Fig. 15). In addition, unpacked 1-MCP (625 ppb) at 20°C during the 9 days of storage inhibited significantly the growth of yeast and molds when compared to unpacked 1-MCP (312.5 ppb) and control group (Fig. 16).

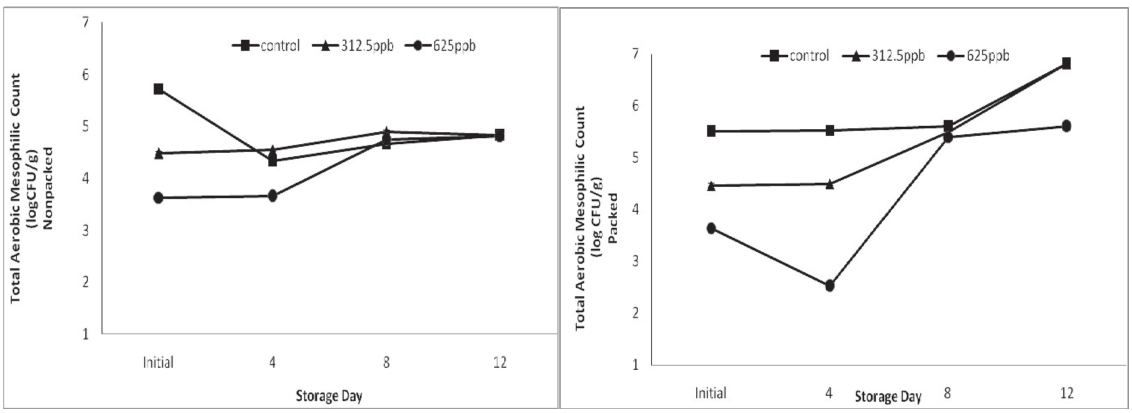

Fig. 13: Changes in total aerobic mesophilic count (log CFU/g) of loquat fruit stored at 5°C for 12 d. Bars indicate ± SE

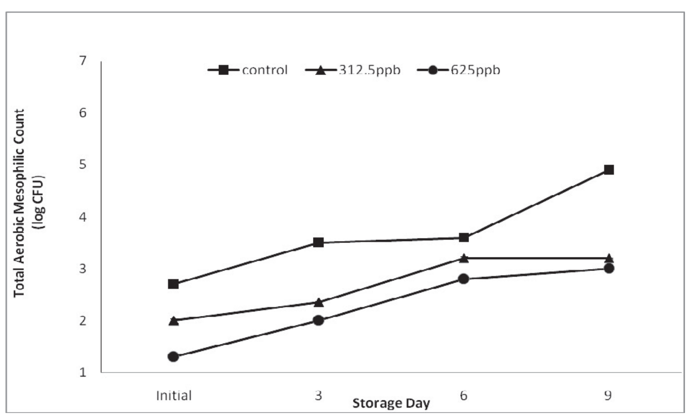

Fig. 14: Changes in total aerobic mesophilic count (log CFU/g) of loquat fruit stored at at 20°C for 9 days. Bars indicate ± SE

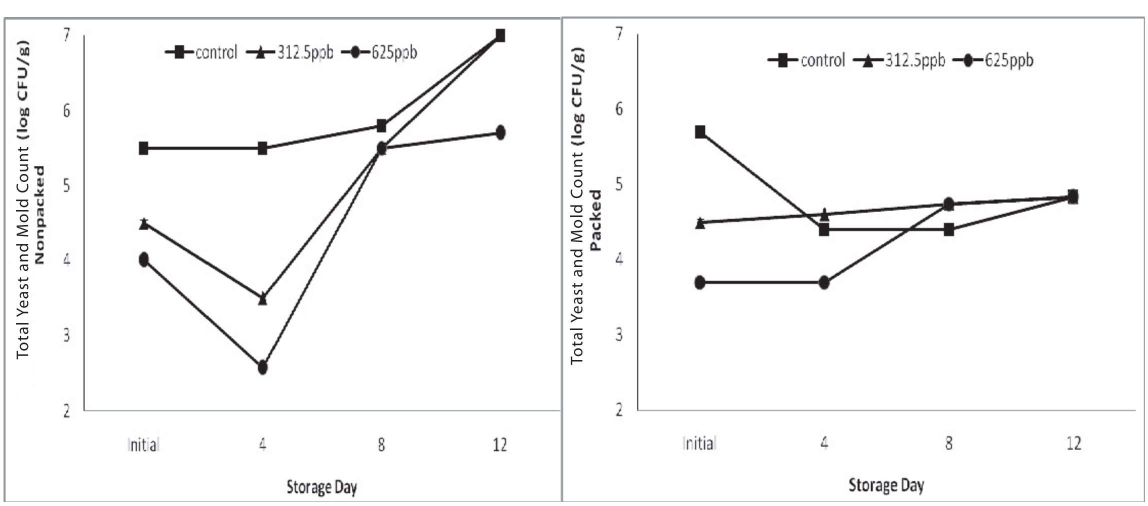

Fig. 15: Changes in total yeast and mold count (log CFU/g) of loquat fruit stored at 5°C for 12 d after harvest. Bars indicate ± SE.

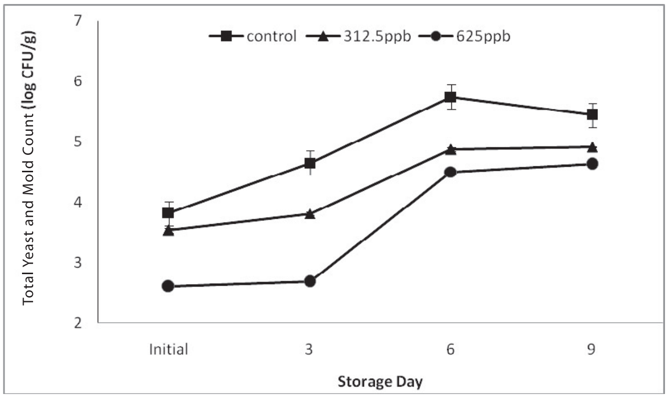

Fig.16: Changes in total yeast and mold count (log CFU/g) of loquat fruit stored at 20°C for 9 d after harvest. Bars indicate ± SE.

Total psychrotrophic bacterial count in unpacked samples increased during 12 days of storage at 5°C (Fig. 17). 1-MCP + MAP and unpacked 1-MCP treatments significantly suppressed the growth of psychrotrophs (Fig. 17). 1-MCP+MAP treatment (especially 625 ppb) stored at 5°C for 12 days significantly decreased psychrotrophic aerobic bacteria count (log CFU/g) of loquat fruit compared to control+MAP treatment. 1-MCP (312.5 and 625 ppb) + MAP reduced the growth of psychrotrophic aerobic bacteria for 4 days after treatment, whereas, population of psychrotrophic aerobic bacteria of MAP control and unpacked control showed an increase during all the storage. Specifically, the combination of 1-MCP (625 ppb)+MAP appeared to be more effective in inhibiting the growth of microorganisms compared to other treatment.

In earlier studies, 1-MCP, MCP+MAP and /or other applications on the growth of microorganism varied with several application parameters including concentration, time, temperature and the nature of commodity (De Ell et al., 2001; Gong et al., 2002). For example, two different research groups indicated that 1-MCP treatment has no beneficial effect in inhibiting the growth of microorganisms on pineapple slices (Budu and Joyce, 2003; Rocculi et al., 2009). Nonetheless, Rupasinghe et al. (2005) observed the beneficial effect of 1-MCP (1 µl L-1 for 24 h) treatment on Empire apples and apple slices, and they reported that the growth of total microorganisms decreased when apple/apple slices were exposed to 1-MCP. The beneficial effect of 1-MCP (for 24 h at 5°C + $CaCl_2$) and the combination of 1-MCP + $CaCl_2$ and CA on fresh-cut strawberries was also observed by Aguayo et al. (2006) who reported that all treatments slowed down the microbial (psychrotrophic and yeast) growth. Moreover, the synergistic effect of 1-MCP and modified atmosphere packaging (MAP) on various fruits such as cantaloupe, honeydew melon, mango and pineapple has been also shown to reduce the growth of molds, mesophiles and psychrotrophic microorganisms (Portela and Cantwell, 1998; Qi et al., 1999; Rattanapanone et al., 2001; Liu et al., 2007). The present results appeared to be in parallel with the findings of Portela and Cantwell (1998); Qi et al. (1999); Rattanapanone et al. (2001); Rupasinghe et al. (2005) Aguayo et al. (2006), Liu et al. (2007).

Conclusion

What the present study suggests is that the combination of 1-MCP and MAP in accordance with low storage temperature delayed senescence processes and at the same time it kept fruit flesh firmness within acceptable range. Furthermore, 1-MCP (625 ppb) treatment with MAP reduced the fruit ripening, browning rate, growth of total aerobic mesophiles, yeast/mold and psychrotrophic aerobic bacteria.

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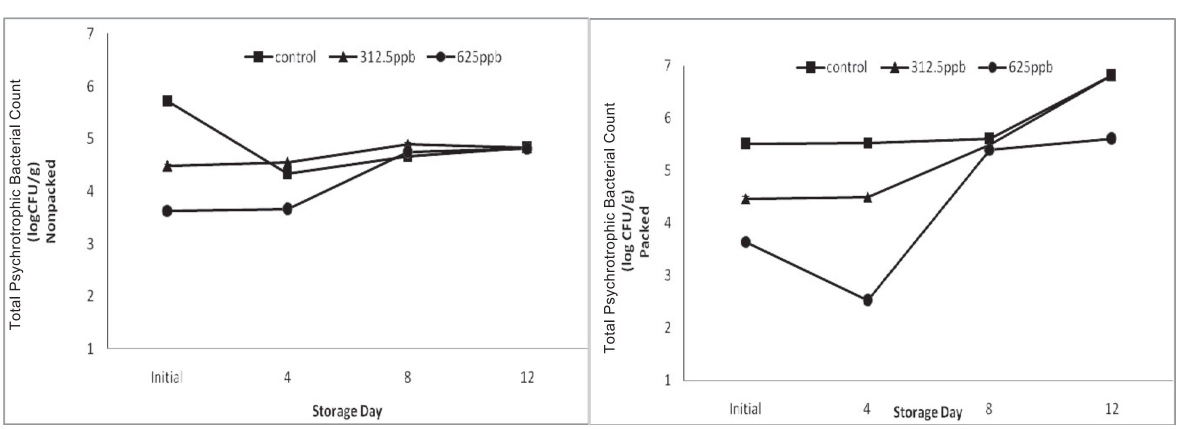

Fig. 17: Changes in psychrotrophic aerobic bacteria count (log CFU/g) of loquat fruit stored at 5°C for 12 d after harvest. Bars indicate ± SE.

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